Distinctiveness : Aurangabad

Organ and Tissue Transplant Programme

Government Recognitions for Organ Transplantation

Government of Maharashtra

सत्यमेव जयते
महाराष्ट्र शासन

Office of the Appropriate Authority

Certificate of Renewal of Registration

No. DHS / THOA / MGMHOSPAUR/KIDNEY **/ F.No** 63 **/ D-20 / 20** 15

This is with reference to the application, dated 13/05/2015 from MAHATMA GANDHI MISSION MEDICAL COLLEGE & HOSPITAL AURANGABAD for renewal of certificate of registration for performing Organ Transplantation, under the Act.

After having considered the facilities and standards of the above-said hospital, the Appropriate Authority hereby renews the Certificate of Registration of the said hospital for the purpose of performing KIDNEY Organ Transplantation for a period of five years from the date of issue.

संचालक आरोग्य सेवा, महाराष्ट्र राज्य, पुणे

Mumbai:

Date: 14/10/2015

Appropriate Authority
and
Director Health Services,
Maharashtra State, Mumbai

समुचित प्रा
तथ
संचालक आर
महाराष्ट्र राज्

Government of Maharashtra

सार्वजनिक आरोग्य विभाग

सत्यमेव जयते
महाराष्ट्र शासन

Certificate Of Registration

In exercise of the power conferred by Sub-Section (1) of section 24 of the Transplantation of Human Organ Act, 1994 (42 of 1994), the Appropriate Authority hereby certify that M.G.M. Medical College and Hospital located at _____ has been inspected by the Appropriate Authority & Certificate of Registration is granted for performing the organ transplantation of the following organs.

1. Kidney
2. —
3. —
4. —

This certificate of registration is valid for a period of 5 years from the date of issue.

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Mumbai

Date 28/7/2010

Appropriate Authority
Director, Health Services,
Maharashtra State, Mumbai

आरोग्य सेवा संचालनालय
(महाराष्ट्र राज्य)
" आरोग्य भवन ", सेंट जॉर्जेस रुग्णालय आवार, पी.डिमेलो रोड, मुंबई- ४०० ००१

	दूरध्वनी	
कार्यालय	२२६२१०३१-३६	Website : http://maha-arogya.gov.in
संचालक (वैयक्तिक)	२२६२१००६	Email : dhs_2005@rediffmail.com
सहसंचालक (रुग्णालये-राज्यस्तर)	२२६११४७१	Email : miscell@rediffmail.com
सहसंचालक (प्राआकेंद्र-जिपस्तर)	२२६२०२४९	Fax No. 022-22621034 / 22620234 (DHS)
सहसंचालक (असंसर्गजन्य रोग)	२२६२११८६	022- 22679044(Hosp.)
सहसंचालक (खरेदी कक्ष)	२२६२६२८२	022-22622155(CAO)
सहसंचालक (अर्थ व आस्थापना)	२२६२६७५५	022-22703785(Control Room)
		022-22621047 (NCD)

क्र.संआसे/डायलेसीस/ प्रशिक्षण/कक्ष ३/२०१३
दिनांक - ४/०४/२०१३

प्रति,

१) विभाग प्रमुख,
नेफ्रॉलॉजी डिपार्टमेंट,
के.ई.एम. हॉस्पीटल मुंबई, बी.जे.एल.नायर रुग्णालय, जे.जे समूह रुग्णालय, बी.जे. वैद्यकिय महाविद्यालय पुणे, के.ई.एम रुग्णालय पुणे, सुपर स्पेशालिटी हॉस्पीटल नागपूर, (अंतर्गत जी.एम.सी.नागपूर), जी.एम.सी औरंगाबाद, एम.जी.एम औरंगाबाद, मिरज मेडिकल कॉलेज मिरज, वैद्यकिय अधिक्षक सुपर स्पेशालिटी हॉस्पीटल अमरावती व नाशिक

" विषय:- राज्यातील जिल्हा रुग्णालये मधे सुरु करण्यात येणाऱ्या डायलेसीस युनिट मधील भिषक/वैद्यकिय अधिकारी /स्टॉफ नर्सेस/ डायलीसीस टेक्नीशियन यांना प्रशिक्षण देणेबाबत...

संदर्भ:- मा. संचालक डी.एम.ई.आर यांचे पत्र क्र.संवैशिवसं/संआसे/डायलेसीस/ तंत्रज्ञ/ प्रशिक्षण/४-३ दि.१५/२/२०१३.

उपरोक्त संदर्भाधिन विषयाव्दारे आपणास कळविण्यात येते की, राज्यातील सर्व जिल्हा रुग्णालये/ सामान्य रुग्णालये व उपजिल्हा रुग्णालय शेगांव व पंढरपूर/ नांदेड स्त्री रुग्णालय या ठिकाणी लवकरच डायलेसीस युनिटस सुरु करण्यात येणार आहेत. तत्पूर्वी या युनिट मधे कार्यरत होणाऱ्या सर्वांचे गुणवत्तापूर्ण प्रशिक्षण होणे गरजेचे आहे. या प्रशिक्षणाचा आराखडा ठरविणे तसेच प्रशिक्षणामध्ये समन्वयाच्या दृष्टीने नेफ्रॉलॉजी युनिट प्रमुख संबंधित हॉस्पीटलस यांची बैठक दिनांक १६/४/२०१३ रोजी दुपारी ३ वाजता आरोग्य सेवा संचालनालय, मुंबई (आठवा मजला,

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

DIRECTORATE OF HEALTH SERVICES.
(MAHARASHTRA STATE)
Arogya Bhavan, St.George's Hospital Compound, P.D'Mello Road, Mumbai-400 001.

	Tel.No.	
Office: Director(Personal) Jt.Director(Hospital) ADHS (HOTA)	22621031-36 22621006 22611471 22703861	Website : http://maha-arogya.gov.in Email : adhthoa@gmail.com Email : jdhs03@gmail.com Fax No. 022-22621034 / 22620234 (DHS) 022-22679044 (Hosp.) 022-22703861 (THAO)
		N0.DHS/THOA/ MGMHsop.Aurangabad /Liver Transp.Team/ D-20/ 17 Date- 27/07/2017

To,
Dr. R. B. Bohra
Dean
Mahatma Gandhi Mission Medical College & Hospital,
N-6, CIDCO
Aurangabad-431003.

Sub:- **Transplantation of Human Organ Act 1994**
Liver Transplant Team

Ref:- Your application dtd. 16/03/2017

With reference to your application, the **Liver Transplant Team** of specialists whose names have been sent to this office for the approval of the State Appropriate Authority under the provision of the Transplantation of Human Organs Act 1994, for the purpose of Liver Transplantations operations in your hospital, the State Appropriate Authority herewith grants recognition to the **Liver Transplant Team** of your hospital as shown as below. **This is valid for the period of five years from the date of issue.**

LIVER TRANSPLANT TEAM

Sr.No.	Designation	Name of Consultant
1	**Transplant Surgeon**	Dr. Ravi Mohanka, Transplant Surgeon Dr. Gaurav Chaubal, Transplant Surgeon Dr. Somnath Chattopadhyay, Transplant Surgeon Dr. Pravin Suryawanshi, Transplant Surgeon
2	**Transplant Physician**	Dr. Samir R. Shah, Gastroenterologist Dr. Akash Shukla, Gastroenterologist Dr. Parijat A. Gupte, Gastroenterologist Dr. Ashok Mohite, Gastroenterologist Dr. Vijay S. Gulwe, Gastroenterologist Dr. Sonali Bhattu, Gastroenterologist
3	**Transplant Anesthesiologist**	Dr. Sanhita Kulkarni, Anaesthesiologist Dr. Vasanthi Kelkar, Anaesthesiologist Dr. Balaji Asegaonkar, Anaesthesiologist Dr. Pramod Apsingekar, Anaesthesiologist Dr. Pramod Bhale, Anaesthesiologist

- If any doctor resigns the institute, then intimate immediately to the Appropriate Authority.

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Govt. of Maharashtra

FORM 16

Certificate of Registration For Performing Organ / Tissue Transplantation / Retrieval And / Or Tissue Banking

[Refer rule 24(2)]

This is to certify that ..MGM MEDICAL COLLEGE &.....
HOSPITAL.....
Hospital/Tissue Bank located at...CIDCO, AURANGABAD.....
has been inspected and certificate of registration is granted for performing the organ/tissue retrieval/transplantation/banking of the following organ(s) /tissue(s) (mention the names) under the Transplantation of Human Organs Act, 1994 (42 of 1994):-

1. LIVER
2. .....
3. .....
4. .....

This certificate of registration is valid for a period of five years from the date of issue.

This permission is being given with the current facilities and staff shown in the present application form. Any reduction in the staff and/or facility must be brought to the notice of the undersigned.

संचालक आरोग्य सेवा, महाराष्ट्र राज्य, मुंबई

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Place :..MUMBAI.....

Date :..27/07/2017.....

Signature of Appropriate Authority

समुचित प्राधिकरण

Seal.....तथा.....

संचालक आरोग्य सेवा,

आरोग्य सेवा संचालनालय

(महाराष्ट्र राज्य)

आरोग्य भवन सेंट जॉर्जेस रुग्णालय आवार, पी.डिमेलो रोड, मुंबई- ४०० ००१

	दूरध्वनी	Website : http://maha-arogya.gov.in Email : adhsthoa@gmail.com Email : jdhs03@gmail.com Fax No. 022-22621034 / 22620234 (DHS) 022- 22679044(Hosp.) 022-22703861 (THAO)
कार्यालय संचालक (वैयक्तिक) सहसंचालक (रुग्णालये-राज्यस्तर) सहा.संचालक (माअप्र)	२२६२१०३१-३६ २२६२१००६ २२६११४७१ २२७०३८६१	
रजिस्टर ए.डी.		क्र.संआसे/माअप्र//एमजीएममेडिकलकॉलेज औरंगाबाद/ईडीसी/नोंदणी/कक्ष-२०/१५ दिनांक:- ०५/०८/२०१५

प्रति,
अधिष्ठाता एमजीएम मेडिकल
कॉलेज ॲन्ड हॉस्पीटल,
एन-६,सीडको, औरंगावाद.

विषय - मानवी अवयव प्रत्यारोपण कायदा १९९४ अंतर्गत आयडोनेशन सेंटर म्हणून नोंदणी मिळणेबाबत.

संदर्भ - आपला प्रस्ताव दि. ६.१०.२०१४

उपरोक्त संदर्भिय विषयाच्या अनुषंगाने आपणास कळविण्यात येते की, आपण मागणी केल्यानुसार आपल्या रुग्णालयास आयडोनेशन सेंटर म्हणून नोंदणी नुतणीकरण प्रमाणपत्र देण्यात येत आहे. सदर प्रमाणपत्राची वैधता प्रमाणपत्र दिल्याच्या दिनांकापासून पुढील पाच वर्षासाठी राहील. नोंदणी प्रमाणपत्राच्या तारखेच्या ३ महिने अगोदर नूतणीकरणचा प्रस्ताव या संचालनालयास सादर करणे आवश्यक राहील याची नोंद घ्यावी.

सोबत: प्रमाणपत्र

5/8/15

समुचित प्राधिकरण तथा
संचालक आरोग्य सेवा
महाराष्ट्र राज्य मुंबई

Dr Rajesh B. Goel

प्रत: सहसंचालक आरोग्य सेवा (अंनिका) मुंबई यांना माहितीसाठी.

9-10-2015
3.40 PM.

Date of Issue - 5/8/2015
Date of ~~Renewal~~ Expiry - 5/8/2020
Date of Renewal - 5/4/2020.

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Government of Maharashtra

सत्यमेव जयते
महाराष्ट्र शासन

Office of the Appropriate Authority

Certificate of Registration

No. DHS/ THOA /MGM MED COLL / EDC /F.No /D-20/2015

This is to certify that MAHATMA GANDHI MISSION, MEDICAL COLLEGE & Hospital located at CIDCO, AURANGABAD has been inspected by the Appropriate Authority and certificate of registration is granted for performing the organ transplantation of the following organs:-

1. EYE DONATION CENTER
2. _____
3. _____
4. _____

This certificate of registration is valid for a period of five years from the date of issue .

Mumbai:

Date: 05/08/2015

संचालक आरोग्य सेवा, महाराष्ट्र राज्य, मुंबई

Appropriate Authority
and
Director Health Services,
Maharashtra State, Mumbai
समुचित प्राधिकरण
तथा
संचालक आरोग्य सेवा,
महाराष्ट्र राज्य, मुंबई

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

DIRECTORATE OF HEALTH SERVICES.
(MAHARASHTRA STATE)

Arogya Bhavan, St.George's Hospital Compound, P.D'Mello Road, Mumbai-400 001.

	Tel.No.	Website : http://maha-arogya.gov.in Email : adhsthoa@gmail.com Fax No. 022-22621034 / 22620234 (DHS) 022-22679044 (Hosp.) 022-22703861(THOA)
Office: Director(Personal) Jt.Director(Hospital) ADHS (THOA)	22621031-36 22621006 22611471 22703861	
		NO.DHS/THOA/MGM med College & Hosp.A'bad./Corneal TranpTeam /19 Date- 23 /04/2019

To,
Dean,
MGM Medical College & Hospital,
N-6, Cidco, Aurangbad-431003.,

Sub:- **Transplantation of Human Organ Act 1994 & Amendment 2011**
Cornea Transplant Team

Ref:- Your application dtd. 15/01/2019

With reference to your application, the **Cornea Transplant Team** of specialists whose names have been sent to this office for the approval of the State Appropriate Authority under the provision of the Transplantation of Human Organs Act 1994, for the purpose of Cornea Transplantations operations in your hospital, the State Appropriate Authority herewith grants recognition to the **Cornea Transplant Team** of your hospital as shown as below. **This is valid for the period of five years from the date of issue.**

CORNEA TRANSPLANT TEAM

Sr.No.	Designation	Name of Consultant
1	**Transplant Surgeon**	Dr. Sarika Gadekar, Ophthalmologist
2	**Transplant Anesthesiologist**	Dr. Vasanti Kelkar, Anesthesiologist Dr. Ajita Annachatre (Dunk), Anesthesiologist Dr. Anuradha Jogdand, Anesthesiologist

- If any doctor resigns the institute, then intimate immediately to the Appropriate Authority.
- If any new doctor is joining to your institute, then before joining the team, the institute has to take the permission on behalf of the doctor from Appropriate Authority, without which the newly joined doctor cannot work in the transplantation program.

Dr. Anupkumar Yadav
Commissioner (Health & Family welfare)
and
Director Health Services, Mumbai

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

DIRECTORATE OF HEALTH SERVICES.
(MAHARASHTRA STATE)

Arogya Bhavan, St.George's Hospital Compound, P.D'Mello Road, Mumbai-400 001.

	Tel.No.	Website : http://maha-arogya.gov.in Email : adisthoa@gmail.com Fax No. 022-22621034 / 22620234 (DHS) 022-22679044 (Hosp.) 022-22703861(THOA)
Office: Director(Personal) Jt.Director(Hospital) ADHS (THOA)	22621031-36 22621006 22611471 22703861	
		NO.DHS/THOA/ MGM med.College & Hosp.A'bad./ /Corneal Tranp.Reg/D-20/19 Date- 23 /04/2019

To,
Dean,
MGM Medical College & Hospital,
N-6, Cidco, Aurangbad-431003..

Sub:- **Transplantation of Human Organ Act 1994 & Amendment 2011**
Cornea Transplant Registration

Ref:- Your application dtd. 15/01/2019

With reference to your application, please find enclosed herewith the approval for following Committee.

1) Certificate of Registration for Cornea Transplantation.
2) Approval for following committees
   a) Cornea Transplant Team

You are instructed to affiliate your hospital with District Blindness Control Society & with Director Regional Organ & Tissue Transplant Organization (ROTTO) Mumbai & Director, National Organ & Tissue Transplant Organization (NOTTO) New Delhi for co-ordination of deceased (cadaver) donor organ transplant activities.

You should regularly submit monthly performance report in the prescribed format.

You are instructed to follow all the provisions in the Transplantation of Human Organs Act 1994 & Rules 1995, Transplantation of Human Organs (Amendment) Rules, 2008 and Transplantation of Human Organs (Amendments) Act, 2011 & Rules 2014.

Please acknowledge the same.

Dr. Anupkumar Yadav
Commissioner (Health & Family welfare)
and
Director Health Services, Mumbai

C.C.to: 1) Joint Director Health Services (NPCB) Mumbai.
2) Secretary, Regional Organ & Tissue Transplant Organization K.E.M. Hospital Parel Mumbai.
3) Director, National Organ & Tissue Transplant Organisation, 4th & 5th Floor, NIOP Bldg., Safdarjung Hospital, New Delhi-110029.

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

Government of Maharashtra

FORM 16

CERTIFICATE OF REGISTRATION FOR PERFORMING ORGAN/TISSUE TRANSPLANTATION/RETRIEVAL AND OR TISSUE BANKING

[Refer Rule No. 24(2)]

This is to certify that MAHATMA GANDHI MISSION MEDICAL COLLEGE & HOSPITAL Hospital/Tissue Bank located at N-6, CIDCO, AURANGABAD-431003 has been inspected and certificate of registration is granted for performing the organ/tissue retrieval/Transplantation/Banking of the following organ(s)/tissue(s) (mention the names) under the Transplantation of Human Organ Act, 1994(42 of 1994):-

1. CORNEA TRANSPLANT CENTRE

This certificate is valid for a period of five years from the date issue.

This permission is being given with the current facilities and staff shown in the present application form. Any reduction in the staff and /or facility must be brought to the notice of the undersigned.

संचालक आरोग्य सेवा, महाराष्ट्र राज्य, मुंबई

Place:- Mumbai

Date :- 23/04/2019

Signature of Appropriate Authority

Seal.....

समुचित प्राधिकरण
तथा
संचालक आरोग्य सेवा,
महाराष्ट्र राज्य, मुंबई

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

[नि.ने.ध.अ./मुं.सा.वि./३म.

No. 4401

सत्यमेव जयते

नोंदणी प्रमाणपत्र

याद्वारे प्रमाणपत्र देण्यात येते की, खाली वर्णन केलेली सार्वजनिक विश्वस्तव्यवस्था ही आज, मुंबई सार्वजनिक विश्वस्तव्यवस्था अधिनियम, १९५० (सन १९५० चा मुंबई अधिनियम २९) या अन्वये Aurangabad Region, Aurangabad. येथील सार्वजनिक विश्वस्तव्यवस्था नोंदणी कार्यालयात योग्य रितीने नोंदण्यात आलेली आहे.

सार्वजनिक विश्वस्तव्यवस्थेचे नाव : Zonal Transplantation Coordination Centee, Aurangabad.

सार्वजनिक विश्वस्तव्यवस्थांच्या नोंदणी पुस्तकातील क्रमांक : E - 1298 (A.bad)

Dr. Sudhir Gajanan Kulkarni यांस प्रमाणपत्र दिले.

आज दिनांक 12.04.2016 २०१- रोजी माझ्या सहीनिशी दिले.

शिक्का : Assistant Charity Commissioner, Aurangabad Region, Aurangabad

सही 12/4/16

पदनाम

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

Organ donation Awareness Programs

Organ Donation Awareness Programs

1½ Mr. Nana Patekar & Mr. Makarand Anaspure pledge organ donation

26 jan. 2016 MGM Medical College & Hospital

लाखभर नागरिक अवयवदानास सज्ज

म. टा. प्रतिनिधी, औरंगाबाद

मागच्या दीड-पावणेदोन वर्षांपासून औरंगाबाद शहरात सुरू झालेल्या अवयवदान मोहिमेत आतापर्यंत १३ ब्रेन डेड रुग्णांनी हृदय, यकृत, मूत्रपिंड, बुब्बुळ आदी ४५ अवयवांचे दान केले आहेच; शिवाय तब्बल दीड लाखापेक्षा जास्त नागरिक अवयवदानासाठी इच्छुक असून, त्यांनी 'झेडटीसीसी'मार्फत, वेगवेगळ्या शासकीय-खासगी रुग्णालयांमार्फत अवयवदानाचे फॉर्म भरले आहेत. काहींनी 'नोटो'च्या वेबसाईटवर अवयवदानाची नोंद करुन अवयवदानाची इच्छा व्यक्त केली आहे. फॉर्म भरले म्हणजे अवयवदान झालेच, असे नसले तरी नागरिक अवयवदानासाठी इच्छुक आहेत आणि त्यांची अवयवदाची सुप्तेच्छा नातेवाईकांना सर्वश्रुत होऊन वेळ आल्यावर त्यांची अखेरची इच्छा पूर्ण होण्याची शक्यता नक्कीच वाढते, असेही मानले जात आहे.

मागच्या वर्षी म्हणजेच १५ जानेवारी २०१६ रोजी मराठवाडा-विदर्भ-खान्देशातील पहिले अवयवदान औरंगाबादेत झाले आणि त्या दिवसापासून अवयवदानाविषयी मोठ्या प्रमाणावर सकारात्मक वातावरण तयार होण्यास सुरुवात झाली. त्याच सुमारास स्थापन झालेल्या 'झोनल ट्रान्स्प्लान्ट कोऑर्डिनेशन कमिटी'मार्फतही (झेडटीसीसी) अवयवदानाचा प्रचार-प्रसार सुरू झाला. मराठवाडा-विदर्भ-खान्देशातील पहिला अवयवदाता राम मगर याच्या अवयवदानानंतर हॉटेल रामा इंटरनॅशनल येथे झालेल्या अवयवदात्याच्या कुटुंबियांच्या सत्कारावेळी अवयवदानाविषयी खास कार्यक्रम घेण्यात आला होता. त्यावेळी अवयवदानाविषयी इत्यंभूत माहिती देण्यात आली होती. त्यानंतरही वेळोवेळी विविध शाळा-महाविद्यालयांमधून तसेच वेगवेगळ्या कार्यक्रमांच्या निमित्ताने अवयवदानाविषयी जनजागृती करण्यात येत आहे. शासकीय स्तरावरही मागच्या वर्षापासून महाअवयवदान उपक्रम राबविण्यात येत आहे. अलीकडे प्रत्येक कार्यक्रमाअंतर्गत अवयवदानाचे फॉर्म वाटप मोठ्या प्रमाणावर होत आहे. त्याचा एकत्रित परिणाम म्हणून आतापर्यंत १३ ब्रेन डेड रुग्णांकडून सुमारे ४५ अवयवांचे दान झाले आहे.

मागच्या वर्षी कार्यक्रमानिमित्त आलेले प्रसिद्ध अभिनेते नाना पाटेकर व मकरंद अनासपुरे यांनीही अवयवदानाचे फॉर्म भरले.

वेगवेगळ्या कंपन्यांमध्ये जनजागृती

सद्यस्थितीत काही औद्योगिक कंपन्यांमध्ये अवयवदानाविषयी जनजागृती केली जात असून, अवयवदानाची शास्त्रशुद्ध माहिती देणे, शंकांचे निरसन करणे, अवयवदानाचे कार्ड वाटप करणे, फॉर्म भरुन घेणे किंवा 'नोटो'च्या वेबसाईटवर नोंद करण्यास प्रोत्साहन देणे, असे विविध उपक्रम हाती घेण्यात आले आहे, असे ट्रान्स्प्लान्ट को-ऑर्डिनेटर मनोज गाडेकर यांनी 'मटा'ला सांगितले.

एखाद्या व्यक्तीने अवयवदानाचा फॉर्म भरला म्हणजे संबंधित व्यक्तीने अवयवदानाची इच्छा प्रकट केली आहे आणि अशी इच्छा फॉर्मच्या किंवा 'डोनर कार्ड'च्या निमित्ताने संबंधितांच्या नातेवाईकांना स्पष्ट झाल्याशिवाय राहात नाही. त्यामुळे तशी वेळ आल्यास नातेवाईकांना अवयवदानाचे स्मरण राहू शकते किंवा अवयवदानाबाबत सुचविल्यास नातेवाईकांची तशी मानसिकता तयार होऊ शकते. त्यामुळे फॉर्म भरणे-भरुन घेण्यातून जनजागृती नक्कीच होत आहे.

– डॉ. सुधीर कुलकर्णी, अध्यक्ष, झेडटीसीसी

Dr. Rajesh B. Goel
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2. Wockhardt India Shendra Unit 16.09.2016

3. Clover Dell School MGM Campus, Aurangabad 19.08.2016

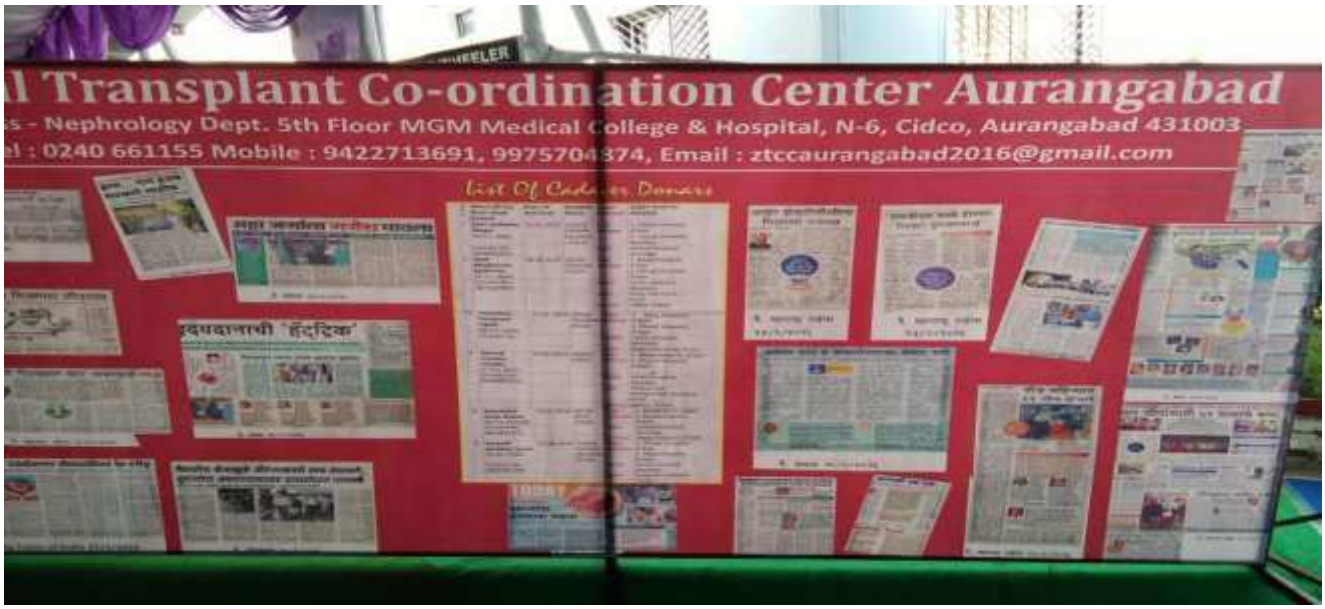
4. Rotary Club Youth Festival Aurangabad

23 Sept. 2016

5. Maharashtra Physician Conferences, Aurangabad

Organ Donation Awareness Stall 21, 22 &23/10/2016

6. Maharashtra Orthopedics Conference 2016, Organ Donation Awareness Stall . Nov. 2016



7. **World kidney day**
   09/03/2017 Rukmini hall MGM Hospital Aurangabad.

- **Dr. Purushottam Bhapkar, IAS , MGM and Govt. Medical College and Hospital authority signing on Donor card.**

- **Felicitation of Donor**

8. **Mahaavayav Daan Abhiyan 2017**

Organ Donation Awareness Rally GMC Hospital Aurangabad. 29.08.2017

9. Indian Medical Associaton, Aurangabad IMA Hall 26.08.2017

10. Vasantrao Naik Maha Vidhyalaya, Aurangabad.

30/08/2017

अवयवदानामुळे दुसऱ्यांना जीवदान मिळू शकते : डॉ. गुंडरे

औरंगाबाद • डीबी स्टार

इतर देशांच्या तुलनेत आपल्या देशात अवयवदानाचे प्रमाण कमी आहे. आज अनेकांना अवयवदानाची गरज आहे. अवयवदानामुळे दुसऱ्यांना जीवदान मिळू शकते, असे प्रतिपादन एमआयटी हॉस्पिटलचे फिजिशियन डॉ. रोहन गुंडरे यांनी केले.

वसंतराव नाईक महाविद्यालयात महाअवयवदान मोहिमेनिमित्त आयोजित व्याख्यानात ते बोलत होते. अध्यक्षस्थानी प्रा. सुभाष चव्हाण, समाजशास्त्र विभागप्रमुख डॉ. डी. के. दराडे उपस्थित होते. डॉ. गुंडरे म्हणाले, नेत्र, त्वचा, हृदय, यकृत, मेंदू दान केल्याने गरजवंताला जीवदान मिळू शकते. जिवंत किंवा मृत व्यक्तीसुद्धा अवयवदान करू शकतात.' कार्यक्रम यशस्वितेसाठी राष्ट्रीय सेवा योजनेचे कार्यक्रम अधिकारी डॉ. महेश कुलथे, डॉ. सुनीता राठोड, प्रा. गजानन हणवते आदींनी परिश्रम घेतले.

वसंतराव नाईक महाविद्यालयात महाअवयवदान मोहिमेनिमित्त आयोजित विविध स्पर्धेतील विजेत्यांना बक्षीस वितरण करताना मान्यवर.

महाअवयवदानानिमित्ताने घेण्यात आलेल्या विविध स्पर्धेतील विजेत्यांना या वेळी पारितोषिके देण्यात आली. रांगोळी स्पर्धेत रिता धानोरकर, प्रगती नागुरे, पूजा बोरा तसेच निबंध स्पर्धेत प्रियंका आहेरराव, पूनम नागुरे, कमलाकर लांडगे यांना बक्षिसे मिळाली. चित्रकला स्पर्धेत दीपक ढाकणे, मयूरी कुलकर्णी, कोमल शेजवळ आणि पोस्टर्स स्पर्धेत आरती गावंडे, कोमल शेजवळ, ऐश्वर्या झिने यांना सन्मानित करण्यात आले.

11. Guruvarya Lahuji Salve Arogya Kendra, Aurangabad. 31/08/2017

12. IANCON Conference 2017 ZTCC Stall . 9&10 September 2017

13. Wockhardt Shendra Unit, Aurangabad 12/09/2017

14. Wockhardt Chikalthana MIDC Unit, Aurangabad 14/09/2017

15. Wockhardt Valuj MIDC Unit, Aurangabad 14/09/2017

16. New High School, Chowka, Dist. Aurangabad
23.09.2017

17. College of Social Work, Dr. B.A.M. Univercity Aurangabad.

25.09.2017

18. Sant Rohidas Arogya Kendra, Mukundwadi, Aurangabad 26.10.2017

19. Central Bank of India, Kranti Chowk, Aurangabad.

12.12.2017

20. Organ Donation (Stall) MAHA_AGRO State Level agriculture Exhibition, Aurangabad. 08.01.2018

21. Sant Savata Mali College, Phulambri. 31.01.2018

22. Nutan Mahavidyalaya, Jintur Road, Parbhani. Organ Donation (Stall) 13.02.2018 to 17.02.201

23.Health awareness Lecture, at. Jolly Board Ltd Chikalthana MIDC Aurangabad

16 May 2018

24.KIDNEYTHON (Kidney awareness marathon) Organized by United CIIGMA Hospital, Aurangabad. 04.03.2018

25.MGM,JNEC Campus Aryabhatta Hall, Aurangabad. World Kidney Day &World Women's Day, 8 March 2018 Organ Donation awareness (Stall).

26. Organ donation awareness campaign at the village of Vihamandava. 31.03.2018

27. (Marriage Ceremony) organ donation awareness Stall. Venue: - Ramchandra mangal Karyalaya, Aurangabad. 07.05.2018

लग्नसोहळ्यात अवयवदानाचा जागर

म. टा. प्रतिनिधी, औरंगाबाद

५० झाडांच्या संवर्धनाचा संकल्प

28. Sai Mandir, Waluj MIDC, Aurangabad. 07.06.2018

ORGAN DONATION AWARENESS PROGRAM 2016

Sr. No.	Date	Venue	Speaker/Guest	Audience	Remark
1	26.01.2016	MGM Medical College & Hospital,Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	50	In Presence of **Mr. Nana Patekar** & **Mr. Makarand Anaspure**
2	19.08.2016	Mahatma Gandhi Mission's Clover Dale School Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	60	
3	28.08.2017	GMC Hospital to Kranti Chowk Aurangabad	Organ Donation Awareness Rally	15000	Maha Avayavdaan Abhiyan 2016
4	31.08.2016	GMC Medical College & Hospital, Aurangabad	Felicitation program of Relatives of Cadaver Donor	100	Maha Avayavdaan Abhiyan 2016
5	16.09.2016	Wockhardt India, Shendra MIDC Unit, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	45	
6	24.09.2016	Sant Eknath Rangmandir, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad		Rotary Club Youth Festival Aurangabad
7	21, 22 & 23 Octo.2016	Jawaharlal Nehru Engineering College, Aurangabad	Organ Donation Awareness Stall	1000	Maharashtra Physician Conferences, Aurangabad
8	Nov. 2016	Jawaharlal Nehru Engineering College, Aurangabad	Organ Donation Awareness Stall	600	Maharashtra Orthopedics Conference 2016,

ORGAN DONATION AWARENESS PROGRAM 2017

Sr. No.	Date	Venue	Speaker /Guest	Audience	Remark
1	09.03.2017	Rukmini Hall MGM Hospital Aurangabad	**Dr. Purushottam Bhapkar, IAS**	200	Felicitation program of Relatives of Cadaver Donor Showed Phir Jindagi movie (Based on organ donation)
2	29.08.2017	GMC Hospital to Kranti Chowk Aurangabad	Organ Donation Awareness Rally	2700	Maha Avayavdaan Abhiyan 2017
3	26.08.2017	IMA Hall, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	35	Organised by Indian Medical Association, Aurangabad
4	30.08.2017	Vasantrao Naik College, Aurangabad	**Dr. Rohan Gundre,** Transplant Physician, MIT Hospital, Aurangabad **Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	210	
5	31.08.2017	Guruvarya Lahuji Salve Arogya Kendra, Aurangabad	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	80	participated slum areas womens & young girls

6	09 & 10 Sap. 2017	MGM Medical College & Hospital,Aurangabad	Organ Donation Awareness Stall	300	IANCON Conference 2017
7	12.09.2017	Wockhardt India, Shendra MIDC Unit, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	55	
8	14.09.2017	Wockhardt India, Chikalthana MIDC Unit, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	48	
9	15.09.2017	Wockhardt India, Valuj MIDC Unit, Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	40	
10	23.09.2017	New High School, Chowka, Tq. Phulambri,Dist. Aurangabad	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	90	
11	25.09.2017	College of Social Work, Dr. B.A.M. Univercity Aurangabad.	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	40	
12	26.10.2017	Sant Rohidas Arogya Kendra, Mukundwadi, Aurangabad	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	60	
13	12.12.2017	Central Bank of India, New Usmanpura, Aurangabad	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	40	Organ Donation camp organised by CBI Group.

ORGAN DONATION AWARENESS PROGRAM 2018

Sr. No.	Date	Venue	Speaker /Guest	Audience	Remark
1	08.01.2018	Ayodhya Nagri, Padampura, Ground, Aurangabad.	**Mr. Jayas Dhabale, Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	800	Organ Donation (Stall) MAHA_AGRO State Level agriculture Exhibition, Aurangabad.
2	31.01.2018	Sant Savata Mali College, Phulambri.	**Mr. Manoj Gadekar** Central Transplant Coordinator ZTCC Aurangabad	58	
3	13.02.1018 to 17.02.2018	Nutan Mahavidyalaya, Jintur Road, Parbhani.	**Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	6000	Organ Donation (Stall) MAHA AROGYA SHIBIR Nutan Mahavidyalaya, Jintur Road, Parbhani.
4	16.02.2018	Health awareness Lecture, at. Jolly Board Ltd Chikalthana MIDC Aurangabad	**Dr. Sudhir Kulkarni** Prof. & Head, Nephrology Dept. MGM. President, ZTCC Aurangabad	90	Topic: How to Keep Healthy & Organ Donation awareness
5	04.03.2018	United CIIGMA Hospital, Aurangabad.	**Mr. Manoj Gadekar, Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	1600	KIDNEYTHON (Kidney awareness marathon) Orgnised by United CIIGMA Hospital, Aurangabad.
6	08.03.2018	MGM,JNEC Campus Aryabhatta Hall, Aurangabad	**Mr. Manoj Gadekar, Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	300	World Kidney day&World Women's Day, 8 March 2018 Organ Donation awareness (Stall)

7	31.03.2018	Vihamandwa Tq. Paithan, Dist, Aurangabad.	**Mr. Manoj Gadekar, Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	95	31.03.2018 on the occasion of Hanuman Jayanti we conducted organ donation awareness campaign at the village of Vihamandva.
8	07.05.2018	Ramchandra mangal Karyalaya (Hall) Beed bypass , Aurangabad.	**Mr. Manoj Gadekar, Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	600	07.05.2018 on the occasion of Deshpande Family (Marriage Ceremony) we conducted organ donation awareness Stall. Venue:- Ramchandra mangal Karyalaya, Aurangabad.
9	07.06.2018	At. Sai Mandir, Waluj MIDC, Aurangabad.	**Mr. Jayas Dhabale** Central Transplant Coordinator ZTCC Aurangabad	1300	Organ Donation awareness (Stall)

ORGAN DONATION AWARENESS PROGRAM 2019

1	09.02.2019	SRM College of Social work Chandrapur/Sant Rohidas arogya jendta aurangabad	Dr. Sudhir Kulkarni, Manoj Gadekar	25	Organ Donation Awareness talk
2	03.02.2019	MASICON 2019/ MGM Medical college & Hospital Aurangabad	Dr. Sudhir Kulkarni, Manoj Gadhekar	400	Organ Donation Awareness talk / Cadaver donor's relatives felicitation and marathon for organ donation
3	21.02.2019	Sanvad setu pratishthan, Aurangabad	Manoj Gadhekar	27	Organ Donation Awareness talk
4	09.03.2019	Gappa katta self healp group, Nandanvan colony, Aurangabad	Manoj Gadekar	47	Organ Donation Awareness talk
5	14.03.2019	Shree rameshwar high school, Waghola, Dist. Aurangabad	Manoj Gadekar	60	Organ Donation Awareness talk / World Kidney day Human chain
6	14.03.2019	Waghola, Tq. Phulambri, Dist. Aurangabad	Manoj Gadekar	34	Organ Donation Awareness talk
8	01.04.2019	Helping Hands, Magal Papers, Supari Hanuman road, Aurangabad	Manoj Gadekar	16	Organ Donation Awareness talk

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Publications

Case Report

Anesthesia for Combined Cesarean Section and Pheochromocytoma Resection

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Abstract

Pheochromocytoma (PCC) is a rare cause of hypertension during pregnancy [1:54000 pregnancies]. Fetomaternal morbidity and mortality is about 58% if the diagnosis is missed. Administration of anesthesia to patients with PCC is challenging. Associated pregnancy adds to the problems. This is a case report of a patient having PCC diagnosed at 26 weeks of gestation. With medical management pregnancy was continued till 34 weeks. She was posted for cesarean section and resection of PCC. Patient underwent surgery lasting for 7 h due to inferior vena cava tear and had stormy intra as well as postoperative course. Mother and baby had uneventful recovery due to continuous invasive monitoring and a good teamwork, despite limited anesthetic resources.

Keywords: *Cesarean section, hypertension, pheochromocytoma, pregnancy*

Introduction

Incidence of pheochromocytoma (PCC) during pregnancy is 1 out of 54,000.[1] Patient may be misdiagnosed as having preeclampsia, leading to increased fetomaternal mortality (58%).[2] This can be reduced by antenatal diagnosis and proper treatment. Anesthetic management of a parturient posted for a cesarean section and PCC resection in a limited resource setting is described.

Case Report

A 22-year-old fourth gravida, having PCC diagnosed at 26 weeks of gestation, was posted for elective cesarean section at 34 weeks. She was asymptomatic and had no pedal edema. Pulse rate was 84/min and blood pressure was 120/80 mm Hg. Her antihypertensive medication consisted of Nifedipine 10 mg TDS, Prazosin 1.5 mg QID, and Metoprolol 50 mg OD. Systemic examination did not reveal any abnormality. Hemoglobin was 9.5 gm%. Ultrasonography abdomen revealed right-sided suprarenal mass [7.6 cm × 5.4 cm]. Urinary vanillylmandelic acid was 30.4 mg (normal range: 2–7 mg) over 24 hours.[3]

There was no albuminuria. Electrolytes, blood sugar, uric acid, coagulation profile, liver, kidney and thyroid function tests, calcitonin levels, fundoscopy, ECG, two-dimensional Echo were within the normal limits.

Patient received oral 10 mg Diazepam and 50 mg Metoprolol at night. Last dose of Nifedipine (10 mg) and 1.5 mg Prazosin was administered 4 h before surgery. We planned epidural anesthesia for cesarean section followed by additional general anesthesia for tumor resection.[4]

Intravenous (IV) Ranitidine and Ondansetron were given. Preoperative Metoclopramide was avoided to prevent hypertensive crisis.[5]

Multiparamonitor was applied. Fetal heart surveillance (FHS) was 130/min. Internal jugular vein and radial artery cannulation was done after giving 50 µgm of Fentanyl and one mg of Midazolam. 500 ml of ringer lactate was administered for preloading. After negative aspiration, a test dose of 2 ml, 1.5% Lignocaine with Adrenaline[6] was administered epidurally, but she became drowsy and developed twitching of muscles with blood pressure of 170/130 mm Hg, 120/min pulse rate, and 90% SpO_2. Suspecting accidental intravascular injection of Lignocaine with Adrenaline, 100% oxygen was administered along with 50 mg of Thiopentone, 1 mg Phentolamine, and 5 mg of Labetalol. After 5 min, the patient was fully conscious and SpO_2 was 98% on air along with pulse rate 110/min, blood pressure 130/90 mm Hg,

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and there were no twitchings. FHS slowed down to 80/min. We planned for general anesthesia to avoid fetal distress. 100 mg Thiopentone, 50 mg Propofol, and 100 mg Suxamethonium were administered for induction and rapid sequence intubation. Oxygen, nitrous oxide, Halothane [0.5%], Vecuronium, and Fentanyl were used for maintenance. A baby (2 kg wt) with APGAR score 8 was delivered without applying fundal pressure and then shifted to neonatal intensive care unit (NICU) with APGAR score 10 (5 min) for monitoring. Patient received Oxytocin to control postpartum hemorrhage and blood transfusion was initiated.

Our aim to maintain mean arterial pressure (MAP) in the range of 70–100 mm Hg, heart rate 80–110/min, central venous pressure (CVP) 8–10 mm Hg was achieved by using phentolamine, metoprolol, and fluid administration. No additional drugs were required to maintain the parameters. Sodium nitroprusside (SNS) drip was added after delivery of baby.

There was a rent in inferior vena cava (IVC) during resection. IVC repair required 5 h and tumor resection another 2 h during which there were a lot of fluctuations in MAP (68–150 mm Hg), heart rate (80–150/min), and CVP (4–12 mm Hg).

Blood loss during this period was about 3000 ml. She required 3500 ml of crystalloids, 500 ml of hydroxy ethyl starch 6%, and 1800 ml of whole blood. Urine output and blood sugar level were within the normal limits.

After resection, CVP was 10 mm Hg but MAP was 40 mm of Hg for which infusion of noradrenalin (1 μg/kg/min) was started. At the end of surgery, while artificial ventilation was still continued, airway pressures progressively increased over 30 min (peak 28 cm and plateau pressure 24 cm) along with fall in SpO_2 90% (FiO_2 1). There were bilateral crepitations in chest. Pink, frothy secretions were seen through endotracheal tube. Blood pressure was 120/90 mm Hg, with pulse rate of 140/min and 14 mm Hg CVP. 60 mg Furosemide was administered and gradually 10 cm PEEP was instituted in stages. Noradrenalin was gradually tapered over 40 min. SpO_2 improved to 100% (FiO_2 0.5) and MAP was stable (60 mm Hg without vasopressor). Two packed cell volumes of blood were administered over 2 h as hemoglobin was 6 gm%. She developed hypoglycemia (50 mg%) during this period, which was promptly treated with 50 ml 50% dextrose and 100 ml/hour 5% dextrose maintenance drip. She was electively ventilated in ICU for 10 hours using Midazolam (1 mg/h) and Vecuron (1micrgm/kg/min) infusion. Postoperative analgesia was provided with 8 hourly IV 100 mg Tramadol and 75 mg diclofenac infusion as the epidural catheter was blocked due to blood clot. As her pulse rate (80–90/min) and MAP (70–80 mm Hg) were stable for next 4 h, she was extubated. Baby was shifted from NICU after 24 hours. Mother was discharged on 12th postoperative day.

Discussion

Goals of management of PCC during pregnancy are early diagnosis, control of blood pressure, and definitive surgery to reduce fetomaternal mortality (<5%).[7]

Hypertension in our patient was controlled with Prazosin, a selective α-1 antagonist. Its short duration helped in titration of dose,[8] without adverse effects on fetus.[9] Nifedipine helped to reduce vasospasm.[10] Terazosin and Doxazosin can also be used.[2] Phenoxybenzamine is commonly used but long duration of action predisposes to postoperative hypotension and needs monitoring of newborn.[11] Our patient had less than 20 mm orthostatic hypotension which assured adequate alpha blockade.[5] Tachycardia was treated with Metoprolol. Other beta-blockers[12] can be used after adequate alpha blockade.

Timing and mode of surgery remains controversial. PCC should be resected in second trimester or after delivery.[13] Cesarean section is preferred over vaginal delivery[14] and PCC should be resected simultaneously or after delivery.[12] Laparotomy is recommended for PCC resection,[12] after 24 weeks of gestation. So we planned cesarean section along with PCC resection.[15,16] If only cesarean section is performed, there can be postoperative hypertensive crisis. There are reports of PCC resection in postpartum period at interval as localization of tumor might be difficult during pregnancy.[17]

These patients may develop hypertension during shifting, induction, intubation, fundal pressure during cesarean section, tumor handling, and hypotension due to hemorrhage and following tumor resection.

Almost every possible anesthetic technique has been advocated by Hull.[17] Anesthetic technique does not have a major impact on surgical outcome.[6] Epidural, general anesthesia, or combined can be used for cesarean section and PCC resection.

We planned epidural for cesarean section to minimize chances of hypertensive crises.[4,18] Test dose of Lignocaine with Adrenalin can be administered as usual.[6] Three ml plain Bupivacaine can also be used. However, we had to switch to general anesthesia in anticipation of impending fetal distress as fetal heart rate decreased following accidental intravascular injection of Lignocaine with Adrenaline (140–80/min).

We added Propofol for induction to reduce intubation pressor response.[19] Rapid sequence intubation was done using Suxamethonium.[20] Fasciculation can compress tumor and stimulate autonomic ganglia.[6] But we had no other option at that time. As time was crucial, prior Magnesium sulfate (2 mg/kg over 10 min) could not be tried. Prior Phentolamine, Labetalol, and Propofol might have prevented hypertension after Suxamethonium in our patient. Rocuronium can be used but may cause hypertensive crises.[21]

Maintenance was done with Halothane 0.5%[22,23] but its usage in patients with PCC is controversial due to its arrhythmogenic property.[12] But that was the only volatile agent available with us 12 years back. All other volatile agents except desflurane can be used. Desflurane produces sympathetic stimulation.[5] Fentanyl was used for our patient. Remifentanyl is preferred due to its shorter half-life in neonates.

We have used phentolamine prior to delivery of baby. Although low-dose SNP infusion (<1 μg/kg/min)[12] is considered safe, keeping possibility of fetal cyanide toxicity, sodium nitroprusside was added later during tumor resection. Postoperative hypotension in our patient could be due to fall in catecholamine levels and due to blood loss. Sudden reductions in catecholamines also lead to hypoglycemia.[5] Pulmonary edema might be due to increased permeability of pulmonary vessels during pregnancy, IV fluids, and noradrenalin. Patient responded to addition of PEEP and furosemide. Gradual tapering of noradrenalin reduced systemic vascular resistance and preload.

PCC is a rare but treatable cause of hypertension during pregnancy leading to high fetomaternal morbidity and mortality. Multidisciplinary approach helps for better outcome. Anesthetic management is challenging and needs a good teamwork.

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Conflicts of interest

There are no conflicts of interest.

References

1. Sarathi V, Lila AR, Bandgar TR, Menon PS, Shah NS. Pheochromocytoma and pregnancy: A rare but dangerous combination. Endocr Pract 2010;16:300-9.
2. Oliva R, Angelos P, Kaplan E, George B. Pheochromocytoma in pregnancy: A case series and review. Hypertension 2010;55:600-6.
3. Memon MA, Aziz W, Abbas F. Surgical management of pheochromocytoma in a 13-week pregnant woman. BMJ Case Rep 2014;2014.
4. Takahashi K, Sai Y, Nosaka S. Anesthetic management for caesarean section combined with removal of pheochromocytoma. Eur J Anaesthesiol 1998;15:364-8.
5. Harish R. Pheochromocytoma resection: Current concepts in anesthetic management. J Anaesthesiol Clin Pharmacol 2015;31:317-23.
6. Bajwa SJ, Bajwa SK. Implications and considerations during pheochromocytoma resection: A challenge to the anesthesiologist. Indian J Endocrinol Metab 2011;15:S337-44.
7. Schenker JG, Chowers I. Pheochromocytoma and pregnancy: Review of 89 cases. Obstet Gynecol Surv 1971;26:739-47.
8. Davi Rittori P, Cinthia C, Claudio M, Rubia M. Pheochromocytoma and pregnancy: A case report and review. J Bras Nefrol 2015;37:496-500.
9. Bourget P, Fernandez H, Ribou F, Edouard D, Frydman R. Weak transplacental passage of prazocin [Alpress] during third trimester of pregnancy – 3 cases. J Gynecol Obstet Biol Reprod 1993;22:871-4.
10. Lehmann HU, Hochrein H, Witte E, Mies SHW. Hemodynamic effects of calcium antagonists. Hypertension 1983;5:1166-73.
11. Santeiro ML, Stromquist C, Wyble L. Phenoxybenzamine placental transfer during the third trimester. Ann Pharmacother 1996;30:1249-51.
12. Lenders JW. Pheochromocytoma and pregnancy: A deceptive connection. Eur J Endocrinol 2012;166:143-50.
13. Mannelli M. Management and treatment of pheochromocytomas and paragangliomas. Ann N Y Acad Sci 2006;1073:405-16.
14. Junglee N, Harries S, Davies N, Scott-Coombes D, Scanlon MF, Rees DA. Pheochromocytoma in pregnancy: When is operative intervention indicated? J Women's Health [Larchmt] 2007;16:1362-5.
15. Oh YS, Lee JJ, Cho GT. Anesthetic management of a patient with pheochromocytoma in pregnancy. Korean J Anesthesiol 1993;26:581-6.
16. Doo AR, Kim D, Cha KN, Han YJ, Kim DC. Anesthetic management of a pregnant woman undergoing laparoscopic surgery for pheochromocytoma-A case report. Korean J Anesthesiol 2013;64:373-5.
17. Hull CJ. Phaeochromocytoma: Diagnosis, pre-operative preparation, and anesthetic management. Br J Anaesth 1986;58:1453-6.
18. Hopkins PM, Macdonald R, Lyons G. Caesarean section at 27 weeks gestation with removal of pheochromocytoma. Br J Anaesth 1989;63:121-4.
19. Virendrakumar RB. Comparison of presser response to induction and endotracheal intubation with thiopentone and propofol –prospective randomized study. IJBAR 2012;3:9.
20. Agarwal A, Khanna P, Narayanawamy S, Ganga Prasad, Borle A. Anaesthetic management for emergency caesarean section in a patient with an untreated recently diagnosed phaeochromocytoma. IJA 2011;55:614-7.
21. Jeong CW, Lee HG, Kim WM, Shin SH, Bae HB. Was a hypertensive crisis in a patient with pheochromocytoma caused by rocuronium? – A case report. Korean J Anaesthesiol 2009;57:249-53.
22. Kim JC, Jeon JK. Halothane anesthesia for pheochromocytoma. Korean J Anesthesiol 1981;14:453-8.
23. Cooperman LH, Engelman K, Mann PE. Anesthetic management of pheochromocytoma employing halothane and beta adrenergic blockade. Anesthesiology 1969;30:29-36.

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Urinary Tract Infection in Chronic Kidney Disease

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Abstract: ***In a study of 120 CKD patients of nephrology unit in MGM Medical College, Aurangabad, a high incidence of urinary tract infection (U.T.I.) i.e. 35% was observed. E.coli was the predominating urinary pathogen. Presence of UTI is found to have associated with increased mortality in CKD patients indicating severe outcome in this study. However, the mortality is attributable only to the presence of UTI cannot be estimated due to presence of other confounding factors like diabetes mellitus and hemodialysis. Moreover, this study indicates the association of Diabetes, hemodialysis and advanced CKD stage as risk factors for U.T.I in CKD patients***

Keywords: UTI, CKD, Diabetes Mellitus, E.Coli, Hemodialysis

1. Introduction

Urinary tract infection is an infection of one or more structure in the urinary system. The urinary tract includes urethra, bladder, ureters, prostate and kidneys which is normally sterile and resistant to bacterial colonization. The body's defense mechanism against uti includes complete emptying of the bladder during urination, urine acidity, the vesicoureteral valve, and various immunologic and mucosal barriers. In cases of renal failure, there is a change in the composition of urine with oliguria, anuria, albuminuria and haematuria. The resultant changes in pH, osmolality and urinary urea definitely have their own effects in urinary infection.[5] The urinary tract is the most common site of nosocomial infection[1,2,3] and most of these infections follow instrumentation of urinary tract, mainly urinary catheterization and is a subsequent cause of significant morbidity, sepsis and death[4.] Most frequently bacteria from the urethral meatus ascend to the bladder between the catheter and urethral surfaces. Alternatively, bacteria may ascend within the urinary drainage systems following contamination of the drainage bag or catheter tubing junction. The presence of bacteria in the bladder constitutes a potential reservoir for multi resistant bacteria.[2,4]

The risk of acquiring a urinary tract infection depends on the method and duration of catheterization, and the quality of catheter care.[2,3]

Intensive care units are a meeting point between the most severely ill patients receiving aggressive therapy and the most resistant pathogens which are selected by the use of broad spectrum antimicrobial therapy.[5]

Epidemiological study suggests that the 3 most commonly seen infectious complications in the CKD population are: urinary tract infection, pneumonia, and sepsis.[1] However, there are few studies of patients with CKD and UTI[6] and the incidence of UTIs in patients with CKD is unclear.[7]

2. Aims & Objectives

1) To study the incidence of urinary tract infections in chronic kidney disease patients
2) To know the microorganisms responsible for urinary tract infections in chronic kidney disease patients
3) To know whether presence of urinary tract infections influences the outcome in chronic kidney disease patients

3. Material and Methods

A total of 120 CKD patients above 18 year old attending nephrology unit were included where as patient having obstructive uropathies (urethral strictures, renal calculi) or patients with indwelling catheter or patients who were on antibiotic therapy prior to hospitalization or on immunosuppressive therapy were excluded. In this study U.T.I was defined as asymptomatic[8] or symptomatic bateriuria[9, 11] with isolation of at least one micro organism in urine culture. A clean catch urine sample-midstream was collected in a wide mouth, leak proof container with straps on lids. Urine samples were sent to laboratory within 2-3 hours of collection. In case of any delay, urine specimens were preserved by using boric acid as preservative or refrigeration at 2 - 4°C up to a span of 24 to 72 hours [12,13]
In the present study diagnosis of U.T.I was established either by

a) Urine microscopy of clean catch urine in which presence of 10 or more white cells per cubic millimeter in a urine specimen, 3 or more white cells per high-power field of unspun urine[8] or leukocytes, leukocyte casts, and other cellular elements were observed directly under the microscope
b) All specimens were inoculated on Mac Conkey agar plate and was incubated at 35°C overnight and specific organism was isolated by inoculating colony on blood agar plates. The antibiotic sensitivity was based on Kirby Bauer method of antibiotic susceptibility
c) The ultrasound image shows a smaller kidney, thinning of the parenchyma and its hyperechogenicity (reflecting sclerosis and fibrosis) except in patients of diabetic

nephropathy in which both renal size and parenchymal thickness are preserved until end-stage renal failure.[17] Ultrasonogram can show evidence of pyelonephritis and cystitis as an additional clue for diagnosis of UTI and rule out obstructive uropathies

4. Results

Table 1: Incidence of UTI in CKD Patients:

UTI	*No. of patients*	*Percentage*
Positive	42	35.0
Negative	78	65.0
Total	120	100%

Out of 120 CKD patients, the total number of patients having urinary tract infection was 42. Hence, the incidence of UTI in CKD patients in this study was 35%.

Table 2: Gender wise distribution of UTI in CKD patients

Gender	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
Male	18	42.9	38	48.7	56	40.0	0.337	P=0.539 NS
Female	24	57.1	40	51.3	64	60.0		
Total	42	100	78	100	120	100		

Out of 120 CKD patients in this study, 56 (40%) were males and 64 (60%) were females. Among the 42 CKD patients having UTI, 18 (42.9%) were males and 24 (57.1%) were females. Among the 78 CKD patients not having UTI, 38 (48.7%) were males and 40 (51.3%) were females. The difference between the two groups was not statistically significant in this study.

Table 3: Age-Group wise distribution of UTI in CKD Patients:

Age-Group	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
19-30	03	7.1	06	7.7	06	5.0	6.68	P=0.246 NS
31-40	04	9.5	11	14.1	15	12.5		
41-50	05	11.9	20	25.6	27	22.5		
51-60	10	23.8	17	21.8	27	22.5		
61-70	16	38.1	22	28.2	38	31.7		
>70	04	9.5	02	2.6	07	5.8		
Total	42	100	78	100	120	100		

Out of 120 CKD patients, 38 (31.7%) were from age group 61-70 years followed by 27 (22.5%) each from age group 41-50 and 51-60 years. Among the 42 CKD patients having UTI, majority were from age group 61 – 70 years i.e. 16 (38.1%) followed by 51 – 60 years i.e. 10 (23.8%). Among the 78 CKD patients without UTI, majority were from age group 61 – 70 years i.e. 22 (28.2%) followed by 20 (25.6%) from 41-50 year age group.

Table 4: Association of Diabetics Mellitus with UTI in CKD Patients:

Diabetic status	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
Diabetic	26	61.9	34	43.6	60	50.0	3.69	P=0.041 S
Non diabetic	16	38.1	44	56.4	60	50.0		
Total	42	100	78	100	120	100		

Out of 120 CKD patients in this study, 60 (50%) were diabetic and 60 (50%) were non diabetic. Among the 42 CKD patients with UTI, 26 (61.9%) were diabetic and 16 (38.1%) were non diabetic. Among the 78 CKD patients not having UTI, 34 (43.6%) were diabetic and 44 (56.4%) were non diabetic. The statistical difference between two groups was significant indicating association between diabetes mellitus and UTI in CKD patients.

Table 5: CKD stage wise distribution of UTI:

CKD stage	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
G1	00	00	00	00	00	00	9.38	P=0.002 S
G2	03	7.1	07	8.9	10	8.3		
G3A	04	9.5	20	25.6	24	20.0		
G3B	06	14.3	20	25.6	26	21.7		
G4	10	23.8	14	17.9	24	20.0		
G5	19	45.3	17	21.8	36	30.0		
Total	42	100	78	100	120	100		

Out of 120 CKD patients, majority belonged to stage G5 i.e. 36 (30%). Among the 42 CKD patients having UTI, majority belonged to stage G5 i.e. 19 (45.3%) followed by G4 i.e. 10 (23.8%). Among the 78 CKD patients without UTI, majority belonged to CKD stage G3a and G3b with each being 20 (25.6%) of patients. The statistical difference between the two groups was significant indicating association of severity of CKD stage and UTI in this study.

Table 6: Association of Hemodialysis with UTI in CKD Patients:

Hemo – dialysis status	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
HD	29	69.0	31	39.7	60	50.0	9.38	P=0.002 S
Non HD	13	31.0	47	61.3	60	50.0		
Total	42	100	78	100	120	100		

Out of 120 CKD patients, 60 (50%) were on maintenance hemodialysis and 60 (50%) were managed conservatively. Among the 42 CKD patients having UTI, 29 (69%) were on maintenance hemodialysis and 13 (31%) were on conservative treatment. Among the 78 CKD patients without UTI, 31 (39.7%) were on hemodialysis and 47 (61.3%) were being managed conservatively. The statistical difference between two groups was significant indicating association between hemodialysis and UTI in CKD patients.

Table 7: Microorganisms isolated in UTI in CKD Patients:

Microorganisms isolated	UTI Positive	
	No	%
E.coli	24	57.1
S.aureus	06	14.3
Klebsiella spp.	08	19.1
Proteus spp.	03	7.1
Candida spp.	03	7.1
Acinetobacter spp.	1	2.4
Cons	03	7.1

The most common microorganism isolated from 120 CKD patients having UTI was E.coli in 24 (57.1%) patients followed by Klebsiella spp. in 8 (19.1%) patients. Other microorganisms were S.aureus in 6 (14.3%), Proteus spp. in

3 (7.1%), Coagulase negative staphylococci in 3 (7.1%), Candida spp. in 3 (7.1%) and Acinetobacter spp. in 1 (2.4%) patients in this study.

Table 9: Deaths in UTI with CKD Patients:

Death status	UTI				Total		Chi-square test	P-value
	Positive		Negative					
	No	%	No	%	No	%		
Dead	14	33.3	10	12.8	24	20.0	7.18	P=0.007 S
Alive	28	66.7	68	87.2	96	80.0		
Total	42	100	78	100	120	100		

Out of 120 CKD patients, 24 (20%) succumbed to death and 96 (80%) were salvaged. Among 42 CKD patients having UTI, 14 (33.3%) patients succumbed to death and 28 (66.7%) were salvaged. Among the 78 CKD patients without UTI, 10 (12.8) succumbed to death while 68 (87.2%) were salvaged. The statistical difference between the two groups is significant indicating association between severity of outcome and UTI in CKD patients.

5. Discussion

1) Incidence of UTI in CKD patients in this study was 35%. In a study conducted by Jadhav SK, et al., high incidence of urinary tract infection i.e. 57.5%, was observed in CKD patients.[5] This difference might be because of Exclusion of obstructive uropathies and catheterized patients in our study. In a cross sectional study conducted by Falah S Manhal, et al., the frequency of UTI in renal failure patients undergoing hemodialysis was found to be 37.5%.[18]
2) Among the 42 CKD patients having UTI, 18 (42.9%) were males and 24 (57.1%) were females. Among the 78 CKD patients not having UTI, 38 (48.7%) were males and 40 (51.3%) were females. The difference between the two groups was not statistically significant in this study. Similar results were observed in a study conducted by Chih-Yen HSIAO, et al. where, 52.2% were females and 47.8% were males out of all CKD patients with lower UTI.[19]
3) Among the 42 CKD patients having UTI, majority were from age group 61–70 years i.e. 16 (38.1%) followed by 51–60 years i.e. 10 (23.8%). Among the 78 CKD patients without UTI, majority were from age group 61 – 70 years i.e. 22 (28.2%). The statistical difference between the two groups was not significant in this study. Similar results were observed in a study conducted by Chih-Yen HSIAO et al. where, average ages of the upper and lower UTI patients with CKD were 59.21 ± 16.54 and 71.18 ± 14.77 years.[19] In a study by Zhang et al., they found a high prevalence (17.4%) of CKD among older adults 50 to 74 years from 9806 participants.[20] Another study done by Gauba C, et al., showed high incidence of urinary tract infections in CKD patients with older age group.[21]
4) Among the 42 CKD patients with UTI, 26 (61.9%) were diabetic and 16 (38.1%) were non diabetic. Among the 78 CKD patients not having UTI, 34 (43.6%) were diabetic and 44 (56.4%) were non diabetic. The statistical difference between two groups was significant indicating association between diabetes mellitus and UTI in this study. In a study conducted by Chih-Yen HSIAO et al., it was observed that 36.9% patients with lower UTI were found to have diabetes mellitus.[19] The difference might be attributable to the large number of diabetics in our study and high prevalence of diabetes mellitus in India.
5) Among the 42 CKD patients having UTI, majority belonged to stage G5 i.e. 19 (45.3%) followed by G4 i.e. 10 (23.8%). Among the 78 CKD patients without UTI, majority belonged to CKD stage G3a and G3b with each being 20 (25.6%) of patients. The statistical difference between the two groups was significant indicating association of severity of CKD stage and UTI in this study. A different study conducted by Gauba C et al. observed high incidence of UTI in patients with advanced CKD stage and low urine flow rates.[21] In a study conducted by Chih-Yen HSIAO et al. it was observed that patients belonging to CKD stage G4 and G5 were 13.4% and 8% respectively.[19] The difference might be due to geographic and genetic differences in Turkish and Indian population and large number CKD patients enrolled in our study belonged to CKD stage G4 & G5. Also all catheterized patients are excluded from our study.
6) Among the 42 CKD patients having UTI, 29 (69%) were on maintenance hemodialysis and 13 (31%) were on conservative treatment. Among the 78 CKD patients without UTI, 31(39.7%) were on hemodialysis and 47 (61.3%) were being managed conservatively. The statistical difference between two groups was significant indicating association between hemodialysis and UTI in this study. In a different study conducted by Jadhav SK, et al., out of 73 CKD patients undergoing hemodialysis 42 had UTI i.e. (57.5%).[5] In a study conducted by Falah S.Manhal et al., 37.5% of the CKD patients on maintenance hemodialysis had UTI.[18] The difference might be attributable to large number of ESRD patients requiring hemodialysis enrolled in our study.
7) The most common microorganism isolated from CKD patients having UTI was E.coli in 24 (57.1%) patients followed by Klebsiella spp. in 8 (19.1%) patients. Other microorganisms were S.aureus in 6 (14.3%), Proteus spp. in 3 (7.1%), Coagulase negative staphylococci in 3 (7.1%), Candida spp. in 3 (7.1%) and Acinetobacter spp. in 1 (2.4%) patients in this study. In a study conducted by FalahS. Manhal et al., (15%) patients had been infected with *E. coli*, (12.5%) patients with Klebsiella spp., and (2.5%) with Acinetobacter, α-hemolytic Streptococci, coagulase negative Staphylococci, and Proteus spp.[18] In a study done by HSIAO et al., Microorganisms isolated in upper and lower UTI were, E.coli (58.9% & 51.2%), Proteus (8.2% & 3%), Klebsiella (4.1% & 7.9%), Enterococcus (0% & 5.9%), Pseudomonas (2.7% & 6.9%) and staphylococcus (0% and 0.5%) respectively.[19] The minor difference may be attributable to exclusion of catheterized patients and obstructive uropathies in our study.
8) Among 42 CKD patients having UTI, 14 (33.3%) patients succumbed to death and 28 (66.7%) were salvaged. Among the 78 CKD patients without UTI, 10 (12.8) succumbed to death while 68 (87.2%) were salvaged. The statistical difference between the two groups is significant indicating association between severity of outcome and UTI in this study. A study done by Reinhard Funfstuck et al. observed that an acute infection can influence the course of a pre-existing renal

disease and enhance the development of renal failure in cases of existing damage of renal parenchyma or anatomical alteration of the urinary tract.[22]

6. Conclusion

This study was conducted on 120 CKD patients attending OPD and/or casualty of nephrology unit in MGM Medical College & Hospital, Aurangabad.

Incidence of urinary tract infection in chronic kidney disease patients is 35%.

E.coli was the most common microorganism isolated from urine cultures.

Presence of UTI is found to have associated with increased mortality in CKD patients indicating severe outcomes in this study. However, whether the mortality is attributable only to the presence of UTI cannot be estimated due to presence of other confounding factors such as diabetes mellitus and hemodialysis.

Moreover, this study indicates the association of Diabetes mellitus, hemodialysis and advanced CKD stage as risk factors for urinary tract infections in CKD patients.

References

[1] KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney inter. Suppl. 2013; 3(1):5-150.
[2] Cohen G, Haag-Weber M, Horl WH. Immune dysfunction in uremia. Kidney Int Suppl. 1997; 62:79–82.
[3] Minnaganti VR, Cunha BA. Infections associated with uremia and dialysis. Infect Dis Clin North Am. 2001; 15:385–406.
[4] Pesanti EL. Immunologic def ects and vaccination in patients with chronic renal failure. Infect Dis Clin North Am. 2001; 15:813–32.
[5] Jadav SK, Sant SM, Acharya VN. Bacteriology of Urinary Tract Infection in Patients of Renal Failure Undergoing Dialysis. J Postgrad Med. 1977; 23(1):10–8.
[6] Bennett WM, Craven R. Urinary tract infections in patients with severe renal disease. Treatment with ampicillin and trimethoprim-sulfamethoxazole. JAMA. 1976; 236: 946–8.
[7] Gilbert DN. Urinary tract infections in patients with chronic renal insufficiency. Clin J Am Soc Nephrol. 2006; (1):327–31.
[8] Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM. Infectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults. Clin Infect Dis. 2005; 40(5):643-54.
[9] Pyuria and urinary catheters. Arch Int Med. 2000; 160(5):673-7.
[10] IDSA Guidelines for treatment of uncomplicated acute bacterial cystitis and pyelonephritis in women. Clin Infect Dis. 1999; 29:745.
[11] Wagenlehner FME, Pilatz A, Naber KG, Weidner W. Therapeutic challenges of urosepsis. Eur J Clin Invest. 2008; 38 (2): 45–9.
[12] Urinalysis and Collection, Transportation, and Preservation of Urine Specimens. Clinical and Laboratory Standards Institute approved Guideline. 2nd ed. Wayne: 2001; 21(19).
[13] Evaluation of Liquid and Lyophilized Preservatives for Urine Culture. Journal of Clinical Microbiology. 1983:912-6.
[14] Ferguson J, Tanner J, Miller MJ. Evaluation of a New, Semiquantitative Screening Culture Device for Urine Specimens. J Ciln Microbiol. 1995; 33(5):1351-3.
[15] Koneman EW. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
[16] Biochemical tests for bacteria identification [image on the internet]. c2007 [updated 2007 Dec 10]. Available from: http://first6weeks.blogspot.in/2007_12_01_archive.html
[17] Ponikvar BJ, Perovic VA. Ultrasonography in chronic renal failure. Eur J Radiol. 2003; 46(2):115-22.
[18] Manhal FS, Mohammed AA and Ali KH. Urinary tract infection in Hemodialysis patients with renal failure. Fac Med Baghdad. 2012; 54: 38-41.
[19] Hsiao CY, Lin HL, Lin YK, et al. Urinary tract infection in patients with chronic kidney disease. Turk J Med Sci. 2014; 44:145-9.
[20] Zhang QL, Koenig W, Raum E, Stegmaier C, Brenner H, Rothenbacher D. Epidemiology of chronic kidney disease: results from a population of older adults in Germany. Prev Med. 2009; 48:122–7.
[21] Gauba C, Agarwal S, Kalra OP, Revathi G. Prevalence of urinary tract infection in patients with chronic renal failure. Indian journal of Nephrology. 1997; 7(4):155-9.
[22] Funfstuck R, Ott U, Naber KG. The interaction of urinary tract infection and renal insufficiency. International Journal of Antimicrobial Agents. 2006; 28:72–7.

Case Report

Trimethoprim-induced Hyperkalemia in Renal Transplant Recipient

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Abstract

Trimethoprim-sulfamethoxazole (TMP-SMX) is an antimicrobial agent used in a variety of infections. Adverse reactions are more common in patients with AIDS but occasionally occur in immunocompetent patients. Renal toxicity is usually a hypersensitivity reaction to the sulfa component and manifests as interstitial nephritis or sulfa crystallization in the renal tubules. Reversible hyperkalemia is a rarely reported side effect of TMP-SMX therapy attributed to TMP inhibition of potassium secretion in the distal renal tubule in a manner similar to the potassium-sparing diuretic, amiloride. We report a case of hyperkalemia associated with TMP-SMX occurring in a 32-year-old renal transplant recipient with no other risk factors for hyperkalemia. He was treated with TMP-SMX (800 mg + 160 mg) two tablets QID for suspected pneumocystis jiroveci pneumonia. He developed severe hyperkalemia on day 9 posttherapy. Hyperkalemia reverted to normal with withdrawal of trimethoprim.

Keywords: Hyperkalemia, kidney transplant, pneumocystis jiroveci pneumonia, trimethoprim

Introduction

Trimethoprim-sulfamethoxazole (TMP-SMX) is an antibiotic of choice for pneumocystis jiroveci pneumonia (PJP). Adverse reactions that may be experienced include gastrointestinal upset and skin lesions.[1,2] Renal toxicity is usually a hypersensitivity reaction to the sulfa component and manifests as interstitial nephritis or sulfa crystallization in the renal tubules.[3,4] Reversible hyperkalemia is a rarely reported side effect of TMP-SMX therapy attributed to TMP inhibition of potassium secretion in the distal renal tubule in a manner similar to the potassium sparing diuretic, amiloride.[5] The occurrence of TMP-SMX-induced hyperkalemia has been reported in patients treated for upper respiratory infections,[6] PJP in non HIV patients,[7] PJP in acquired immunodeficiency syndrome[1] and as a standard dose prophylaxis in renal transplant recipients.[8] We present a case of life-threatening TMP-SMX-induced hyperkalemia in a renal transplant recipient.

Case Report

A 32-year-old male underwent second kidney transplant 2 years after failed graft due to biopsy-proven recurrence of immunoglobulin A nephropathy. In the present kidney transplant, the donor was a father-in-law with 5/7 mismatch. The donor's age was 65 years. He received antithymocyte globulin (2 mg/kg/day for 2 days) as induction therapy followed by standard triple immunosuppression. The patient was also given pneumocystis jiroveci prophylaxis with TMP-SMX, which was later omitted because of pancytopenia. Pancytopenia recovered after stopping TMP-SMX. The patient received cytomegalovirus (CMV) prophylaxis with valganciclovir 450 mg orally once a day. The patient was alright for 3-month posttransplant, with creatinine stable at 1.8 mg/dl. He presented with fever and cough for 7 days, for which he had received antibiotic (amoxicillin + clavulanate). In spite of antibiotic course, he developed sudden onset of breathlessness for which he was admitted. Chest X-ray on day 1 of admission showed bilateral opacities [Figure 1]. The patient's serum creatinine was 3.2 mg/dl when he developed PJP. His serum potassium was 3.8 meq/L at that time. ABG

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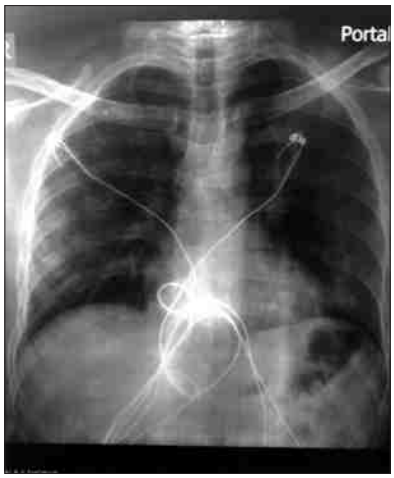

Figure 1: Chest X-ray: Day 1 of admission

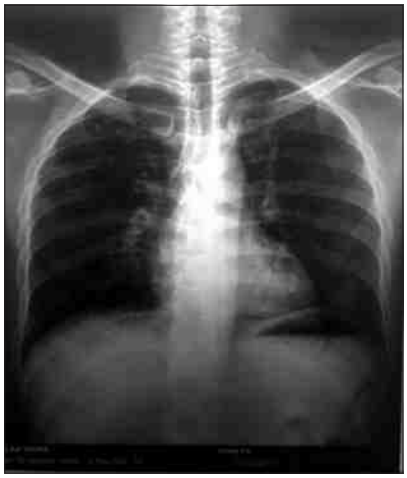

Figure 2: Chest X-ray: Day 14 of admission shows bilateral opacities; original photograph shows clearance

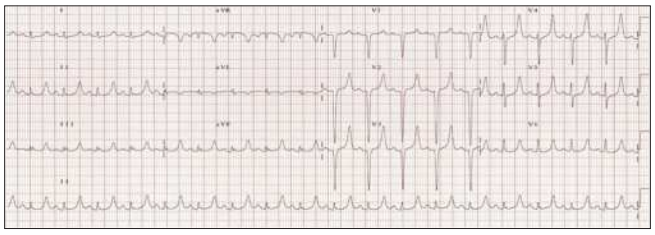

Figure 3: Electrocardiography: Signs of hyperkalemia

parameters were pH – 7.39, HCO3 – 13.2, PCO2 – 22.30, and PO2 – 79.70. Lactate level was not measured. His X-ray chest showed ground-glass opacities in both basilar areas. His serum lactate dehydrogenase levels were elevated to 890 mg/dl. CMV polymerase chain reaction (PCR) was negative. He did not produce sputum initially, and later, when sputum was available, it was sent for pneumocystis jiroveci PCR. The test was negative. He was started on TMP-SMX (two tablets QID). The dose of oral steroid was increased to 15 mg QID. The patient was given noninvasive ventilation as his oxygen saturation levels were low. The patient was maintained on oxygen therapy for 10 days. He showed gradual improvement in general condition. On the 9th day, his serum creatinine was 2.8 mg/dl, but he had serum potassium of 6.2 meq/L with electrocardiography evidence of hyperkalemia. Figure 2 the patient was not on any other confounding medications such as angiotensin receptor blockers/angiotensin-converting enzyme inhibitor which could contribute to hyperkalemia. His creatinine was 3.2 mg/dl at the time of admission, but it was improving gradually, so the possibility of hyperkalemia due to graft was not suspected. The possibility of trimethoprim-induced hyperkalemia was entertained, after ruling out tacrolimus as a cause of hyperkalemia. Tacrolimus levels were in normal range (9.76 ng/dl). The dose of TMP-SFX was reduced to two tablets BD. He was treated with intravenous calcium gluconate, glucose-insulin drip, and sodium bicarbonate. His serum potassium levels returned to normal on day 18. His serum creatinine had also come down to 2.5 mg/dl. The patient had symptomatic relief, and chest X-ray showed resolution of pneumonia. Figure 3 the patient was put on pneumocystis jiroveci prophylaxis once again with TMP-SMX double strength once a day. The dose of mycophenolate was reduced to 500 mg twice a day from 500 mg three times a day. Immunosuppression was restored to previous levels at discharge (tacrolimus 1.5 mg BD, mycophenolate 500 mg TDS, and oral steroid 20 mg BD).

Discussion

Our patient underwent second kidney transplantation (KT) with antithymocyte globulin as induction therapy. He did not receive pneumocystis jiroveci prophylaxis posttransplant completely due to thrombocytopenia. The donor was a father-in-law who was 65 years of age. The patient had reached serum creatinine of 1.8 mg/dl 15-day posttransplant. Three-month post-KT, his serum creatinine was 1.8 mg/dl. A British study by Akoh and Rana[9] showed that transplant patients who had donor above 60 years of age had mean serum creatinine of 2.0 mg/dl at the end of 3 months. Furthermore, our patient had good renal output, DSA was negative, graft rejection was not suspected, and renal biopsy was not performed during his initial 3 months after renal transplant. The patient developed PJP 3 months after transplant. The incidence of PJP in renal transplant recipient has been reported to be around 1%–6% in different studies.[10-12] The standard treatment for PJP is high-dose sulfamethoxazole-trimethoprim (TMP-SMX).[10] As bioavailability of co-trimoxazole is excellent, it can be used orally. Up to date, Thomas and Limper[13] recommended that co-trimoxazole has excellent bioavailability and oral administration is appropriate for all patients who have a functioning gastrointestinal tract. They also recommended the use of oral steroids in moderate-to-severe PJP. A study by Goto *et al*.[14] concluded that dose, duration, and timings of steroids had not been fully studied in transplant patients for treating PJP. Sometimes, hyperkalemia can be life threatening and levels can be as high as >6.5 mE/L, so it is mandatory to monitor potassium in patients receiving sulfamethoxazole + trimethoprim for PJP.

The mechanism of hyperkalemia with trimethoprim is blocking of potassium secretion from distal convoluted tubule.[3,4]

Conclusion

Potassium levels should be monitored when using high-dose SMX-TMP in patients of PJP. Reduction of dose and proper treatment of hyperkalemia will revert this complication.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Choi MJ, Fernandez PC, Patnaik A, Coupaye-Gerard B, D'Andrea D, Szerlip H, *et al.* Brief report: Trimethoprim-induced hyperkalemia in a patient with AIDS. N Engl J Med 1993;328:703-6.
2. Marinella MA. Case report: Reversible hyperkalemia associated with trimethoprim-sulfamethoxazole. Am J Med Sci 1995;310:115-7.
3. Perazella MA. Trimethoprim-induced hyperkalaemia: Clinical data, mechanism, prevention and management. Drug Saf 2000;22:227-36.
4. Velázquez H, Perazella MA, Wright FS, Ellison DH. Renal mechanism of trimethoprim-induced hyperkalemia. Ann Intern Med 1993;119:296-301.
5. Perazella MA. Trimethoprim is a potassium-sparing diuretic like amiloride and causes hyperkalemia in high-risk patients. Am J Ther 1997;4:343-8.
6. Nickels LC, Jones C, Stead LG. Trimethoprim-sulfamethoxazole-induced hyperkalemia in a patient with normal renal function. Case Rep Emerg Med 2012;2012:815907.
7. Monteagudo M, Sola J, Mestre J, Conesa D. Hyperkalemia during therapy of *Pneumocystis carinii* pneumonia with trimethoprim-sulfamethoxazole. Rev Clin Esp 1995;195:198-9.
8. Koç M, Bihorac A, Ozener CI, Kantarci G, Akoglu E. Severe hyperkalemia in two renal transplant recipients treated with standard dose of trimethoprim-sulfamethoxazole. Am J Kidney Dis 2000;36:E18.
9. Akoh JA, Rana TA. Impact of donor age on outcome of kidney transplantation from controlled donation after cardiac death. Saudi J Kidney Dis Transpl 2013;24:673-81.
10. Gerrard JG. *Pneumocystis carinii* pneumonia in HIV-negative immunocompromised adults. Med J Aust 1995;162:233-5.
11. Basu G. Infections after kidney transplantation: The bug bear of kidney transplantation in tropics. Open Urol Nephrol J 2015;8:76-87.
12. Borstnar S, Lindic J, Tomazic J, Kandus A, Pikelj A, Prah J, *et al. Pneumocystis jirovecii* pneumonia in renal transplant recipients: A national center experience. Transplant Proc 2013;45:1614-7.
13. Thomas CF, Limper AH. Treatment and Prevention of *Pneumocystis pneumonia* in HIV-Uninfected Patients. Uptodate; 2017.
14. Goto N, Futamura K, Okada M, Yamamoto T, Tsujita M, Hiramitsu T, *et al.* Management of *Pneumocystis jirovecii* pneumonia in kidney transplantation to prevent further outbreak. Clin Med Insights Circ Respir Pulm Med 2015;9:81-90.

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CASE REPORT

Hyperhomocysteinemia with Anticoagulant-related Acute Kidney Injury

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ABSTRACT

A case of 40-year-old young woman with an extensive, acute thrombosis of left distal brachial artery following an elective laparoscopic cholecystectomy was reported. The patient underwent urgent surgical intervention for brachial artery thrombosis and was started on oral anticoagulant. Within a week, the patient presented with bleeding diathesis and acute renal insufficiency with sepsis. She was found to have markedly increased serum homocysteine level. No other thrombophilic factors could be found. On investigation, a genetic defect of homocysteine metabolism was found to be the underlying cause. The patient recovered completely on treatment with pyridoxine, cyanocobalamin, and folate.

Keywords: Acute kidney injury, Anticoagulant, Heterozygous methylenetetrahydrofolate reductase gene mutation, Thromboembolic event.

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INTRODUCTION

Thrombophilias, inherited or acquired, are conditions associated with hypercoagulable state and increased risk of arterial and venous thrombosis, which represent a significant cause of mortality and morbidity worldwide.[1] There may be interaction of genetic and environmental factors.[2] Investigating for thrombophilia requires an initial evaluation of classical prothrombotic risk factors, such as smoking, dyslipidemias, arterial hypertension, or diabetes mellitus. Extended profile of investigations is necessary in patients with arterial or venous thrombosis, which occurs repeatedly in unusual sites or at young age, also when family aggregation of thrombotic events is identified, as well as in women with recurrent idiopathic pregnancy loss. It must include a complete blood count and erythrocyte sedimentation rate, blood film examination, prothrombin time (PT) and activated partial thromboplastin time, factor V Leiden, antithrombin and fibrinogen levels, protein C and S, prothrombin gene mutations, homocysteinemia, methylenetetrahydrofolate reductase (MTHFR) gene mutations, and antiphospholipid antibodies.[3]

Mild to moderate hyperhomocysteinemia (HHC), meaning mildly to moderately increased plasma homocysteine (15–50 µmol/L), is uncommon in the general population. This condition is caused by either genetic factors (mutations of homocysteine metabolism enzymes) or acquired conditions, such as deficiencies in B vitamins, renal insufficiency, and some medications.[4] Two common mutations involving the MTHFR gene have been identified: C677T and A1298C.

CASE REPORT

The case of a 40-year-old nondiabetic and nonhypertensive female admitted with clinical picture of acute kidney injury (AKI) has been presented. Her medical history started 2 weeks prior to her admission, when she underwent an elective laparoscopic cholecystectomy. On the second postoperative day, she developed painful swelling of the left arm with clawing of the hand and ischemia of the fingers. Color Doppler study of left upper limb revealed acute thrombosis in left distal brachial, radial, ulnar, and median arteries with absent flow. She underwent urgent vascular intervention with left brachial artery embolectomy. She was heparinized and started on oral anticoagulant Acenocoumarol 3 mg twice a day. 1 week after embolectomy, she developed generalized swelling involving the face, arms and lower limbs, oliguria, hematuria, bleeding gums, hematemesis, shortness of breath, and fever.

On examination, she was febrile, conscious, oriented with pulse rate 124/minute, blood pressure 120/70 mm Hg, and relative risk 28/minute. She was pale, with facial and pedal edema. She had bleeding gums and left upper limb swelling. Her systemic examination was normal except bilateral fine crepitations on chest auscultation. Her investigations revealed hemoglobin (Hb) 2.9 gm/dL, total leukocyte count (TLC) 28,100/mm^3, platelets 600,000/mm^3, urea 124 mg/dL, creatinine 6.6 mg/dL, Na 118 mEq/L, K 4.4 mEq/L, PT 120 seconds, international

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normalized ratio (INR) 7.5, total serum proteins 5.2 gm/dL, serum albumin 1.8 gm/dL, Ca 9.5 mg/dL, PO_4 7.2 mg/dL, and uric acid 9.7 mg/dL. Urine analysis showed 4+ proteinuria, plenty of red blood cells (RBCs), and 30 to 40 pus cells/hpf. Liver function test, lipid profile, electrocardiogram, and two-dimensional echocardiogram were normal. Anticardiolipin antibodies and lupus anticoagulant tests were negative. Thrombophilia tests showed that protein C, protein S, and antithrombin III levels were within normal limits. Serum C3 complement was normal. Her homocysteine level was 43.08 µmol/L (normal 3.36 to 20.44 µmol/L in females).

The patient was negative for factor V Leiden and prothrombin gene mutation. However, she was found to have MTHFR gene polymorphism in the form of compound heterozygous for C677T and A1298C. Abdominal ultrasound found both normal-sized kidneys with increased echogenicity. Kidney biopsy was not performed due to risk of bleeding. She was treated conservatively with five packed cell volume (PCV) and eight fresh frozen plasma transfusions, intravenous vitamin K, injection Meropenem, injection Tranexamic acid, pyridoxine, cyanocobalamin, and folate supplements. Anticoagulants were discontinued. She did not need hemodialysis. By the end of 2 weeks, patient showed gradual improvement and was discharged with normal clinical and biochemical parameters. At the time of discharge, her laboratory tests showed PT 27.5 seconds, INR 2.29, normal renal functions, Hb 8.7 gm/dL, TLC 12,600/mm^3, PCV 26%, mean corpuscular volume 70 fL, mean corpuscular hemoglobin 18 pg, mean corpuscular hemoglobin concentration 26 gm/dL, and normal urinalysis.

DISCUSSION

The mechanism by which MTHFR gene mutations produce prothrombotic states is represented by elevated levels of plasma homocysteine due to decreased enzymatic activity of MTHFR that participates in regulating homocysteine metabolism, and a mutation of MTHFR may be a marker for possible elevated homocysteine levels. At present, HHC is considered to represent a risk factor for deep vein thrombosis and a common risk factor for recurrent venous thrombosis.[5] Heterozygotic status for two polymorphisms of the MTHFR gene, the C677T and A1298C, was found in our case. There are studies which suggest supplementation with folic acid; vitamin B6 and B12 may help in lowering the homocysteine concentrations, and even in reversing endothelial dysfunction regardless of the underlying cause of HHC.[5]

Acute kidney injury resulting from glomerular hemorrhage has been described in patients with glomerular lesions in the absence[6-8] and presence[9,10] of coagulopathy (INR 6–9 range). A biopsy study in patients who developed otherwise unexplained AKI in association with anticoagulant overdose revealed the predominant lesion of distal tubular injury and obstruction with RBCs and RBC casts.[11] The glomeruli show little or no abnormalities by light, immunofluorescence, or electron microscopy.[11] The recognition of a characteristic histologic lesion that was associated with the clinical presentation of otherwise unexplained AKI in the setting of overanticoagulation led to the term "anticoagulant-related nephropathy."

The pathogenesis event appears to be glomerular hemorrhage[12,13] resulting in the formation of obstructing RBC casts within renal tubules, which is the most conspicuous histologic feature of anticoagulant-related nephropathy.[11] The diagnosis of anticoagulant-related nephropathy should be suspected among patients who present with AKI in the setting of excessive anticoagulation. A definitive diagnosis is made by renal biopsy. However, biopsies are usually not performed, at least initially, among patients who are anticoagulated because the risk of bleeding is high.

Among patients who develop AKI and are on anticoagulant therapy, a presumptive diagnosis of anticoagulant-related nephropathy may be made if a severe warfarin coagulopathy is present and if other causes of AKI have been excluded by clinical features and serologic tests. Restoration of a therapeutic INR may limit the extent of AKI and chronic kidney injury that results from glomerular hemorrhage. The patient was discharged from the hospital with folic acid, vitamin B6, and vitamin B12 supplements. The peculiarities of the present case were the thrombotic events and the extensive arterial thrombosis in a young patient with HHC due to two heterozygotic mutations in the MTHFR gene. Anticoagulant nephrotoxicity presented as AKI, which was successfully treated conservatively.

CONCLUSION

In patients with unexplained arterial or venous thrombosis, it is appropriate to investigate for the possible coexistence of multiple predisposing factors for thrombosis, including measurement of the serum homocysteine level, in addition to investigations for mutations of the MTHFR, the prothrombin, and the factor V genes.

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REFERENCES

1. Khan S, Dickerman JD. Hereditary thrombophilia. Thromb J 2006 Sep;4:15 [cited 2006 Sep 12]. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1592479/.
2. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999 Apr;353(9159):1167-1173.
3. Hoffbrand, AV.; Moss, PAH. Essential haematology. 6th ed. Oxford: Wiley Blackwell; 2011. p. 363-371.
4. Omar S, Ghorbel IB, Feki H, Souissi M, Feki M, Houman H, Kaabachi N. Hyperhomocysteinemia is associated with deep venous thrombosis of the lower extremities in Tunisian patients. Clin Biochem 2007 Jan;40(1-2):41-45.
5. Ünlü Y, KeleşS, Becit N, Koçogşulları CU, Koçak H, Bakan E. Hyperhomocysteinaemia as a risk factor for deep-vein thrombosis. Eur J Vasc Endovasc Surg 2005 Sep;30(3):315-318.
6. Kincaid-Smith P, Bennett WM, Dowling JP, Ryan GB. Acute renal failure and tubular necrosis associated with hematuria due to glomerulonephritis. Clin Nephrol 1983 Apr;19(4):206-210.
7. Clarkson AR, Seymour AE, Thompson AJ, Haynes WDG, Chan YL, Jackson B. IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. Clin Nephrol 1977 Nov;8(5):459-471.
8. Praga M, Gutierrez-Millet V, Navas JJ, Ruilope LM, Morales JM, Alcazar JM, Bello I, Rodicio JL. Acute worsening of renal function during episodes of macroscopic hematuria in IgA nephropathy. Kidney Int 1985 Jul;28(1):69-74.
9. Kabir A, Nadasdy T, Nadasdy G, Hebert LA. An unusual cause of gross hematuria and transient ARF in an SLE patient with warfarin coagulopathy. Am J Kidney Dis 2004 Apr;43(4):757-774.
10. Abt AB, Carroll LE, Mohler JH. Thin basement membrane disease and acute renal failure secondary to gross hematuria and tubular necrosis. Am J Kidney Dis 2000 Mar;35(3):533-536.
11. Brodsky SV, Satoskar A, Chen J, Nadasdy G, Eagen JW, Hamirani M, Hebert L, Calomeni E, Nadasdy T. Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. Am J Kidney Dis 2009 Dec;54(6):1121-1126.
12. Ryan M, Ware K, Qamri Z, Satoskar A, Wu H, Nadasdy G, Rovin B, Hebert L, Nadasdy T, Brodsky SV. Warfarin-related nephropathy is the tip of the iceberg: direct thrombin inhibitor dabigatran induces glomerular hemorrhage with acute kidney injury in rats. Nephrol Dial Transplant 2014 Dec;29(12):2228-2234.
13. Kadiyala D, Brewster UC, Moeckel GW. Dabigatran induced acute kidney injury. Paper presented at the American Society of Nephrology Annual Meeting; November 1-4, 2012, San Diego, California. p. FR-PO1122.

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Study of Intradialytic Hypertension: A Single Centre Analysis

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ABSTRACT

In India around 20,000 people are dependent upon hemodialysis. The greatest burden of morbidity and mortality for hemodialysis patients are cardiovascular diseases (CVDs) including fluctuations in blood pressure, as CVDs account for approximately 50% of all deaths. Intradialytic hypertension (IDH) is one such complication responsible for increased morbidity and mortality in chronic kidney disease (CKD) patients undergoing hemodialysis. In India, there is limited data available in the literature for the incidence of IDH in CKD patients on hemodialysis. In this observational study, we evaluated the incidence of IDH in Indian CKD patients undergoing hemodialysis. We found a higher incidence of IDH (34.51%) in our cohort than in Western studies. These patients were further evaluated for the association of IDH with contributory factors and patient outcomes after one year of follow-up. This analysis yielded a novel finding of a higher incidence of IDH in patients with lower creatinine, which needs to be confirmed with multicenter trials.

KEY WORDS: Intradialytic hypertension; Chronic kidney disease (CKD); Hemodialysis.

ABBREVIATIONS: CVDs: Cardiovascular diseases; IDH: Intradialytic hypertension; CKD: Chronic Kidney Disease; ESA: Erythropoietin-stimulating agent;

INTRODUCTION

In India around 20,000 people are dependent upon hemodialysis.[1] The greatest burden of morbidity and mortality for hemodialysis patients are cardiovascular diseases (CVDs), which accounts for approximately 50% of all deaths.[2] Fluctuations in blood pressure (BP) is one of the most common complication that occurs in patients taking hemodialysis. A recent study from South Africa showed that intradialytic hypertension (IDH) may affect as many as 28% of the dialysis population.[3] The only Indian study, reported from south India in 2016, looked at the incidence of IDH on the Indian hemodialysis population but did not study the factors responsible for IDH and the impact of IDH on mortality.[4] The aim of this study was to evaluate the incidence of IDH, to study factors responsible for IDH and effect of IDH on mortality in patients attending regular dialysis sessions at a single dialysis unit.

MATERIALS AND METHODS

The present study was conducted on 142 patients with chronic kidney disease (CKD) undergoing maintenance hemodialysis at the dialysis centre of MGM Medical College & Hospital, Aurangabad, Maharashtra, India. This was a prospective observational cohort study over a period of 2.5 years from January 2013 to June 2015. All CKD patients over 18 years of age were included in the study. Patients of acute kidney injury were excluded. Primary end point was the development of IDH by patients undergoing regular hemodialysis. Secondary end points were potential biological markers associated with IDH and mortality in all patients. IDH was defined as an increase in systolic blood pressure of more than 10 mmHg during hemodialysis more than

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two hemodialysis sessions.[5] Using this definition, patients were stratified into IDH & Non-IDH categories for analysis.

Potential associated factors which were studied include age, sex, known case of hypertension (HTN) or diabetes mellitus, serum creatinine level, IV erythropoietin use, oedema, serum albumin level, and number of anti-hypertensive drugs. All patients were observed for one year to determine the mortality rate.

Dialysis prescription used for patients included in our study was as follows:

- Dialyser- Nipro elision 13 M synthetic polynephron
- Time- 4 hours
- Blood flow rate- 200-300 ml/hour
- Dialysate flow rate- 500 ml/hour
- Ultra filtration rate- as per weight gain
- Dialysate composition- Na^{++} 135-145 meq/L, K^{+} 0-4 meq/L, Ca^{++} 2.5-3.5 meq/L, Mg^{++} 0.5-0.75 meq/L, Cl 98-124 meq/L, Acetate 2-4meq/L, HCO_3 30-40 meq/L, Dextrose 11 g, pH 7.1-7.3
- Dialysate temperature- 36 °C to 37 °C
- Anticoagulation- Heparin 2000 IU at start of hemodialysis & 1000 IU per hour

Dialysis prescription was modified for IDH patients in the form of ultrafiltration rate and time of hemodialysis session. Patients who had IDH were treated either with injectable metoprolol 5 mg or injectable labetalol 20 mg with dose modified as per need. Effect of these drugs on IDH and on outcome was not studied in our study. The study was conducted in accordance with the ethical principles set out by the declaration of Helsinki and approval for the study was granted by the Human Research Ethics Committee of MGM University of Health Sciences (Registration Number- ECR/581/Inst/2013).

Statistical Analysis

SPSS software version 20 was used for the analysis of this data. Chi-square test was applied to check significant association between different groups and outcome of different attributes. *p*-value was checked at 5% level of significance.

RESULTS

Out of 142 patients, 49(34.51%) patients were found to have IDH (Figure 1). Among them 33(67.34%) were male, 30(61.22%) were above 60 years of age, 39(79.59%) were hypertensive, 10(20.41%) were diabetic, 34(69.38%) patients were receiving IV erythropoietin, 33(67.35%) were oedematous, 26(53.06%) had a serum albumin less than 2.5 mg/dL. 21(53.85%) patients

Graph 1: Incidence of IDH.

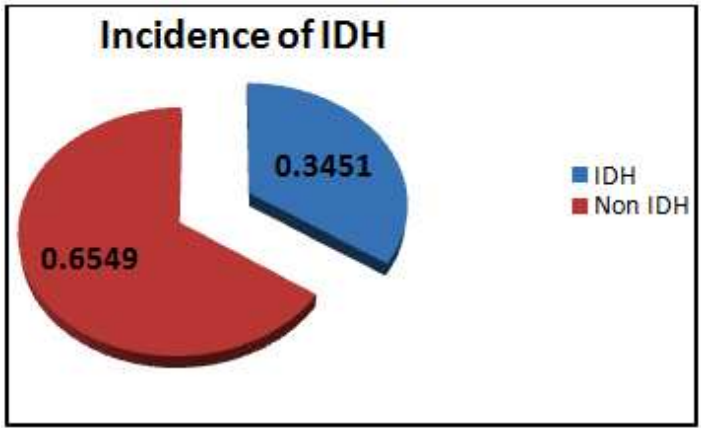

Table 1: Results Showing Non-modifiable Factors Responsible for IDH.

Factor		Results: IDH	Results: NON IDH	*p*-value
Age	>60 years	30 (61.22%)	60 (64.52%)	*p*=0.699
	<60 years	19 (38.78%)	33 (35.48%)	NS
Sex	Male	33 (67.34%)	70 (75.27%)	*p*=0.315 NS
	Female	16 (32.66%)	23 (24.73%)	

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Table 2: Results Showing Modifiable Factors Responsible for IDH.

Factor		Result		
		IDH	Non-IDH	*p*-value
Hypertension	HTN	39 (79.59%)	69 (74.19%)	*p*=0.474
	Non-HTN	10 (20.41%)	24 (25.81%)	NS
Diabetes mellitus	Diabetic	10 (20.41%)	16 (32.66%)	*p*=0.639
	Non-Diabetic	39 (79.59%)	77 (82.80%)	NS
Serum creatinine	<10 mg/dL	42 (85.71%)	61 (65.60%)	*p*=0.003
	>10mg/dL	7 (14.29%)	32 (34.40%)	S
Erythropoietin IV	EPO	34 (69.38%)	41 (44.09%)	*p*=0.004
	Non-EPO	15 (30.62%)	52 (55.91%)	S
Oedema	Oedematous	33 (67.35%)	30 (32.26%)	*p*<0.0001
	Non-Oedematous	16 (32.65%)	63 (67.74%)	S
Serum albumin	<2.5 mg/dL	26 (53.06%)	30 (32.26%)	*p*=0.016
	>2.5 mg/dL	23 (46.94%)	63 (67.74%)	S
No. of antihypertensive drugs	<2 drugs	21 (53.85%)	49 (71.01%)	*p*=0.073
	>2 drugs	18 (46.15%)	20 (28.99%)	NS

IDH Intradialytic hypertension; HTN Hypertension.

were taking less than two anti-hypertensive drugs. 19(38.77%) patients died within 1 year of study initiation. Novel finding was 42(85.71%) patients among IDH population had serum creatinine less 10 mg/dL A detailed description of all factors is listed in Tables 1 and 2.

DISCUSSION

The present study is a prospective observational study of the incidence of IDH in CKD patients undergoing maintenance hemodialysis. A total of 142 CKD patients were studied over a period of 2.5 years. Out of 142 patients, 49 were found to have IDH with incidence of 34.51%, which was greater than the incidence found in western studies by Stidley et al[6], Mees D[7], in which it was found to be 5-15%. A recent study from South Africa showed that IDH may affect as many as 28% of the dialysis population.[3] None of these studies focused in the Indian patient population. One study from South India by Darimireddi SK et al[4] found the incidence of IDH to be 49% in 100 patients studied. This supports our finding that the incidence of IDH is higher in Indian population.

Out of 142 patients in our study, 103 were males and 39 were females. 90 patients were above 60 years of age. In this study, no relation was found between the incidence of IDH and the age or sex of the patient. No previous studies demonstrated a statistically significant difference among males and females. According to a study by Inrig et al[5] on 32,295 patients, incidence of IDH was found more amongst the elderly. Similarly, in our study we found that IDH occurred more frequently in the elderly but it was not statistically significant.

No significant relationship was found between the incidence of IDH and previous HTN or diabetes in CKD patients. Although, no study examined the burden of IDH in known hypertensive patients, removal of anti-hypertensive medications during hemodialysis is one of the proposed mechanisms for IDH.[8] No comparable data regarding the relationship of diabetes mellitus and IDH was found in previous studies.

A significant inverse relationship was found between the incidence of IDH and serum creatinine level, with a higher incidence of IDH in patients with lower serum creatinine levels (Figure 2). In a study previously published by Inrig et al[9] patients who experienced IDH were thinner, had lower muscle mass (lower serum creatinine) and were more likely to experience a rise in blood pressure (BP) with minimal volume excess. Similarly, in our study occurrence of IDH among patients with lower creatinine value was statistically significant with greater incidence of IDH in lower creatinine group. Majority of our study population were frail, mostly due to malnutrition and the disease itself. Acute intradialytic changes in endothelial cell function have been proposed as etiologies for the increase in vascular resistance, although it is unclear if endothelin-1 or some other vasoconstrictive peptide is responsible. Chou et al[10] demonstrated imbalances in endothelial cell-derived mediators after dialysis in the IDH patients. Specifically, there were higher levels of the vasoconstrictor endothelin-1 (ET-1) and smaller ratios of the vasodilator nitric oxide to ET-1. Karakelleoglu et al[11] showed that the patients with malnutrition have higher endothelin-1 levels. Recent study by Park et al[12] indicate that lower creatinine levels in patients undergoing hemodialysis are associated with lower muscle mass, malnutrition, and mortality. These findings sup-

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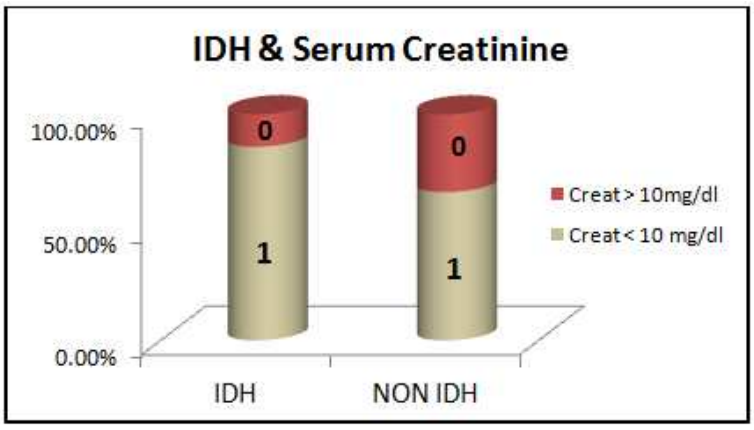
Graph 2: IDH and Serum Creatinine Co-Relation.



port that lower creatinine level which is a marker for malnutrition and is responsible for IDH through ET-1 mediated IDH.

A study by Sarkar SR et al[13] found an increased incidence of IDH among patients receiving IV erythropoietin therapy. A study by Abraham et al[14] found that 21% of patients had clinically important increases in BP during treatment of anaemia with erythropoietin. In a small investigation of the acute effects of erythropoietin-stimulating agent (ESA) in hemodialysis patients within 30 minutes following intravenous ESAs, there was a significant increase in ET-1 and a concomitant rise in mean arterial pressure (MAP) which was not demonstrated in patients given subcutaneous ESA or placebo.[12] In addition, 53% (10/19) of patients given intravenous ESA, had an increase in MAP>10 mmHg in the intradialytic period. Thus, if ESA is given intravenously, prior to the end of hemodialysis, it is possible that this may contribute to development of IDH in susceptible patients. It is possible that vasoconstriction, arising due to improved tissue oxygenation may result in IDH in some patients. In our study, incidence of IDH was higher in patients receiving IV erythropoietin during hemodialysis sessions, which was found to be statistically significant. This result was similar to studies done by Abraham et al[14] and Buckner et al.[15]

A significant relationship was also found between incidence of IDH and presence of oedema, with greater incidence of IDH in oedematous patients secondary to volume overload. Similar findings were shown in studies by Inrig et al[9] and Agarwal et al.[16] It was also observed that BP may paradoxically rise with ultrafiltration, when patients are volume overloaded. In a study by Inrig et al[9] it was found that incidence of IDH was higher in patients having low serum albumin levels. Similar results were found in our study. The mechanism of IDH may be due to reduced blood viscosity causing high cardiac output and increased peripheral vascular resistance.

In the Inrig et al[9] study, it was found that IDH incidence was greater in hypertensive patients who were prescribed greater number of anti-hypertensive drugs compared to those who were given standard regimen. In our study, incidence of IDH was more with patients receiving more than two anti-hypertensive drugs than patients receiving less than two anti-hypertensive drugs but it was statistically not significant. It is studied in literature that there is an association between dialysate to serum sodium gradients and BP increase during dialysis in patients with IDH, although it is unclear if this is related to endothelial cell activity or acute osmolar changes. In addition to probing the dry weight of patients with intradialytic hypertension, other management strategies include lowering dialysate sodium and changing anti-hypertensives to include carvedilol or other poorly dialyzed anti-hypertensives will help to reduce IDH.[17] All patients in our study were prescribed similar dialysis prescription to remove this confounding factor, and all patients of IDH were prescribed non-dialyzable anti-hypertensives for treatment of IDH. Except angiotensin receptor blocker (ARB) and angiotensin-converting enzyme (ACE) inhibitors, most of other drugs are dialyzed and hence incidence of IDH is more when more drugs are prescribed.

A significant relationship was found between incidence of IDH and survival of CKD patients. Patients having IDH had a more frequent occurrence of deaths at one year (Figure 3). Inrig et al[18] in a cohort of 438 prevalent hemodialysis patients, demonstrated that systolic BP elevations of more than 10 mmHg with hemodialysis are associated with a 20% increased odds of death or hospitalization at 6 months. Inrig et al[9] also demonstrated that increasing systolic BP in incident hemodialysis patients was associated with poorer 2-year survival.

CONCLUSION

Incidence of IDH in our study was 34.51% which was higher than what was found in the African study.[3] Only one Indian study showed incidence of 49% in 100 patients studied.[4] This suggests incidence of IDH is more in Indian population. Prognosis of CKD patients was worse among IDH group as 38.77% patients died within 1 year. We found a statistically significant relation between incidence of IDH and serum creatinine level, use of IV erythropoietin, oedema, and serum albumin level. However, no

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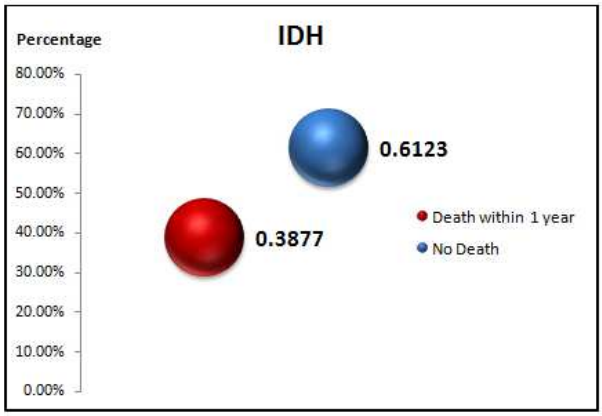
Graph 3: Results Showing Death within 1 Year of IDH.



significant relation was found between incidence of IDH and age of the patient, sex of the patient, hypertension, diabetes mellitus and number of anti-hypertensive drugs.

IDH is preventable if we control modifiable risk factors such as avoiding use of IV erythropoietin, reducing interdialytic weight gain, correction of serum albumin. This will reduce cardiovascular morbidity and mortality in CKD patients on maintenance hemodialysis. This is the first study in India which showed factors responsible for IDH and impact of IDH on mortality in Indian population. The other Indian study[4] has studied only incidence of IDH without studying factors responsible for IDH and its impact on mortality. No studies in literature till date evaluated the role of previous hypertension or diabetes with incidence of IDH. There was no association found between IDH and hypertensive or diabetic status of patient in our study. Higher incidence of IDH in patients with lower creatinine is a novel finding which needs to be confirmed with multicenter trials. Thus, IDH is an important cardiovascular event in Indian hemodialysis population which contributes to increased mortality in patients on hemodialysis although its exact pathogenesis is not known. Measures should be taken to reduce its incidence in hemodialysis patients.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Dash SC, Agarwal SK. Incidence of chronic kidney disease in India. *Nephrol Dial Transplantation*. 2006; 21: 232-233. doi: 10.1093/ndt/gfi094

2. Van Buren PN, Kim C, Toto RD, Inrig JK. The prevalence of persistent intradialytic hypertension in a haemodialysis population with extended follow-up. *Int J Artif Organs*. 2012; 35(Suppl 12): 1031-1038. doi: 10.5301/ijao.5000126

3. Sebastian S, Filmalter C, Harvey J, Chothia M-Y. Intradialytic hypertension during chronic haemodialysis and subclinical fluid overload assessed by bio impedance spectroscopy. *Clinical Kidney J*. 2016; 9 (Suppl 4): 636-643. doi: 10.1093/ckj/sfw052

4. Darimireddi SK, Salla SP, Bayya AB, Gedda JP. A study on incidence of acute cardiovascular complications during maintenance haemodialysis of end-stage renal failure patients. *J Evid Based Med Health*. 2016; 3: 4230-4235. Web site. http://www.jebmh.com/data_pdf/Surya%20Prakash%20-%201%20--%20FINAL.pdf. Accessed January 29, 2017.

5. Inrig JK, Patel UD, Gillespie BS. Relationship between interdialytic weight gain and blood pressure among prevalent haemodialysis patients. *Am J Kidney Dis*. 2007; 50(1): 108-118. doi: 10.1053/j.ajkd.2007.04.020

6. Stidley CA, Hunt WC, Tentori F. Changing relationship of blood pressure with mortality over time among hemodialysis patients. *J Am Soc Nephrol*. 2006; 17: 513-520. doi: 10.1681/ASN.2004110921

7. Mees D. Rise in blood pressure during hemodialysis-ultrafiltration: A "paradoxical" phenomenon? *Int J Artif Organs*. 1996; 19(10): 569-570.

8. Li Z, Lacson E, Lowrie EG. The epidemiology of systolic blood pressure and death risk in hemodialysis patients. *Am J Kidney Dis*. 2006; 48(4): 606-615. doi: 10.1053/j.ajkd.2006.07.005

9. Inrig JK, Patel UD, Toto RD, Szchzech LA. Association of

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-SE-2-101

blood pressure increases during haemodialysis with 2-year mortality in incident haemodialysis patients: A secondary analysis of the dialysis morbidity and mortality wave 2 study. *Am J Kidney Dis*. 2009; 54(5): 881-890. doi: 10.1053/j.ajkd.2009.05.012

10. Chou KJ, Lee PT, Chen CL, et al. Physiological changes during hemodialysis in patients with intradialysis hypertension. *Kidney Int*. 2006; 69: 1833-1838. doi: 10.1038/sj.ki.5000266

11. Karakelleoglu C, Orbak Z, Ozturk CF, Kosan C. Hypomagnesemia as a mortality risk factor for protein energy malnutrition. *J Health Popul Nutr*. 2011; 29(2): 181-182.

12. Park J, Mehrotra R, Rhee CM, et al. Serum creatinine level, a surrogate of muscle mass, predicts mortality in peritoneal dialysis patients. *Nephrol Dial Transplant*. 2013; 28(8): 2146-2155. doi: 10.1093/ndt/gft213

13. Sarkar SR, Kaitwatcharachai C, Levin NW. *Complications during Haemodialysis*. USA: McGraw-Hill Professional; 2005.

14. Abraham PA, Macres MG. Blood pressure in hemodialysis patients during amelioration of anemia with erythropoietin. *J Am Soc Nephrol*. 1991;2: 927-936. Web site. http://jasn.asnjournals.org/content/2/4/927.short. Accessed January 29, 2017.

15. Buckner FS, Eschbach JW, Haley NR, Davidson RC, Adamson JW. Hypertension following erythropoietin therapy in anemic hemodialysis patients. *Am J Hypertens*. 1990; 3: 947-955. doi: 10.1093/ajh/3.12.947

16. Agarwal R, Light RP. Intradialytic hypertension is a marker of volume excess. *Nephrol Dial Transplant*. 2010; 25(Suppl 10): 3355-3361. doi: 10.1093/ndt/gfq210

17. Van Buren PN, Inrig JK. Mechanisms and treatment of intradialytic hypertension. *Blood Purif*. 2016; 41: 188-193. 10.1159/000441313

18. Inrig JK, Oddone EZ, Hasselblad V. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. *Kidney Int*. 2007; 71: 454-461. doi: 10.1038/sj.ki.5002077

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Case Report

Fusarium Peritonitis an Uncommon Complication in a Patient on Continuous Ambulatory Peritoneal Dialysis - A Case Report

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ABSTRACT

Fungal Peritonitis is a serious complication of treatment with peritoneal dialysis, with high rates of morbidity and mortality. In majority of the cases cause of fungal peritonitis is *Candida* species, with *Candida albicans* predominating. Infections by *Fusarium* species can be superficial or limited to single organs in otherwise healthy patients. In contrast, disseminated fusariosis affects the immunocompromised host. Fusarium infection is uncommon cause of peritonitis among patients on Continuous Ambulatory Peritoneal Dialysis [CAPD]. Here, we report a case of peritonitis due to *fusarium* species in a patient on Continuous Ambulatory Peritoneal Dialysis. Fusarium infection in patients on CAPD can be life threatening

Key words: *Fusarium;* Fungal peritonitis, Continuous Ambulatory Peritoneal Dialysis [CAPD].

INTRODUCTION

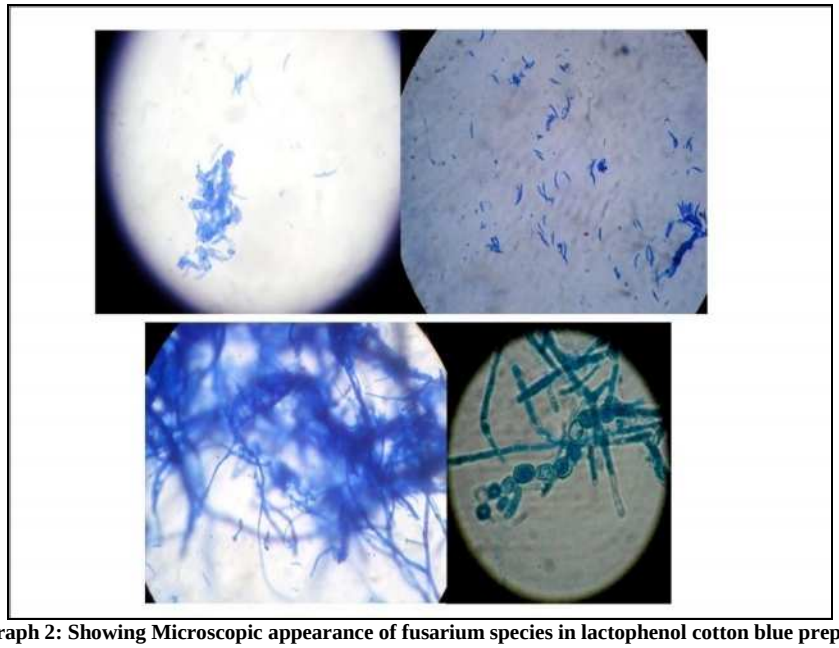
Peritonitis is the main complication of continuous ambulatory peritoneal dialysis. Fungal Peritonitis accounts for 1 - 16 % episodes in various studies. [1-3] Patients with previous bacterial peritonitis and antibiotic usage are at greater risk of developing fungal peritonitis. [3] Predominant cause of Fungal Peritonitis is Candida species. [2,4,5] The genus Fusarium is a common soil saprophyte and important plant pathogen that causes a broad spectrum of human disease, including mycotoxicosis, and infections which can be superficial, locally invasive or disseminated. [6] Fusariosis is an invasive mold infection associated with *Fusarium* species, most commonly *F. solani*. The skin and respiratory tract are the primary portals of entry. Localized skin infections may occur at sites of trauma in immunocompetent hosts. [7] Fusarium infection in immunocompromised patients has been reported in various studies. [8,9] Fusarium infection is uncommon cause of peritonitis among patients on CAPD. [4] This report presents the first known case of *Fusarium* peritonitis in a patient on CAPD in MGM Medical College, Aurangabad.

CASE REPORT

A 27 year young female patient had been on CAPD for one & half years. She presented in Medicine OPD of our Institute with H/o fever, vomiting, cough, pain in epigastric region & decreased urine output four days prior to hospitalization in 14 March 2013. She was a known case of chronic kidney disease with hypertension with hypothyroidism. She was on treatment for hypertension and hypothyroidism for last one year duration.

The diagnosis of peritonitis was based on clinical manifestations. She had not documented any episode of peritonitis prior to admission in our Institute. She was non diabetic. In the hospital she received inj metronidazole 400 mg TDS for 5 days, inj ceftazidime 1 gm after each cycle of peritoneal dialysis, inj Vancomycin 1 gm every 48 hrs, followed by Tab metronidazole 400 mg TDS, tab rifaximin 400 mg TDS & Tab fluconazole 150 mg once a day and vancomycin powder locally QID prior to availability of fungal culture report. Analysis of CAPD fluid was carried out, which showed total leucocyte count of 100 cells per mm. [3] It showed predominant [80%] polymorphs. The percentage of lymphocytes in CAPD fluid was 20%.

Photograph 1: showing growth of fusarium species on Sabraoud's dextrose agar.



Photograph 2: Showing Microscopic appearance of fusarium species in lactophenol cotton blue preparation

We received CAPD fluid of this patient for Gram stain, routine bacterial culture & fungal culture in Microbiology department. No organism could be detected on gram staining of the specimen. Routine culture was negative for growth of bacterial pathogen. Direct microscopic examination of CAPD fluid revealed no fungal element but culture of CAPD fluid on Sabraoud's agar without cycloheximide yielded growth which was identified on the basis of their macroscopic [photograph1] and microscopic appearance. Microscopic examination of colony showed presence of sickle shaped multicelled microconidia having 3 - 5 septae typical of fusarium species [photograph 2].

The patient was discharged with treatment advised which included inj fluconazole 200 mg on alternate day, inj vancomycin 1 gm after 48 hrs intra

peritoneally, in addition to other supportive treatment. She was advised to continue CAPD and come for follow up after 7 days. While on treatment, patient died due to sepsis in May 2013.

DISCUSSION

Peritoneal dialysis has been shown to be practical, safe & cost effective alternative to chronic haemodialysis. Bacterial peritonitis is most commonly encountered in these patients. The definition of CAPD peritonitis includes at least two of the following criteria: symptoms or signs (or both) of peritonitis, a cloudy dialysate (effluent) and a positive culture (and / or Gram stain of the dialysate). [10] The criteria for diagnosis of fungal peritonitis do not differ from those of bacterial peritonitis. The isolation of fungal organism on gram stain and or culture is diagnostic of fungal peritonitis. [4] Patients with previous bacterial peritonitis and antibiotic usage are at greater risk of developing fungal peritonitis. [3] Various studies report that fungal peritonitis accounts for 1-16 % episodes of peritonitis in patients on peritoneal dialysis. [1-3] *Fusarium* species are commonly found as saprophytes on organic debris & in soil. [11] *Fusarium* species cause a broad spectrum of infections in humans, including superficial, locally invasive, and disseminated infections. The clinical form of fusariosis depends largely on the immune status of the host and the portal of entry of the infection. [8] The principal portal of entry for Fusarium *spp*. is the airways, followed by the skin at site of tissue breakdown and possibly the mucosal membranes. [8] The duration of peritoneal dialysis treatment before the diagnosis of fungal peritonitis in our patient is also similar to the range reported by other studies [1,12] In our study Gram staining of the fluid revealed no organisms this finding is in concordance with study by Joseph et al [13] The organism has a propensity to attach to foreign bodies such as intravascular and intraperitoneal catheters. Therefore, successful treatment of infections caused by *Fusarium* may require catheter removal in addition to systemic antifungal therapy. [1,12,13] Prasad et al in their study reported that abdominal pain, abdominal pain with fever, and catheter in situ are the most commonly noted risk factors for mortality. [4,14] Fungal peritonitis, though uncommon, has great morbidity and is more difficult to treat successfully than bacterial peritonitis. In present study, the patient died of sepsis.

CONCLUSION

Fungal agents cause significant morbidity and mortality in patients with CAPD peritonitis and are usually more difficult to treat. Fusarium infection in patients on CAPD can be life threatening Fungal infections may be clinically suspected on the basis of clinical and laboratory findings, which should lead to prompt therapy.

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REFERENCES

1. Evangelia Bibashi, Dimitrios Memmos Elizabeth Kokolina et al Fungal peritonitis complicating peritoneal dialysis during an 11 – year period : Report Of 46 Cases Clinical Infectious Diseases 2003; 36 : 927–31
2. Vikrant S., Guleria R.C , Kanga A et al Microbiological aspects of peritonitis In patients on continuous ambulatory peritoneal dialysis Indian J Nephrol 2013 ; 23(1) : 12–17.
3. Indhumathi E, Chandrasekaran V, Jagadeswaran D et al The risk factors and outcome of fungal peritonitis in continuous ambulatory peritoneal dialysis patients. Indian J Med Microbiol. 2009; 27(1): 59-61.
4. Narayan Prasad And Amit Gupta, Fungal peritonitis in peritoneal

dialysis patients Perit Dial Int, 2005; 25: 207–222.
5. Predari Sc, De Paulis An, Verón D.et al Fungal peritonitis in patients on peritoneal dialysis : Twenty five years of experience in a teaching hospital in Argentina Revista Argentina de Microbiología (2007) 39: 213-217.
6. Mariya Cecelia Dignani, Elias N. Kiwan, Elias J Anissie. Hyalohyphomycoses In: Anaissie, McGinnis, Pfaller ed, Clinical Mycology Ist ed. Elsevier Science (USA) 2003; 309 -324.
7. Stanley W. Chapman, Donna C Sullivan, Miscellaneous Mycoses and Algal Infections In: Fauci, Braunwald, Kasper, Hauser, Longo, Jameson. Loscalzo, ed. Harrison Harrison's Principle of internal Medicine 17 th ed. McGraw-Hill Companies, Inc (USA) 2008.
8. Marcio Nucci And Elias Anaissie. Fusarium infections in immunocompromised patients Clin. Microbiol. Rev. 2007;20 (4) : 695.
9. Banerji J And Singh J .Cutaneous fusarium infection in a renal transplant recipient: a case report, Journal Of Medical Case Reports 2011; 5:205
10. Alexander Von Graevenitz and Daniel Amsterdam. Microbiological aspects of peritonitis associated with continuous ambulatory peritoneal dialysis Clinical Microbiology Reviews, 1992: 36-48
11. Caroline B Moore and David W. Denning, Deep Hyalohyphomycosis In: Borriello SP, Murray PR, Funke G. ed. Topley and Wilson's. Microbiology and Microbial Infection. Mycology 10 th ed. Wiley J and Sons (UK) 2009.
12. David J. McNealy, Stephen I. Vas Nicholas Dombros et al Oreopoulos Fusarium peritonitis: an uncommon complication of continuous ambulatory peritoneal dialysis Downloaded From Http: //Www Pdiconnect. Com on 29 April 2013.
13. Joseph T. Flynn, Debrah Meislich, Bruce A. Kaiser, et al Fusarium peritonitis in a child on peritoneal dialysis: case report and review of the literature Peritoneal Dialysis International, 1995 (16): 52-57.
14. Prasad KN, Prasad N, Gupta A et al Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis: a single centre Indian Experience. J Infect 2004; 48: 96-101.

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Role of Iron Deficiency Anemia in Patients with Chronic Kidney Disease

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Abstract:
Background: *Chronic kidney disease (CKD) is a worldwide health problem. CKD is a progressive condition and ultimately end up with kidney failure. A normocytic, normochromic anemia is observed in CKD. The primary cause in patients with CKD is insufficient production of erythropoietin (EPO) by the diseased kidneys. Anemia is both a complication of CKD as a part of uremic syndrome and a risk factor which influences the adverse outcomes of CKD, So evaluation and management of anemia is important to prevent the progress of CKD and for the general well being of the patient. As the renal function worsens, there is a progressive increase in the percentage of CKD patients with anemia.*
Aims and Objectives*: To determine the prevalence of iron deficiency anemia in patient with chronic kidney disease and study the effect of iron deficiency anemia on survival of CKD patients.*
Results: *In present study the overall prevalence of iron deficiency anemia in chronic kidney disease was 42.63% whereas in males, the prevalence of iron deficiency anemia was 44.4% which was less than female patients (55.6%). The observed values of iron deficiency anemia in CKD in relation to age group, hypertension and type of iron therapy have been found satistically nonsignificant, however iron deficiency anemia outcome with sex group, stage of CKD, diabetes and dialysis therapy were found to be satistically significant.*
Conclusion: *Iron deficiency anemia is common in CKD patients (42.63%). Functional Iron deficiency is seen in 39.03%. Iron deficiency is related to stage of CKD, Sex, Diabetes mellitus, erythropoietin therapy and dialysis therapy. There was no relation of Iron deficiency anemia with age, hypertension, and type of iron therapy. However mortality was not related to iron deficiency in CKD patients.*
Keywords: *Chronic kidney disease, Iron deficiency anemia, Hemodialysis, Erythropoietin.*

I. Introduction

Chronic kidney disease is a significant cause of morbidity and mortality world wide[1]. In India, there is a rising incidence and prevalence of kidney failure, with poor outcomes and high cost. The hallmark of CKD is structural and functional damage of the glomeruli of the kidney. The most important outcomes of this kidney damage are loss of kidney function and cardiovascular disease leading to premature death. In CKD, Erythropoietin (EPO)is produced in the peritubular cells of the kidney and is the major hormone involved in the production of red blood cells (erythropoiesis) when erythrocyte cells are produced. Anaemia starves the body of Oxygen and causes decreased capacity, cognitive impairment, and diminished quality of life[2]. Management of anemia in chronic kidney disease involves use of EPO. However EPO is effective only when iron is available in sufficient quantity. Hence evaluation of iron status in patients with chronic kidney disease is very vital. This study is under taken to study haematological profile of iron deficiency anemia in chronic kidney disease.

Defining CKD[3] as Kidney damage for 3 months or more as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifests by either : a) Pathological abnormalities detected by histopathological studies; or b) Markers of kidney damage, including abnormalities in the composition of the blood or urine , or abnormalities in imaging tests. GFR less than 60ml/min/1.73 m^2 for 3 months or more, with or without kidney damage.

Fishbane et al. found high rates of iron deficiency in adult men (57.8 to 58.8%) and women (69.9 to 72.8%) with CKD stages 3-5 in the NHANES 3 and 1999-2004 survey[4].

The prevalence of anemia in stage 3-5 CKD patients aged >64 in 2007-2010 NHANES survey was 24.4%. The prevalence of CKD in the SEEK-India cohort was observed to be 17.2% with 6% have CKD stage 3 or worse. Prevalence of CKD stages 1, 2, 3, 4 and 5 was 7%, 4.3%, 4.3%, 0.8% and 0.8% respectively[5].

National kidney foundation kidney dialysis outcomes quality initiative
(NKF K/DOQI 2012) DEFINES IRON DEFICIENCY ANEMIA[6, 7] :
1. **Absolute Iron Deficiency:** Ferritin <100 ng/ml and TSAT <20%.
2. **Functional Iron Defeciency:** Ferritin > 100 ng/ml and TSAT <20%.

Iron deficiency is common in patients with CRF. 25-37.5% patients of CRF have iron deficiency[8]. The cause's are : Decreased iron absorption as a part of uremic syndrome, loss of RBCs and Fe due to bleeding tendencies in uremic syndrome, dialysis related loss of RBCs and Fe, blood loss due to frequent blood tests.

Serum ferritin reflects body stores of iron[9]. A level of <100 ng/ml indicates absolute iron deficiency in CKD. But this is not very sensitive; it can reflect depleted stores only when the depletion is very low. Serum ferritin values < 30ng/ml indicate severe iron deficiency and are highly predictive of absent iron stores in bone marrow. It is not very specific as serum ferritin is also an acute phase reactant and elevated in conditions such as hyperthyroidism, inflammation/infection, hepatocellular disease, malignancies, alcohol consumption and oral contraceptives[10]. Transferrin saturation reflects the amount of available iron for erythropoiesis. In iron deficiency, elevated transferrin levels maintain the circulating iron pool despite the marked decrement in Tsat. A level of <20% in CKD indicates absolute iron deficiency. Transferrin saturation is decreased only when serum ferritin is decreased in absolute iron deficiency.

The ESRD National Cooperative Anemia Project, a personal communication, J. Wish, April;1997 - 60% of patients were found to be iron deficient. Recent analysis of the National Health and Nutrition Examination Survey 4 suggests that up to 50% of patients with CKD stages 2-5 have absolute or relative (functional) iron deficiency. In CKD, both absolute and relative iron deficiency are common[11].

Aims and Objectives :
- To determine the prevalence of iron deficiency in patient with chronic kidney disease.
- To study the effect of iron deficiency anemia on survival of CKD patients.

II. Material And Methods

It is an open non randomized prospective, cross sectional observational study to determine the prevalence of iron deficiency anemia with CKD and the effect of IDA on survival of CKD patients. The data obtained was studied on 9 parameters and chi-square test was applied and P value was calculated to the attributes to test their significance at 5 % level of significance.
No. Of cases: 190

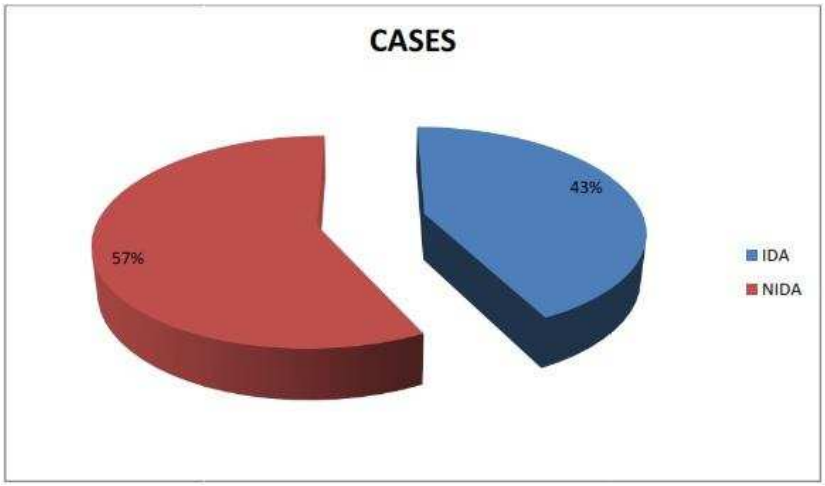
Case definition: Patient was defined as having Iron deficiency anemia in chronic kidney disease if there was: Serum ferritin >100ng/ml and transferring saturation <20%.

Inclusion criteria: All cases diagnosed as chronic kidney disease.

Exclusion criteria: Cases of acute kidney injury, bleeding diathesis, acute bleeding (urological or gastro enterological).
Methodology : The patients under study evaluated as per protocol.
1- **History :** Age, sex, history of hypertension / diabetes mellitus, EPO therapy/ Iron therapy, Hemodialysis.

2. Laboratory investigations :
a) Hemoglobin
b) Serum creatinine
c) Iron studies (serum iron, serum ferritin, TIBC, Transferrin saturation)

Results: Pia chart : showing prevalence of iron deficiency anemia (IDA)



Out of 190 patients, 81(43%) patients were found to have iron deficiency anemia whereas 109(57%) patients were NON-IDA.

Table 1 : Association of different parameters (satistically significant) with iron deficiency anemia (IDA) :

PARAMETERS	IDA	NIDA	TOTAL	CHI-SQUARE	P- VALUE
SEX : MALE	36(44.4%)	73(66.9%)	109	9.6427	0.001 S
: FEMALE	45(55.6%)	36(33.1%)	81	S (>3.84)	(<0.05)
DIABETES	30(37%)	23(21.1%)	53	5.8676	0.015 S
NON-DIABETIC	51(63%)	86(78.9%)	137	S	
EPO THERAPY	55(67.9%)	56(51.4%)	111	5.224	0.022 S
NON-EPO	26(32.1%)	53(48.6%)	79	S	
HEMODIALYSIS	70(86.4%)	78(71.6%)	148	5.9593	0.014 S
NON-HD	11(13.6%)	31(28.4%)	42	S	

The observed values of iron deficiency anemia in CKD in relation to sex group, diabetes and erythropoietin therapy and hemodialysis therapy have been found satistically significant.

Table 2: Association of different parameters (satistically nonsignificant) with iron deficiency anemia (IDA)

PARAMETERS	IDA	NIDA	TOTAL	CHI- SQUARE	P- VALUE
AGE: 21-40	20(24.69%)	26(23.85%)	46	0.0373	0.9981 S
: 41-60	30(37.03%)	40(36.69%)	70	NS	(>0.05)
: > 60	31(38.28%)	41(39.46%)	74	(<3.84)	
HYPERTENSION	54(66.7%)	83(76.1%)	137	2.0763	0.14 NS
NON-HTN	27(33.3%)	26(23.9%)	53	NS	
IRON : ORAL	10(12.34%)	20(18.34%)	30		
: I. V.	27(33.33%)	43(39.445)	70	2.7376	0.6026 NS
NOT ON IRON	44(54.32%0	46(42.22%)	90	NS	
CKD STAGE : 4	8(10.1%)	6(5.7%)	14	1.2485	0.2616 NS
: 5	71(89.9%)	99(94.3%)	170	NS	
DIED	27(33.3%)	36(33%)	63	0.002	0.9612 NS
ALIVE	54(66.7%)	73(67%)	127	NS	

The observed values of iron deficiency anemia in CKD in relation to age group, hypertension and type of iron therapy, CKD staging and prognosis have been found satistically non-significant.

III. Discussion

This study was undertaken in view of prevalence of iron deficiency anemia in chronic kidney disease patients. In the present study, prevalence of iron deficiency anemia, factors responsible for it and its effect on survival of chronic kidney disease patients was studied. Total 190 patients diagnosed as chronic kidney disease were studied. Out of 190 patients, 81 (42.63%) patients were found to have iron deficiency anemia (IDA). The observed values of iron deficiency anemia in CKD in relation to age group, hypertension and type of iron therapy have been found satistically non significant, however iron deficiency anemia outcome with sex group, stage of CKD, diabetes and dialysis therapy were found to be satistically significant.

According to a study by Bowling CB, Inker LA et al[12] , Prevalence of iron deficiency anemia was found more in elderly age group. In this study, contradictory results are found probably because of more young patients developing CKD in Indian population.

According to a study by Singh et al[5] . Prevalence of iron deficiency anemia is higher in males than females. In this study, prevalence of iron deficiency anemia is higher in females than males.

In a a study previously published by New JP, Aung T, Baker PG et al[13] and Fishbane et al[14]. Prevalence of iron deficiency anemia was found to be higher in patients with Diabetes Mellitus. In this study, Prevalence of iron deficiency anemia is found to be higher in patients with Diabetes Mellitus, which is similar to previous studies. According to a study by James B. Post et al[15] and Saul Nurko et al[16] , there was increased prevalence of iron deficiency anemia among the patients receiving erythropoietin. In the present study, there is a direct relation between erythropoietin therapy and IDA i.e. prevalence of IDA is higher in patients receiving erythropoietin therapy, which is similar to previous studies.

According to a study by Melissa E. Stauffer, Tao Fan et al[17] , Prevalence of anemia increased with stage of CKD, from 8.4% at stage 1 to 53.4% at stage 5. In the present study, prevalence of iron deficiency anemia is increased with stage of CKD, which is similar to previous studies.

In a study previously published by Allen R. Nissenson and Jur Strobes et al[18] , 60% of patients were found to be iron deficient on hemodialysis. In our study, There is a direct relation between Hemodialysis and IDA i.e. prevalence of IDA is higher in patients on Hemodialysis, which is similar to previous studies.

IV. Conclusion

Iron deficiency anemia is common in CKD patients (42.63%). Functional Iron deficiency is seen in 39.03%. Iron deficiency is related to stage of CKD, Sex, Diabetes mellitus, erythropoietin therapy and dialysis therapy. There was no relation of Iron deficiency anemia with age, hypertension, and type of iron therapy. However mortality was not related to iron deficiency in CKD patients.

References

[1]. National Kidney Foundation- DOQI clinical practice guidelines on chronic kidney disease. Executive summary. Am J Kidney Dis 2002; 39(2): suppl 1:S18.
[2]. National Kidney Foundation- DOQI clinical practice guidelines on chronic kidney disease. Executive summary. Am J Kidney Dis 2002;39(2):suppp1:S16.
[3]. National Kidney Foundation- DOQI clinical practice guidelines on chronic kidney disease. Background. Am J Kidney Dis 2002;39(2) suppl 1:S32.
[4]. Nissenson AR, Wade S, Goodnough T et al. (2005) Economic burden of anemia in an insured population. J Manag Care pharm 11: 565:574.
[5]. Singh et al.Epidermiology and risk factors of chronic kidney disease in india. BMC Nephrology 2013,14:114, pg-1-10.
[6]. NKF K/DOQI National Kidney Foundation kidney dialysis outcomes quality initiative . Am J Kidney Dis 1997;30(4) supp 3:S16.
[7]. KIDGO Clinical Practice Guidelines for Anemia in Chronic Kidney Disease.volume 2,issue 4, Augest (2) 2012.
[8]. Fishbane S, Maesaka JK. Iron management in end-stage renal diseases. Am J Kidney Dis 1997;29(3): 319-333.
[9]. Fernandez-Rodrigauz AM, Guindeo-Casasus MC, Molero-Labarta T et al. Diagnosis of iron deficiency in chronic renal failure.Am J Kidney Dis 1999; 34:508-513.
[10]. Kalantar-zadeh K, Hofken B, Wunch H et al. Diagnosis of iron deficiency in chronic renal failure patients during the post-erythropoietin era. Am J Kidney Dis 1995;26:292-296.
[11]. UNITED STATES RENAL DATA SYSTEM: USRDS 1996 Annual data report. Bethesda, National Institute of Health, April 1996.
[12]. Bowling CB, Inker LA, Gutierrez OM, Allman RM, Warnock DG, et al.(2011) Age specific association of reduced estimated glomerular filtration rate with concurrent chronic kidney disease complications. Clin J Am Soc Nephrol 6: 2822-2828.
[13]. New JP, Aung T, Baker PG et al. The high prevalence of unrecognised anemia in patient with diabetes and chronic kidney disease: populationbased study. Diabetic Medicine 2008; 25:564-569.
[14]. Fishbane S, Pollack S, Feldman HI, Joffe MM. Iron indices in chronic kidney disease in the national health and nutrition examination survey 1998-2004. Clin J Am Nephrol 2009;4:57-61.
[15]. James B. Post, Barry M. Wilkes and Michael F. Michelis. International Urology and Nephrology (2006)38:719-723.
[16]. Eschbach JW, Haley NR, Egrie JC, Adamson JW. A comparision of the responses to recombinant human erythropoietin in normal uremic subjects. Kidney Int 1992; 42: 407-416.
[17]. Melissa E. Stauffer, Tao Fan, prevalence of anemia in chronic kidney disease in the United States. Am J Kidney Dis 2014; 9(1):5 92.
[18]. Allen R. Niessenson and Jur Strobos. Iron deficiency in patients with renal failure. Kidney International 1999;vol.55,suppl(69):340-382.

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Study of Clinical Profile and Prognostic Factors of Acute Kidney Injury (AKI) In Tertiary Referral Centre in Marathwada

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Abstract: *Mortality in acute kidney injury (AKI) remains impressively high despite technical and medical advances made following the advent of dialysis. Previous studies of prognosis in AKI have analyzed the influence of demographic factors, severity of AKI, nature of diseases causing AKI, coexisting diseases, treatment received and complications. Several other factors also affect prognosis of AKI viz. oliguria, a rise in serum creatinine greater than 3 mg%, older debilitated patients, multiorgan failure, associated comorbid conditions, need of dialysis, suspected or proven sepsis. Hence cases of AKI were studied at tertiary health centre in Marathwada region of Maharashtra to study the clinical profile and factors affecting the prognosis of acute kidney injury.*

Keywords: *Acute Kidney Injury (AKI), Dialysis, Oliguria.*

I. Introduction

The kidney is a highly vascular organ. The kidneys are important organs of our body, which deals with the excretion of the waste products of protein catabolism from the body and maintenance of water and electrolyte balance. The portion of the total cardiac output that passes through the kidneys, called as renal fraction is about 20%. Kidneys are prone to develop ischemic injury whenever blood supply to them is decreased. They are also susceptible to nephrotoxic injury by virtue of their rich blood supply and the ability to concentrate toxins in the medullary interstitium and renal epithelial cells.

Acute kidney injury (AKI), previously known as acute renal failure (ARF), is a syndrome characterized by rapid decline of glomerular filtration rate (hours to weeks), retention of nitrogenous waste products and perturbation of extracellular fluid volume and electrolyte and acid base homeostasis[1].

The term failure reflects only part of the spectrum of damage to the kidney that occurs clinically. In most cases of damage, the reduction in kidney function is modest. Nevertheless, this modest change has been documented to be associated with negative effects on outcome, albeit not nearly as ominous as seen with large decreases in kidney function associated with frank kidney failure that often requires acute dialysis therapies. Furthermore, the term renal is not well understood in the general population and this makes communication with patients and family more challenging; hence "kidney" has replaced "renal". Hence the name acute renal failure was changed to "acute kidney injury."[1]

Urine output is generally reduced to < 400 ml/day called as oliguric AKI but some patients continue to pass > 400 ml of urine per day, called as non-oliguric AKI. In AKI nitrogenous waste products accumulate in the body and renal mechanisms responsible for maintaining water and electrolyte balance is disturbed. In addition the renal contribution to the control of acid base balance is deficient; hydrogen ions accumulate in the body producing metabolic acidosis. Loss of excretory function leads to hyperkalemia and oedema, with or without pulmonary oedema, if dietary intake of potassium and water is not restricted.

AKI is defined as any of the following[2]:

1. Increase in Serum Creatinine by 0.3 mg/dl within 48 hours; or
2. Increase in Serum Creatinine to 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or
3. Urine volume < 0.5 ml/kg/h for 6 hours.

It is one of the most common clinical syndrome encountered in the clinical practice. Most AKI is reversible, the kidneys being relatively unique among major organs in its ability to recover in function.

Acute Kidney Injury is sub classified into three categories, viz prerenal, intrinsic renal and post renal failure. Prerenal failure the most common form and is characterized by renal hypoperfusion without compromising to integrity of renal parenchyma. Intrinsic renal failure is produced by disorders that directly involve renal parenchyma. These are sepsis, ischemic, various nephrotoxins and diseases of glomeruli. Post renal failure is produced by urinary tract obstruction[1].

As there are no specific therapies for treatment of ischemic and nephrotoxic AKI and mortality is so high, prevention is of paramount importance. Early identification of patients at risk with prompt elimination of potential insults is the golden rule. Aggressive restoration of intravascular volume has been shown to reduce the incidence of ARF dramatically in volume depleted states. Injudicious use of nephrotoxic drugs like aminoglycosides and NSAIDs should be avoided especially in elderly and in combination with diuretics. Dose modification should be done when renal failure has already developed. Maintenance of volume status to optimum during operative and post operative periods is very important.

Sepsis is by far the commonest cause of death in AKI, so all steps should be taken to avoid or limit it. Many a times, doctors are responsible for development of AKI in hospitals, with a little more awareness about the precipitating factors, avoidance of the injudicious use of nephrotoxic drugs, proper maintenance of intake and output records in post operative cases and a watchful readiness to act promptly if unavoidable circumstances arises, can prevent and minimize the number of AKI cases. Factors that predispose AKI are renal insult, myeloma, diabetes mellitus, proteinuria, previous cardiac or renal insufficiency, diuretic use, volume depletion, advanced age.[3]

Mortality rate among patients of AKI approximates 50 percent and mortality rate vary greatly, depending on the cause of AKI[4].

Several factors affect prognosis of AKI viz oliguria, a rise in serum creatinine greater than 3 mg%, older debilitated patients, multiorgan failure, associated comorbid conditions, need of dialysis, suspected or proven sepsis[5]. Hence cases of AKI were studied at Tertiary Health Centre in Marathwada region of Maharashtra MGM Medical College and Hospital, Aurangabad to study the clinical profile and factors affecting the prognosis of acute kidney injury.

Observations

Table – 1 Age And Gender Distribution

Age Group	Male	Female	Total
0-20	6(4.16)	5(3.47)	11(7.63)
21-40	21(14.58)	20(13.88)	41(28.47)
41-60	36(25)	16(11.11)	52(36.11)
≥ 61	25(17.36)	15(10.41)	38(26.38)
Total	88(61.11)	56(38.88)	144(100)

Figures in ( ) indicates percentage.

Graph – 1 Age & Gender Distribution

Male preponderance was seen in all age groups.

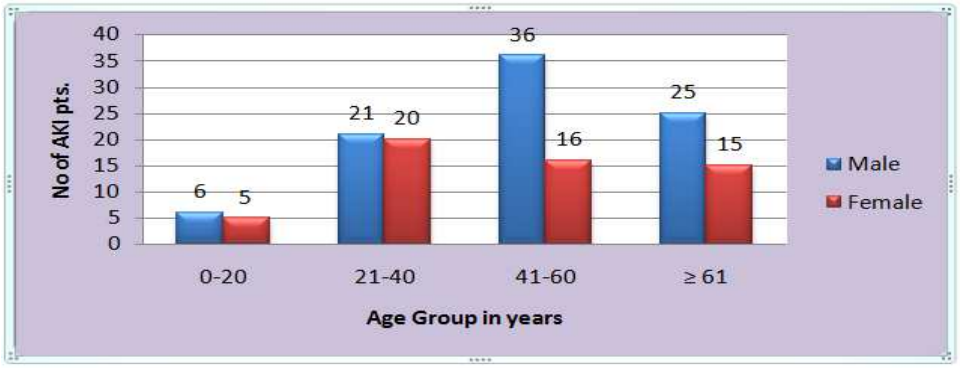
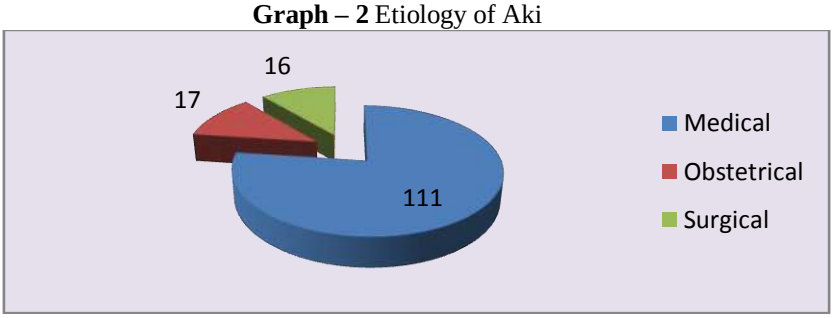


Table 2 – Etiology Of Aki

Etiology of AKI	No. Of pts.	Percentage (%)
Medical AKI	111	77.08
Obstetric AKI	17	11.80
Surgical AKI	16	11.11
Total	144	100

Graph – 2 Etiology of Aki



The commonest cause of AKI was Medical (77.08%), followed by Obstetrical (11.80%) and Surgical causes (11.11%).

Table - 3 Clinical And Laboratory Data In 144 Patients Of Aki

Parameters	Mean ± SD, Ratio
Age (years)	48 ± 18.24
Sex (M: F)	88:56 (1.57:1)
Oliguria/Nonliguria	61/83
Hypotension	41 (28.47%)
Bleeding tendency	11 (7.63%)
Hyperkalemia	40 (27.77%)
Peak Blood Urea (mg %)	131.50 ± 69.94
Peak serum Creatinine (mg %)	4.96 ± 3.23
Mortality	29.16 %

Out of 144 patients of AKI studied, male to female ratio was 1.57:1. Mean age was 48 ± 18.24. Overall mortality was 29.16 %.

Table – 4 Causes Of Oliguric V/S Nonoliguric Aki

Causes	Oliguric patients (n=61)	Non-oliguric patients (n=83)
Surgical	11(18.03)	5(6.02)
Obstetrical	5(8.19)	12(14.45)
Medical	45(73.77)	66(79.51)
Medical causes		
Sepsis	16(35.55)	20(30.30)
Acute Gastroenteritis	6(13.33)	12(18.18)
Acute Pancreatitis	7(15.55)	10(15.15)
ContrastInduced Nephropathy	5(11.11)	5(7.57)
Hepatorenal Syndrome	6(13.33)	3(4.54)
Malaria	2(4.44)	3(4.54)
Dengue Fever	1(2.22)	4(6.06)
Snake Bite	2(4.44)	1(1.51)
Leptospirosis	0(0)	2(3.03)
HUS	0(0)	1(1.51)

Figures in ( ) indicates percentage.

Non-oliguric AKI was more common than Oliguric AKI. Non-oliguric AKI was seen in all etiologies.

Table - 5 Age And Medical Etiology Of Aki

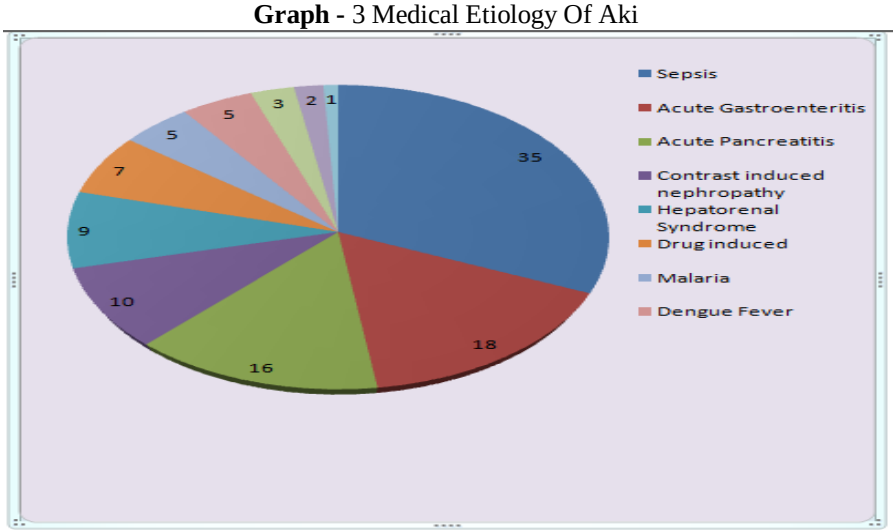
Age	0-20	21-40	41-60	≥ 61	Total	Percentage (%)
Sepsis	0(0)	6(17.14)	12(34.28)	17(48.57)	35(31.53)	31.53
Acute Gastroenteritis	2(11.11)	3(16.66)	8(44.44)	5(27.77)	18(16.21)	16.21
Acute Pancreatitis	0(0)	10(62.5)	6(37.5)	0(0)	16(14.41)	14.41
Contrast Induced Nephropathy	0(0)	1(10)	4(40)	5(50)	10(9)	9
Hepatorenal Syndrome	1(11.11)	0(0)	4(44.44)	4(44.44)	9(8.10)	8.10
Drug Induced	1(14.28)	0(0)	5(71.42)	1(14.28)	7(6.30)	6.30
Malaria	1(20)	3(60)	1(20)	0(0)	5(4.50)	4.50
Dengue Fever	0(0)	2(40)	1(20)	2(40)	5(4.50)	4.50
Snake Bite	1(33.33)	1(33.33)	1(33.33)	0(0)	3(2.70)	2.70

Leptospirosis	0(0)	0(0)	2(100)	0(0)	2(1.80)	1.80
HUS	1(100)	0(0)	0(0)	0(0)	1(0.69)	0.69
Total	7(6.30)	26(23.42)	44(39.63)	34(30.63)	111(100)	100

Figures in ( ) indicates percentage.

AKI secondary to sepsis and contrast induced nephropathy were common in age group more than 61 years. AKI secondary to acute pancreatitis was more common in age group 21-40.

Graph - 3 Medical Etiology Of Aki



Sepsis was the commonest cause of medical AKI (31.53%). Other causes of medical AKI were as follows: Acute Gastroenteritis (16.21%), Acute pancreatitis (14.41%), Contrast induced nephropathy (9%), Hepatorenal syndrome (8.10%), Drug induced (6.30%), Malaria (4.50%), Dengue fever (4.50%), Snake bite (2.70%), Leptospirosis (1.80%), HUS (0.69%).

Table-6 Age And Etiology Of Aki

Age	Medical	Obstetric	Surgical	Total
0-20	7 (4.86)	4(2.77)	0(0)	11(7.63)
21-40	26(18.05)	13(9.02)	2(1.38)	41(28.47)
41-60	44(30.55)	0(0)	8(5.55)	52(36.11)
≥ 61	34(23.61)	0(0)	6(4.16)	40(27.77)
Total	111(77.08)	17(11.80)	16(11.11)	144(100)

Figures in ( ) indicates percentage.

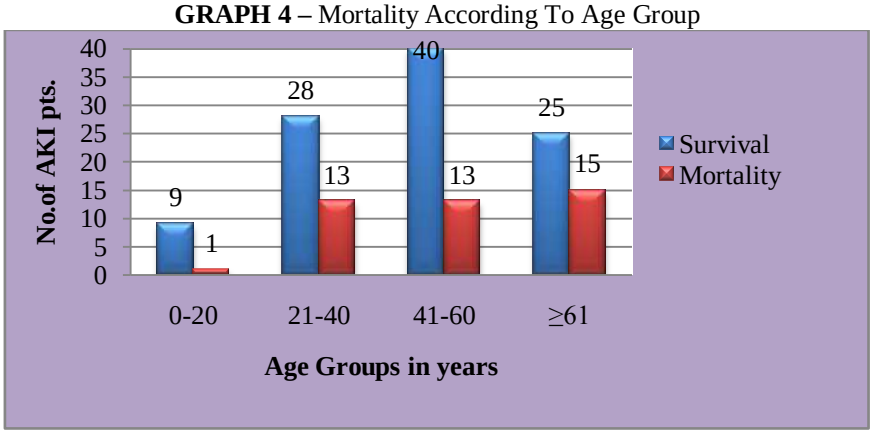
AKI due to medical and surgical causes were more common in patients with age more than 41 years. AKI due to obstetric causes was more common in age group 21-40 years.

Table – 7 Relation Of Age Distribution To Mortality

Age (yrs)	Survival	Mortality	Total patients	Percentage Mortality
0-20	9(90)	1(10)	10(6.94)	10
21-40	28(68.29)	13(31.70)	41(28.47)	31.70
41-60	40(75.47)	13(24.52)	53(36.80)	24.52
≥ 61	25(62.5)	15(37.5)	40(27.77)	37.5
Total	102(70.83)	42(29.16)	144(100)	29.16

(Chi square=2.79) $p > 0.05$ (non-significant)

GRAPH 4 – Mortality According To Age Group



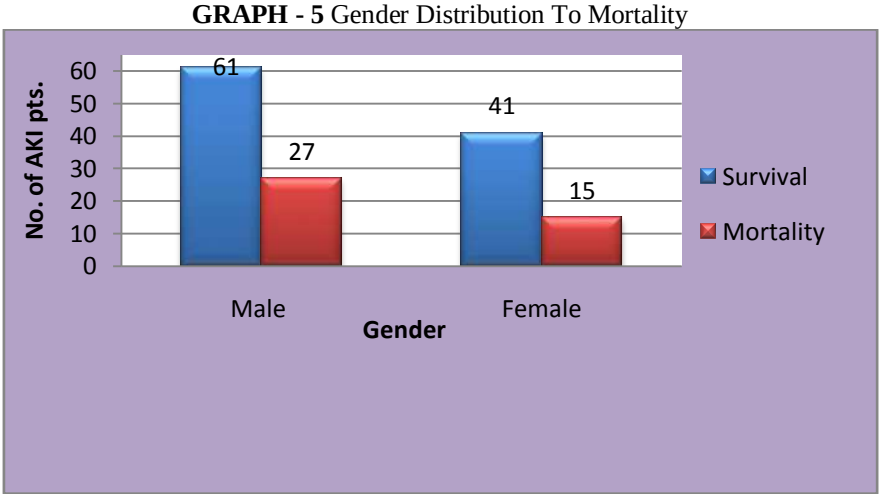
Although the mortality was maximum (37.5%) in age group more than 61years as compared to other age groups, chi square test showed no difference in mortality among various age groups. Overall mortality was 29.16 %.

TABLE – 8: Relation Of Gender Distribution To Mortality

Gender	Survival	Mortality	Total patient	Percentage Mortality
Male	61(69.31)	27(30.68)	88(61.11)	30.68
Female	41(73.21)	15(26.78)	56(38.88)	26.78
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =0.2517) p > 0.05 (non-significant)

GRAPH - 5 Gender Distribution To Mortality



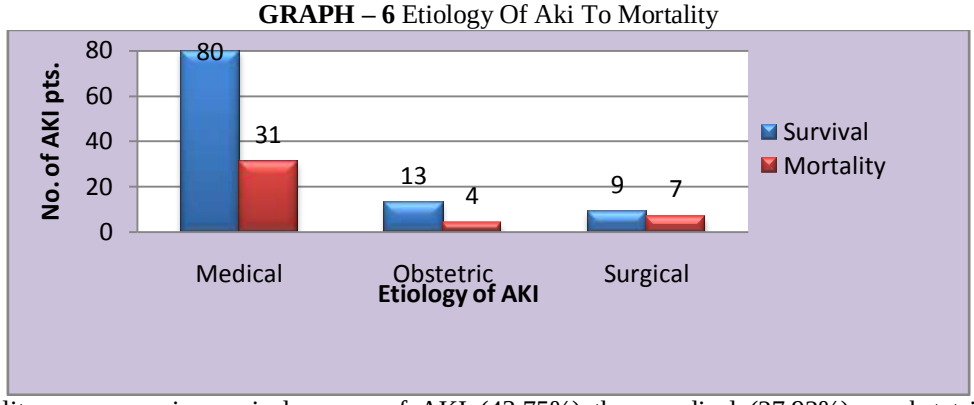
Although mortality was more in males (30.68%) than females (26.78%) but statistically there was no significant difference in mortality.

TABLE – 9: Relation Of Etiology Of Aki To Mortality

Etiology	Survival	Mortality	Total Patients	Percentage Mortality
Medical	80(72.07)	31(27.92)	111(77.08)	27.92
Obstetric	13(76.47)	4(23.52)	17(11.80)	23.52
Surgical	9(56.25)	7(43.75)	16(11.11)	43.75
Total	102(70.83)	42(29.16)	144(100)	29.16

Figure in ( ) indicates percentage (Chi square =1.995) p > 0.05 (Non Significant)

GRAPH – 6 Etiology Of Aki To Mortality



The mortality was more in surgical causes of AKI (43.75%) than medical (27.92%) or obstetrical causes (23.52%) of AKI, but the difference in mortality among these three groups was statistically not significant.

TABLE – 10: Relation Of Medical Causes Of Aki To Mortality

Etiology	Survival	Mortality	Total	Percentage Mortality
Sepsis	25(71.42)	10(28.57)	35(31.53)	28.57
Acute Gastroenteritis	17(94.44)	1(5.55)	18(16.21)	5.55
Acute Pancreatitis	8(50)	8(50)	16(14.41)	50
Contrast Induced Nephropathy	5(50)	5(50)	10(9)	50
Hepatorenal Syndrome	6(66.66)	3(33.33)	9(8.10)	33.33
Drug Induced AKI	6(85.71)	1(14.28)	7(6.30)	14.28
Malaria	4(80)	1(20)	5(4.50)	20
Dengue fever	3(60)	2(40)	5(4.50)	40
Snakebite	3(100)	0(0)	3(2.70)	0
Leptospirosis	2(100)	0(0)	2(1.80)	0
HUS	1(100)	0(0)	1(0.90)	0
Total	80(72.07)	31(27.92)	111(100)	27.92

Among medical causes of AKI highest mortality was seen in AKI secondary to Acute Pancreatitis (50%) and Contrast induced nephropathy (50%) followed by Hepatorenal syndrome (33.33%). Mortality in sepsis induced AKI was 29.57%. Overall mortality in medical causes of AKI was 27.92%. High mortality in contrast induced nephropathy was due to associated multiple comorbid conditions were present in 70% of contrast induced nephropathy cases.

TABLE – 11: Relation Of Obstetric Causes Of Aki To Mortality

Etiology	Survival	Mortality	Total Patients	Percentage Mortality
Puerperal sepsis	6(66.66)	3(33.33)	9(52.94)	33.33
Eclampsia	4(100)	0(0)	4(23.52)	0
PPH	1(50)	1(50)	2(11.76)	50
Abortion	2(100)	0(0)	2(11.76)	0
Total	13(76.47)	4(23.52)	17(100)	23.52

Figures in ( ) indicates percentage. (Chi square =3.1207) $p > 0.05$ (non-significant)

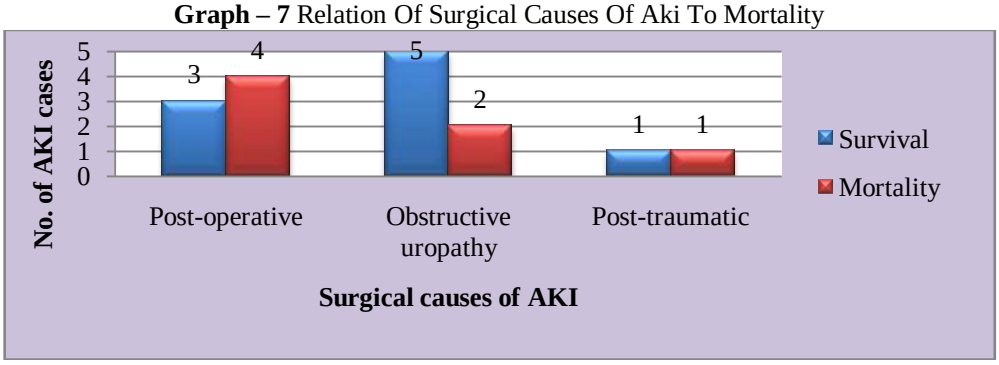
Puerperal sepsis was the major cause of Obstetric AKI (52.94%). Among obstetric causes maximum mortality was seen in PPH (50%). But the difference in mortality among various causes of obstetric AKI was statistically non-significant. Overall mortality in obstetric AKI was 23.52%.

TABLE – 12: Relation Of Surgical Causes Of Aki To Mortality

Etiology	Survival	Mortality	Total	Percentage Mortality
Postoperative	3(42.85)	4(57.14)	7(43.75)	57.14
Obstructive Uropathy	5(71.42)	2(28.57)	7(43.75)	28.57
Post-traumatic	1(50)	1(50)	2(12.5)	50
Total	9(56.25)	7(43.75)	16(100)	43.75

Figures in ( ) indicates percentage (Chi square =2.47) $p > 0.05$ (non-significant)

Graph – 7 Relation Of Surgical Causes Of Aki To Mortality



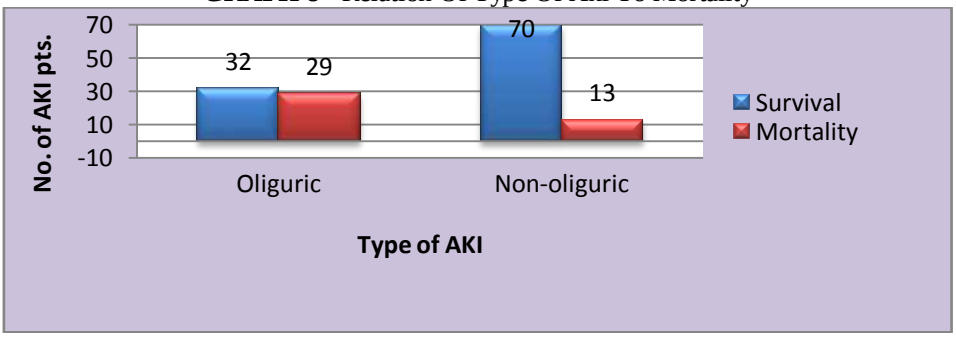
Mortality was more in post-operative cause of surgical AKI (57.14%) than other causes of surgical AKI, but it was statistically non-significant. The overall mortality in surgical group of AKI was 43.75%.

TABLE – 13: Relation Of Type Of Aki To Mortality

Type of AKI	Survival	Mortality	Total Patients	Percentage Mortality
Oliguric	32(52.45)	29(47.54)	61(42.36)	47.54
Non-oliguric	70(84.33)	13(15.66)	83(57.63)	15.66
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =17.28) $p < 0.001$ (Significant)

GRAPH 8 - Relation Of Type Of Aki To Mortality



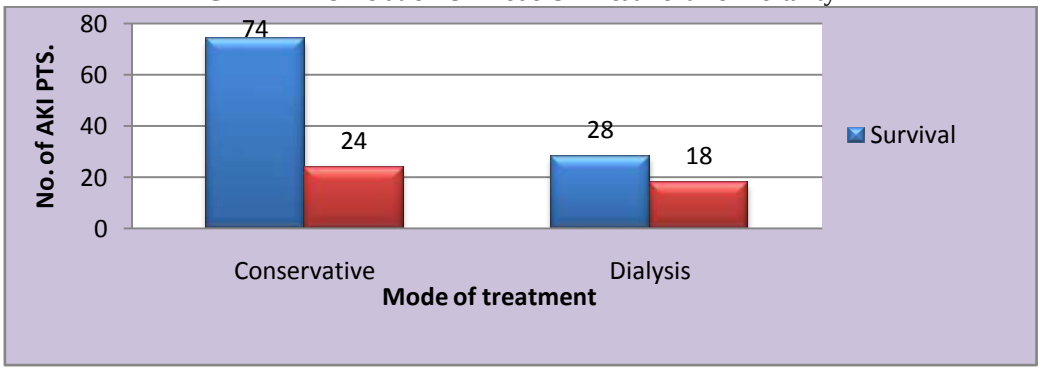
Non-oliguric AKI (57.63%) was more common than oliguric AKI (42.36%). Mortality was more in oliguric AKI (47.54%) than in non-oliguric AKI (15.66%). This difference in mortality among oliguric and non-oliguric AKI was statistically significant.

TABLE – 14 Relation Of Mode Of Treatment To Mortality

Mode of treatment	Survival	Mortality	Total	Percentage Mortality
Conservative	74(75.51)	24(24.49)	98(68.05)	24.49
Dialysis	28(60.87)	18(39.13)	46(31.94)	39.13
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =3.24) $p < 0.05$(Significant)

GRAPH – 9 Relation Of Mode Of Treatment To Mortality



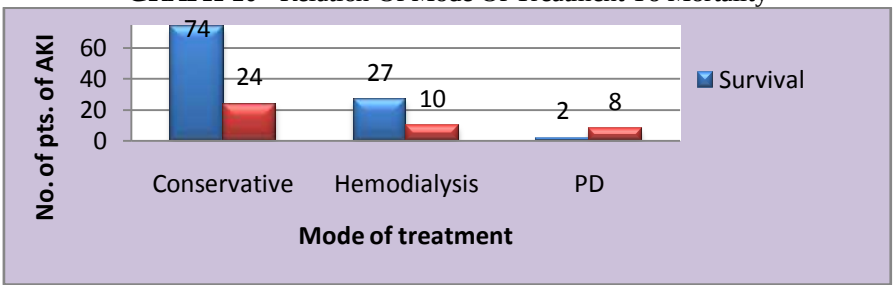
Mortality in patients who needed dialysis was 39.13% while mortality in patients who were treated conservatively was 24.49%. This difference in mortality was statistically significant. Thus mortality was more in patients who needed dialysis.

TABLE – 15: Relation Of Mode Of Treatment To Mortality

Mode of treatment	Survival	Mortality	Total Patients	Percentage Mortality
Conservative	74(75.51)	24(24.49)	98(68.05)	24.49
Hemodialysis	27(72.97)	10(27.02)	37(25.69)	27.02
Peritoneal Dialysis	2(20)	8(80)	10(6.94)	80
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =13.66) $p < 0.001$ (Significant)

GRAPH 10 - Relation Of Mode Of Treatment To Mortality



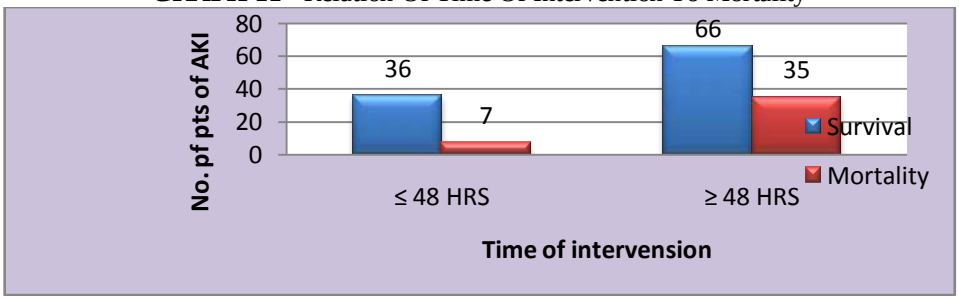
Majority of patients were treated conservatively (68.05%). Mortality was more in patients treated with peritoneal dialysis (80%) than in patients treated conservatively (24.49%) and with hemodialysis (27.02%). This different in mortality with different modes of treatment was statistically significant. This was because in our institute peritoneal dialysis was considered in patients who were hemodynamically not suitable for hemodialysis.

TABLE – 16 Relation Of Time Of Intervention To Mortality

Time	Survival	Mortality	Total Patients	Percentage Mortality
Within 48 hrs	36(83.72)	7(16.27)	43(29.86)	16.27
After 48 hrs	66(65.34)	35(34.65)	101(70.13)	34.65
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =4.94) $p < 0.05$ (Significant)

GRAPH 11 - Relation Of Time Of Intervention To Mortality



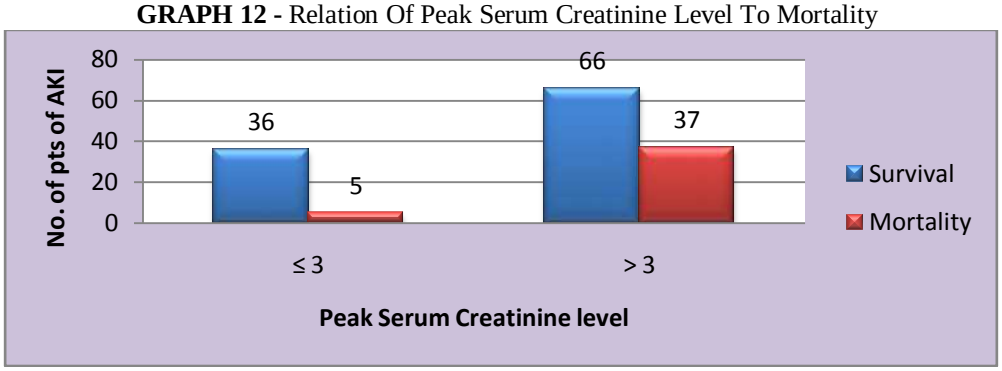
Majority of patients were treated after 48 hrs of diagnosis of AKI. Mortality was more in patients treated after 48 hours of diagnosis of AKI (34.65%) as compared with patients treated within 48 hours of diagnosis of AKI (16.27%). This difference in mortality due to time of intervention was statistically significant.

TABLE – 17: Relation Of Peak Serum Creatinine Level To Mortality

Peak Serum Creatinine (mg %)	Survival	Mortality	Total Patients	Percentage Mortality
< 3	36(87.80)	5(12.19)	41(28.47)	12.19
≥ 3	66(64.07)	37(35.92)	103(71.52)	35.92
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =7.98) $p < 0.05$ (Significant)

GRAPH 12 - Relation Of Peak Serum Creatinine Level To Mortality



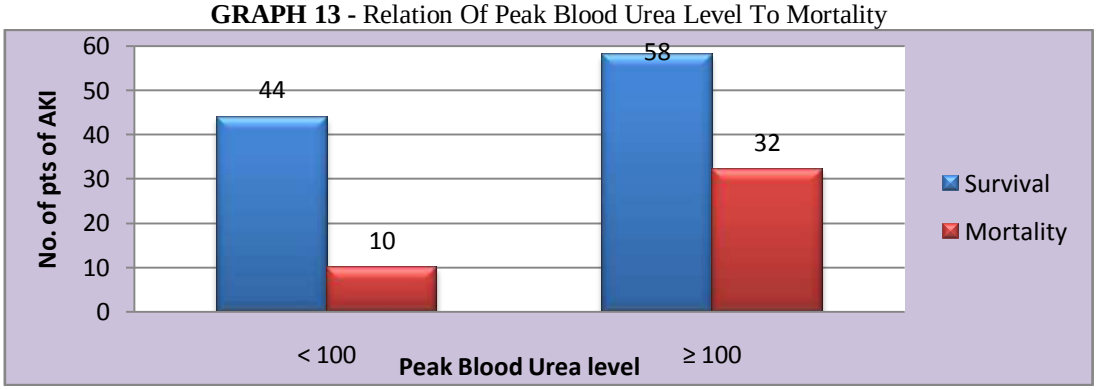
Majority of patients had peak serum creatinine more than 3 mg % (71.52 %). Mortality was more in patients who had peak serum creatinine more than 3 mg % (35.92%) than in patients who had peak serum creatinine less than 3 mg% (12.19%). This difference in mortality was statistically significant.

TABLE – 18: Relation Of Peak Blood Urea Level To Mortality

Peak Blood Urea level (mg %)	Survival	Mortality	Total Patients	Percentage Mortality
< 100	44(81.48)	10(18.51)	54(37.5)	18.51
≥ 100	58(64.44)	32(35.55)	90(62.5)	35.55
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =4.74) $p < 0.05$ (Significant)

GRAPH 13 - Relation Of Peak Blood Urea Level To Mortality



Majority of patients had peak blood urea level more than 100 mg % (62.5 %). Mortality was more in patients who had peak blood urea level more than 100 mg % (35.55%) as compared to patients who had peak blood urea level less than 100 mg% (18.51%). This difference in mortality was statistically significant.

TABLE – 19: Relation Of Serum Sodium Level To Mortality

Serum Sodium (meq/L)	Survival	Mortality	Total Patients	Percentage Mortality
< 136	57(75)	19(25)	76(52.77)	25
≥ 136	45(66.17)	23(33.82)	68(47.22)	33.82
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =1.35) $p > 0.05$ (Non Significant)
Hyponatremia was seen in 76 patients (52.77 %). There was no difference in AKI mortality among patients with or without hyponatremia.

TABLE – 20: Relation Of Serum Potassium Level To Mortality

Serum Potassium level (meq/L)	Survival	Mortality	Total Patients	Percentage Mortality
≤ 5.0	75(72.81)	29(28.15)	103(71.52)	28.15
>5.0	27(67.5)	13(31.70)	40(27.77)	31.70
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage (Chi square =0.4924) $p > 0.05$ (Non Significant)
Hyperkalemia was seen in 40 patients (27.77 %). There was no difference in mortality in patients with or without hyperkalemia.

TABLE – 21: Relation Of Serum Bilirubin Level To Mortality

Serum Bilirubin level (meq/L)	Survival	Mortality	Total Patients	Percentage Mortality
< 2	73(73)	27(27)	100(69.44)	27
≥ 2	29(65.90)	15(34.09)	44(30.55)	34.09
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =0.7431) p > 0.05 (Non Significant)

Serum bilirubin more than 2 mg % was seen in 44 patients (30.55 %). Hyperbilirubinemia had statistically no significant effect on mortality.

TABLE – 22 : Relation Of Platelet Level To Mortality

Serum Platelet level (/mm3)	Survival	Mortality	Total Patients	Percentage Mortality
< 50,000	14(60.87)	9(39.13)	23(15.97)	39.13
≥ 50,000	88(72.72)	33(27.27)	121(84.02)	27.27
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi Square=0.7844) p > 0.05 (Non Significant)

Platelet count less than 50,000 was seen in 23 patients (15.97 %). There was no statistically significant difference in mortality in AKI patients with or without thrombocytopenia.

TABLE – 23: Relation Of Total Leucocyte Count To Mortality

Total Leucocyte Count(/mm3)	Survival	Mortality	Total Patients	Percentage Mortality
< 11000	30(73.17)	11(26.82)	41(28.47)	26.82
≥ 11000	72(69.90)	31(30.09)	103(71.52)	30.09
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =0.1501) p > 0.05 (Non Significant)

Leucocytosis (TLC≥ 11,000 / cmm) was seen in 103 patients (71.52 %). Mortality was more in patients with leucocytosis (30.09%) as compared with patients without leucocytosis (26.82%), but the difference in mortality was statistically insignificant.

TABLE – 24: Relation Of Bleeding Tendency To Mortality

Bleeding Tendency	Survival	Mortality	Total Patients	Percentage Mortality
Present	5(45.45)	6(54.54)	11(7.63)	54.54
Absent	97(72.93)	36(27.06)	133()	27.06
Total	102(70,83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =3.73) p < 0.05 (Significant)

Bleeding tendency was seen in 11 patients (7.63 %). Mortality was more in patients who had bleeding tendency (54.54%) as compared with patients without bleeding tendency (27.06%). This difference in mortality was statistically significant.

TABLE – 25: Relation Of Hypotension To Aki Mortality

Hypotension	Survival	Mortality	Total Patients	Percentage Mortality
Present	13(31.70)	28(68.29)	41(28.47)	68.29
Absent	89(86.40)	14(13.59)	103(71.52)	13.59
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =42.49) p < 0.001 (Significant)

Hypotension was seen in 41 patients (28.47 %). Mortality was more in patients who had hypotension (68.29%) than in patients without hypotension (13.59%). This difference in the mortality was statistically significant.

TABLE – 26: Relation Of Other Organ Involvement (Associated Comorbid Condition To Aki Mortality)

Other organ involvement	Survival	Mortality	Total Patients	Percentage Mortality
Present	36(53.73)	31(46.26)	67(46.52)	46.26
Respiratory	10(38.46)	16(61.53)	26(38.80)	61.53
Hepatic	22(62.85)	13(37.14)	35(52.23)	37.14
CNS	4(66.66)	2(33.33)	6(8.95)	33.33
Absent	66(85.71)	11(14.28)	77(53.47)	14.28
Total	102(70.83)	42(29.16)	144(100)	29.16

Figures in ( ) indicates percentage. (Chi square =17.73) $p < 0.001$ (Significant)
Associated comorbid conditions were seen in 67 patients (46.52%). Associated comorbid conditions like hepatic, respiratory failure or CNS involvement increased the mortality in AKI. This difference in mortality was statistically significant.

TABLE –27complications Related To Etiology Of Aki

Etiology	Hypotension	Bleeding Tendency	Hyperkalemia	Respiratory failure	CNS (Encephalopathy)
Medical					
Sepsis	13	2	9	9	2
Acute Gastroenteritis	0	0	6	0	3
Acute Pancreatitis	6	1	7	2	0
Contrast Induced Nephropathy	3	1	3	5	1
Hepatorenal Syndrome	4	2	1	0	0
Drug Induced	1	0	4	1	0
Malaria	1	0	0	1	0
Dengue Fever	1	1	1	1	0
Snake Bite	1	2	0	0	0
Leptospirosis	0	0	0	0	0
HUS	0	0	0	0	0
Obstetrical	5	2	6	2	0
Surgical	6	0	3	5	0
Total(144)	41(28.47%)	11(7.63%)	40(27.77%)	26(18.05%)	6(4.16%)

Hypotension (28.47%) was the commonest complication of AKI followed by hyperkalemia (27.77%).

TABLE – 28recovery Of Renal Function

Recovery	Total Patients	Percentage
Mortality	42	29.16
Incomplete recovery	6	4.16
Complete recovery	96	66.66

Most of the patients had complete recovery from AKI (66.66%). Incomplete recovery was observed in (6/144) 4.16% of patients.

II. Conclusion

Total 144 cases of AKI were studied at Tertiary Health Centre (MGM Medical College and Hospital, Aurangabad) of Marathwada region of Maharashtra, during the period from June 2011 to Nov 2013 to study clinical profile, etiology and prognostic factors of AKI.

Majority of cases were in the age group 41-60 years and male to female ratio was 1.57:1. Medical causes of AKI were more common than obstetrical and surgical. In medical causes sepsis was the most common cause. In medical causes, Acute Pancreatitis and Contrast induced nephropathy had the highest mortality (50%). High mortality in contrast induced nephropathy in present study was due to 70% patients of CI-AKI had associated multiple comorbid conditions, 80% patients of CI-AKI had baseline serum creatinine more than 3 mg% and 40% patients needed dialysis. The mortality was more in surgical AKI than medical and obstetric AKI, but the difference in mortality among surgical, medical and obstetrical AKI was statistically not significant. Sepsis and contrast induced nephropathy were more common in age more than 61 years. Hypotension, hyperkalemia, bleeding tendency and respiratory failure were the common complications observed. Non-oliguric AKI was more common than oliguric AKI. Non-oliguric AKI was seen in all etiologies. Mortality in oliguric AKI was more than nonoliguric AKI. Mean peak blood urea and Sr. creatinine level were 131.50 ± 69.94 mg% and 4.96 ± 3.23 mg% respectively. Overall mortality was 29.16%. Maximum mortality was seen in patients with age more than 61 years but there was no statistically significant difference in mortality among various age groups. Thus age was a weak determinant of mortality in AKI. Mortality was more in males than females however, this was not statistically significant. Thus gender was a weak determinant of mortality in AKI. In obstetrical AKI, Puerperal Sepsis was the commonest cause of AKI (52.94%). Overall mortality in obstetric cases was 17.64%. There was no significant statistical difference in mortality among obstetric causes of AKI. In surgical AKI, post-operative AKI and obstructive uropathy were the commonest causes of AKI (43.75%). Overall mortality in surgical causes was 43.75%. Among surgical causes maximum mortality was seen in postoperative causes of surgical AKI. But statistically there was no difference in mortality among various surgical causes of AKI. Most of the cases were treated conservatively (68.05%). The difference in mortality with various modalities of treatment i.e. conservative, peritoneal dialysis and hemodialysis was statistically significant. Mortality was more in patients who needed dialysis. Mortality was more in patients

treated with peritoneal dialysis than in patients treated conservatively and with hemodialysis. This was because in our institute peritoneal dialysis was considered in patients who were hemodynamically unstable for hemodialysis. Delay in the initiation of treatment was found to be important factor in deciding outcome of AKI. Patients who received treatment after 48 hours of onset of AKI had higher mortality than those who received treatment within 48 hours. Our institute is the tertiary referral centre. Patients are referred from various primary and secondary canters to our institute; therefore there was delay in the treatment of AKI. Peak serum creatinine ≥3 mg% and peak blood urea ≥100 mg% were associated with high mortality rate. In our institute patients come mainly from poor socioeconomic strata, therefore they were not affording for costly investigations like biomarkers of acute kidney injury. Hence peak serum creatinine and peak blood urea levels were still the primary investigations for prognostic indicators of AKI.Hypotension, Bleeding tendency were found to be significantly associated with high mortality. Presences of hyponatremia, hyperkalemia, increased serum bilirubin (≥2 mg %), low platelet count (≤50,000), leucocytosis were not found to be predictor of high mortality. Other organ involvement in the form of respiratory, liver, CNS and cardiac was associated with high mortality. Most of the cases had complete recovery from AKI. Thus the only factors which were statistically significant in deciding mortality were peak serum creatinine ≥ 3 mg%, peak blood urea ≥ 100 mg%, oliguric AKI, delay in initiation of treatment after onset of AKI (≥48 hours), bleeding tendency, hypotension, need of dialysis and associated other organ involvement. Thus most cases of the AKI are reversible, if the etiology is identified and treated early.

Reference

[1]. Sushrut S Waikar, Joseph V Bonventre : Acute Kidney Injury, Harrison's Principles of Internal Medicine, 18th ed., Fauci et al (eds), McGraw Hill, PP. 2293-2308.
[2]. Kidney International Supplements (2012) KDIGO 2, 8–12.
[3]. Fernando Liano, Julio Pascual and the Acute Renal Failure Study Group. Epidemiology of Acute Renal Failure: A Prospective, Multicenter, Community based Study. Kidney International, Vol. 50 (1996): 811-818.
[4]. Hugh R Brady, Gary G Singer: Acute Renal Failure. The Lancet, vol. 346 December 1995: 1533-1539.
[5]. SL Chew, RL Lins, R Daelemans and ME DeBroe: Outcome in Acute Renal Failure. Nephrology Dialysis Transplantation (1993) 8:101-107.

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A Study of Various Angioaccess in Haemodialysis Patients

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Abstract: *Contemporary societies are in the midst of an epidemic of chronic non communicable diseases including that of chronic kidney disease (CKD). India is no exception to this rule. Hemodialysis is still the main treatment given for patients of Chronic Kidney Disease, although renal transplantation is slowly changing the trends with better immunosuppression and better surgical techniques with wider acceptance of the masses. The provision of effective and timely hemodialysis requires a stable and reliable vascular access. Thus, vascular access is not only the obvious 'Achilles heel' of hemodialysis (HD) but it is also the quiet undercurrent of trends in patient outcomes. Data on the comparisons between various angioaccess are very limited in literature. So we undertook a study to compare the various angioaccess used for hemodialysis in patients of CKD.*
Key Word: *CKD- Chronic Kidney Disease, HD- Hemodialysis*

I. Introduction

Contemporary societies are in the midst of an epidemic of chronic non communicable diseases including that of chronic kidney disease (CKD). India is no exception to this rule. Many parts of India are undergoing rapid epidemiological transition as a consequence of economic and social changes.[1] The increase in non communicable diseases is real and not simply due to better diagnosis. This epidemiologic transition is partially attributable to better nutrition, control of infectious disease and gain in life expectancy. An untoward consequence of this extension of life is the emergence of chronic diseases as leading causes of death.[2]

Chronic kidney disease is a worldwide health problem. According to World Health organization (WHO) Global Burden of Disease project, diseases of the kidney and urinary tract contribute to global burden with approximately 850,000 deaths every year and 115,010,107 disability adjusted life years. CKD 12th leading cause of death and 17th cause of disability.[3] This global prevalence, however, may be grossly underestimated for a number of reasons. Patients with CKD are at high risk for cardiovascular disease (CVD) and cerebrovascular disease (CBVD), and they are more likely to die of CVD than to develop end-stage renal failure. Moreover, patients with CVD often develop CKD during the course of their disease, which may go unrecognized. Therefore, an unknown proportion of people whose death and disability attributed to CVD have kidney disease as well.[4]

Moreover, most epidemiological data (prevalence, incidence, patient demography, morbidity, and mortality) on CKD are derived from renal registries. However, most registries record data of patients who are at late stage of kidney disease. Much less is known about the prevalence of the earlier stages of the CKD. Indeed, it has been acknowledged that the majority of the individuals at early stages of CKD have gone undiagnosed and under treated.[4] The number of end stage of renal disease patients that need dialysis or renal transplantation increased in the world. The overall magnitude and pattern of chronic kidney disease (CKD) in India has been studied sporadically. The CKD Registry of India is a new initiative that has been started to document CKD and its course in our country.

Modern haemodialysis therapy started on 17 March 1943, when Willem Kolff, a young doctor in the small hospital of Kampen (The Netherlands), treated a 29-year-old housemaid suffering from malignant hypertension and 'contracted kidneys'. Kolff had constructed a 'rotating drum kidney' with the support of Mr Berk, the director of the local enamel factory. First, Kolff used only venipuncture needles to obtain blood from the femoral artery and to reinfuse it by puncturing a vein. Later, he performed surgical cut-down of the radial artery which caused severe bleeding during heparinization.

In the years that followed, substantial technical developments are linked to the names of Nils Alwall in Lund (Sweden) and John P. Merril in Boston (USA). In the 1950s, the technical devices were available for regular haemodialysis treatments, e.g. Kolff's so-called twin-coil kidney[5] but, the Achilles heel was a reliable access to the circulation for multiple use which did not yet exist. Vascular access is not only the obvious 'Achilles heel' of hemodialysis (HD) but it is also the quiet undercurrent of trends in patient outcomes.

It took another 16 years before Quinton and Scribner introduced the first permanent vascular access: the Scribner shunt.[6] This device consisted of 2 Teflon® tubes connecting the patient to the dialyser; one tube was inserted into a suitable peripheral artery and one into a suitable vein. After treatment, the circulatory access

was kept open by connecting the two tubes outside the body using a small U-shaped Silastic device over a stainless steel plate. The major disadvantages of Scribner shunts were high thrombosis and infection rates resulting in a limited shunt and hence patient life span.

In 1962, Cimino and Brescia reported on veno-venous access for HD which used a sphygmomanometer to dilate an accessible forearm vein and a blood pump, and in which blood was returned through another vein, usually in the ankle.[6] This experience led them to make one of the most important developments in HD - the arteriovenous fistula.[7] Even though this required a blood pump for dialysis, the blood access problem was solved and use of the shunt declined rapidly. In 1966, Brescia and Cimino solved the blood access problem with a surgically created arteriovenous fistula (AVF) between the radial artery and a vein.[7] This new vascular access was able to deliver flow rates of 250-300 mL/min for unlimited intervals. Results were satisfactory, 13 AVFs (87%) functioned without any complication and two failed before cannulation. Nowadays, the Brescia-Cimino (radio-cephalic) AVF is still the preferred type of vascular access.[8-9]

In 1961, Shaldon Higgs and Chiandussi introduced temporary HD catheters and these catheters continue to be the primary means of achieving acute hemodialysis access. The ready availability of the CVC as a vascular access (VA) for HD often makes them the access of choice, especially when urgent or emergent HD is required either at the time of initiation of renal replacement therapy or when a permanent access becomes dysfunctional.[10]

Central Venous Catheter remain an important method to obtain VA as a bridge to the placement and maturation of an arteriovenous fistula (AVF) or arteriovenous graft (AVG), pending renal transplantation, and as the sole access in many patients. The use of CVCs has several advantages in short term: It does not require the integrity of the peripheral blood vessels, a number of sites are available for immediate insertion, it can be used immediately and for prolonged periods, and it provides painless access.

A catheter conundrum' remains in existence where we hate catheters, but cannot live without them.[11] Thus, while advantageous in very short term, unavoidable and often necessary, CVC are a hazard in most other situations, especially if used for longer periods.

It has been convincingly argued that there is a disproportionately high use of Central venous catheters for dialysis in the US. According to the Dialysis Outcomes and Practice Patterns Study (DOPPS), CVC were the major type of vascular access for initiation of hemodialysis in the US in comparison to countries in Europe and in Japan.[12]

There is a lack of pre dialysis care by nephrologists in US and this seems to have an important correlation with the use of catheters as incident access. According to USRDS 2009 report, in year 2007, 43% of ESRD patients were not followed by a Nephrologist prior to the initiation of HD.[13] The early referral to nephrologist by primary care physicians, and an early referral to surgeon for fistula placement by nephrologists are the key interventions to improve incident CVC use. Preoperative vascular mapping can improve fistula placement rates, and perhaps the fistula maturation rates, which has the potential of reducing prevalent CVC rates.[14]

The provision of adequate hemodialysis is dependent on repeated and reliable access to central circulation. An ideal access delivers a flow rate adequate for the dialysis prescription, has a long use-life and has a low rate of complications (e.g. stenosis, thrombosis, aneurysms, limb ischemia, and infections). Although no current access type fulfils all of these criteria, the native arteriovenous (AV) fistula comes the closest to doing so.

For prevalent patients, it would be important to consider placing secondary AVF as a conscious strategy to reduce use of CVC in patients with failing primary access. It has been shown that a significant number of patients using CVC as their access have suitable veins for AVF creation.[15] Special attention should be paid to the patients using TDC on a 'permanent' basis, as a significant percentage of these patients tend to have suitable veins for AVF creation. Continual evaluation and patient education regarding their next access is extremely important, as it is a challenge for dialysis staff and nephrologists to convince patients to give up the "ease" of catheter use for the safety and long term benefits of an AVF or AVG.

Basic principles of using distal sites first and preferring autogenous fistulae over grafts are the mainstay of decision making. Among autogenous fistulae, direct fistulae, transpositions and translocations should be considered in that order with an aim of performing simpler and less-morbid procedures first. Lower limb and body wall sites should be considered after all upper limb options are exhausted. Use of non-dominant hand first holds true only when access opportunities are equal on both sides, otherwise hand with more suitable veins gets preference.[16]

Aims and Objectives

1. To study the percentages and frequencies of the various types of angioaccess used in our hospital.
2. To study the failure rates of various types of angioaccess in our hospital and associated co-morbidities.
3. To identify the prevalence of various complications of the angioaccess under consideration.

II. Materials And Methods

Study centre: Department of Nephrology and Renal Transplant Centre, Mahatma Gandhi Mission's Medical College & Hospital, Aurangabad.
Sample size: 211
Sampling technique: outpatient and inpatients visiting the hospital for dialysis
Duration of study: 2 ½ years

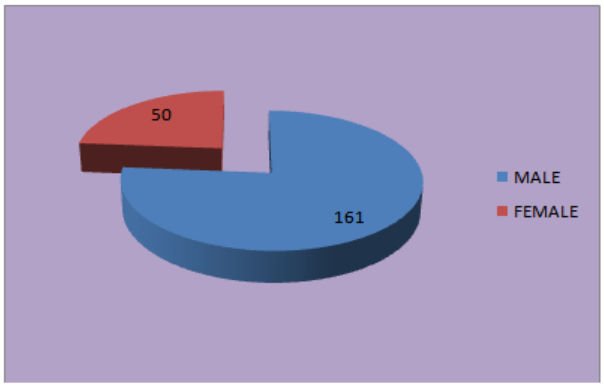
Study Design: observational prospective, single centre, non-randomized prospective study.
Inclusion criteria: Testing and evaluation was carried out on all OPD and IPD patients above the age of 18 years of chronic kidney disease and candidates on dialysis (maintenance or first time or emergency basis).
Exclusion criteria: All patients who were below the age of 13 years, those patients with acute renal failure and those with normal kidney function tests.

Methodology: Detailed history taking, regarding the condition: onset, duration, symptoms, first time diagnosed, personal history, co-morbid conditions, mode of angioaccess used, surgical measures undertaken such as AV Fistula creation.

- Assessment of patency and frequency of change of angioaccess and follow up along with complications.
- Assessment of serum creatinine at onset of dialysis.
- Assessment of eGFR was done by the Cockcroft-Gault equation.
- Chi square test was used to find any statistical significance amongst the groups under comparison.

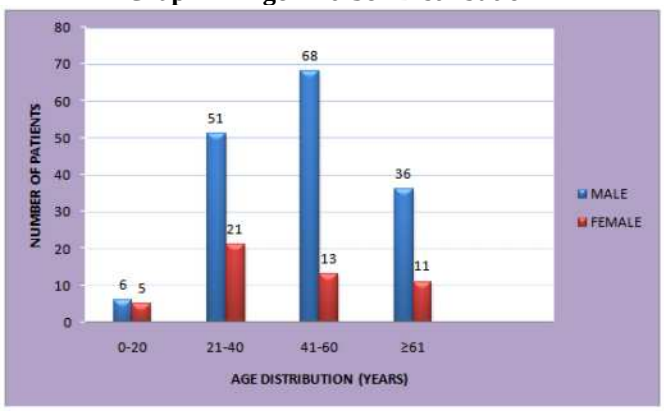
III. Observations

Graph 1 - Gender Distribution



In this study, total number of patients were 211. Of these, 161 (76.30%) participants were male and 50 (23.69%) were female. The Male : Female ratio was 161:50 = 3.22:1. Thus, a male preponderance was seen in our study.

Graph 2 - Age And Sex Distribution

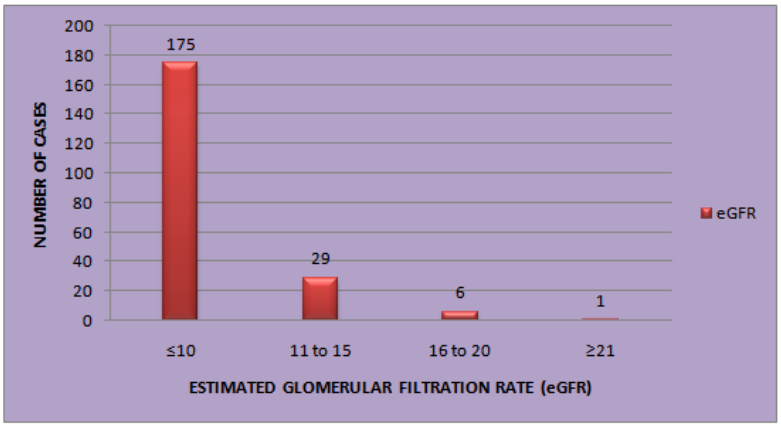


The mean age in the present study was 46.84 years with a range of 13-80 years. The maximum number of patients were from the 41-60 age group, i.e. 81/211(38.38%). The maximum number of male participants were from the 41-60 age group, i.e. 68/161 (42.23%). The maximum number of female patients were seen in the 21-40 age group, i.e. 21/50(42%). Male preponderance was seen as shown in the above graph.

Table No. 1 -Estimated Glomerular Filtration Rate (Egfr) In The Study Population

eGFR	NUMBER OF PATIENTS	PERCENTAGE %
≤10	175	82.93%
10-15	29	13.74%
15-20	6	2.84%
≥21	1	0.47%
TOTAL	**211**	

Graph-3 - Comparison Of Estimated Glomerular Filtration Rate (Egfr) In The Study Group

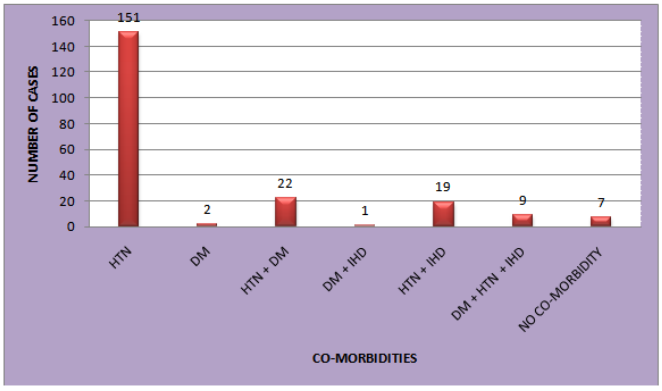


The maximum number of patients had eGFR ≤10, i.e 175 (82.93%) in the study population.

Table No.2 - Co-Morbidities Present In The Study Population

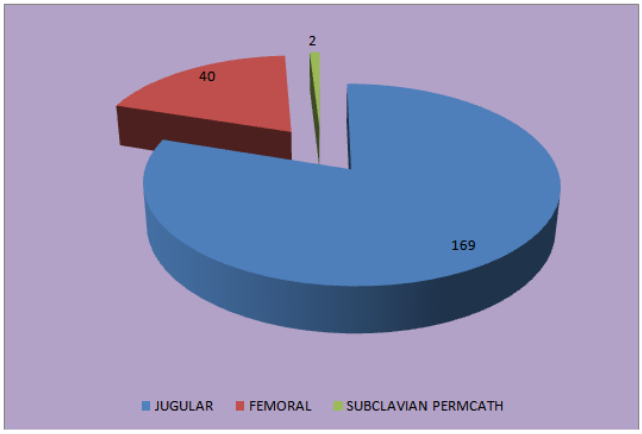
CO-MORBDITIY	NUMBER OF PATIENTS	PERCENTAGE %
HTN	151	71.56%
DM	2	0.94%
HTN + DM	22	10.42%
DM + IHD	1	0.47%
HTN + IHD	19	9.00%
DM + HTN + IHD	9	4.26%
NO COMORBIDITY	7	3.31%
TOTAL	**211**	**100%**

Graph 4 - Co-Morbidities Present In The Study Population



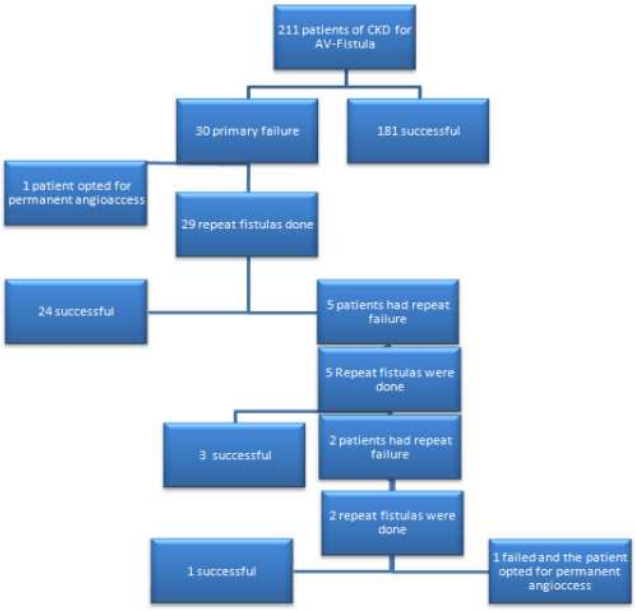
In the study population, 71.56% of the study population had hypertension as the single most common co-morbidity as compared to diabetes and ischemic heart disease.

Graph 5 - Type Of Artificial Angioaccess Used In Ckd Patients On Hemodialysis

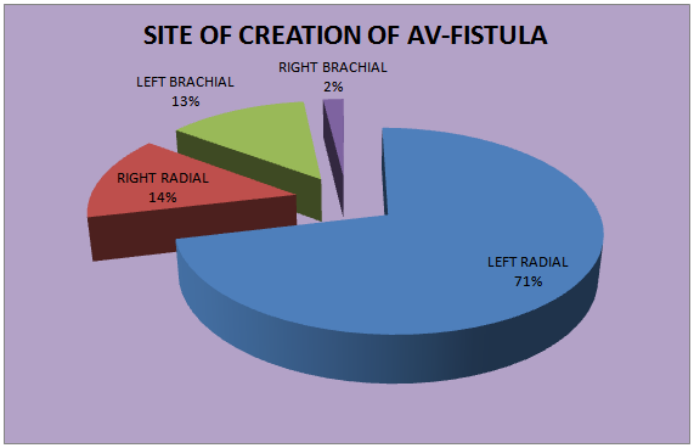


The total number of artificial angioaccess established was 211. In this study, the most commonly used angioaccess was the Internal Jugular 169 (80.09%) as compared to the femoral route 40 (18.95%) and subclavian permanent catheter 2(0.94%)

Flow-Chart



Graph 6 - Site Of Arterio-Venous Fistula Creation In Ckd Patients On Hemodialysis



The total number of AV-Fistulas created in 211 patients during the duration of the study was 248. Amongst these there were 39 documented primary failures and repeat fistulas were done. In one patient, after primary AV-fistula failure, the patient opted for permanent angioaccess. In a second patient, after 4 failed AV-fistulas, the patient opted for permanent artificial angioaccess. Thus only in 209 patients AV-fistula was used as the primary medium for CRRT. Out of 211 patients in whom AV-fistula was the access used for providing CRRT, 170 never had any primary failures. Thus there was a primary success rate of 80.56%(170/211). In this study, the preferred site for AV-fistula creation was the left radial; 149(71.29%) and the least preferred site was the right brachial with 4(1.9%).

Table No. 3 -Complications Of Artificial Angioaccess

COMPLICATION	JUGULAR (NO.)	FEMORAL (NO.)	TOTAL
INFECTION	14 (8.28%)	10 (25%)	24(11.48 %)
BLEEDING	17 (10.05%)	6 (15%)	23(11 %)
POOR FLOW	15 (8.87%)	8 (20%)	22(10.52%)

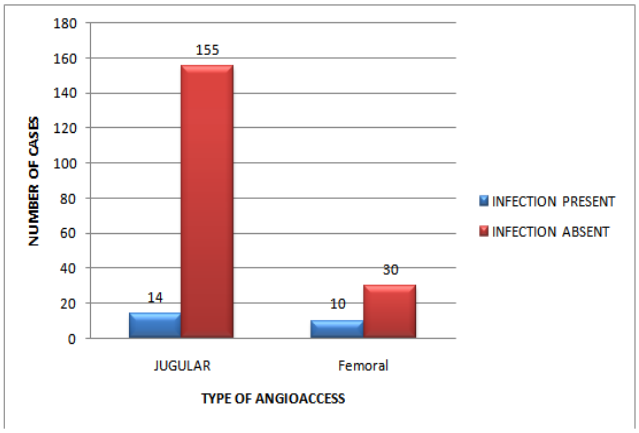
In the present study, infection was the commonest complication associated with use of artificial angioaccess in CKD patients. The incidence of complications were higher with femoral as compared with the internal jugular HD catheter.

Table No.4 - Relation Of Infection With Artificial Angioaccess.

ANGIOACCESS	INFECTION PRESENT	INFECTION ABSENT	TOTAL
JUGULAR	14	155	169 (8.28%)
FEMORAL	10	30	40(25%)
TOTAL	**24**	**185**	**209(11.48%)**

Chi square = 8.89 $p < 0.01$ (Significant)

Graph 7 - Relation Of Infection With Angioaccess



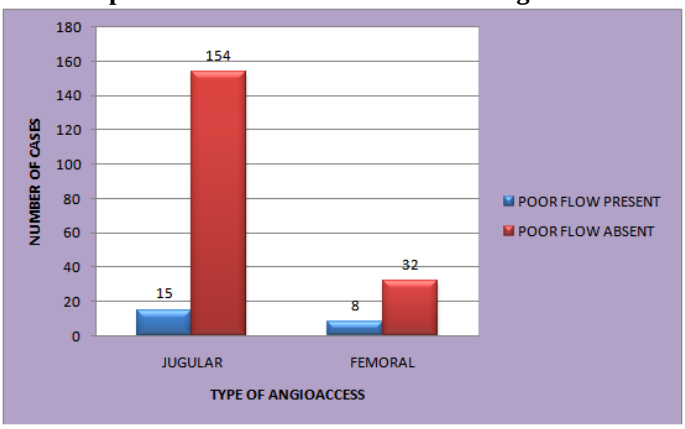
In this study, the total number of infections were 11.48% (24/209). It was noted that a significant higher rate of infection occurred amongst femoral HD catheters, i.e. 10/40 (25%), as compared to the jugular access 14/169 (8.28%). This increased percentage of infection in femoral angioaccess as compared to jugular angioaccess was statistically significant.

Table No.5 - Relation Of Poor Flow To Angioaccess

ARTIFICIAL ANGIOACCESS	POOR FLOW PRESENT (NO.)	POOR FLOW ABSENT (NO.)	TOTAL
JUGULAR	15	154	169 (8.87%)
FEMORAL	8	32	40 (20%)
TOTAL	**23**	**186**	**209(11%)**

Chi Square = 4.09 $p < 0.05$ (Significant)

Graph 8 - Relation Of Poor Flow To Angioaccess



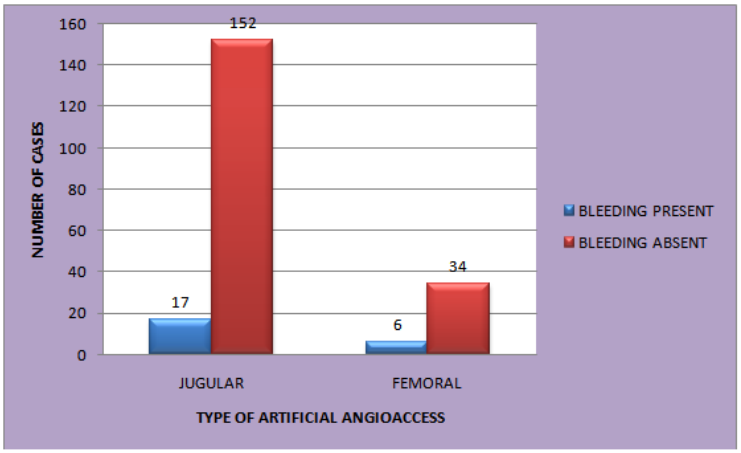
In the study, poor flow was recorded in 11.00% (23/209) of artificial angioaccess. A higher incidence of poor flow was associated with the femoral type, i.e. 8/40(20%) of artificial angioaccess as compared to the jugular 15/169 (8.87%), which was statistically significant.

Table No.6 - Relation Of Bleeding With Artificial Angioaccess

ANGIOACCESS	BLEEDING PRESENT (NO.)	BLEEDING ABSENT (NO.)	TOTAL
JUGULAR	17	152	169 (10.05%)
FEMORAL	6	34	40 (15%)
TOTAL	**23**	**186**	**209 (11 %)**

Chi Square= 0.806 p > 0.05 (not significant)

Graph 9 - Incidence Of Bleeding In Various Artificial Angioaccess



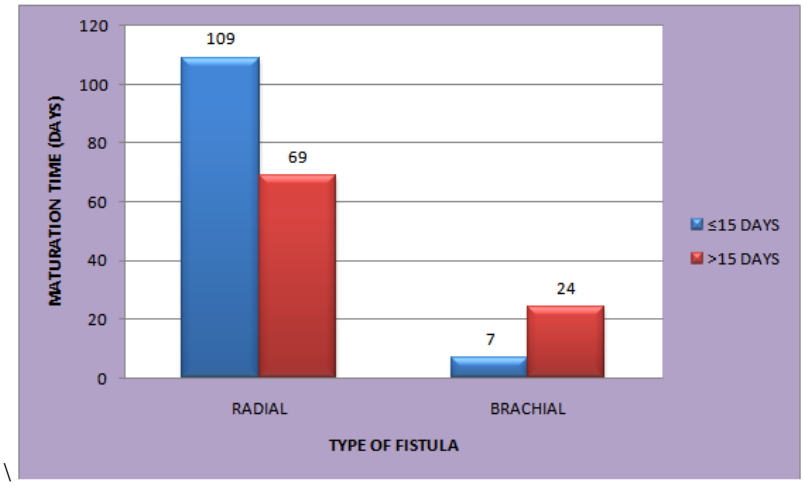
The overall incidence of bleeding was in 11.00% (23/209). Although the percentage of bleeding was more in femoral catheter (15%) as compared to jugular, (10.05%), it was not statistically significant.

Table No. 7 - Relation Of Fistula With Maturation

SITE OF AV-FISTULA	MATURATION ≤15 DAYS	MATURATION >15 DAYS	TOTAL
RADIAL	109	69	178 (38.76%)
BRACHIAL	7	24	31 (77.41%)
TOTAL	**116**	**93**	**209 (44.49%)**

Chi Square =15.9 p< 0.05 (Significant)

Graph 10 - Relation Of Fistula With Delayed Maturation



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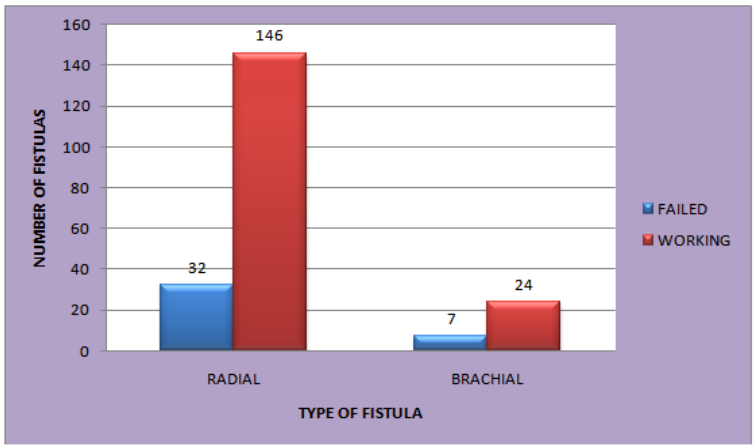
In the study, of 44.49% (93/209) of AV-Fistulas matured after 15 days. There was a higher significant incidence of delayed maturation (>15 days) with Brachial fistulas (77.41%) as compared to Radial fistulas (38.76%).

Table No.8 - Relation Of Site Of Fistula With Failure.

SITE OF AV-FISTULA	FISTULA FAILED ≥ 1 TIME	FISTULA NOT FAILED	TOTAL
RADIAL	32	146	178 (17.98%)
BRACHIAL	7	24	31(22.58%)
TOTAL	**39**	**170**	**209 (18.66%)**

Chi Square = 0.368 p>0.05 (Significant)

Graph 11 - Relation Of Site Of Fistula With Failure



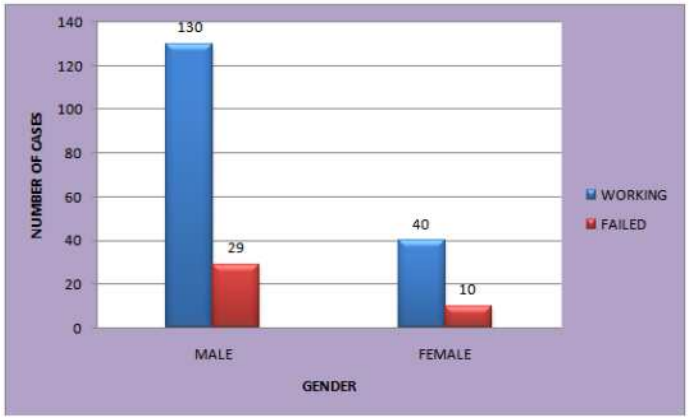
In the study, AV-Fistula failure was seen in 18.66% (39/209) of patients. Higher failure rates were noted with brachial fistulas, i.e. 7/31 (22.58%) as compared to radial AV-Fistulas32/178(17.98%), which was statistically significant.

Table No.9 - Fistula Failure Rate Comparison In Gender

GENDER	FISTULA FAILED (NO.)	FISTULA WORKING (NO.)	TOTAL (NO.)
MALE	29	130	159 (18.23%)
FEMALE	10	40	50 (20%)
TOTAL	**39**	**170**	**209 (18.66 %)**

Chi Square = 0.07 p > 0.05(not significant)

Graph 12 - Gender Comparison Of Av-Fistula Failure Rates



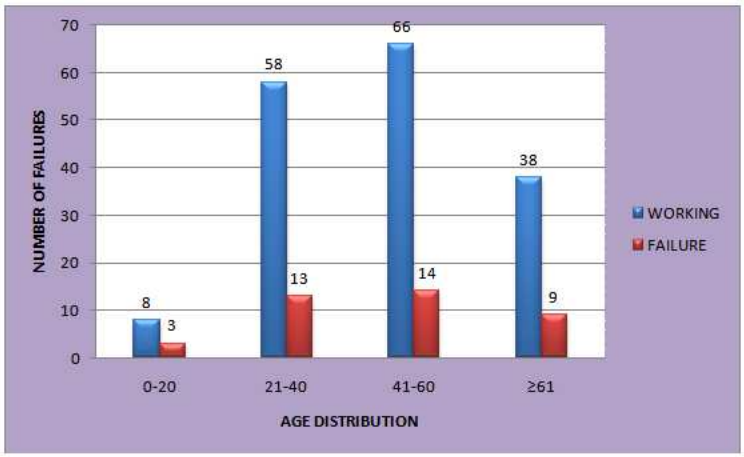
The AV-Fistula failure rate amongst males was 18.23% while that in females was 20%. Although the failure rate was more in females, there was no statistically significant difference in failure rates recorded between male and female gender.

Table No.10 - Relation Of Age Distribution To Failure Rates

AGE GROUP	FAILURE (NO.)	WORKING (NO.)	TOTAL
0-20	3	8	11 (27.27%)
21-40	13	58	71(18.31%)
41-60	14	66	80 (17.5%)
61-80	9	38	47 (19.14%)
TOTAL	**39**	**170**	**209 (18.66%)**

Chi Square = 0.59 $p > 0.05$ (not significant)

Graph 13 - Comparison of Av-Fistula Failure Rates In Various Age Groups



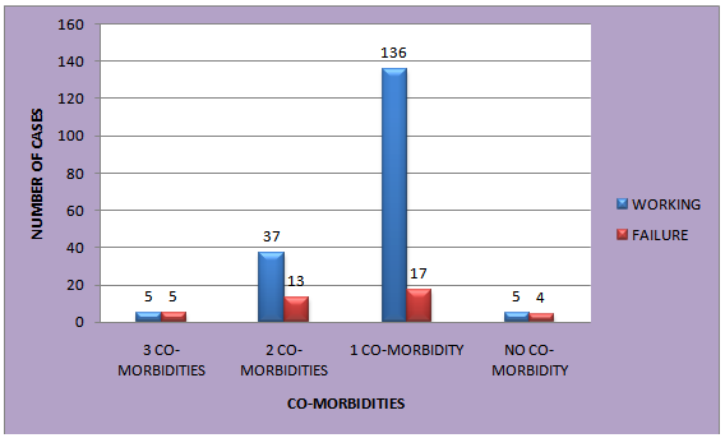
Although the failure rate was more in the age group of 0-20 years (27.27%) as compared to other age groups, it was not statistically significant.

Table No.11 - Relation Of Fistula Failure With Number Of Co-Morbidities

NUMBER OF CO-MORBIDITIES	FAILURE (NO.)	WORKING (NO.)	TOTAL
3 CO-MORBIDITIES	5	5	10(50%)
2 CO-MORBIDITIES	13	37	50(26%)
1 CO-MORBIDITY	17	136	153(11.11%)
NO CO-MORBIDITY	5	5	9(44.44%)
TOTAL	**39**	**183**	**222**

Chi Square = 18.65 $p < 0.001$(significant)

Graph 14 - Relation Of Number Of Co-Morbdities With Fistula Outcome

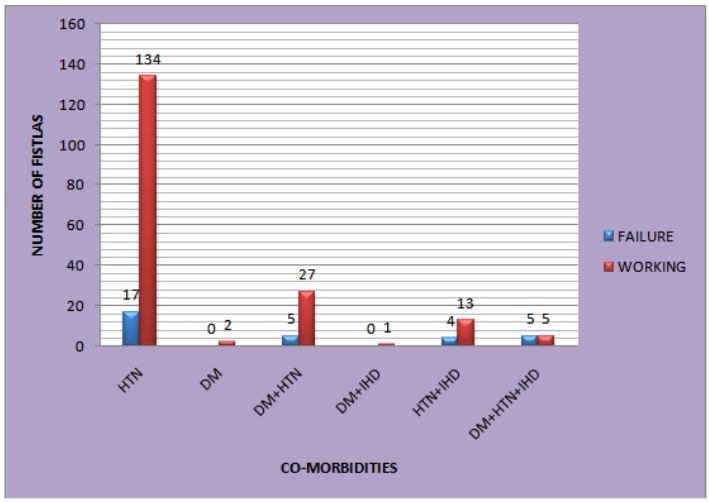


In the present study, the presence of 3 co-morbidities resulted in a greater incidence of failure 5/10 (50%) for AV-fistula. A significant incidence of failure in patients with no underlying co-morbidities (44.44%), but the number of subjects in that subset were small. The cause of failure was not evaluated.

Table No.12 - Relation Of Fistula Outcome With Co-Morbidities

CO-MORBIDITY	FISTULA FAILED (NO. OF CASES)	FISTULA WORKING (NO. OF CASES)	TOTAL
HTN	17	134	151 (11.25%)
DM	0	2	2 (0%)
HTN + DM	9	23	32 (28.12%)
DM + IHD	0	1	1(0%)
HTN + IHD	4	13	17 (23.52%)
DM + HTN + IHD	5	5	10 (50%)
NO CORMOBIDITY	4	5	9 (44.44%)
TOTAL	**39**	**183**	**222**

Graph 15 - Relation Of Co-Morbidities With Fistula Outcome



In the study, there was a significant higher incidence of failure in patients with co-existence diabetes, hypertension and ischemic heart disease - 5/10 (50%), as compared to each of these co-morbidities alone. Also noted were failure in 44% of patients with no co-morbidities, but the cause of failure was not further evaluated.

Table No.13 - Complications Of Av-Fistula

COMPLICATION	RADIAL (NO.)	BRACHIAL (NO.)	TOTAL
INFECTION	5 (2.80%)	2 (6.45%)	7 (3.34%)
BLEEDING	16 (8.98%)	1 (3.22%)	17 (8.13 %)
POOR FLOW	13 (7.30%)	4 (12.90%)	17 (8.13 %)

In the present study, poor flow was seen in 8.13% (17/209) fistulas. The complication of bleeding was similarly noted at 8.13%(17/209) and infection was less noted at 3.34%(7/209) of AV-Fistulas. There was greater incidence of infection (6.45% V/s 2.8%) and poor flow (12.90% V/s 7.30%) associated with brachial fistulas as compared to radial fistulas. The incidence of bleeding however was more with radial fistulas as compared to brachial fistulas (3.22% V/s 8.98%).

Table No.14 -Incidence Of Complications In Native Angioaccess (Av-Fistula) Vs Artificial Angioaccess

COMPLICATION	AV-FISTULA (NO.)	ARTIFICIAL ANGIOACCESS (NO.)	TOTAL
INFECTION	7(3.3%)	24 (11.48%)	31
BLEEDING	17(8.13%)	23(11.00%)	40
POOR FLOW	17(8.13%)	22(10.52%)	39

Complications of infection rate, poor flow and bleeding were higher amongst artificial angioaccess as compared to AV-fistulas (69 V/s 41).Thus, AV-Fistula is associated with lesser incidence of complications as compared to artificial angioaccess.

IV. Summary And Conclusions

The present study was done in Department of Nephrology and Renal Transplant Centre, Mahatma Gandhi Mission's Medical College & Hospital, Aurangabad in CKD patients who underwent AV-fistula creation and temporary angioaccess insertion and the associations with various risk factors.

- A male preponderance was seen in our study with the Male : Female Ratio 3.22:1
- The mean age in the study was 46.84 years with a range of 13-80 years.
- The maximum number of patients were from the 41-60 age group (38.38%). The maximum number of male participants were from the 41-60 age group (42.23%). The maximum number of female patients were seen in the 21-40 age group (42%).
- The maximum number of patients had eGFR < 10 (82.93%) in the study population.
- Hypertension was the most prevalent (71.56%) single co-morbidity in the study population.
- The most commonly used angioaccess was the Internal Jugular (80.09%).
- The primary success rate of AV-Fistula creation was 84.27%.
- The preferred site for AV-fistula creation was the left radial (71.29%) and the least preferred site was the right brachial (1.9%).
- Infection was the most commonly seen (11.48%) complication associated with the use of artificial angioaccess.
- A significant higher rate of infection was noted in femoral HD catheters (25%), when compared to the jugular access (8.28%).
- Poor flow was more commonly associated with the femoral HD catheters (20%) as compared to the jugular (8.87%).
- Although the percentage of bleeding was more in femoral catheter (15%) as compared to jugular (10.05%), but it was not statistically significant.
- A higher incidence of maturation (>15 days) was observed in our study with Brachial fistulas (77.41%) as compared to Radial fistulas (38.76%).
- There was equal incidence of bleeding and poor flow as complications of native angioaccess(8.13%).
- Infection was the least common complication seen in AV-Fistulas (3.34%).
- Higher failure rates were noted with brachial fistulas (22.58%) as compared to radial AV-Fistulas (17.98%).
- There was no statistically significant difference in failure rates recorded between male and female gender.
- The failure rate was highest in the 0-20 age group (27.27%), but this was not statistically significant.
- The presence of 3 or more co-morbidities resulted in a greater incidence of failure (50%)for AV-fistula.
- Although there was a significant incidence of failure in patients with no underlying co-morbidities (44.44%), the number of subjects in that subset were small. This was not statistically significant. The cause of failure was not further evaluated.

- The complications of bleeding and poor flow were more commonly seen (8.13%) as compared to infection (3.34%) among AV-fistulas.
- Bleeding however was seen more with radial fistulas (3.22% V/s 8.98%), whereas greater incidence of infection (6.45% V/s 2.8%) and poor flow (12.90% V/s 7.30%) was associated with brachial fistulas as compared to radial fistulas.
- The incidence of complications were more associated with artificial angioaccess as compared to native angioaccess. Thus, AV-Fistula was associated with lesser incidence of complications as compared to artificial angioaccess.

References

[1]. Joshi R, Magnolia C, Srinivas I, et al. : Chronic diseases now leading cause of death in rural India-mortality data from the Andhra Pradesh rural health initiative; Int J Epidemiol. 2006;35 (6):1522-9.
[2]. McClellam WM : The Epidemic of renal disease: What drives it and what can be done; Nephrol Dial Transplant 2006;21(6):1461-4.
[3]. World Health Organisation – Global Burden of disease project available at: http://www.3.who_int/whosis/menu.cfm?path, March 2006.
[4]. Schieppati A, Giuseppe R. Chronic renal diseases as a public health problem: Epidemiology, Social and conomic implications. Kidney Int 2005;68 (supp98):s7-10.
[5]. Kolff WJ. : First clinical experience with the artificial kidney; Ann Int Med 1965; 62: 608–19.
[6]. Quinton W, Dillard D, Scribner BH. : Cannulation of blood vessels for prolonged hemodialysis; Trans Am Soc Artif Intern Organs 1960; 6: 104-113.
[7]. Brescia MJ, Cimino JE, Appel K, Hurwich BJ. : Chronic hemodialysis using venipuncture and a surgically created arteriovenous fistula; N Engl J Med 1966; 275: 1089-1092.
[8]. NKF-K/DOQI Clinical practice guidelines for vascular access. Am J Kidney Dis 2006; 48 Suppl 1: S248-273.
[9]. Dagher F, Gelber R, Ramos E, Sadler J. : The use of basilic vein and brachial artery as an A-V fistula for long term hemodialysis; J Surg Res 1976; 20: 373-376.
[10]. The Open Urology & Nephrology Journal, 2012, 5, (Suppl 1: M3) 12-18.
[11]. Schwab SJ, Beathard G. : The hemodialysis catheter conundrum: hate living with them, but can't live without them; Kidney Int 1999; 56: 1-17.
[12]. Rayner HC, Pisoni RL, Gillespie BW, et al.: Creation, cannulation and survival of arteriovenous fistulae: data from the Dialysis Outcomes and Practice Patterns Study; Kidney Int 2003; 63: 323-30.
[13]. USRDS. Annual Data Report 2009-http://www.usrds.org/atlas09.aspx.
[14]. Silva MB Jr, Hobson RW, Pappas PJ, et al.: A strategy for increasing use of autogenous hemodialysis access procedures: impact of preoperative noninvasive evaluation; J Vasc Surg 1998; 27: 302-8. 4.
[15]. Asif A, Unger SW, Briones P, et al.: Creation of secondary arteriovenous fistulas: maximizing fistulas in prevalent hemodialysis patients; Semin Dial 2005; 18: 420-4.
[16]. Srivastava A, Sharma S. : Hemodialysis vascular access options after failed Brescia-Cimino arteriovenous fistula; Indian J Urol 2011;27:163-8

Pleuroperitoneal fistula complicating peritoneal dialysis

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Department of Nephrology and Surgery, MGM Medical collegeCIDCO, Aurangabad 431003

[1]Tapadiya Diagnostic Centre , Railway Station Road, Aurangabad 431001

Abstract: 66 years female with ESRD, treated with CAPD started complaining of dyspnoea, dry cough and back ache within 2 days of commencing CAPD. Chest X-ray revealed massive hydrothorax on right side .Investigations eliminated infective, cardiac and primary respiratory causes .CAPD related hydrothorax was suggested by biochemistry and a pleuroperitoneal leak was confirmed by peritoneal scintigraphy using -99 m Tc -MAA (Macro aggregated albumin).Laparoscopic suturing of Pleuroperitoneal fistula was done and CAPD initiated successfully.

Key Words: CAPD, Pleuroperitoneal fistula, peritoneal scintigraphy, 99 m TC-MAA, laparoscopic repair of fistula

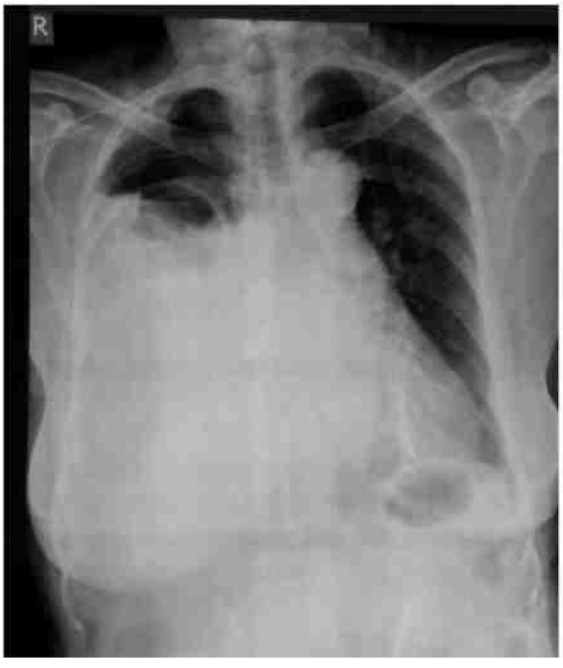
Introduction

PD-related hydrothorax was first reported in 1967 [1]. The reports of prevalence rates of hydrothorax vary, ranging from 1.6 to 10% of PD patients [2]. Usually, these patients present with sudden dyspnea, a decrease in ultrafiltration and chest pain. Some of them may remain asymptomatic or just complain of a dry cough [3]. An early and accurate diagnosis of pleural leakage is important. Here we report a case of pleural leakage which was diagnosed by scintigraphy and repaired laparoscopically.

Case History:

66 year female with ESRD secondary to hypertension was treated with CAPD. After 2 days she started complaining of shortness of breath, dry cough and back ache. On physical examination she was tachypneac and chest examination showed signs of fluid on right side. X-ray chest revealed right sided hydrothorax (Fig.1)

Fig 1 : X-ray chest showing right hydrothorax



Address for Correspondence

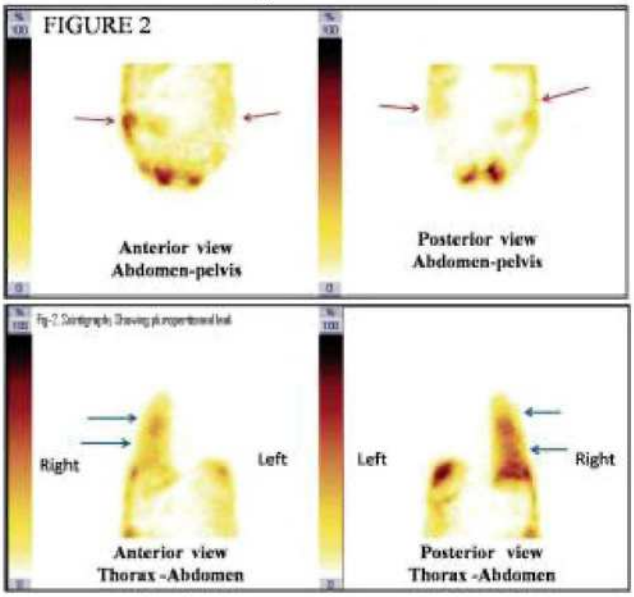
Sudhir G. Kulkarni

Department of Nephrology ,
MGM Medical college
CIDCO, Aurangabad 431 003

.Thoracocentesis was done and 1 L of clear fluid was removed. The fluid examination showed glucose concentration 278 mg %, much higher than serum glucose, which raised suspicion of leakage of peritoneal fluid in the pleural space. Report of pleural fluid showed protein-0.3mg/dl, WBC-60/cu mm, Polymorph-60%, lymphocytes-40% and ADA level was 11.0U/L

For confirmation of peritoneal leak peritoneal scintigraphy using radioisotope 99mTc-MAA was performed. 3mci of 99mTc was instilled with 300ml of dialysate in the peritoneal cavity via CAPD catheter under asepsis. Images of thorax and abdomen demonstrated abnormal tracer accumulation in right pleural cavity suggesting a pleuroperitoneal fistula (Fig.2). Decision to withhold PD was taken after the scan.

Fig 2 : Scintigraphy showing leakage of isotope on right side of chest



Conservative approach with small volume exchanges was tried but failed, so patient was considered for other form of renal replacement therapy. As patient was reluctant to continue on heamodialysis , minimal invasive surgery with Laparoscopic suturing of peritoneopleural fistula was performed .CAPD was continued after 4 days with ICD drain, which was removed after 7 days as the repeat chest X-ray confirmed no further leak .The patient is on CAPD for last two years.

Discussion:

Hydrothorax due to the migration of dialysis fluid from the abdominal space into the pleural space creates a serious complication of PD; it is normally not life-threatening. Hydrothorax frequently presents as shortness of breath or even dyspnea, Hydrothorax is almost always on the right side (4). Our patient also suffered from right pleural effusion.

The sensitivity of radionuclide scans (such as Tc-99m DTPA) to demonstrate peritoneo-pleural fistula is reported to be between 40 and 50% (4,5). Although the leakage location could not be located in this case, pleuroperitoneal communication was definitively diagnosed through scintigraphy.

Fig 3 : Laparoscopic picture showing pleuroperitoneal fistula.

There are a lot of ways of treating pleuroperitoneal communication. First of all, PD should be interrupted for a period of time. Resting the membrane for at least 4-6 weeks will allow the mesothelium to reconstitute itself over the defect, and the pleuroperitoneal communication may reseal. A temporary interruption of PD was associated with a 53% success rate in resuming PD (4). Furthermore, invasive procedures like pleurodesis and thoracotomy as well as video-assisted thoracic approaches can be applied if the conservative treatment is failing. Nearly 60% of patients with pleural defects resume maintenance PD after either conservative or interventional treatment (6) but the others have to permanently transfer to hemodialysis.

PD-related hydrothorax should be recognized and treated timely, although most of the diagnostic strategies that confirm it is unsatisfactory, CT peritoneography provide a non-invasive and useful diagnostic tool for pleuroperitoneal communication. Conservative treatment generally precedes invasive intervention. The discontinuation of PD for a few weeks often leads to the successful resolution of hydrothorax.

References:

1. Edward SR, Unger AM: Acute hydrothorax: a new complication of peritoneal dialysis. JAMA 1967;199:853-855.

2. Lew SQ: Hydrothorax: pleural effusion associated with peritoneal dialysis. Perit Dial Int 2010;30:13-18.

3. Kay H, Doreen R, Thomas K, Peter G: Dry cough in a CAPD patient. Nephrology Dial Transplant 2003;18:1027-1029.

4. Wu PS ,Lee BF,Chiu NT ,Yao WJ ,Huang JJ, Wang MC .Peritoneal scintigraphy for the assessment of Dialysate Leakage in patients on Continuous Ambulatory Peritoneal Dialysis .Ann Nucl Med Sci 2001:14:11-18

5. Chow KM, Szeto CC, Li PK: Management options for hydrothorax complicating peritoneal dialysis. Semin Dial 2003;16:389–394

Recognitions and Awards

Recognitions/Awards

Felicitation by Health Minister Of Odisha and VC Kalinga University(Dr.Samantha) 2018

(Retd) Justice Venkatachalaya felicitating Dr.Sudhir Kulkarni July 2013

Award from Vice President of India

DISTINCTIVENESS: NAVI MUMBAI

INITIATIVE FOR A TUBERCULOSIS-FREE INDIA

Clinical Facilities for Tuberculosis

MGM Medical College, Navi Mumbai

Biosafety Cabinet

CBNAAT-Gene-Xpert

Cooling Microcentrifuge

MDR TB Ward

Microscopy

TB Lab Microscopy

Thermocycler

TwinCubator

Video Bronchoscope

ACTIVITIES OF THE DOTS-PLUS CENTRE

CME AND WORLD TB DAY 2017

JYOTHISH CARE CENTRE (HIV AIDS) TB AWARENESS 2017

TALOJA JAIL INMATES SCREENING FOR TB 2017

JNPT TB SCREENING CAMP 2017

KALAMBOLI POLICE STATION TB SENSITIZATION 2018

NTI BANGALORE INSPECTION IN MGM TB LAB 2018

CME FOR PHARMACIST 2018

KHOPOLI RURAL AREA CAMP 2018

WORLD TB DAY 2019

OUT-REACH TB SCREENING CAMP IN KHOPOLI 2019

WORLD TB DAY 2019 DR. CAMILLA RODRIGUES

WORLD TB DAY 2019 STATE TUBERCULOSIS OFFICER DR. PADAMAJA JOGEWAR

WORLD TB DAY QUIZ COMPETITION 2019

WORLD TB DAY 2019

MOU with RNTCP Dec 2017

MS Copy

महाराष्ट्र MAHARASHTRA ◐ 2016 ◐ AB 905158

8 NOV 2017

MEMORANDUM OF UNDERSTANDING

Between

Chief executive officer, Zila Parishad Raigad & District Revised National Tubersulosis Control Programme society, District Raigad

AND

MGM Medical College Hospital, Kamothe

This MOU is executed on 15th December 2017 between **Chief executive officer, Zila Parishad Raigad & District Revised National Tubersulosis Control Programme society, District Raigad** having its office at In front of Civil Hospital, Alibag (Hereinafter called **"the Grantor"** which expression shall unless exclude by or repugnant to the context include its successors in-interest, executors, administrators and legal representatives) And **MGM MEDICAL COLLEGE**

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

M.G.M. MEDICAL COLLEGE NEW MUMBAI 410209 KAMOTHE

Dean.
M.G.M. Medical College & Hospital
Kamothe, Navi Mumbai - 410209

HOSPITAL, KAMOTHE hence forth referred to as PPP Partner, having its office at Plot No 1&2, Kamothe, Navi Mumbai acting through its Hereinafter called **"the Grantee"**, which expression shall unless excluded by or repugnant to the context include its successors it, interest, executors, administrators and legal representatives).

WHEREAS the Grantor plans to implement "RNTCP (Revised National TB Control Programme) ie DR TB center with Indoor & Outdoor facilities through Grantee on partnership (PPP partener).

AND WHEREAS the Grantor has agreed to engage the services of the Grantee, subject to terms and as hereunder.

1. **DRTB center (under):** The activities would be implemented in the District/s of **Raigad & Navi Mumbai , Maharashtra** for performance of the following activities in accordance with RNTCP policy;

2. Project Location

The PPP Partner would be provicing the services as specified above at the following location/ (s) as decided in consultation with concerned CTO/DTO

a. Urban/ Rural: **Urban/ Rural**

b. District/ TU/ Block/ (s): **Raigad & Navi Mumbai**

c. Urban Wards/ Panchayats covered: Yes

d. Population Covered: App. 40 lacs

3. Period of Co-operation:

The PPP Partner agrees to perform all activities outlined in the guideline for partnerships in above mentioned area. The duration of cooperation will be from day signing of MOU or the day of the starting the activity / function whichever is later.

Duration

Contract is signed for a period of three year 15th December 2017 to 14th November 2020, renewable as per the needs of the programme, subject to satisfactory performance. The contract can be terminated by the District Health Society/ State Health Society or the PPP Partner any time with one month prior notice by either side.

4. Terms, conditions and specific services during the period of the MOU.

A. **The District Health Society shall** (please strike out whichever is not applicable)

i. Provide financial and material support to the PPP for carrying out the activities as mentioned in the partnership guideline.

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Chief Executive Officer
Zilla Parishad,

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
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Navi Mumbai- 410 209

ii. Provide relevant copy of technical guidelines, updates, manuals & circulars, etc.)

iii. Provide RNTCP drugs, logistics and laboratory consumables for use as per RNTCP policy as outlined the partnership guideline

iv. Periodically review the performance and activities being undertaken by the PP Partner

B. MGM will: -

i. Perform all activities as agreed upon and signed under the partnership as mentioned below.

Outdoor DRTB center Scheme:

1. Institute should be tertiary care hospital with the pulmonologist will be available round the clock.
2. Separate designated clinic for DR TB patient management should be available and comply with the National Guidelines for Air –borne infection control for out patient settings
3. Relevant speciaiists like Pulmonologist, Physician, Psychiatrist, Dermatologist & gynecologist etc should be available.
4. DR TB center Committee to be formed with the above group of doctors.
5. To renovate (in keeping with the National Airborne Infection Control Guidelines and National Guidelines for Programmatic Management of Drug Resistant TB (PMDT) provided for the purpose) and designate a special clinic area designated for MDR TB out patient service with earmarked well ventilated preferably open air waiting area separate from other waiting areas, away from clinics managing immune suppressed and venerable cases where the patients who will be eligible to avail DR TB services under RNTCP will be fast tracked , segregated and counseled in accordance with RNTCP guidelines.
6. Doctors and Nursing staff should be available from institute round the clock consultation services made available , if required by the patients.
7. Management of adverse drug reactions (ADRs) as per National PMDT Guidelines.
8. The diagnostics services to be provided by the partner organization would include at least.

Sl No	Investigations	Minimum No. of times test will be done	Rate for tests** (In Rs.)
1	Complete blood count	1	138
2	Blood sugar	1	25
3	LFT. OT/PT/Bilirubin	1	275
4	Blood Urea Nitrogen	1	55
5	Serum Creatinine	6	56
6	TSH	1	125
7	Urine routine & microscopy		39

Chief Executive Officer
Raigad Zilla Parishad, Alibag

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8	Pregnancy test		69
9	Chest X ray		70
10	ECG		100

*Rates are based on rate of CGHS Delhi rates (per test) and are subject to revision as and when updated in CGHS website

Indoor DR TB Center scheme :

The terms and condition are as follows.

1. To designate a special ward compliant with national AIC guidelines and at least 10 beds earmarked for indoor management of DRTB patients according to National PMDT Guidelines.
2. Routine clinical laboratory investigation facility to be made available for pretreatment evaluation and monitoring.
3. Doctors and Nursing staff should be available from institute round the clock to the DRTB patients
4. Ancillary drugs to be provided as per DR TB center Committee's advised services / facilities to diagnose and manage adverse drug reaction (ARDs) as per National PMDT Guidelines
5. Services /facilities to diagnose and manage the comorbid condition
6. Records and reports to be maintained for PMDT registration , follow up, referral and transfer (if required) \of patients as per guidelines update the same on the day basis using Nikshay
7. Quarterly reports to be submitted electronically
8. All doctors in the hospital should be following Standards fore TB care in India and notify all TB cases through Nikshay
9. Ensure coordination with implementing District officers and staff as well as laboratory for proper follow up of patients till outcome.
10. The diagnostics services to be provided by the partner organization would include at least.

Sl No	Investigations	Minimum No. of times test will be done	Rate for tests** (In Rs.)
1	Complete blood count	1	138
2	Blood sugar (RBS)	1	25
3	LFT. OT/PT/Bilirubin	1	275
4	Blood Urea Nitrogen	1	55
5	Serum Creatinine	1	56
6	TSH	1	125
7	Urine routine & microscopy	1	39
8	Pregnancy test	1	69
9	Chest X ray	3	70
10	ECG	1	100
11	Indoor stay for maximum of 7days	1	
12	Food for maximum of days	1	

Chief Executive Officer

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13	Specialist consultation	As required
14	Ancillary drugs for management \of adverse drug reaction and comrbidities	As required

11. The DR TB Centre cannot deny services to any eligible patient from the geographical area assign to the centre
12. This does not restrict the DR TB Centre from extending any further services to the patients, if clinically deemed necessary
13. DR TB Centre committee doctors will have to be trained in PMDT at National Level.
14. Management of MRD/XRD TB patients is to be done as per RNTCP Guidelines second line anti TB drugs will be provided from RNTCP.
15. The performance review of the PPM partner would be done bi annually and in case lack of satisfactory performance the contract may be terminated by either party with one month written notice

Gant-in-Aid

Fund shall be released by the respective health society in the name of the MGM for initial six months and subsequently biannually, within 30 days of the satisfactory completion activities and submission of required documents. The MGM will submit utilization certificate indicating expenditure during the particular quarter and available unspent balance to the respective State/District Health Society on quarterly basis. The subsequent release will depend on the unspent balance and committed liability (if any).

1. Remuneration for following posts on contractual basis will be provided :
   - Counselor –Rs.10,000/-pm
2. For diagnostic test of MRD –TB patients on outdoor basis, private partner would be reimbursed as per rates given above (applicable for area) by RNTCP.
3. In case ambulatory care of MRD TB patients Rs 200/day/per patients consultation charges would be applicable
4. Package cost per day for admitted MRD-TB cases will be Rs 800/- including pre treatment evaluation (as per list above),bed charges ,meals and ancillary drugs.
5. In house Specialist Consultation charges would be applicable at Rs 200/day/per patient for indoor patients
6. For patients convenience, if he/she is partially or completely managed on ambulatory basis at the district level under guidance of DR TB Centre Committee
7. Rs 500/- per day if pre treatment investigation is done at the district level and patient is admitted to the ward hospital

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8. Rs 500/- one time for only DR TB Centre decision based on reports sent from the districts, if pre treatment investigation and treatment initiation is done at the district level in case patient refuses to get admitted. This will also be applicable if the district's request for follow up advise over email/phone/post on decision of DR TB Centre for either charges in regimen, adverse drug reaction management, co-morbidity management etc. without patient admission to the DR TB centre
9. In case of re-admission/ extension of stay due to cause /s secondary to TB or side effects of second line anti-TB drugs or co-morbidity management .
10. Charges up to Rs.800/day/patient(including bed and meals + investigations and ancillary drugs)
11. To provide Training, formats and registers for PMDT
12. To provide Computer and Internet Facility
13. To Provide access & training to NIKSHAY for online data management and patient tracking

6. Fund Management

Funds under this MOU shall be placed at the disposal of the Grantee in separate account opened by it, subject to its furnishing to the Grantor a letter of commitment containing such conditions as may be approved by the Grantor from the bank that the bank shall not exercise a lien over the said account or may right to set off or adjust any amount due to payable under any loan or credit arrangement which the Grantee may be having or may have with the bank against the amounts standing to the credit of the Grantee in the said amount.

The Grantee shall install and maintain separate books of accounts on cash basis accounting along with proper vouchers for expenditure incurred and with details of outstanding liabilities, if any. The Grantor shall have the right to inspect by its authorized officers of independent agencies the books of accounts and other records relating to the project fund kept by the Grantee any time during the agreement period or thereafter.

7. Grievance Redressal Mechanism

All grievances will be addressed within a period of thirty days, by DTO of the concerned district. Final decision will rest with district Health Societies. Annual review would be a platform for addressing grievance of PPM partners.

8. Right over Information/data

All documents, information, statistics and data collected by the Grantee in the discharge of the obligation under the MOU incidental or related to it (whether or not submitted to the Grantor) shall be the joint property of the Grantor, and the Grantee

Chief Executive Officer
Zilla Parishad, Alibag

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9. Indemnity

The Grantee hereby agrees to always keep the Grantor indemnified and harmless from all claims /demands / action and proceedings which may arise by reason of any activity undertaken by Grantee if the activity is not in accordance with the approved guidelines.

This MOU shall be enforceable in courts situated at [Mumbai, Maharashtra] ; any suit or application for enforcement of the above shall be filed in the competent court at Mumbai and no other district of Maharashtra or outside Maharashtra shall have any Jurisdiction in the matter

10. Termination Mechanism

The partnership may be terminated by either side through written notice of one month. In case services of PPM partner are discontinued, unspent balance, if any will be refunded by the partner.

If the Grantor at any stage decides that the Grantee has misutilised the amounts (or any part thereof) already received from the Grantor or has fraudulently claimed any covenants, stipulation or obligations hereunder a commits a breach of any of the terms, conditions or provision of this MOU on its part to be observed and performed, or it at any stage reasonable ground exist to apprehend the breach of the terms and condition of the MOU in future or that the continuance of this project

may be prejudiced or be in jeopardy he/she may revoke this MOU wholly or partially and ask the Grantee to refund the amount received till then along with interest accrues, if any after giving at least fifteen days' notice and an opportunity of being heard to the Grantee

11. The programmatic and financial review of the partnership will be conducted every quarter.

12. Necessary approval of State Health Society has been obtained: Yes

Dean,
M.G.M. Medical College & Hospital
Kamothe, Navi Mumbai - 410209

Dr G S Narshetty
Dean
MGM Medical College & Hospital,
Kamothe, Navi Mumbai

Seal

28/12/17

Signature of authorised signatory
Chief executive officer, Zila Parishad
Raigad & District Revised National
Tubersulosis Control Programme
society, District Raigad

Seal

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
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EXTENSION ACTIVITIES OF THE CENTRE

MAHATMA GANDHI MISSION HOSPITAL
Sector-1, Plot No.1 & 2, Kamothe, Navi Mumbai – 410 209
Tel.: 022- 2743 7900/ 01, Fax: 2743 1723

MGMH/ Respi. Med/2018/22

Date – 25th January 2018.

To,
Dean,
MGM Medical College,
Kamothe, Navi Mumbai.

Subject –Tuberculosis Screening Camp.
Ref.- Khopoli Nagar Parishad letter O. N./KMC/NUHM-41/dated 10/01/2018.

Respected Sir,

This is to inform you that Tuberculosis Screening Camp was held on dated 23-01-2018 at 10.00 am to 2.00 pm in Mohan wadi Village, under Khopoli, Nagar Parishad. Following members of the respiratory medicine department (DOTS PLUS CENTRE) were activity involved.

1) Dr. C. D. Kulkarni – Assistant Professor,

2) Shri. Kalpesh Khawas, PRO/Counselor cum Computer Operator, DDR-TB Center.

Total Screening of 48 OPD patients was done and 9 were referred to MGM.

Yours sincerely,

Dr. P. V. Potdar
Nodal Officer
DDR – TB Center
Professor & HOD
Department of Respiratory Medicine
MGM Medical College & Hospital

TALOJA CENTRAL PRISON
Navi Mumbai 410210
Tel: 022 27762251 / 52 / 54 | Fax: 022 27762253
Email: talojajail@gmail.com / talojacp-mh@gov.in

O/w No.: TCP/HOSP/ MGMCER/ 2419 /2018 Navi Mumbai Date: 24.07.2018

CERTIFICATE OF APPRECIATION

We appreciate your efforts, MGM Hospital Kamothe DOT Plus Centre and RNTCP Dist. Raigad, Alibaug for screening of Taloja Prison inmates on 22/08/2017 along with X-Ray and HIV testing which helped in diagnosing 10 patients of TB who were put on treatment as well it has formed strong coordination in diagnosis and follow up of inmates in TB which is contributing in the global and Vision of India to end TB by 2025.

I sincerely congratulate team of Doctors and RNTCP staff for such an initiative.

(Dr.Sunil M. Kale)
Chief Medical Officer
Taloja Central Prison
Navi Mumbai

(Shri S.N.Gaikwad)
Superintendent,
Taloja Central Prison
Navi Mumbai

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

MGM INSTITUTE OF HEALTH SCIENCES
(Deemed University u/s 3 of UGC Act, 1956)
Grade 'A' Accredited by NAAC

MGM NEW BOMBAY COLLEGE OF NURSING
5th Floor, MGM Educational Campus, Plot No. 1& 2, Sector-1
Kamothe, Navi Mumbai–410 209.

Ref no.: MGMNBCON/41/3/18

28.03.2018

To,
Dr. Alka Patnaik
RNTCP Medical Officer,
MGM Hospital,
Kamothe, Navi Mumbai.

Dear Madam,

I take this opportunity to express our heartfelt thanks to you and Dr. P.V. Poddar (HOD, Department of Respiratory Medicine) for conducting valuable session on 'Tuberculosis and its prevention' on 15/01/18 at seminar Hall of MGM New Bombay College of Nursing. The information shared was very valuable and informative.

Looking forward for continued association in future.

Thanking you,
Yours truly,

Dr. (Mrs.) Prabha K. Dasila
Professor & Director

MGM New Bombay College of Nursing Kamothe, Navi Mumbai 410209

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

OFFICE OF THE CHIEF MEDICAL OFFICER, 102 RAF, NAVI MUMBAI

No. M-III-1/2018-RAF-102

Dated, the 23rd July' 2018

To,

Dean ,
MGM Hospital,
Kamothe, navi Mumbai.

Sir,

We want to extend our gratitude and special thanks to Dr. Shreeja Nair, Asst. Professor TB & Chest Department, MGM, Kamothe for delivering a very informative lecture followed by interactive session to our troops on tobacco use & deaddiction on the eve of World health day i.e.07/05/2018.

Looking forward to more of such useful sessions & talks.

Thanking you.

Place:- Navi Mumbai.
Date:- 23/07/2018

Yours Sincerely,

23/7/18

Chief Medical Officer
102 RAF
Navi Mumbai

(Dr. [redacted] Choudhary)
Chief Medical Officer,
102 BN, RAF, CRPF,
Taloja, Navi Mumbai

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

Phone : 022 - 2774 4406
Fax : 2774 9895
E-mail : ymtayurved@drgdpolfoundation.org

DR. G. D. POL FOUNDATION'S
Y.M.T. AYURVEDIC MEDICAL COLLEGE & HOSPITAL
NAVI MUMBAI

Institutional Area, Sector - 4, Kharghar, Navi Mumbai - 410 210.

Ref. No.:YMTA/900/16

Date : 1/9/2016

Certificate

This is to certify that Dr. Alka Patnayak, Medical Officer, RNTCP officer, has conducted discussion session in lecture series of RNTCP, organized by Rognidan Dept, Dr. G. D. Pol Foundation's YMT Ayurvedic Medical College.

V D SANJEEV YADAV
Dean
Y.M.T. Ayurvedic Medical
College & Hospital
Sec. 4, Kharghar
Navi Mumbai 410 210

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

Tel. : 2742 6967
2742 3399

A.H.F. JYOTHIS CARE CENTRE

Plot No. 4, Sector - 11, Kalamboli, Navi Mumbai - 410 218

Ref. No.

Date 23 3 18

To,

Whomsoever it may concern,

This is to certify that Dr. Alka Patnaik , the medical officer DOTS at MGM ,Kamothe has been working in the field of TB and HIV co-infection by actively participating in taking care of PLHAs registered at AHF-Jyothis clinic, Kalamboli. Along with her team workers, she has conducted the world TB day successfully at Jyothis care center on the 23rd March. She gave informative lecture to the 25 such patients and their families on the importance of TB drugs adherence, need of family and society support in the eradication of TB from India. The interactive session thus conducted by her was beneficial to all those who attended it.

AHF-JYOTHIS CLINIC
PLOT NO - 4, SECTOR - 11,
KALAMBOLI, NAVI MUMBAI
PIN - 410218

DR. DIVYA MITHAL
M.B.B.S., T.D.D., F.R.M.
REGN. NO. 55931
MEDICAL OFFICER
JYOTHIS CARE CENTRE

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Khapoli Camp conducted on 23.01.18

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Khapoli Camp conducted on 23.01.18

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Lecture 23.03.18 A.H.F. Jyothis Kalamboli

TB Screening 22.08.17 Taloja Central Prison

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Extension Activities, JNPT Uran

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Extension Activities, JNPT Uran

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Extension Activities, Kalamboli Police Station

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Meeting with District TB Officer

Photographs of Extension Activities and Activities at DOTS-Plus Centre

Activities at DOTS-Plus Centre

RESEARCH

PATENTS

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते

क्रमांक : 022106609
SL No :

भारत सरकार
GOVERNMENT OF INDIA
पेटेंट कार्यालय
THE PATENT OFFICE

पेटेंट प्रमाणपत्र
PATENT CERTIFICATE
(Rule 74 Of The Patents Rules)

पेटेंट सं. / Patent No.	:	325554
आवेदन सं. / Application No.	:	201721040727
फाइल करने की तारीख / Date of Filing	:	15/11/2017
पेटेंटी / Patentee	:	MGM Institute Of Health Sciences (MGMIHS), Deemed University u/s 3 of UGC Act, 1956

प्रमाणित किया जाता है कि पेटेंटी को उपरोक्त आवेदन में यथाप्रकटित QUANTUM DOT POWERED IP-10 ANTIBODY BASED KIT FOR LATENT TB AND TB ANTIGEN DETECTION नामक आविष्कार के लिए, पेटेंट अधिनियम, १९७० के उपबंधों के अनुसार आज तारीख 15th day of November 2017 से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled QUANTUM DOT POWERED IP-10 ANTIBODY BASED KIT FOR LATENT TB AND TB ANTIGEN DETECTION as disclosed in the above mentioned application for the term of 20 years from the 15th day of November 2017 in accordance with the provisions of the Patents Act,1970.

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

पेटेंट कार्यालय
THE PATENT OFFICE
GOVT.OF INDIA
भारत सरकार

अनुदान की तारीख : 22/11/2019
Date of Grant :

पेटेंट नियंत्रक
Controller of Patent

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

Application Details

APPLICATION NUMBER	201721023794
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	06/07/2017
APPLICANT NAME	1 . MGM- COLLEGE OF ENGINEERING AND TECHNOLOGY (MGMCET) 2 . MGM INSTITUTE OF HEALTH SCIENCES (MGMIHS)
TITLE OF INVENTION	A BIOLOGICAL SAFETY CABINET AND A KIT THEREOF
FIELD OF INVENTION	BIO-MEDICAL ENGINEERING
E-MAIL (As Per Record)	info@krishnaandsaurastri.com
ADDITIONAL-EMAIL (As Per Record)	patent@krishnaandsaurastri.com
E-MAIL (UPDATED Online)	
PRIORITY DATE	NA
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	11/01/2019

Application Status

APPLICATION STATUS	Application Published

WPTR
world Patent and Trademark Register

REGISTRATION OF THE INTERNATIONAL PATENT

Contract Number: 1192101984

AI 2019/21

Sent Date: 29.05.2019

MGM INSTITUTE OF HEALTH SCIENCES (MGMIHS),
DEEMED UNIVERSITY U/S 3 OF UGC ACT, 1956
Sector -1, Kamothe,
Navi Mumbai, Maharashtra 410209
India

Applicant

WPTR s.r.o.
Prikop 843/4
602 00 Brno
Czech Republic

Tax number: 07659776

Provider

REGISTRATION DETAILS

Title:

QUANTUM DOT POWERED IP-10 ANTIBODY BASED KIT FOR LATENT TB AND TB ANTIGEN DETECTION

Int. Class:

G01N 33/569 (2006.01)

Int. Application No:	PCT/IN2018/050688
Publication Number:	WO/2019/097536
Publication Date:	23.05.2019
Int. Filing Date:	25.10.2018

Sign the document within 14 days and send it back by e-mail to **office@wptr.biz** or by mail to:
WPTR s.r.o., Prikop 843/4, 602 00 Brno, Czech republic.

Registration Fee	Amount	
Registration Fee for 1192101984	2,329.00	USD
Processing Fee	27.00	USD
Total Registration Fee	**2,356.00**	**USD**

Registration of the International Patent:

The international patent has been published in the WIPO-Gazette, which is edited by Bureau of the World Intellectual Property Organization. This publishing forms the basis of our offer. Please note, registration is not affiliated with the publication of the official International Patent Application registration and is not a registration by a government entity. By signing this Agreement, the Applicant signs a binding "WPTR Registration" service provided by the provider specified in the GTB article 3 paragraph 1 and undertakes to pay the provider the price stated on this form. Given that this form is exclusively an offer for the conclusion of a contract, the contractual relationship created by this contract arises at the moment of the delivery of this contract to the provider. Effective delivery is deemed to be the delivery of the contract to the address of the provider and the delivery of the contract to the email address of the provider. By signing this contract, the Contracting Authority agrees that the contractual relationship is governed by the General Business Terms and Conditions of the Provider, which are listed on the other side of this Form and are governed by the Act No. 89/2012 Coll. Civil Code. The Applicant declares that he has read and read these General Business Terms and the scope of the service provided, and he further declares that they agree with their wording.

Applicant

Date	Full name
Signature	

Provider

WPTR s.r.o.
Příkop 843/4, 602 00 Brno
Czech Republic
IČ: 07659776

-3-

470E0860

Received AABhagil 27/06/2019 (3:10PM)

Dr. Rajesh B. Goel
Registrar
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(Deemed University u/s 3 of UGC Act, 1956)
Navi Mumbai- 410 209

INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS
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सत्यमेव जयते
GOVERNMENT OF INDIA

Controller General of Patents,Designs and Trademarks
Department of Industrial Policy and Promotion
Ministry of Commerce and Industry

(12) PATENT APPLICATION PUBLICATION
(19) INDIA

(21) Application No. : 3620/MUM/2015

(22) Date of filing of Application :23/09/2015

(43) Publication Date : 05/02/2016
Journal No. - 06/2016

(54) Title of the invention : MICRO-TRENCH BASED BIOCHIP DEVICE FOR SCREENING OF INFECTIOUS DISEASES USING METAL NANO PARTICLES / NANO COATING

(51) International classification	:A61B 8/14
(31) Priority Document No	:NA
(32) Priority Date	:NA
(33) Name of priority country	:NA
(86) International Application No	:NA
Filing Date	:NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number	:NA
Filing Date	:NA
(62) Divisional to Application Number	:NA
Filing Date	:NA

(71)**Name of Applicant :**
1)Department of Atomic Energy
Address of Applicant :Anushakti Bhavan, C.S.M. Marg, Mumbai - 400 001, Maharashtra, India Maharashtra India
2)MGM Institute of Health Sciences
3)Birla Institute of Technology and Science, Pilani
(72)**Name of Inventor :**
1)SURI, V. K. (India)
2)R. Balasubramaniam (India)
3)MISHRA, Shivam (India)
4)GHILDIYAL, Shrinkhla (India)
5)JOSHI, D. S. (India)
6)THAKUR, Mansee (India)
7)PAI, Girish (India)
8)BHAND, Sunil (India)
9)PAL, Souvik (India)

(57) Abstract :
The present disclosure relates to a medical micro-trench based diagnostic device and method for screening of infectious disease causing pathogens using DNA hybridization of the disease causing pathogen. In an aspect, the device includes a chip having a substrate that is configured with a plurality of micro trenches that are configured to facilitate hybridization of a target nucleic acid of a disease causing pathogen, and an oligonucleotide that bears signature of the target nucleic acid of a disease causing pathogen, wherein the plurality of micro trenches are configured with micro metal coating and the oligonucleotide is coupled to the micro metal coating to prevent diffusion and spreading of the oligonucleotides from the confined space of the plurality of micro trenches and to facilitate entrapment of high concentration of the oligonucleotide in the trench to increase the hybridization with target nucleic acid.

Number of Pages = 27

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

RESEARCH PROJECTS

తెలంగాణ तेलंगाना TELANGANA

B 506817

Sl. No. 5798 Date 14/05/2015 Rs. 100/-

Sold to Nagendra

S/o Narayana R/o Seribad

For whom M/s IKP Knowledge Park

NAKKA NAGESHWAR
Licensed Stamp Vendor. Lic. No. 15-07-010/2013
Flat No. 211, 2nd Floor, Silver Oak Apartments,
CHERLAPALLY - 500 051 (R.R. Dist.)
Cell : 9949 110 435

For IKP Knowledge Park
Authorised Signatory

Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act, 1956)
KAMOTHE, NAVI MUMBAI

GRAND CHALLENGES IN TB CONTROL AWARD AGREEMENT

THIS AGREEMENT is executed at Hyderabad on this 14th day of December 2015

BETWEEN

IKP KNOWLEDGE PARK, a Company registered under the Company's Act, 1956, having its registered office at Genome Valley, Turkapally, Shameerpet, Ranga Reddy District, Hyderabad 500 078 hereinafter referred to as 'IKP' or 'IKP Knowledge Park' (which expression shall mean and include unless repugnant to the context, its successors, assigns and legal representatives) of the ONE PART represented by its authorized representative, Mrs Deepanwita Chattopadhyay, Chairman & CEO.

AND

MGM Institute of Health Sciences, a deemed university registered under Section 3 of UGC Act, 1956, and having its registered office at MGM Institute of Health Sciences, MGM Educational Campus, Sector - 1, Kamothe, Navi Mumbai 410 209 referred to as the 'Recipient' (which expression shall mean and include unless repugnant to the context, its successors, assigns and legal representatives) of the SECOND PART, represented by its authorized representative, Dr. Sudhir N Kadam, Vice Chancellor

For IKP Knowledge Park
Authorised Signatory

Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act, 1956)
KAMOTHE, NAVI MUMBAI

ANNEXURE 3

AWARD NO: GCTBC/C2P1/2015/.12/14/06.....

RECIPIENT: "Non-Invasive TB Triage and patient mapping platform using breath via low-cost Titanium Dioxide Nanotube Sensor"

Expense Heads / Month	1	2	3	4	5	6	7	8	9	10	11	12	Total
Capital Expenses	1,520,000												1,520,000
Rentals													-
Consumables	245,000			500,000		425000							1,170,000
Salaries	80000	80000	80000	80000	80000	80000	80000	80000	80000	110000	110000	110000	1,050,000
IP / Legal Expenses													-
Travel	37500	37500	37500	37500	37500	37500	37500	37500	37500	37500	37500	37500	450,000
Test setup													-
Contingency			50000			50000			50000			50000	200,000
Volunteer Compensation			120000			120000			120000				360,000
Utilities	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	20000	240,000
												Total	4,990,000
Milestone 1		Approvals & Design of trials											
Milestone 2				Healthy volunteers tested									
Milestone 3							Groups 2 to 5 tested						
Milestone 4										Completion of data collecction			
Milestone 5												Final Report	

Budget:

*Excluding service tax

** Regarding Host Government taxes refer 17(C)(1)(C)

B. Funds Disbursement:

Disbursement will be made in 6 tranches at the following times -

Signing of contract - 40%

Month 2 - 20%

Month 4 - 15%

Month 7 - 10%

Month 10 - 10%

Completion of project - 5% **

Expenses will be reimbursed at cost. Recipient will invoice IKP Knowledge Park with service tax as applicable prior to each tranche. Payment to the Recipient shall be subject to deduction of taxes and levies, as applicable from time to time under various laws in India. Payment to the Recipient by IKP shall be released after verification of the Milestones and expenses incurred. In the normal course payment will be released within 30 days from the date of receipt of the Invoice/Bill along with the requisite documents, complete in all respects.

For IKP Knowledge Park

Authorised Signatory

Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s GC Act, 1956)
KAMOTHE, NAVI MUMBAI

All amounts will be credited by IKP Knowledge Park to the no lien current account No. 0183104000236140 of MGM Institute of Health Sciences, IDBI Bank, IFSC Code IBKL0000183

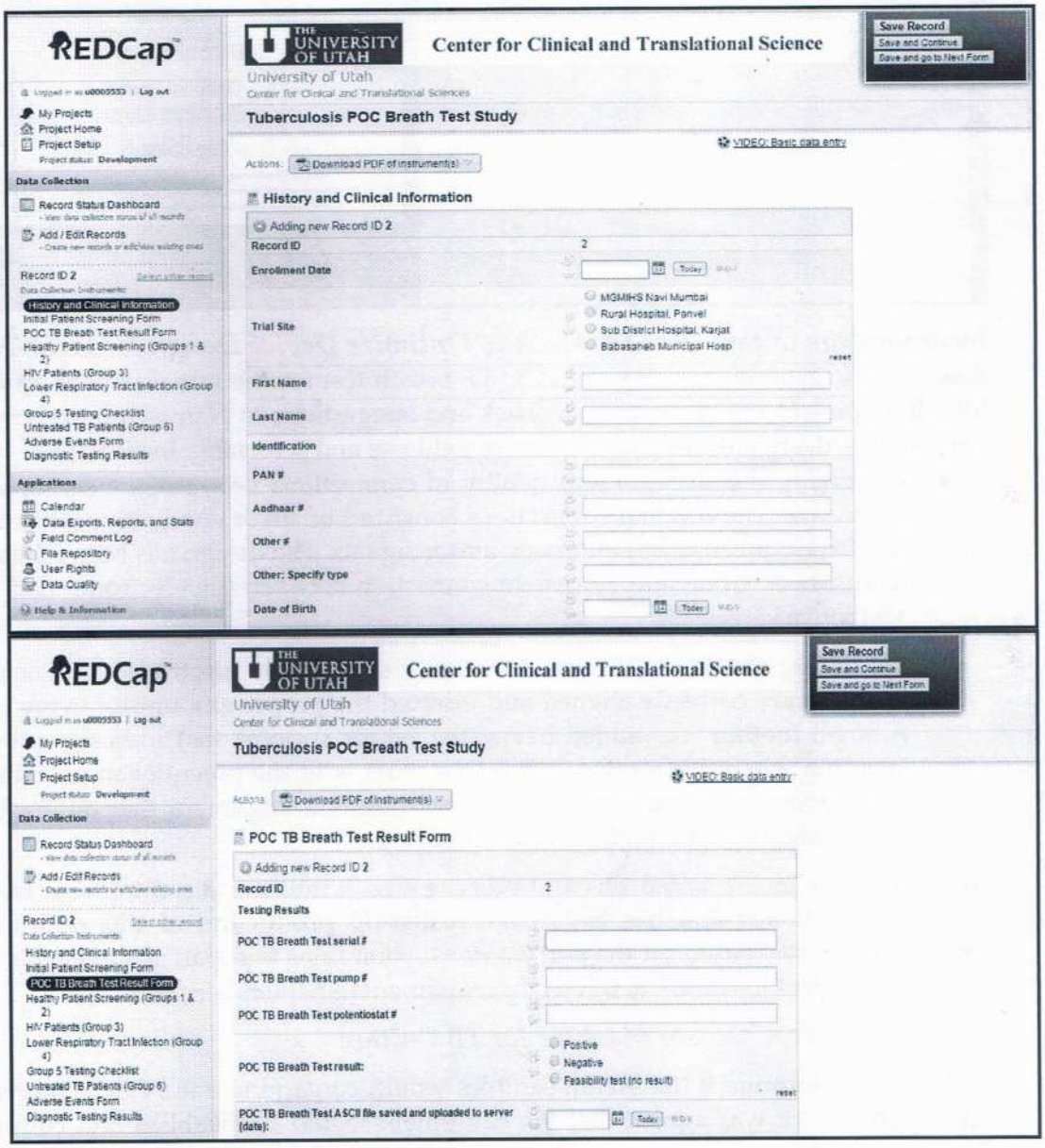
For IKP Knowledge Park

Authorised Signatory

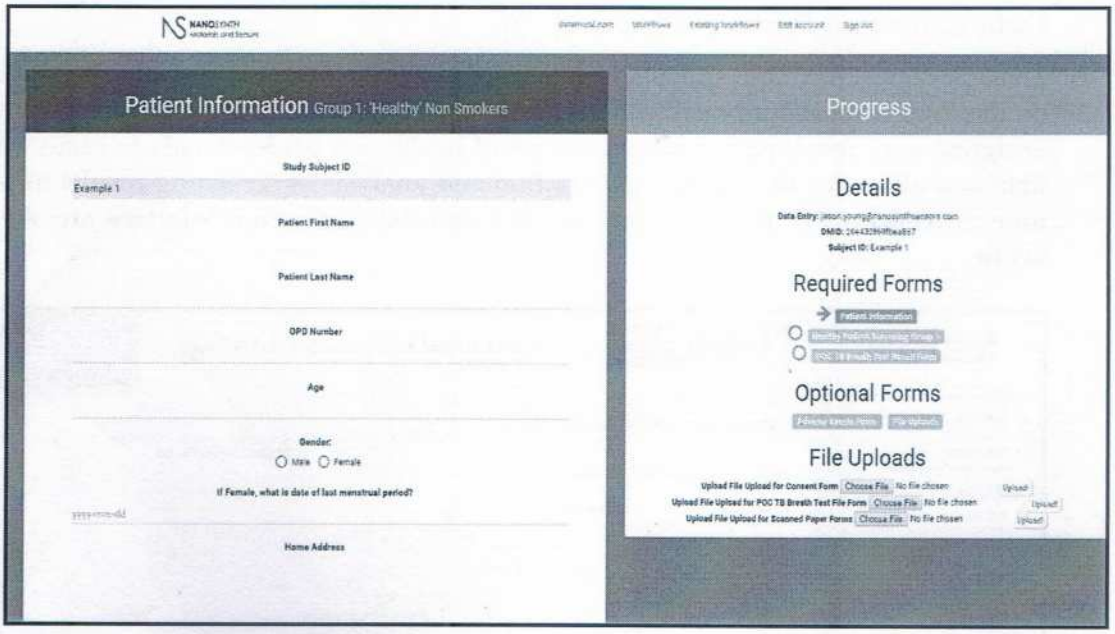
Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s ... UGC Act, 1956)
KAMOTHE, NAVI ... MBAI

Data Collection Protocol Finalized

The case report form questions and REDCap database structure were finalized based on the final clinical study protocol. An easy to use data collection interface was also designed and developed to allow a range of healthcare professionals to easily enter data and allow for the secure aggregattion and analysis of screening results in real-time. Screenshots of the REDCap database and data collection interface are shown below.



Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209



Incorporation of End User Feedback to Optimize Device Design for Field Use

Based on the experience using the POC TB breath test for the initial patient testing, MGMIHS project staff provided feedback and suggestions to Nanosynth on how to optimize the design of the POC TB test for field use and screening, including:

- Improving the strength and quality of connections between the sensor and electrodes: The original connections consisted of silver conductive paint that resulted in inconsistent and poor sensor signals. The design has been adjusted to ensure a strong and consistent connection between the electrodes and the TB biomarker sensor.
- Modifying the POC TB sensor housing so that the electrodes and sensor supports are correctly aligned and inserted the proper amount for every test: A small footing was added below the sensor support that indicates proper insertion when this footing comes in contact with the potentiostat port. Prior to this, there was variability in the insertion and alignment that diminished the consistency and quality of the sensor signal.

Nanosynth has incorporated this and other feedback into the current design of the POC TB breath test and has begun the scaled-up production of this optimized version of the test. Based on the current production rates and patients available for testing, we expect to complete full study enrollment in September.

Swab the TB POC Sensor Machine for TB Culture

In order to determine if the breath samples would contaminate the POC TB breath test equipment, it was agreed that the equipment would be swabbed and cultured for 5 breath samples from TB patients. This testing is being done as per standard protocols in TB laboratory testing (KoneMan's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition by Elmer W. Koneman 2005;page 1070):

"There is virtually no indication for obtaining material for mycobacterial culture with a swab because of the hydrophobic nature of the lipid containing cell wall of the bacteria inhibits transfer of the organisms from the swab to the aqueous culture medium. Still if a swab is

received should be placed directly on the surface of culture medium or into the tube containing 5 ml of 7H9 broth and incubated for 4-6 weeks. Mycobacteria if present may be found forming the colonies in the fibers of the swab at the junction with the culture media."

Even though surface swabs are not ideally acceptable, in this study they are being used to check for surface contamination while testing breath bags by POC test device. The protocol for culture of surface swabs for TB culture near and around the surface of POC device is as follows:

1. Swabs will be collected from the surface near and around the POC testing device.
2. Swabs will be immediately placed directly into the tube containing 5 ml of MGIT culture tubes and incubated for 42 days
3. Fibers of the swab at the junction with the culture media will be checked for any growth.
4. Report of the findings at the end of 42 days

5Five culture swabs were collected from near around the surface of POC device and cultured in MGIT along with positive controls as per protocol. All the 5 swabs showed no growth for Mycobacterium tuberculosis.

Data Collection and Analysis

Patient Enrollment

Overall 92 patients have been enrolled in this project as shown in the table below, including TB suspect patients from all of the rural clinical sites.

Group	Site	Enrollment
Group 1 Healthy Nonsmoker	MGM Hospital	40
Group 2 Healthy Smoker	MGM Hospital	2
Group 5 TB suspect	MGM Hospital	11
	Karjat Subdistrict Hospital	26
	Panvel Rural Hospital	7
	Muncipal Hospital, Khopoli	6
Total Subjects Enrolled		92

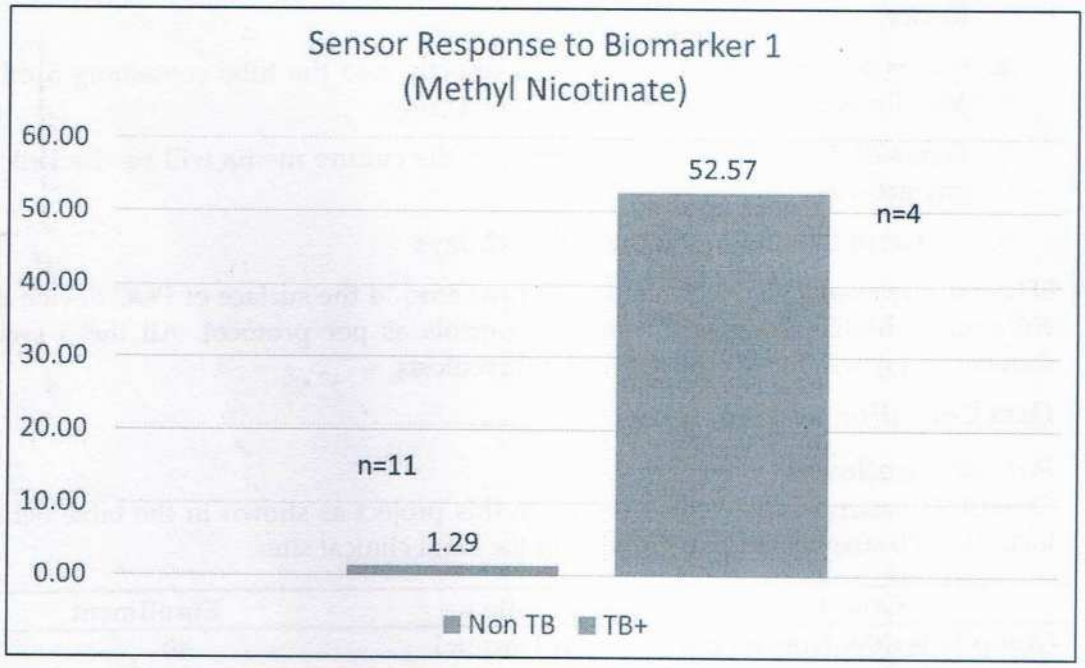
Patient Testing

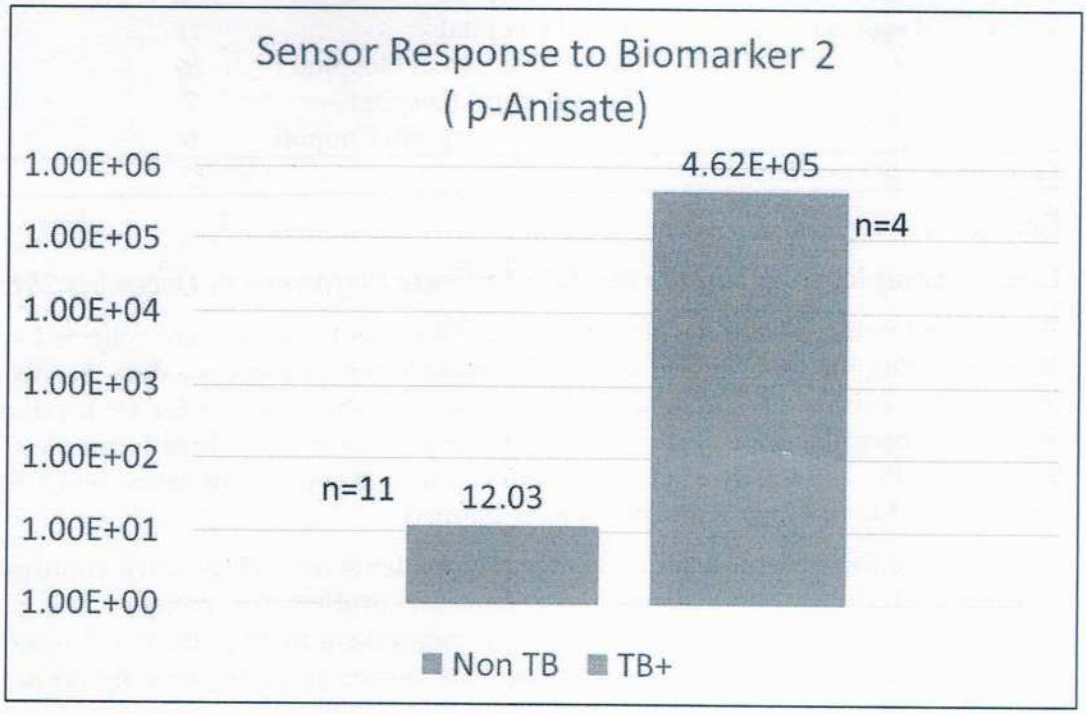
Control Samples from Known Healthy Patients Performed in December 2015

Ten healthy patients' and 1 known positive TB patient breath were collected and analyzed using the POC device to provide baseline sensor response data. Feedback from field use during this test resulted in improved pump design for air handling. Solid extraction fibers were used to extract compounds in those breath samples for transport to the University of Utah for further analysis and identification by GCMS. Based on GCMS findings, Algorithms were defined.

Initial testing focused on healthy TB-negative patients and TB-positive confirmed patients in order to understand the biomarker profiles for patients living in Mumbai. The sensor response to the four TB biomarkers in 15 patients (11 healthy patients, 4 TB patients) are shown below. The sensor response was significantly

higher for the TB positive subjects for all four biomarkers compared to the healthy patients that were tested. The largest difference was seen for biomarkers 3 and 4, methyl phenylacetate and o-phenyl anisole, with a sensor response many orders of magnitude larger for the TB-positive subjects relative to the TB-negative subjects. Methyl nicotinate had the smallest difference in sensor response between the two patient groups but still showed a difference of approximately 50x higher for TB positive patients. These results are consistent with earlier findings for all four of the biomarkers.





Since, MGIT culture results are awaited for a number of tests, so considering GenXpert as Gold Standard, POC test (55%) has higher sensitivity than Smear AFB (36%), which is current screening tool for PTB diagnosis.

Study exceptions

As per our study Protocol, in order to maximize the utility of this feasibility study and prevent events unrelated to the sensor's accuracy from influencing the initial estimates of sensitivity and specificity, the following data points will be excluded from the analysis:

- The results from patients that were found not to meet necessary inclusion criteria but were incorrectly included in any of the study groups.
- The results from patients where equipment failure (other than the sensor) had a significant impact on the testing procedure and results, including a pump failure resulting in breath sample not reaching the sensor, a computer malfunction that prevented data acquisition from a functioning sensor, or power disruption.
- Results from patients indicating improper use of the potentiostat (including not ensuring proper voltages are set) or failure to properly deliver the breath sample to the sensor.

There were some issues with testing of first lot of sensors and as per our protocol for study exceptions, we plan to exclude these subjects from the study.1
Further, issues related to sensors and connections to potentiostat and voltage stabilization was handled and with new modified lots of sensors, consistent results were obtained.

Groups	Excluded Subjects
Group1	5
Group 5 KARJAT	9
Group 5 MGM	6
Group 5 PANVEL	0
Group 5 KHOPOLI	1
TOTAL	21

User feedback to optimize POC test for field use

The sensor is designed to work with the moisture found in the breath. The presence of moisture does not contribute significantly to the sensor signal when detecting the VOCs of interest. All sensors (i.e. carbon nanotube, metal oxide sensors) generate response to moisture however in our case the contribution of moisture is on the pico-amp scale. When VOCs are detected, the signal ranges from microamps to milliamp range. Water vapor (moisture) does not donate electrons to the sensor in the manner that specific VOCS do when they are interacting with a specific metal of on the surface of our nanostructure at a specific bias voltage (as they do in our sensing system.) Electron donation leads

Since, MGIT culture results are awaited for a number of tests, so considering GenXpert as Gold Standard, POC test (55%) has higher sensitivity than Smear AFB (36%), which is current screening tool for PTB diagnosis.

Study exceptions

As per our study Protocol, in order to maximize the utility of this feasibility study and prevent events unrelated to the sensor's accuracy from influencing the initial estimates of sensitivity and specificity, the following data points will be excluded from the analysis:

- The results from patients that were found not to meet necessary inclusion criteria but were incorrectly included in any of the study groups.
- The results from patients where equipment failure (other than the sensor) had a significant impact on the testing procedure and results, including a pump failure resulting in breath sample not reaching the sensor, a computer malfunction that prevented data acquisition from a functioning sensor, or power disruption.
- Results from patients indicating improper use of the potentiostat (including not ensuring proper voltages are set) or failure to properly deliver the breath sample to the sensor.

There were some issues with testing of first lot of sensors and as per our protocol for study exceptions, we plan to exclude these subjects from the study.1
Further, issues related to sensors and connections to potentiostat and voltage stabilization was handled and with new modified lots of sensors, consistent results were obtained.

Groups	Excluded Subjects
Group1	5
Group 5 KARJAT	9
Group 5 MGM	6
Group 5 PANVEL	0
Group 5 KHOPOLI	1
TOTAL	21

User feedback to optimize POC test for field use

The sensor is designed to work with the moisture found in the breath. The presence of moisture does not contribute significantly to the sensor signal when detecting the VOCs of interest. All sensors (i.e. carbon nanotube, metal oxide sensors) generate response to moisture however in our case the contribution of moisture is on the pico-amp scale. When VOCs are detected, the signal ranges from microamps to milliamp range. Water vapor (moisture) does not donate electrons to the sensor in the manner that specific VOCS do when they are interacting with a specific metal of on the surface of our nanostructure at a specific bias voltage (as they do in our sensing system.) Electron donation leads

UNIVERSITY OF UTAH SUBCONTRACT

NO. 10040842-01

BY AND BETWEEN

THE UNIVERSITY OF UTAH

AND

SUBCONTRACTOR

This subcontract (Subcontract) is entered into and effective as of Jan. 27th, 2016 by and between the University of Utah, an institution of higher education for the State of Utah ("University") and Mahatma Gandhi Mission Institute of Health Sciences having their principal place of business at MGM Educational Campus, Sector-1 Kamothe, Navi Mumbai 410209("Subcontractor").

RECITALS

WHEREAS, University wishes to have certain services performed in accordance with the scope of work outlined in this Subcontract; and

WHEREAS, the performance of such services is consistent, compatible and beneficial to the role and mission of Subcontractor; and

WHEREAS, Subcontractor is qualified to provide such services required under this Subcontract.

AGREEMENT

NOW, THEREFORE, for and in consideration of the mutual covenants, conditions and undertakings herein set forth, the parties agree as follows:

1. Scope of Work: Subcontractor agrees to perform for University certain services ("Services") described in the Scope of Work set forth in Appendix A, which is attached hereto and incorporated herein by this reference.

2. Period of Performance. This Subcontract commences on January 27, 2016 and will continue until January 31, 2017("Project Period").

Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act, 1956)
KAMOTHE, NAVI MUMBAI

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Budget

Sr. No.	Staff	Cost US $
1	Staff Salary, Compensation, Honorarium	44,400
2	Chemicals , Consumables, Test	30,000
3	Transportation of samples, Travel by Investigators	10,600
4	Volunteer Compensation	8,000
5	Ethical Committee Fee	750
6	Insurance	750
7	Overhead	5,550
	Total	1,00,000

The total budget for the project is $1,00,000 and will be allocated based on the milestones as follows:

- Subcontract $ 50,000
- Milestone 2 $ 45,000
- Milestone 3 $ 5,000

Exhibit B – Background IP

IP Owned by the University

- PCT Patent Application: PCT/US2013/067319
  - Titled: Functionalized nanotube sensors and related methods (filed October 29, 2013)
- Trade secrets
  - Titanium dioxide nanotube sensor production and manufacturing
- Data Platform:
  - IP related to the storage, transmission, or mapping of TB data.

IP Owned by Subcontractor

Vice Chancellor
MGM INSTITUTE OF HEALTH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act, 1956)
KAMOTHE, NAVI MUMBAI

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Government of India
Department of Atomic Energy
BRNS Secretariat

Central Complex, 1st floor
BARC, Mumbai 400 085

Date: 25-7-2012

No. 2012/36/15-BRNS/

Office Memorandum

Sub: R/P entitled "Development of micro biosensor for rapid diagnosis of tuberculosis (TB)" under Dr. D.S. Joshi, MGM College of Engineering and Technology, Sector 18, Kamothe, Navi Mumbai 410 209

On the recommendations of the Board of Research in Nuclear Sciences (BRNS), I am directed to convey the administrative approval and sanction of the President of India for the captioned project for two years beginning from financial year 2012-13 with a total grant of Rs.23,82,750/- as detailed below:

	Item of expenditure	I year (2012-13)	II year (2013-14)
*	Equipment	Rs.12,00,000	--
	Consumable	Rs. 4,00,000	Rs.1,35,000
	Travel : PI	Rs. 1,25,000	Rs.1,25,000
	Contingency	Rs. 50,000	Rs. 50,000
&	Overhead	Rs. 1,29,375	Rs. 19,500
		Rs.19,04,375	Rs.3,29,500

* (1) Biosafety cabinet level II/III, (2) Deep freezer, (3) Photomultiplier with fiber optics adaptors, (4) Laser light source with filter tubes, (4) Lyophilizer

& Overhead calculated @ 7.5% of other heads **except** contingency. The remaining 7.5% towards overhead (Rs.1,48,875) shall be released only on meeting the requirements specified (**see Annex-B**).

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

Government of India
Department of Atomic Energy
BRNS Secretariat

Central Complex, 1st floor
BARC, Mumbai 400 085

No.36(2)/14/26/2014-BRNS/ 1367

Date: 18 AUG 2014

Office Memorandum

Sub: R/P entitled "Development of prototype micro-PCR device for identification of MDR MTB" under Dr. Mansee Thakur, M.G.M. Institute of Health Sciences, Secator 18, Kamothe, Navi Mumbai 410 209

On the recommendations of the Board of Research in Nuclear Sciences (BRNS), I am directed to convey the administrative approval and sanction of the President of India for the captioned project for two years beginning from financial year 2014-15 with a total grant of Rs.24,98,900/- (Rupees twenty four lakh ninety eight thousand nine hundred only) as detailed below:

	Item of expenditure	I year	II year
		(2014-15)	(2015-16)
#	Staff : JRF(1)	Rs. 1,92,000	Rs. 1,92,000
$	Technical Assistance	Rs. 96,000	Rs. 96,000
	Consumable	Rs. 8,00,000	Rs. 6,10,000
	Travel : PI	Rs. 50,000	Rs. 50,000
	Contingency	Rs. 50,000	Rs. 50,000
&	Overhead	Rs. 85,350	Rs. 71,100
	Total	**Rs.12,73,350**	**Rs.10,69,100**

JRF fellowship calculated @ Rs.16,000/- per month for two years.

$ Technical Assistance includes equipment hire charges, computer charges and charges for hiring services.

& Overhead calculated @ 7.5% of other heads **except** contingency. The remaining 7.5% towards overhead (Rs.1,56,450) shall be released only on meeting the requirements specified (**see Annex-B**).

Dr. Rajesh B. Goel
Registrar
MGM INSTITUTE OF HELATH SCIENCES
(DEEMED UNIVERSITY u/s 3 of UGC Act,1956)
NAVI MUMBAI- 410 209

2. I am also directed to convey the sanction of the President of India to incur an expenditure of Rs.12,73,350/- (Rupees twelve lakh seventy three thousand three hundred fifty only) towards grant for the financial year 2014-15.

3. The expenditure involved is debitable to :

Grant No.	:	04	- Atomic Energy
Major Head	:	3401	- Atomic Energy Research
Minor Head	:	00 004	- Research & Development
Sub head	:	08 02	- BRNS
Detailed Head	:	08 02 31	- Grant-in-Aid

4. This issues with the concurrence of Scientific Secretary, BRNS and IFA.

Sd
(Dr. Debanik Roy)
Programme Officer, BRNS

Pay and Accounts Officer
Department of Atomic Energy
CSM Marg
Mumbai 400 001

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

No.36(2)/14/26/2014-BRNS/ Date :

Copy forwarded to:

1. Director of Audit, Scientific Department, AEAP, OYC, CSM Marg, Mumbai - 400 001.
2. Joint Secretary (R&D), DAE, Anushakti Bhavan, CSM Marg, Mumbai-400 001.
3. Director, M.G.M. Institute of Health Sciences, Sector 18, Kamothe, Navi Mumbai 410 209
4. ** Principal Investigator : Dr. Mansee Thakur, M.G.M. Institute of Health Sciences, Sector 18, Kamothe, Navi Mumbai 410 209

A. First year grant is being released in full vide ECS through Pay & Accounts Officer, Department of Atomic Energy, Anushakti Bhavan, CSM Marg, Mumbai-400 001 directly.

i) Receipt of this sanction letter and grant for the amount sanctioned for the first financial year may please be acknowledged (Form-I)**.**

ii) THIS SANCTION IS FURTHER SUBJECT TO THE CONDITIONS STIPULATED IN ANNEX-A, ANNEX-B AND ANNEX-C **(ENCLOSED)**, WHICH MAY BE GONE THROUGH CAREFULLY.

B. Second year grant will be released after the PI submits the following documents to the Programme Officer (NRFCC):

a) Claim in Form-II **(enclosed)** quoting the reference of the sanction issued for the first year.

b) Utilisation Certificate (UC) as on **31st March** of the preceding financial year in Form-III **(enclosed)** showing bank interest accrued and duly audited by the Internal Auditor of the University/ Institution or a Chartered Accountant.

c) Statement of Accounts (SA) as on **31st March** of the preceding financial year in Form-IV **(enclosed)** showing bank interest accrued and duly audited by the Internal Auditor of the University/ Institution or a Chartered Accountant.

d) Copy of appointment order and joining report of the staff appointed for the project along with minutes of the Selection Committee.

e) An inventory of equipment in Form-V **(enclosed)**.

f) A One Page report on the progress of work during first year.

C. Grant for the third year and subsequent years (if any), will be released only after the Principal Investigator (PI) fulfills the following requirement:

i) The Department will issue a fresh sanction for the third and subsequent years after receiving the recommendations of the BRNS after scrutiny of the Renewal Application in Form 4R.

Hence, **2 copies** of renewal request in the **Form 4R (enclosed)** and **2 copies** of detailed Progress Report must reach to **Dr. Vivekanand Kain, (MS, NRFCC), Materials Science Division, BARC, Mumbai-400 085** and one copy of **Form 4R** to **Dr. Debanik Roy, Programme Officer (NRFCC), BRNS Secretariat, First Floor, Central Complex, BARC, Trombay, Mumbai-400 085** on or before **30th November of the second or subsequent year of the project as the case may be**.

ii) If the progress is found to be satisfactory the renewal sanction for the year will be issued in the beginning of that financial year.

iii) On receipt of the renewal sanction, the PI shall claim the funds sanctioned by submitting the following documents to **Programme Officer (NRFCC)**:

a) Claim in Form II **(enclosed)** quoting reference of the renewal sanction.

b) Utilisation Certificate (UC) as on **31st March** of the preceding financial year in Form-III **(enclosed)** showing bank interest accrued and duly audited by the Internal Auditor of the University/ Institution or a Chartered Accountant.

c) Statement of Accounts (SA) as on **31st March** of the preceding financial year in Form-IV **(enclosed)** showing bank interest accrued and duly audited by the Internal Auditor of the University/ Institution or a Chartered Accountant.

d) However, the final consolidated Statement of Accounts/ Utilization Certificate showing bank interest accrued to be submitted at the end of the Terminal year shall be audited by a Chartered Accountant or the Statutory (Govt.) Auditor

e) Copy of appointment order and joining report of the staff appointed for the project along with minutes of the Selection Committee.

f) An inventory of equipment in Form-V **(enclosed)**.

g) Final consolidated Progress Report for settling the Terminal Grant.

5. AAO (Bills II), DAE, Anushakti Bhavan, CSM Marg, Mumbai - 400 001 – With a request that the amount granted for the first year of the project may be released immediately.

6. Member Secretary, NRFCC : Dr. V. Kain, MSD, BARC, Mumbai-400 085

7. Member Secretary, TSC-2, NRFCC : Shri V. Bhasin, RSD, Hall-7, BARC, Mumba-85

8. Principal Collaborator : Dr. V.K. Suri, Head, PED, BARC, Mumbai 400 085 - You or your nominee may please be the DAE representative for selection of Research Fellow / Research Associate for the project.

9. Co-Principal Investigator (1) : Dr. D.S. Joshi, M.G.M. Institute of Health Sciences, Sector 18, Kamothe, Navi Mumbai 410 209

10. Co-Principal Investigator (2) : Dr. Sudhir Kadam, M.G.M. Institute of Health Sciences, Sectpr 18, Kamothe, Navi Mumbai 410 209

Debanik Roy

(Dr. Debanik Roy)
Programme Officer, BRNS

** Note : Please quote the Sanction Number (**No.36(2)/14/26/2014-BRNS)** in all your correspondence with BRNS.

Govt.of Maharashtra, Health Services
Jt.Director of Health Services (Leprosy & TB)
"AROGYA BHAVAN" Opp. Vishrantwadi Police Station,
Alandi Road, Yerwada, Pune-411006.

Jt.Director - ☎ (020) 26686955
Dy.Director - 26686951
Office - 26686952-54
Fax – 26686956

Section wise e-mail
TB section- stomh@rntcp.org
Lep section - jtlepnms@rediffmail.com
Est section - jdhsest99@gmail.com

No.Jt.DHS/TB&L/ Desk- RNTCP/TB/ 7405-10 /18
Date 19/5/2018

To,
The Dean,
Mahatma Gandhi Mission Medical College,
Navi Mumbai

Sub:- Sanction of grant-in-aid for the Operational Research proposal under RNTCP.

Ref:- The State Operational Research Committee meeting held on 26th September, 2017 at Disha Hall, Parivartan Building, Arogya Bhavan, Pune.

The following Operational Research proposal submitted by the Principal Investigator (PI) of your institute was discussed in State Operational Research Committee Meeting held on 26th September, 2017 under RNTCP and it has been approved.

Sr. no	Name of the PI	Name of the Department & Medical College	Topic
1	Dr. P. V. Potdar, Principal Investigator	Department of Respiratory, MGM Hospital, Navi Mumbai	Study, evaluate and analysis of MGM - TB detector system

The Principal Investigator (PI) will sign a Memorandum of Undertaking (MOU) with the TB programme manager on behalf of the society for the release of funds. The MOU will include the objects for which he will utilize the funds and the timeline for the study. It will also include the commitment from him to return the funds if the study cannot be taken up due to any reason, and other relevant causes. Funds will be released on the name of the institution of the Principal Investigator, so that the College / Department can ensure the study of its completion / return the funds in the event that the Principal Investigator is moved from the college during the course of the study.

A Grant-in-aid of **Rs. 2,00,000 (Rs. Two lac only)** for the above OR proposal will be released from the "Medical College Budget Head" from RNTCP funds by City TB Officer, Navi Mumbai Municipal Corporation 50% of the grant-in-aid will be released initially and remaining 30%

after receiving the report of data analysis and 20% will be released after receipt of the four hardcopies of the final documents.

Joint Director of Health Services
(Leprosy & TB) Pune

Copy to –

1. The CTO Navi Mumbai– To follow up with the respective medical college & Principal Investigator and release the grant-in-aid amount of Rs. 2,00,000 (Rs. Two lac only) from the "Medical College Budget Head" from RNTCP funds as per the guidelines.

2. Dr. P. V. Potdar, Principal Investigator, Department of Respiratory, MGM Hospital, Navi Mumbai

3. The RNTCP Medical Consultants by email – mhconsultants@rntcp.org

4. The OR Committee Members ..... (All)

Copy with complements to –

Dr. Babaji Ghewade, Chairman State OR Committee, Maharashtra & Professor Respirator Medicine, Chief Medical Superintendent, Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Wardha

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
(Deemed University u/s 3 of UGC Act)
Navi Mumbai- 410 209

PUBLICATIONS

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Research Article
Biological Sciences

HYPOCALCEMIA AND HYPERPHOSPHATEMIA IN TUBERCULOSIS

S Bhandari*[1], S Gautam[2], A K Parajuli[3], Z G Badade[4], P Potdar[5]

[1]Lecturer, Department of Biochemistry, Gandaki Medical College, Pokhara, Nepal
[2]Lecturer, Department of Community Medicine, Gandaki Medical College, Pokhara
[3]Medical Lab Technologist, Department of Biochemistry, Gandaki Medical College, Pokhara
[4]Professor, Department of Biochemistry, MGM Medical College, Navi Mumbai, India
[5]Professor, Department of TB and Respiratory Medicine, MGM Medical College, Navi Mumbai,
*Corresponding Author Email: princesureesh@hotmail.com

ABSTRACT

Tuberculosis (TB), a major public health problem, is a granulomatous infection caused by Mycobacterium tuberculosis. One third of world's population is infected with M. tuberculosis. This study was aimed to estimate serum calcium and phosphorous levels in TB patients before during and after DOTS (Directly Observed Treatment Short course). Calcium and phosphorous are essential macro-minerals required for various physiological functions; changes may cause a detrimental effect on those functions.92 subjects (42 normal healthy controls and 50 patients with TB) were studied and their serum calcium and phosphorous levels were estimated. Serum calcium level was observed to be low and Serum phosphorous level was found to be high; both being statistically significant (p<0.001) at diagnosis and after two months of therapy whereas both the levels became normal on completion of therapy.This study helps in marco-minerals level changes during TB infection and may suggest need for therapy targeted at normalizing those levels for early prognosis of the disease.

KEY WORDS

Tuberculosis (TB), serum calcium, serum phosphorous, DOTS

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* which is an aerobic non motile bacillus. The tubercle bacilli can attack any part of the body, most commonly lungs. It spreads through droplet infection. When infectious people cough, sneeze, talk or spit they propel TB germs known as bacilli into air. Inhalation of very small numbers of these bacilli will lead to *M. tuberculosis* infection. One third population of the world is infected with *M. tuberculosis*. The vast majority of these have latent infections. Annually more than 8 million people develop tuberculosis and approximately 1.8 million cases results in death.[1] Most of the estimated number of cases in 2010 occurred in Asia (59%) and Africa (26%). India alone accounted for an estimated one quarter (26%) of all TB cases worldwide, and China and India combined accounted for 38%.[1]

Calcium and phosphorous are important macro-minerals required for vital functions. Calcium is important for growth and development of bones and teeth, action of enzymes, mediation of hormonal responses, blood coagulation, muscle contractility and normal neuromuscular irritability.[2] Phosphorous is required for formation of bone and teeth, production of high energy phosphate compounds such as ATP, GTP, creatine phosphate etc, synthesis of nucleoside co-enzymes like NAD and NADP, DNA and RNA synthesis and formation of phosphate esters like glucose-6-phosphate and phospholipids.[3] Any imbalance in the serum calcium and phosphorous concentration will have a detrimental effect on the physiological functions they perform.

MATERIALS AND METHODS

A prospective study was carried out in 92 subjects, of which 42 were normal healthy individuals and 50 suffering from tuberculosis. The subjects were selected from OPD, IPD and DOTS centre of MGM group of Hospitals, Navi Mumbai, India. The estimation of serum calcium and phosphorous was carried out. Out of 50 tuberculosis patients, 31 were assessed after two months and after six months (completion) of DOTS therapy. DOTS therapy comprises of combination of drugs – Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. The regimen was administered as per the RNTCP (DOTS) guidelines[4]. The control group comprised of healthy subjects of both sexes. The diagnosis of TB was based on clinical, radiological, sputum Acid Fast Bacilli (AFB) smear positivity and tuberculin skin test positivity. Venous blood samples were obtained from tuberculosis patients (3 times- at the time of diagnosis, after two months and on completion of DOTS therapy) and healthy controls. Serum calcium and phosphorous levels were estimated by Trinder's Method[5] and Fiske Subbarow Method[6] respectively.

Statistical analysis: Comparison of serum calcium and phosphorous levels were performed between control group and study groups (newly diagnosed TB patients, patients after 2 months of therapy and patients on completion of therapy) by using MS Office Excel 2007 and the software 'GraphPad Quick Cals t-test calculator'. Results were expressed as mean (M), standard deviation (S.D) and considered significant when $p<0.05$ determined by Student's t-test and Paired t-test.

RESULTS

Results were expressed as Mean ± S.D for each parameter

Control group: 42 normal healthy individuals aged between 20 to 60 years of age [27 males (64.28%) and 15 females (35.72%)] were included as controls. In this group Mean ± S.D of serum calcium and phosphorous were found to be 10.31 ± 0.40 mg/dl and 3.53 ± 0.37 mg/dl respectively.

Study group: 50 newly diagnosed TB patients were included in the study. Out of those, in 31 patients serum calcium and phosphorous levels were also estimated during treatment and on completion of therapy.

In the study, mean serum calcium level in newly diagnosed patients was 8.91 ± 0.94 mg/dl. The values were estimated to be 9.28 ± 0.46 mg/dl and 10.09 ± 0.58 mg/dl in patients after two months of therapy and on completion of therapy respectively.

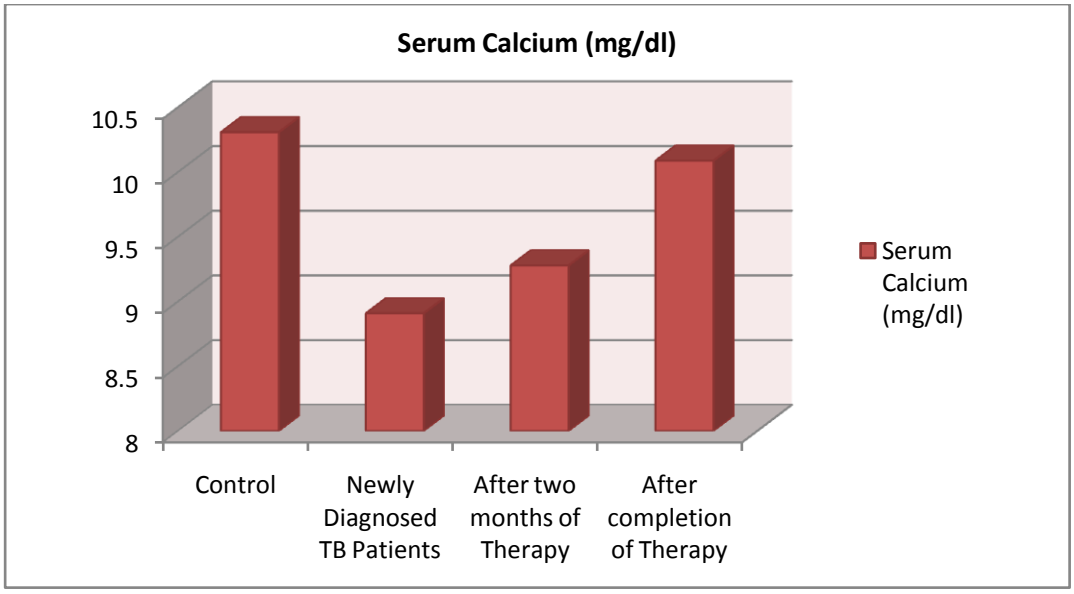
In the study, mean serum phosphorous level in newly diagnosed patients was 4.85 ± 0.38 mg/dl. The values were estimated to be 4.40 ± 0.30 mg/dl and 3.49 ± 0.62 mg/dl in patients after two months of therapy and on completion of therapy respectively.

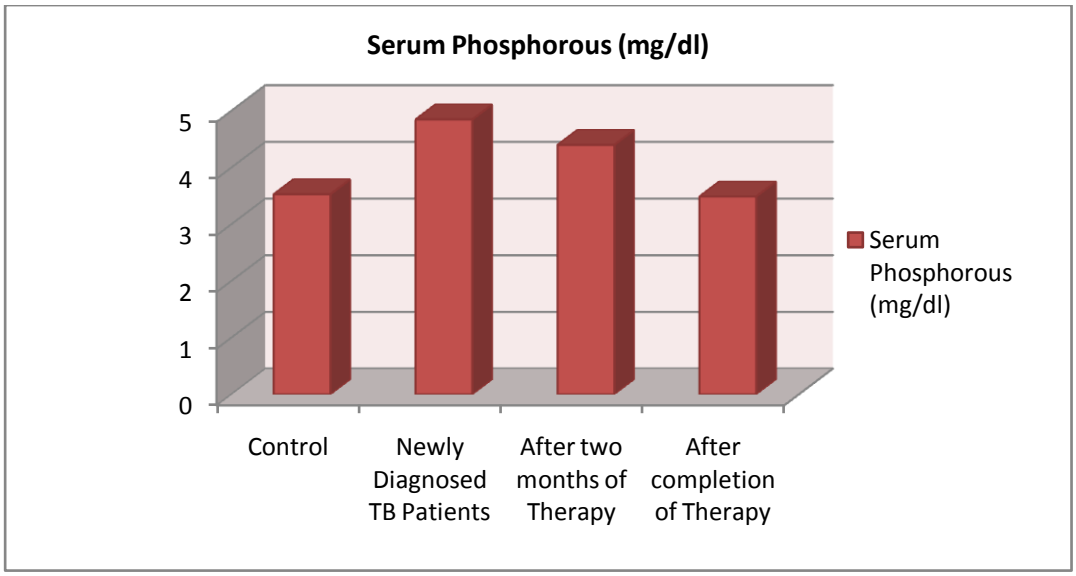
No significant difference was observed in mean serum calcium and phosphorous levels in different sex and age group among the patients.

Table 1: Comparison of serum calcium and phosphorous in control group and different study groups

Parameter	Control Group (Mean ± S.D)	Study Groups		
		Newly diagnosed TB patient (Mean ± S.D)	Patients after two months of therapy (Mean ± S.D)	Patients on completion of therapy (Mean ± S.D)
Calcium (mg/dl)	10.31 ± 0.40	8.91 ± 0.94 *	9.28 ± 0.46 #	10.09 ± 0.58 *
Phosphorous (mg/dl)	3.53 ± 0.37	4.85 ± 0.38 *	4.40 ± 0.30 *	3.49 ± 0.62 *

*** p<0.001 (high statistically significant) # P<0.05 (statistically significant)**





DISCUSSION

In present study, out of 50 newly diagnosed tuberculosis patients 20 were found to have a low level of serum calcium (i.e. hypocalcemia) and rest were normo-calcemic. We found that the serum calcium levels were significantly low (P<0.001) in newly diagnosed TB patients as compared to the control group. There was a significant increase (P<0.05) in the mean calcium level after two months of anti-tubercular treatment. The mean calcium level in patients after the completion of DOTS therapy was highly statistically significant (P<0.001) as compared to the newly diagnosed patients. A significant increase (P<0.001) in the mean calcium level in patients after completion of therapy was observed as compared to the same patients after two months of anti-tubercular therapy. This finding is consistent with the earlier studies from Pakistan[7], Japan[8], Egypt [9, 10] and Nigeria[11]. Controversial reports of changes in serum calcium levels in tuberculosis patients have also been reported. L. Lind et al[12] reported that out of 67 patients with pulmonary tuberculosis 25% were hypercalcemic before the initiation of therapy. The mean ±SD value for serum calcium in the newly diagnosed patients was 2.51±0.16 mmol/L which was significantly higher (P<0.001) that the mean level

found in the control group (2.43±0.07 mmol/L). They also found that after one year of successful treatment the serum calcium values had normalized. Similar observation was also reported by Abbasi A.A et al.[13], Subash C Sharma et al.[14], C.K. Liam et al[15] and Kitrou MP et al[16]. It could be explained by influence of many factors like ethnic differences, malabsorption and malnutrition associated with patients of pulmonary tuberculosis.

The serum phosphorous level was significantly high (P<0.001) in newly diagnosed TB patients as compared to the control. There was a significant decrease (P<0.05) in the mean phosphorous level after two months of anti-tubercular treatment. The mean phosphorous level in patients after the completion of anti-tubercular therapy (six month) was statistically significant (P<0.001) as compared to newly diagnosed patients. A significant decrease (P<0.001) in the mean phosphorous level in patients on completion of therapy (six months) was observed as compared to the same patients after two months of DOTS therapy. Similar observations were reported by Well H.G. et al[17], Sweany H.C. et al[18] and Sharma et al[19]. This finding could be due to the distribution of the intracellular phosphate which is liberated due to the destruction of the cells.

CONCLUSION

In the present study, mean serum calcium level was significantly decreased in newly diagnosed patients as compared to control which became normal after completion of DOTS therapy. However, mean serum phosphorous level was significantly increased in newly diagnosed patients as compared to control which came to normal levels after the anti-tubercular therapy. This may signify the changes in different macro-minerals' levels due to TB infection. Studies can be carried out on the prospects of use of calcium based phosphate binders as adjuvant to DOTS for TB treatment.

ACKNOWLEDGEMENT

The authors would like to acknowledge MGM Medical College and group of hospitals, Kamothe, Navi Mumbai, India for their invaluable support. We are also thankful to Dr. Gyanendra Raj Joshi, Demonstrator, Department of Pharmacology, Gandaki Medical College, Pokhara, Nepal.

REFERENCES

1. The Global Tuberculosis Control 2011.2011 Geneva: World Health Organization.
2. Thomas M. Devlin. Textbook of Biochemistry with Clinical Correlations. Seventh edition 2010. Pg no 1085-86
3. DM Vasudevan, Sreekumari S, Kannan Vaidyanathan. Textbook of Biochemistry for medical students. Sixth edition 2011. Pg no. 419-20
4. Fdsha
5. Trinders P. Colorimetric Micro determination of Calcium in serum. Analyst, 1960, 85: 889-894.
6. Fiske C.H. and Subbarow Y. The colorimetric determination of phosphorous. J Biol chem. 1925, 66: 375-400.
7. Aamir Ijaz, Tariq Mehmood, Waseem Saeed, AH Qureshi. Calcium abnormalites in pulmonary tuberculosis. Pakistan J Med Res. 2004, Vol. 43 No.4.
8. Shirai M, Sato A, Suda T, Shichi I, Yasuda K, Iwata M, Okano A, Genma H, Chida Calcium metabolism in tuberculosis. Kekkaku. 1990 Jun; 65(6):415-20.(Abstract).
9. Hafiez AA, Abdel-Hafez MA, Salem D, Abdou MA, Helaly AA, Aarag AH. Calcium homeostasis in untreated pulmonary tuberculosis. I--Basic study. Kekkaku, 1990 May;65(5):309-16.
10. Hafiez AA, Abdel-Hafez MA, el-Khashab M, Abdou MA, Al-Helaly AA, el-Aarag AH. Calcium homeostasis in untreated pulmonary tuberculosis II--dynamic study. Kekkaku. 1990 Jun;65(6):391-5.
11. Ali Gombe A, Onadeko BO. Serum calcium levels in patients with active pulmonary tuberculosis. Afr. J. Med Med Sci. 997 Mar-Jun; 26(1-2): 67-8.
12. L. Lind, **S.** Ljunghall. Hypercalcaemia in pulmonary tuberculosis. Upsala J Med Sci 95: 157-160, 1990.
13. Abbasi, A.A., Chemplavil, J.K., Farah, S., Muller, B.F. & Arnstein, A.R.: Hypercalcemia in active pulmonary tuberculosis. Ann Int Med 90: 324-328, 1979.
14. Subhash C. Sharma. Serum calcium in pulmonary tuberculosis. Postgraduate Medical Journal (November 1981) 57, 694-696.
15. Liam CK, Lim KH, Srinivas P, Poi PJ. Hypercalcaemia in patients with newly diagnosed tuberculosis in Malaysia. Int J Tuberc Lung Dis 1998 Oct; 2(10):818-23.
16. Kitrou MP, Phytou-Pallikari A, Tzannes SE, Virvidakis K, Mountokalakis TD. Serum calcium during

chemotherapy for active pulmonary tuberculosis. Eur J. Respir Dis. 1983 Jul; 64(5):347-54.
17. Wells H.G., DeWitt L.M. & Long E.R. The Chemistry of Tuberculosis. Bailliere, Tindall and Cox 1923, London.
18. Sweany H.C., Weathers A.T. & McCluskey K.L. The chemistry of blood in tuberculosis. American Review of Tuberculosis, (1923-4), 8,405.
19. Sharma S.C. Serum calcium in pulmonary tuberculosis. Postgraduate Medical Journal, 1981, 57, 694.

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Original Research Article

Study of clinical profile of tuberculosis patients admitted in respiratory medicine ward at a tertiary care hospital in Marathwada

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ABSTRACT

Background: Tuberculosis is one of the most important cause of most of the respiratory diseases. It is estimated that about one-third of the world's population is infected with mycobacterium tuberculosis. It is important to know about the clinical profile of these patients. There are many studies which are done among OPD patients but fewer among indoor patients hence, the current study was planned.
Methods: Retrospective study of the patients admitted in the inpatient department of the study area were taken as the sample size from May 2016 to April 2017, who were diagnosed as TB patients.
Results: a retrospective study was conducted among admitted patients, which included data of one year. There was male predominance with male: female ratio of 2.89. major cause of admission was extrapulmonary causes. The HIV patients were more predisposed to extrapulmonary and diabetes than pulmonary tuberculosis.
Conclusions: There is male predominance for admission cases. There is also increased cases of extrapulmonary TB admitted than pulmonary cases.

Keywords: Extrapulmonary tuberculosis, Pleural effusion, Pulmonary tuberculosis, Tuberculosis

INTRODUCTION

Tuberculosis is chronic infectious disease cause by M. tuberculosis. it affects primarily lungs and causes pulmonary tuberculosis (85%).[1,2] There could be affection of other organ systems called extrapulmonary tuberculosis.

Tuberculosis is one of the dreaded diseases which accounts for 9.6 million cases globally as per the WHO Global TB Report 2015. Among these cases India contributes to 2.2 million incidence cases. It has not only high morbidity but also the mortality is high with 0.22 million deaths in India in 2015.[3]

One third of the world population is currently infected with TB and 1.8 million new cases of TB arise annually in India alone[4-7] Tuberculosis is one of the most important cause of most of the respiratory diseases. The prevalence of tuberculosis was estimated to be 10.5 million. In India alone 1.8 million new cases of TB arise annually.[6,7] It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent TB rather than TB disease.[8] There are many factors which determine the conversion of latent TB to TB disease, one the most important factor which determines is host factors. The most important factors are immunity which is determined by age and genetic factors.[9]

India, home for around 2.5 million HIV/AIDS patients. As with HIV, there is an immuno-suppression in diabetes also due to impaired phagocytosis and cellular immunity.[10,11] Some studies have reported that the proportion of TB that is EPTB is on the rise due to the HIV epidemic.[12,13]

To reduce the incidence and prevalence, India has introduced National Tuberculosis Control Programme (NTP) in 1962, followed by Revised National Tuberculosis Control Programme (RNTCP) 1993 -1996 and with Directly Observed Treatment Short-Course chemotherapy (DOTS) strategy in 1997, WHO released Stop TB strategy in 2006 India adopted in 2007. There are continuous efforts made to decrease the incidence and also prevalence of tuberculosis, continuous change in the strategies under RNTCP which are made. Further there was adoption of Goals of NSP with a vision of TB Free India in 12th Five-year plan in (2012-17). The current adoption of end TB strategy has a vision of WORLD FREE OF TB. [14] And with the goal to END TB EPIDEMIC. So, it is important to know the reasons for admission and also the profile of TB patients who get admitted so that we prevent further incidence and for early diagnosis, so, the above study was undertaken.

TB affecting other sites-known as extra-pulmonary TB is rarely smear-positive; it is generally accepted that the contagious potential of this form is negligible and it has, therefore, never been a priority in the campaigns undertaken by national TB control programs.[15,16] Lymph nodes are the most common site of involvement followed by pleural effusion and virtually every site of the body can be affected.[17]

There is limited knowledge about the host –related factors responsible for admissions of tuberculosis patients especially extra pulmonary TB cases. So, this study was planned to study in-depth about the distribution of host related factors such as age and sex among the admitted TB patients.

Objective of this study was to distribution of some of the host related factors (age and sex wise distribution) and also clinical profile of cases of tuberculosis admitted at respiratory medicine department at MGM Hospital.

METHODS

The study is a retrospective descriptive study conducted at MGM college Aurangabad after obtaining permission of the institutional ethical committee.

The study used data of 1 year from May 2016 to April 2017 . The data is specifically only of indoor patient's admitted under the department of respiratory medicine.

The study subjects consisted of all the tuberculosis patients admitted under department of respiratory medicine. The patients on treated on OPD basis were excluded from the study. It was a record based study so consent of the patients for inclusion criteria was not taken in consideration.

The data consisted of patient profile and confirmed clinical diagnosis. The final diagnosis was taken into consideration as per records. The patients are categorized into pulmonary and extra-pulmonary tuberculosis on the basis of site of lesion.

Statistical analysis was done using Microsoft excel. Confidentiality of the patients was maintained.

RESULTS

There were 113 patients admitted, and all were included in the study. The study consisted of 29 females and 84 males. The mean age of the patients admitted was 41.17 years with a standard deviation of 16.66 years. The mean age of females was 40.44 with a standard deviation of 16.44. the mean age of males was 41.34 and standard deviation was 16.63. Though the ratio of males: females was 2.89 the mean age was found to be similar. Among the females their range of age group were 14 - 65 years. The range of age group among males were 18 - 87 years.

Table 1: Sex wise distribution of study subjects.

Sex	Mean age (in yrs)	Standard deviation (in yrs)	Range (in yrs)
Males	41.34	16.63	18 - 67
Females	40.44	16.44	14 - 65

Patient clinical profile

There were 34 (30.08%) cases of pulmonary tuberculosis and 79 (69.9%) cases of extra- pulmonary cases. Figure one shows percentage wise distribution of cases according to diagnosis. There were further subdivision of cases among extra pulmonary cases, Table 2 shows diagnosis wise distribution of extra-pulmonary cases.

Table 2: Distribution of extra pulmonary cases.

Diagnosis	Number of cases N (%)
Pleural effusion	73 (92.4)
Miliary tuberculosis	2 (2.53)
Tubercular lymphadenopathy	2 (2.53)
Tubercular empyema	2 (2.53)

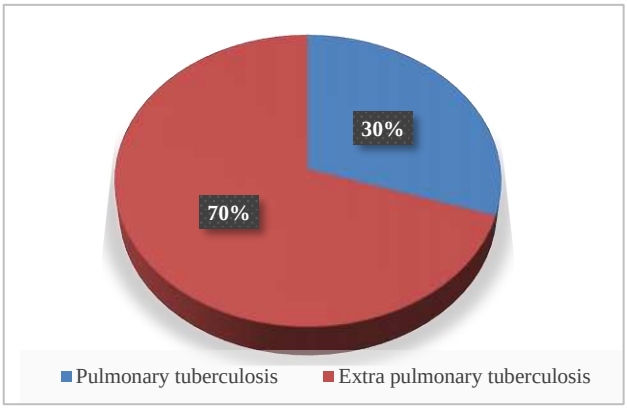

Figure 1: Percentage-wise distribution of patients according to diagnosis.

Profile of pulmonary tuberculosis patients

Among the total 34 pulmonary tuberculosis patients there were 13 (38.23%) females and 21(61.76) male patients. The mean age of pulmonary TB cases was 41.3 with standard deviation of 16.78. the figure 2 shows age wise distribution of pulmonary tuberculosis cases.

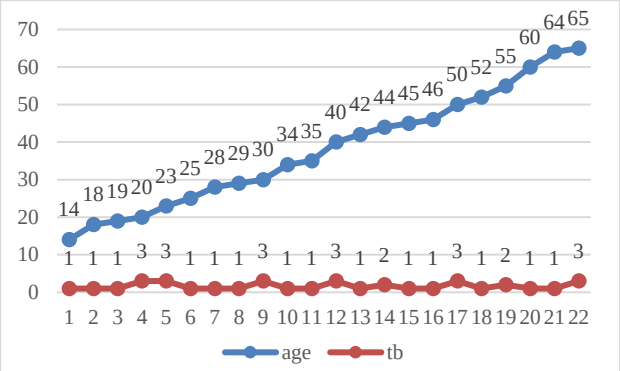

Figure 2: Age wise distribution of pulmonary tuberculosis patients.

There is no correlation between age group and number of pulmonary cases. There is no increase in admission rates with increase in age.

Profile of extra-pulmonary tuberculosis patients

There were more male patients than female. The male: female ratio was found to be 3.94. most of the patients were of pleural effusion. The mean age of admissions among extra pulmonary cases was found to be 41.7 with a standard deviation of 16.6 years. Figure 3 shows diagnosis wise distribution of patients of extra pulmonary tuberculosis.

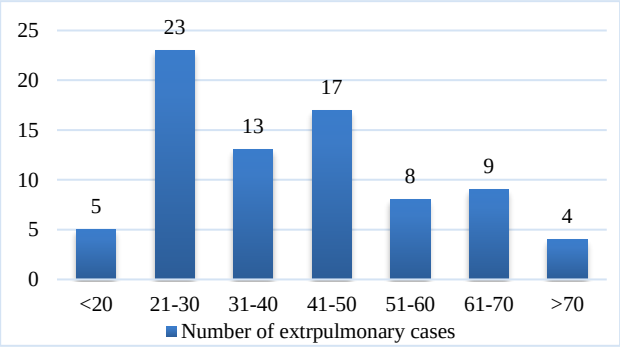

Figure 3: Age-wise distribution of extrapulmonary cases.

Distribution of study patients according to HIV serological profile

Among the 113 patients admitted, there were 3 (2.65%) who were serologically positive for HIV antibodies. Among these further there were 2 who were previously diagnosed and on antiretroviral therapy and 1 who were newly diagnosed to be having HIV. The rest 110 (97.34%) were HIV negative. Among the 3 HIV positive patients there were 2 (66.6%) of extrapulmonary cases and 1 (33.3%) of pulmonary TB case.

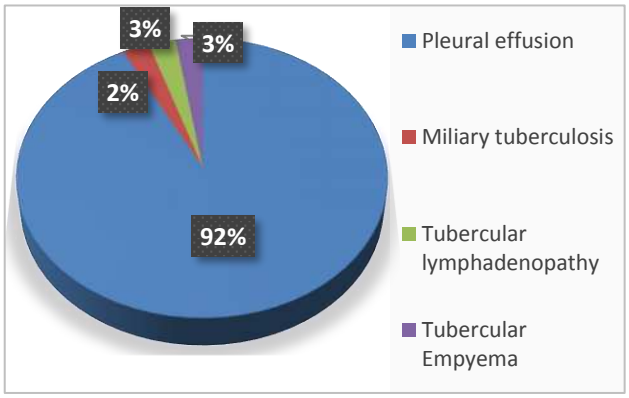

Figure 4: Distribution of extrapulmonary cases based on diagnosis.

Table 3: Distribution of patients according to HIV status.

HIV status	Number N	Percentage (%)
Positive	3	2.65
Negative	110	97.34
Total	113	100

Distribution of patients based on diabetic status

There were 24 (21.23%) patients who were know cases of diabetes admitted for tuberculosis. While there were 89 (78.76%) who were non diabetic cases. Among the diabetic cases there were 10 (41.66%) patients were cases of extra pulmonary TB and rest 14 (58.33%) were pulmonary tuberculosis.

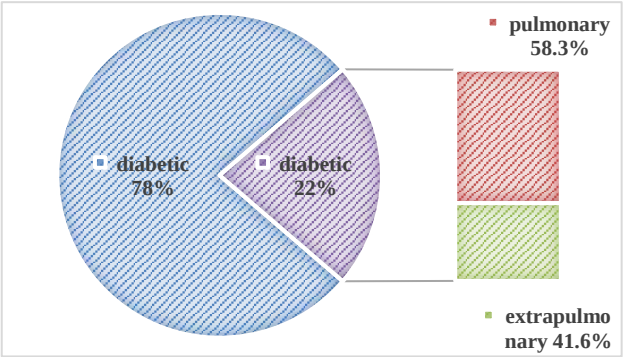

Figure 5: Distribution of patients based on diabetic status.

DISCUSSION

The current study included 113 patients who were included in the study. The study consisted of 29 (25.66%)

females and 84 (74.33%) males. The mean age of the patients admitted was 41.17±16.66 years. The ratio of males: females was 2.89. Male predominance was seen in other studies too with ratios of males: females to be 3.6:1 and 3.1:1 in pulmonary and extrapulmonary cases respectively.[17]

Another study too showed male predominance with ratio 1.6. these findings could be due to the fact that the exposure to pollution and minute dust is more among men then women with cutting down of indoor air pollution.[18,19]

The mean years was found to be 41.3±16.78 in pulmonary TB cases and 41.7±16.6 in extrapulmonary cases with maximum number of patients in age group of 21- 30 years. Few studies had bimodal peak in pulmonary TB cases during 15-25 years and 60-70 years.[18] The mean was found similar in other studies too around 41.11±15.7 yeas in pulmonary TB and 34.62±12.9 among extrapulmonary. Most commonly TB is seen to affect the productive age group this will lead to further deterioration of already susceptible lower socio economic condition.[17]

The most common type of extrapulmonary case was pleural effusion 73 (92.4 %) followed by Miliary tuberculosis 2 (2.53%), Tubercular lymphadenopathy2 (2.53%) and Tubercular Empyema 2 (2.53%). In most of the studies there were higher percentages of lymph node involvement and in few disseminated TB.[18-22] Since, this is a respiratory medicine department the type of patients treated in the indoor department are pulmonary related hence pleural effusion patients are found to be more.

There were 3 (2.65%) of HIV positive patients and among them 2 (66.6%) were of extrapulmonary TB cases and among the 24 (21.23%) diabetic patients maximum were of 14 (58.33%) pulmonary TB. This correlation is seen in other studies too with diabetes significantly higher among pulmonary TB cases and HIV more commonly associated with extrapulmonary cases.[18,23-27]

CONCLUSION

In the study we found more admissions among males in the productive age groups with higher incidence of extrapulmonary TB among 21-30 years of age group. The most common presentation among extrapulmonary tuberculosis was found to be pleural effusion.

The cases of HIV more predisposed to extrapulmonary and diabetes patients to be more predisposed to pulmonary tuberculosis.

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REFERENCES

1. Fundamental of tuberculosis. Global tuberculosis institute. Available from URL: globaltb.njms.rutgers.edu/downloads/products/Mantoux.../FundamentalsofTB.ppt.last accessed on 12/12/2017.
2. Hospital unit. Indian institute of technology Delhi. Available from URL: http://hospital.iitd.ac.in/content/tuberculosis; last accessed on 13/12/2017
3. Tuberculosis facts sheet. India . 2014 -2015 . available from URL: http://www.tbfacts.org/tb-statistics-india/ Accessed on 9/6/2017.
4. Sudre P, Tendam G, Kochi A. Tuberculosis: a global overview of the situation today. Bull World Health Organ. 1992;70:149-59.
5. World Health Organization. Global Tuberculosis Report 2016.Geneva: WHO; 2016.
6. World Health Organization. Tuberculosis Fact Sheet. Fact Sheet No. 104. Available: http://www.who.int/mediacenter/factsheets/fs104/en/print.html; 2007[accessed 15.02.09].
7. Steinbrook R. Tuberculosis and HIV in India. New Engl J Med. 2007;356:1198-9.
8. Global tuberculosis Control, Surveillance, Planning, Financing- WHO Report 2005: WHO/HTM/TB/2005. 49, WHO Geneva; 2005.
9. Grippi MA. Fishman's Pulmonary diseases and disorders. 5th ed. United States. Mc Graw Hill education; 2015.
10. Swaminathan S, Narendran G. HIV and tuberculosis in India J Biosci. 2008;33:527-37.
11. Ljubic S, Balachandran A, Pavlic-Renar I, Barada A, Metelko Z. Pulmonary infections in diabetes mellitus. Diabetologia Croatica. 2004;33:115-24.
12. Narain JP, Lo YR. Epidemiology of HIV-TB in Asia. Indian J Med Res. 2004;120:277-89.
13. Reported tuberculosis in the United States 1999 Division of Tuberculosis Elimination. National Centre for HIV, STD and TB prevention, Centre for Diseases Control [http://www.cdc.gov/nchhstp/tb/surv99].
14. Park K. Park's Textbook of Preventive and Social Medicine. 24th ed. Jabalpur: M/s Banarsidas Bhanot Publishers; 2017.
15. World Health Organization. Tuberculosis programme: Framework for effective tuberculosis control. Geneva, Switzerland: WHO; 1994. p. 179.
16. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004;120(4):31653.
17. Gupta S, Shenoya VP, Bairya I, et al. Diabetes mellitus and HIV as co-morbidities in tuberculosis patients of rural south India. Journal of Infection and Public Health. 2011;4:140-4.
18. Sreeramareddy CT, Panduru KV, Verma SC, Joshi HS, Bates MN. Comparison of pulmonary and extrapulmonary tuberculosis in Nepal- a hospital-based retrospective study. BMC Infectious Diseases. 2008:1-7.

19. Prakasha SR, Suresh G, D'sa IP, Shetty SS, Kumar SG. Mapping the Pattern and Trends of Extrapulmonary Tuberculosis. J Glob Infect Dis. 2013;5(2):54-9.
20. Nissapatorn V, Kuppusamy I, Jamaiah I, Fong MY, Rohela M, Anuar AK. Tuberculosis in diabetic patients: a clinical perspective. Southeast Asian J Trop Med Public Health. 2005;36:213-20.
21. Devi SB, Naorem S, Singh TJ, Singh KB, Prasad L, Devi TS. HIV and TB coinfection. J Ind Acad Clin Med. 2005;6:220-3.
22. Barthwal MS, Rajan KE, Deoskar RB, Sharma SK. Extrapulmonary tuberculosis in human immunodeficiency virus infection. MJAFI. 2005;61:340-1.
23. Shetty N, Shemko M, Vaz M, D'Souza G: An epidemiological evaluation of risk factors for tuberculosis in South India: a matched case control study. Int J Tuberc Lung Dis. 2006;10:80-6.
24. Alisjahbana B, van Crevel R, Sahiratmadja E, den Heijer M, Maya A, Istriana E, et al. Diabetes mellitus is strongly associated with tuberculosis in Indonesia. Int J Tuberc Lung Dis. 2006;10:696-700.
25. Yang Z, Kong Y, Wilson F, Foxman B, Fowler AH, Marrs CF, et al. Identification of risk factors for extra pulmonary tuberculosis. Clin Infect Dis. 2004;38:199-205.
26. Reported tuberculosis in the United States 1999 Division of Tuberculosis Elimination. National Centre for HIV, STD and TB prevention, Centre for Diseases Control Available from: http://www.cdc.gov/nchhstp/tb/surv99.
27. Solomon SS, Kumarasamy N, Celentano DD, Yepthomi TH, ArvindVP, Solomon S: Trends in HIV-related morbidity among patients admitted to a South Indian tertiary hospital between 1997 and 2003. AIDS Care. 2006;18:366-70.

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Original Article

Bronchoscopic evaluation in endobronchial tuberculosis

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Abstract

Endobronchial tuberculosis (EBTB) is defined as tuberculous infection of the tracheobronchial tree with microbial and histopathological evidence. It is seen in 10-40% of patients with active pulmonary tuberculosis. More than 90% of the patients with EBTB have some degree of bronchial stenosis. 10 to 20 % have normal chest radiograph. Therefore, a clear chest radiograph does not exclude the diagnosis of EBTB. Bronchoscopic sampling has been the key to the diagnosis producing more than 90% yield on smear as well as on culture. Bronchoscopy is the gold standard for diagnosis of EBTB. Early diagnosis and prompt treatment, before the development of fibrosis is important to prevent complications of endobronchial tuberculosis, such as bronchostenosis. EBTB is classified into seven subtypes by bronchoscopic findings according to Chung Classification: actively caseating, edematous-hyperemic, fibrostenotic, tumorous,granular, ulcerative, nonspecific bronchitic. The Aim of the Study is to evaluate varied findings of Bronchoscopic presentations of Endobronchial Tuberculosis.

Key Word: Bronchoscopic evaluation, endobronchial tuberculosis.

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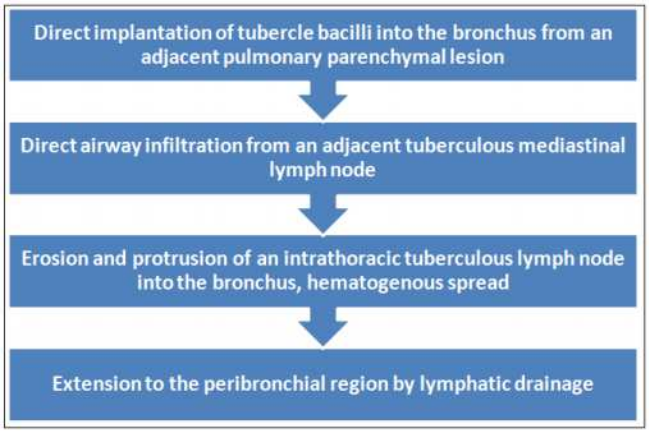
INTRODUCTION

Pulmonary tuberculosis is one of the major health problems worldwide. In spite of much progress in diagnosis and therapy, this problem still remains. Moreover, there has been a resurgence of pulmonary tuberculosis recently, which is related to the HIV epidemic, multidrug-resistant strains, poverty, immigration, and shortness in the prevention and treatment system. About 10% to 40% of patients with active pulmonary tuberculosis had EBTB.It has been defined as tuberculous infection of the tracheobronchial tree with microbial and histopathological evidence or a complication of progressive primary tuberculosis. EBTB continues to be a health problem because of the following:[1] Its diagnosis is frequently delayed because the decreased incidence itself diminishes the suspicion of tuberculosis;[2] bronchostenosis may develop as a serious complication despite efficacious antituberculosis chemotherapy; and[3] it is often misdiagnosed as bronchial asthma or lung cancer.

Pathogenesis of EBTB[4,5,6,7,8]



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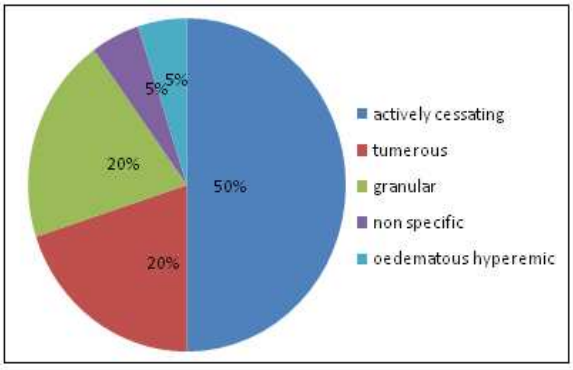
MATERIALS AND METHODS

The study was conducted in Pulmonary medicine Department MGM Medical College and Hospital Aurangabad, Maharashtra from January 2015 to march 2016,of age above 18 years with constitutional symptoms like fever, cough, loss of appetite and weight. All the patients who were Sputum Negative were subjected to Bronchoscopy.

RESULTS

Analysing the data from 20 patients, EBTB were classified into subtypes by initial bronchoscopic findings, the most common bronchoscopic classification was Actively Cessating were found in 10 patients (50%), Tumerous were found in 4 patients (20%), Granular in 4 patients (20%), non specific in 1 patient (5%),Oedematous Hyperemic in 1 patient (5%). Lesions were totally recovered in all the patients with treated with standard anti-tuberculous treatment.



DISCUSSION

EBTB contains rather high amounts of tuberculosis bacilli. Early diagnosis and treatment is important for prevention of the spread of tuberculosis and complications like cicatricial bronchostenosis due to endobronchial involvement.[10] In this study actively caseous type was reported to be most common . In recent studies it was reported that most common bronchoscopic finding was edematous-hyperemic type.[11,12] This is the most common type of bronchoscopic finding in middle lobe syndrome.[13] It is also reported that while active caseous type recovers almost completely, edematous or active caseous + edematous type changes to fibrostenotic type in more than 60% of cases.[14] Four of the subtypes—actively caseating, edematous-hyperemic, fibrostenotic, and tumorous EBTB—show varying degrees of luminal narrowing of the bronchus, while the other three subtypes—granular, ulcerative, and nonspecific bronchitic EBTB—do not.[14] Similarly, it is reported in a study that early stage exudative, granular and ulcerative lesions recovered without sequel; caseous and tumorous lesions of advanced disease might lead complications such as bronchostenosis causing bronchectasia.[15] A study conducted by Um SW *et al.* revealed that factors related to development of bronchial stenosis were age over 45 years, pure or combined fibrostenotic subtype and symptoms lasting more than 90 days prior treatment.[16] In our study most common lesions observed bronchoscopically were active caseous lesions. These were followed by granular, tumorous, non specific and oedematous lesions with decreasing frequencies. In this study all the Lesions were totally recovered in all the patients treated with standard anti-tuberculous treatment.

CONCLUSION

The most common EBTB was found to be actively cessating type. Bronchoscopy should be performed as soon as possible in suspected patients, especially when patients present the relatively long duration of symptoms . Therefore, the bronchoscopic approach is benefitial, not only for the prompt diagnosis of EBTB, but also for the prevention of further bronchostenosis.

REFERENCES

1. Hoheisel G, Chan BKM, Chan CHS, Chan KS, Teschler H, Costabel U. Endobronchial tuberculosis: diagnostic features and therapeutic outcome. Respiratory Medicine. 1994; 88: 593-597.
2. Indian J Chest Dis Allied Sci 2003; 45 : 247-256
3. Chung HS, Lee JH, Han SK, *et al*. Classification of endobronchial tuberculosis by the bronchoscopic features. Tuberc Respir Dis 1991; 38:108–115 (in Korean)
4. Matthews JI, Matarese SL, Carpenter JL. Endobronchial tuberculosis simulating lung cancer. Chest 1984; 86:642– 644
5. Smith LS, Schillaci RF, Sarlin RF. Endobronchial tuberculosis: serial fiberoptic bronchoscopy and natural history. Chest 1987; 91:644–647 11 Judson MA, Sahn SA. End
6. 16 Myerson MC. Tuberculosis of the trachea and bronchus. Springfield, IL: Charles C. Thomas, 1944; 250–275
7. Lee JH, Park SS, Lee DH, *et al*. Endobronchial tuberculosis: clinical and bronchoscopic features in 121 cases. Chest 1992; 102:990–994
8. Kim YH, Kim HT, Lee KS, *et al*. Serial fiberoptic bronchoscopic observations of endobronchial tuberculosis before and early after antituberculosis chemotherapy. Chest 1993; 103: 673–677
9. Chan HS, Pang JA. Effect of corticosteroids on deterioration of endobronchial tuberculosis during chemotherapy. Chest 1989; 96:1195–1196
10. Yanardag H, Tetikkurt C, Tetikkurt S, *et al*. Computed tomography and bronchoscopy in endobronchial tuberculosis. Can Respir J 2003;10:445-8
11. An JY, Lee JE, Park HW, *et al*. Clinical and bronchoscopic features in endobronchial tuberculosis. Tuberc Respir Dis 2006;60:532-9
12. Morrone N, Abe NS. Bronchoscopic findings in patients with pulmonary tuberculosis. J Broncho12007;14:15-8

13. Kim HC, Kim HS, Lee SJ, *et al*. Endobronchial tuberculosis presenting as right middle lobe syndrome: clinical characteristics and bronchoscopic findings in 22 cases. Yonsei Med J 2008;49:615-9
14. Chung HS, Lee JH. Bronchoscopic assessment of the evolution of endobronchial tuberculosis. Chest2000;117:385-92
15. Um SW, Yoon YS, Lee SM, *et al*. Predictors of persistent airway stenosis in patients with endobronchial tuberculosis. Int J Tuberc Lung Dis 2008;12:57-62
16. Yanardag H, Tetikkurt C, Tetikkurt S, *et al*. Computed tomography and bronchoscopy in endobronchial tuberculosis. Can Respir J 2003;10:445-8
17. Swallow CE, McAdams HP, Colon E. Tuberculosis manifested by laryngeal mass on CT scans. Am J Roentgenol 1994; 163 : 179-80.

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Research Article

Study of mycobacterium culture and sensitivity pattern of various anti-tubercular drugs in suspected multidrug resistant tuberculosis patients

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Abstract

Resistance to the first line drugs is the most difficult part of treating tuberculosis. Different studies have found upto 3% previously treated patient and Upto 17% patients who have taken Antitubercular treatment previously as being multidrug resistant MDR TB(Multi drug resistant tuberculosis) is defined as resistance to isoniazid and rifampicin. More and more patients are attending chest OPDs with pulmonary. tuberculosis and not responding to primary line of antituberculosis drugs. We undertook a study in our institution to find the drug susceptibility pattern in 50 patients who were suspected to be MDR (resistant to INH + Rifampicin) and found only 2% of such patients to be sensitive to both INH and RFM (i.e. 98% were MDR-TB patient),no patient was sensitive to all the first line drugs,21patients(42%)were sensitive to only one first line drug and 4% patients sensitive to only one drug cycloserine. Our study endeavoured to find the sensitivity and resistance patterns of the mycobacterium to the various antituberculous drugs. This study will be useful in identifying and choosing which drugs are more effective in management of MDR Tuberculosis. **Abbreviation**: MDR: Multi Drug resistant Short Running Head: Drug Sensitivity pattern in M.D.R Tuberculosis

Keywords: MDR TB, Mycobacterium culture, Drug Resistance, Sensitivity Pattern

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INTRODUCTION

Transmission of tuberculosis occurs by air borne spread of infection droplets and droplet nuclei containing the tubercle bacilli. The source of infection is a person with sputum smear positive pulmonary tuberculosis. Transmission often occurs indoor where droplets and droplet nuclei can stay in the air for a longer time. With the advent of various Anti-Tubercular drugs, Anti-Tubercular treatment has become more and more effective. Short course chemotherapy and DOTs/RNTCP have made it possible to achieve the goals of cure rate of 85% among newly detected infectious cases and maintain detection rate of at least 70% of all such cases in the population. The emerging trend of resistance to various first line Anti-Tubercular Drugs is disturbing. Multi Drug Resistant Tuberculosis is a laboratory diagnosis and should not be a clinical impression of treating doctor. Resistance to Isoniazid and Rifampicin is called Multi Drug Resistance. Resistant to one drug is called monoresistance; resistance to two or more drugs is called polyresistance. Another type of resistance called Extensive. Drug Resistance is resistance to INH, rifampicin, at least one injectible amino glycoside and a fluroquinolone. About 3 % previously untreated patients and up to 17% previously treated patients have multidrug resistant tuberculosis. The diagnosis and appropriate treatment of multidrug resistant tuberculosis patients is very important not only because they will spread the multidrug resistant strain of tuberculosis to other patients but also because MDR tuberculosis leads to severe

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morbidity and has high mortality rate since it is difficult to treat. Our study attempts to find drug resistant cases and to see the sensitivity pattern of mycobacteria in our area to the various antitubercular drugs.

MATERIAL AND METHODOLOGY

SUBJECT: Patient above 15 years of age who have taken Anti- Tubercular drug at least one time.

INCLUSION CRITERIA

1. Patients above the age of 15 yrs.
2. Taken Anti- Tubercular Drug at least 1 time and still Sputum positive after 5 months.
3. Initially Sputum negative but positive after 5 months.

EXCLUSION CRITERIA

1. Severely ill patients / end stage diseases.
2. Patients >60yrs and <15yrs of age.

STUDY PROCEDURE

1. 50 patients of suspected drug resistant tuberculosis were studied on the basis of the above inclusion and exclusion criteria.
2. Informed consent was taken.
3. Appropriate information, including symptoms about the patients was collected.
4. Detailed history regarding their treatment, checking of old X-rays and Sputum reports.
5. Family history of TB.
6. History of TB in Contacts.
7. Sputum collection in a sterile container.
8. Culture on LJ Medium/BACTEC METHOD and sensitivity testing to

The following antitubercular drugs was carried out:Isoniazid, Rifampicin, Pyrazinamide, ethambutol, Streptomycin, Kanamycin, Ofloxacin, Cyloserine, Ethionamide, Paraaminosalicylic acid (PAS).

RESULTS

Table 1: Sex distribution

Sex	Number of Patients	Percentage
Male	29	58%
Female	21	42%
Total	**50**	**100%**

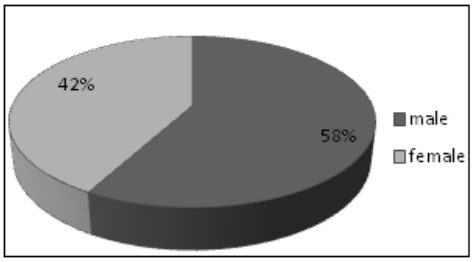

Figure 1: Pie Chart- Sex Distribution

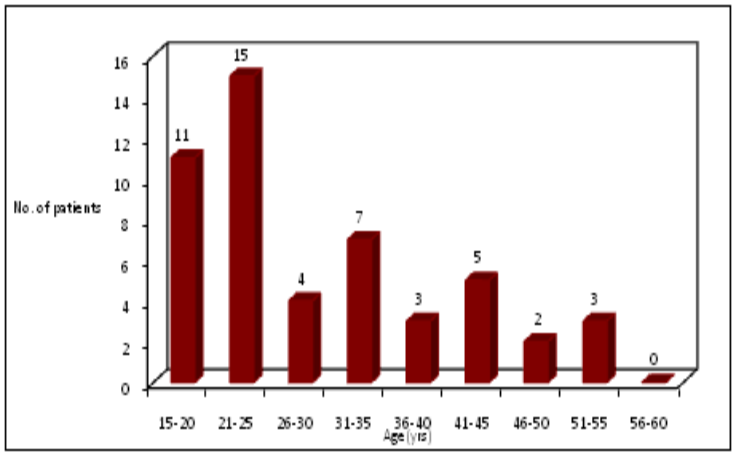

Figure 2: Age Distribution Bar Diagram

Table 2: Resistance and Sensitivity to Antitubercular Drugs

Drug	Resistance	Sensitivity
Streptomycin	30	20
Isoniazid	47	3
Rifampicin	49	1
Etnambutamol	31	19
Pyrizinamide	30	20
Paraaminosalicylic	11	39
Ethionamide	11	39
Cycloserine	20	30
Kanamycin	7	43
Ofloxacin	27	23

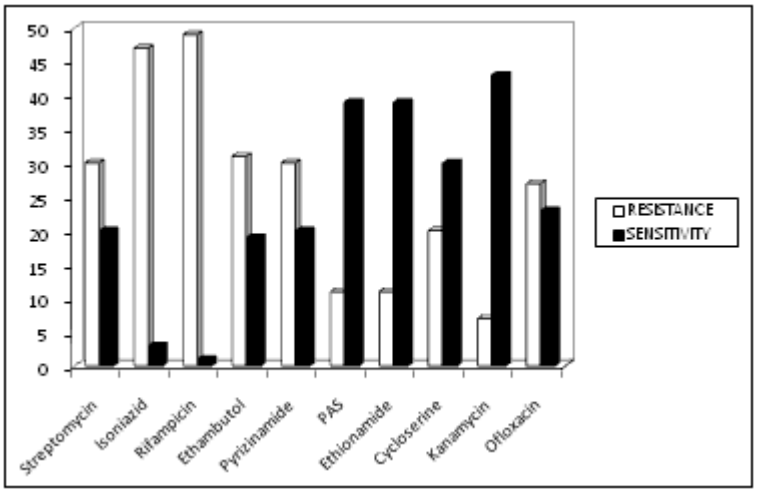

Figure 3: Resistance and Sensitivity Pattern Bar Diagram

DISCUSSION

The study was done to know the sensitivity pattern of various Antitubercular. Drugs in Aurangabad (TB U -2) in suspected Multidrug Resistant. Tuberculosis patients by performing sputum culture of mycobacterium Tuberculosis. It comprised of 50 patients of pulmonary tuberculosis, who previously had taken tuberculosis treatment and remain sputum positive at the end of five

months, who show radiological deterioration after 8 months or those who have become sputum positive after being sputum negative initially. Multidrug-resistant tuberculosis (MDR TB) is resistance to both isoniazid and rifampicin with or without resistance to other drugs. Globally, about 3% of all newly diagnosed patients have MDR-TB. The proportion is higher in patients who have previously received antituberculosis treatment reflecting the failure of programmes designed to ensure complete cure of patients with tuberculosis. While host genetic factors may probably contribute, incomplete and inadequate treatment is the most important factor leading to the development of MDR-TB. The definitive diagnosis of MDR-TB is difficult in resource poor low income countries because of non-availability of reliable laboratory facilities and efficiently run tuberculosis control programmes based on Directly Observed Treatment Short courses (DOTS). For newly diagnosed patients, the frequency of resistance to at least one antituberculosis drug ranged from 1.7 per cent in Uruguay to 36.9 percent in Estonia (media, 10.7%). The median prevalence of MDR-TB among new cases of tuberculosis was only 1.0% but the prevalence was much higher in Estonia (14.1%), Henan province in china (10.8%), Latvia (9%), the Russian oblasts of Ivanovo (9%) and Tomsk (6.5%), Iran (5%) and Zhejiang Province in china (4.5%). In our study of 50 patients who were suspected Multidrug Drug Resistant Tuberculosis

1. Sex Ratio Male: Female 58%: 42% Males are more commonly affected as compared to female. (58% - 42%)
2. Age Group Most commonly seen in 21 – 25 age group (30%) 2nd Most commonly seen in 15 – 20 age group (22%) 3nd Most commonly seen in 31 – 35 age group (14%)
3. Resistance Pattern to single drug It revealed a resistance in 47 patients to isoniazid (94%), 49 patients to rifampicin (98%), 30 patients to streptomycin (60%), 31 patients to ethambutol (62%), 30 patients to pyrazinamide (60%). Resistance was seen in 27 patients (47%) to ofloxacin.
4. Sensitivity Pattern to single second line drug Second line drugs mainly injectible (Kanamycin) has highest sensitivity in 43 patients (86%), followed by Para amino salicylic acid in 39 patients (78%), Ethionamide in 39 patients (78%) followed by cycloserine in 30 patients (60%) Only 3 patients of suspected multidrug resistance were sensitive to isoniazid (6%) and 1 patient (2%) sensitive to rifampicin.
5. Sensitivity to first line drugs (H R Z E S) in this study of suspected multidrug resistance No patient was sensitive to all the first line drugs 2 patients were sensitive to four first line drugs 6 patients were sensitive to three first line drugs 8 patients were sensitive to two first line drugs 21 patients were sensitive to only one first line drug.
6. Sensitivity to isoniazid and rifampicin 3 patients were sensitive to isoniazid (6%) 1 patient was sensitive to rifampicin (2%) 1 patient of the above was sensitive to both isoniazid and rifampicin
7. Sensitivity to only single or two drugs 2 patients (4%) were sensitive to single drug – cycloserin 2 patients were sensitive only to two drugs out of whom – One patient sensitive to (ofloxacin + cycloserine) and the other was sensitive to (pyrinazamide + para amino salicylic acid)

In India, prevalence of primary MDR-TB in newly diagnosed cases has been observed to be 3.4 percent or less. Data meticulously collected at the Tuberculosis research center (TRC), Chennai over the last three decades suggest that rifampicin resistance started appearing in the early 1990s and MDR-TB levels in newly diagnosed patients has been one percent or less[1, 8.] Mutations occurring in MTB confer resistance to anti tuberculosis drugs. Mutations in rpoB (rifampicin) Kat/G and the ribosomal binding site of inhA (isoniazide) ,gyrA and gryB (ofloxacin) and r/A and rrs (streptomycin) causes resistance to the drugs. In a recent study from India[2]. Patients with HLA-DRB 1*13 and DRB 1*14 were found to have two fold increased risk of developing MDR-TB. Park *et al*[3] found that susceptibility to MDR-TB in Korean patients was strongly associated with HLADRB1*08032-DQB1*0601 haplocytes. The exact role of these factors is not known. It is likely that these loci or the alleles linked with them play a permissive role in conferring increasing susceptibility to the development of MDR-TB.

Factors related to previous antituberculosis treatment

Incomplete and inadequate treatment: Review of published literature strongly suggest that the most powerful predictor of the presence of MDR-TB is a history of treatment of tuberculosis. TB patients in India get treated not only through the Revised National Tuberculosis Control Programme (RNTCP), but also receive treatment from private medical practitioners. Irregular, incomplete, inadequate treatment is the commonest means of acquiring the drug resistant organism. Use of a single drug to treat TB is another cause of MDR TB in the Indian setting. This could have occurred because of ignorance, use of penicillin/streptomycin combination, use of rifampicin for other diseases, and economic constrains. Futhermore, there is a problem of using unreliable combinations with

an appreciable failure rate such as thiacetazone/isoniazid as initial treatment. Another common error in prescription practice is the "addition syndrome", if another drug is added to the existing regimen when the patients appears to deteriorate clinically and if resistance had developed to the drugs in use, adding another drug effectively amounts to monotherapy with the drug. Unreliable drugs with poor bioavailability from unregulated companies may pose a risk (e.g. rifampicin, isoniazid, pyrazinamide combination). The important problem in our country is the bizarre regimen for inadequate periods given by alternate medicine practitioners. OTC availability of antitubercular drugs adds to this. Inadequate treatment compliance: treatment compliance is significantly affected due to change over from fully supervised sanatorium treatment to unsupervised domiciliary treatment. Non compliance is because too many drugs including antacid, multivitamin, heamatinics, etc. are given to the patients. Thus, the physician finds it difficult to identify noncompliance in patients. Considering the changing epidemiological scenario DOTS is presently being advocated by the WHO to be the only effective way to control tuberculosis[4,5,6]. However, DOTS has not been adopted universally and the control programme in several parts of the world is chaotic[7]. In my study of 50 patients of suspected multidrug resistance, resistance to isoniazid and rifampicin alone was seen in 47 and 49 patients respectively. Resistance to all first line drugs was seen in 13 patients. A study to find the trend of drugs resistant Mycobacterium in a tertiary Tuberculosis centre concluded that a significant increase in the isoniazid and streptomycin resistance in the last few years would present a serious challenge to effective management of tuberculosis. To conclude, first line antitubercular drug should be reserved for the treatment of tuberculosis and second line drug especially quinolones should be reserved for management of M.D.R T.B patients.

CONCLUSION

Our study of 50 patients highlights the increasing trends of resistance to various first line anti tuberculosis drugs, and sensitivity pattern of second line drugs. Only one patient (2%) was sensitive to both isoniazid and rifampicin, 98% were resistant to isoniazid and rifampicin, no patient was sensitive to all the first line drugs; and 4% patients were sensitive to a single drug only i.e. cycloserine. This raises the issue of presence of Extreme Drug Resistance and the difficulty in managing such patients in clinical settings in India. There is an urgent need for a proper nationwide survey to evaluate the true picture of resistance and stronger implementation of RNTCP guidelines.

REFERENCE

1. Paramasivan CN. Status of drug resistance in tuberculosis after the introduction of rifampicin in India. J Indian Med Assoc 2003; 101: 154-6.
2. Sharma SK, Turaga KK, Balamurugan A, Saha PK, Pandey RM, Jain NK, *et al*. Clinical and genetic risk factors for the development of multidrug-resistant tuberculosis in non-HIV infected at a tertiary care center in India: a case-control study. Infect Genet Evol 2003; 3: 183-8.
3. Park MH, Song EY, Park HJ, Kwon SY, Han SK, Shim YS. HLA-DRB1 and DQB1 gene polymorphism is associated with multidrug-resistant tuberculosis in Korean patients. Hum Immunol 2002; 63: S33.
4. World Health Organization. Tuberculosis fact sheet. Available from URL: http://www.who.int/gtb/ publications/factsheet/index.htm. Accessed on 1 July 2003.
5. Frieden TR. Direct observed therapy short course: The strategy that ensures cure of tuberculosis. In: Sharma SK, Mohan A, editors. Tuberculosis. New Delhi: Jaypee Brothers Medical Publishers; 2001 p. 536-46.
6. Khatri GR, Frieden TR. Controlling tuberculosis in India. N Engl J Med 2002; 347: 1420-5.
7. Bastian I, Rigouts L, Van Deun A, Portaels F. Directly Observed Treatment, short-course strategy and multidrug resistant Tuberculosis: are any modifications required? Bull World Health Organ 2000; 78: 238-51.
8. S.K.Sharma and A.Mohan. Multidrug resistant tuberculosis. Review Article in Indian J Med Res 120, October 2004, pp 354-376.

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PLOS ONE

Alarming Levels of Drug-Resistant Tuberculosis in HIV-Infected Patients in Metropolitan Mumbai, India

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Abstract

Background: Drug-resistant tuberculosis (DR-TB) is a looming threat to tuberculosis control in India. However, no countrywide prevalence data are available. The burden of DR-TB in HIV-co-infected patients is likewise unknown. Undiagnosed and untreated DR-TB among HIV-infected patients is a major cause of mortality and morbidity. We aimed to assess the prevalence of DR-TB (defined as resistance to any anti-TB drug) in patients attending public antiretroviral treatment (ART) centers in greater metropolitan Mumbai, India.

Methods: A cross-sectional survey was conducted among adults and children ART-center attendees. Smear microscopy, culture and drug-susceptibility-testing (DST) against all first and second-line TB-drugs using phenotypic liquid culture (MGIT) were conducted on all presumptive tuberculosis patients. Analyses were performed to determine DR-TB prevalence and resistance patterns separately for new and previously treated, culture-positive TB-cases.

Results: Between March 2013 and January 2014, ART-center attendees were screened during 14135 visits, of whom 1724 had presumptive TB. Of 1724 attendees, 72 (4%) were smear-positive and 202 (12%) had a positive culture for *Mycobacterium tuberculosis*. Overall DR-TB was diagnosed in 68 (34%, 95% CI: 27%–40%) TB-patients. The proportions of DR-TB were 25% (29/114) and 44% (39/88) among new and previously treated cases respectively. The patterns of DR-TB were: 21% mono-resistant, 12% poly-resistant, 38% multidrug-resistant (MDR-TB), 21% pre-extensively-drug-resistant (MDR-TB plus resistance to either a fluoroquinolone or second-line injectable), 6% extensively drug-resistant (XDR-TB) and 2% extremely drug-resistant TB (XDR-TB plus resistance to any group-IV/V drug). Only previous history of TB was significantly associated with the diagnosis of DR-TB in multivariate models.

Conclusion: The burden of DR-TB among HIV-infected patients attending public ART-centers in Mumbai was alarmingly high, likely representing ongoing transmission in the community and health facilities. These data highlight the need to promptly diagnose drug-resistance among all HIV-infected patients by systematically offering access to first and second-line DST to all patients with 'presumptive TB' rather than 'presumptive DR-TB' and tailor the treatment regimen based on the resistance patterns.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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Introduction

India is a high burden country for tuberculosis (TB) and multidrug-resistant TB (MDR-TB). The World Health Organization has estimated that India accounted for 26% of the total number of TB cases worldwide in 2012, with 2.2% and 15% of the new and retreatment cases respectively being caused by multidrug-resistant strains [1]. Further, India is home to approximately 2.4 million people living with HIV [2] and considered to have a high burden on account of the large absolute numbers of people living with HIV in the country.

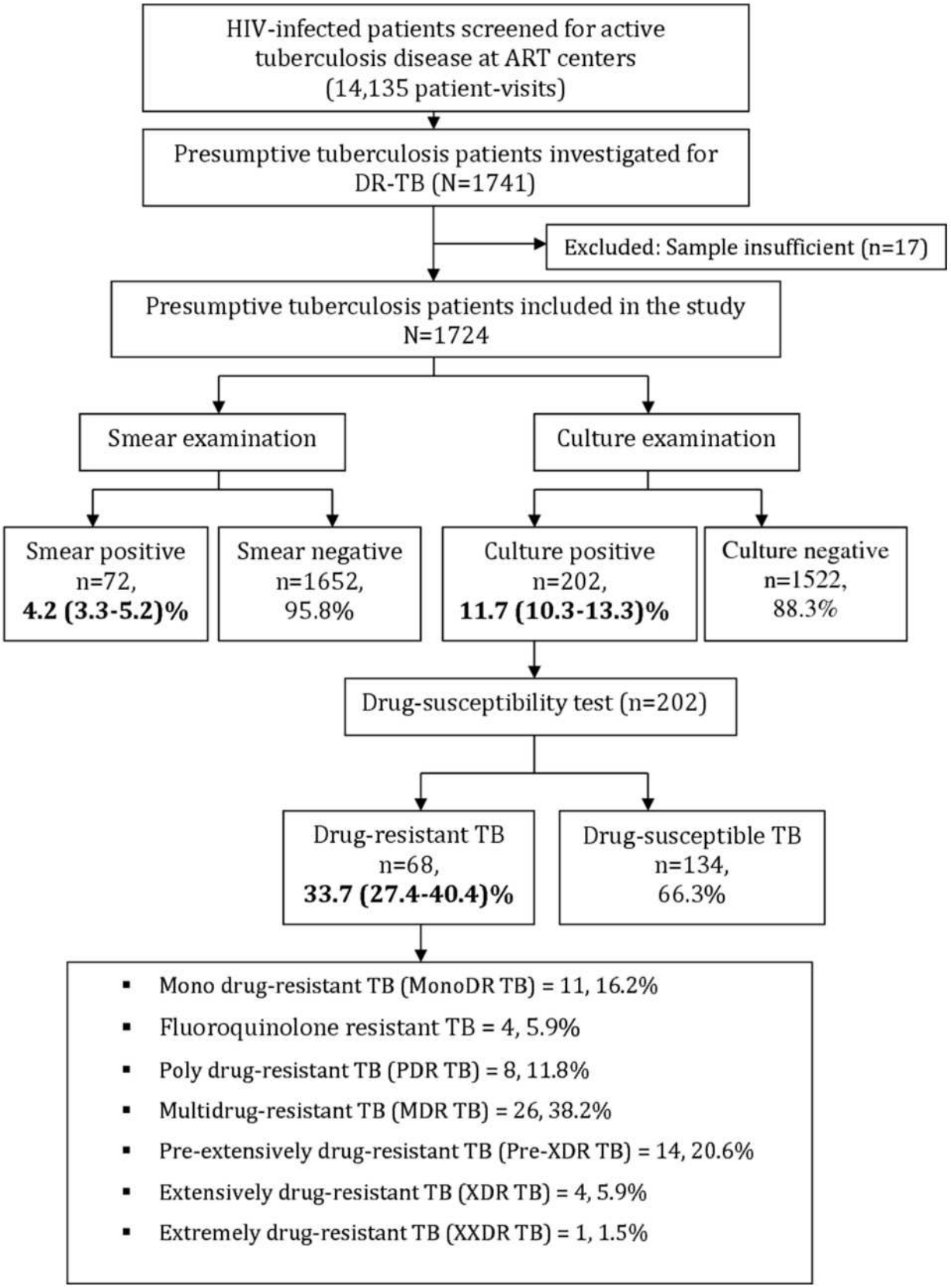

Figure 1. Drug-resistant tuberculosis among HIV-infected patients with presumptive tuberculosis, Mumbai, India.
doi:10.1371/journal.pone.0110461.g001

The dual burden of HIV and TB/DR-TB in India is significantly high with a combined rate of 5.2%, ranging from 0.4% to 28.8% in various studies, with increasing trends noted in states having a higher burden of HIV infection [3–7]. However, nation-wide studies do not exist and previous studies have occurred mainly in hospitals and tertiary care centres [2,6–11]. A crude estimate from these studies suggests that 2500–3000 HIV-infected persons develop MDR-TB annually in India.

Country-wide or state-wide drug resistance surveys (DRS) aim to estimate the DR-TB burden at the country or state level. While this approach is scientifically and operationally acceptable, it may mask significant and important variance in the magnitude of the epidemic in different localities, communities or specific populations. For India, a vast country with an enormous burden of TB and a relatively large burden of HIV in absolute numbers, this statement seems to hold true; from an overcrowded impoverished slum in Mumbai to a small isolated village in the Northern Eastern Provinces of the country, one can assume that several different epidemics may exist. A description of such local epidemics is necessary so as to complement the country-wide prevalence estimate. While there is an urgent need for a nationally representative, country-wide DRS in India, specific studies to identify pockets of extremely high DR-TB prevalence or extensive drug resistance patterns are equally needed in order to advocate for and implement effective control strategies.

The overall aim of this study was to assess the burden of drug-susceptible and drug-resistant tuberculosis among HIV-infected patients attending antiretroviral treatment (ART) centers in the metropolitan area of Mumbai. The specific objectives were a) to determine the proportion of HIV-infected patients with DR-TB among those attending public ART centers, b) to describe drug susceptibility patterns among *Mycobacterium tuberculosis* isolates from this population, and c) to identify factors associated with TB and drug-resistant TB among HIV patients. We aimed to contribute to the evidence base that informs policies and practices and help to estimate the resources needed to control the epidemic in this specific group, as well as the community.

Methods

Ethics

The study was approved by the Institutional Ethics Committee of Grant Medical College and Sir J.J. Group of Hospitals (Mumbai, India), the Ethics Review Board of Médecins Sans Frontières (Geneva, Switzerland) and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (Paris, France). The study protocol was approved by the Indian

Table 1. Demographic and clinical characteristics of HIV-infected patients with presumptive TB, Mumbai, India.

Characteristics	HIV-infected patients with presumptive TB (N = 1724)
	n (%)
Age [years, median (IQR)]	35.0 (24.3–44.0)
Sex of patients	
Male	1042 (60.4)
Female	671 (38.9)
Transgender	11 (0.6)
Family income per month (in Rupees)	
Less than 3500	88 (5.1)
3500–6999	910 (52.8)
7000 and above	454 (26.3)
Patient did not disclose	272 (15.6)
TB site	
Pulmonary	1688 (97.9)
Extra-pulmonary	36 (2.1)
ART status	
On ART	1386 (80.4)
Pre-ART	338 (19.6)
CD4 count, last visit (in cells/μl)	
Less than 200	258 (15.0)
200–349	351 (20.4)
350–499	289 (16.8)
500 and above	684 (39.7)
No information	142 (8.2)
ART duration* [months, median (IQR)]	26.0 (10.7–47.5)
Previous episode of TB	
Yes	933 (54.1)
No	791 (45.9)

ART: Antiretroviral treatment* Patients on ART with available information about ART initiation date, N = 1370.
doi:10.1371/journal.pone.0110461.t001

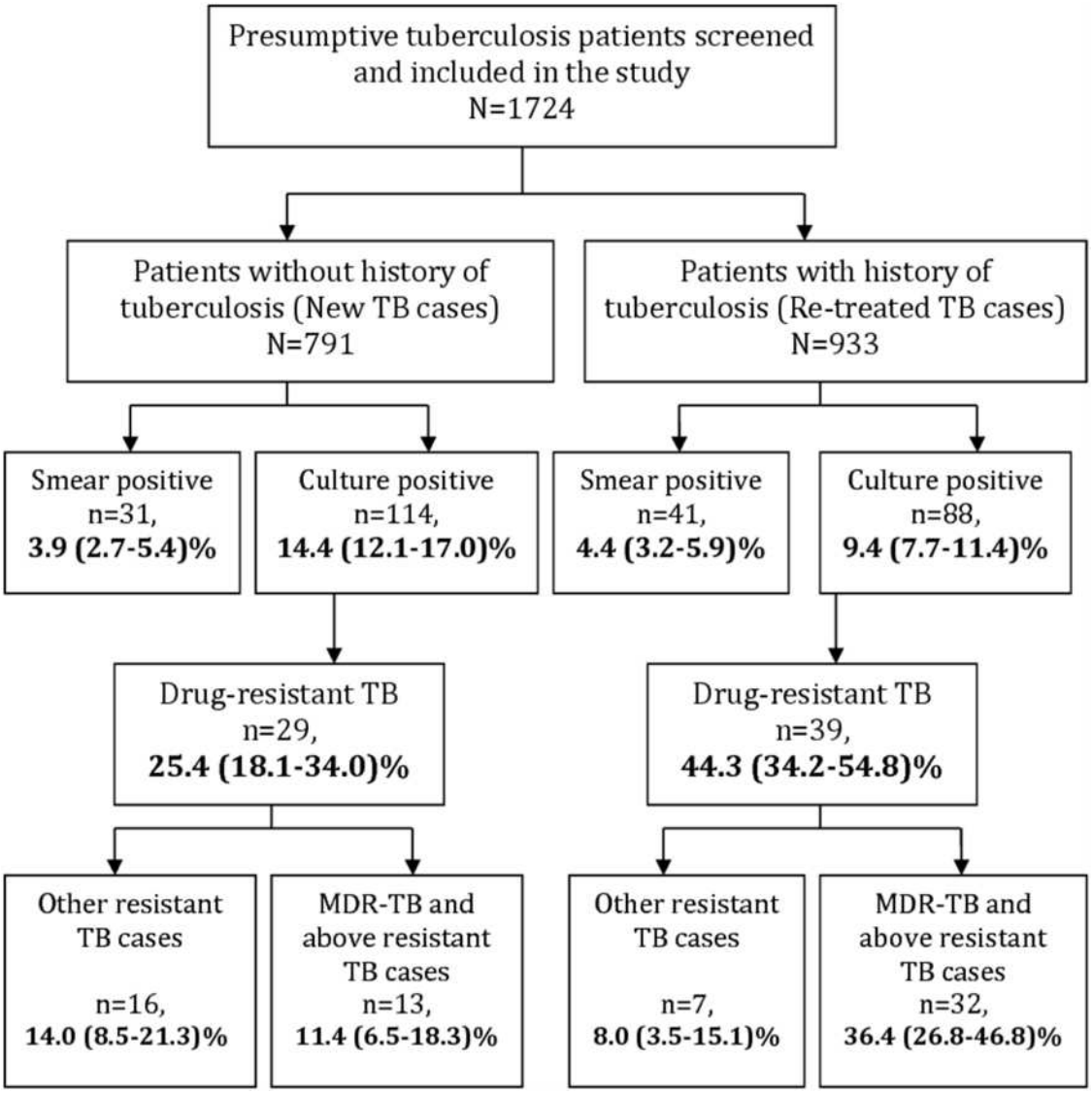

Figure 2. Distribution of Drug-resistant tuberculosis among HIV-infected (new and previously treated) with presumptive tuberculosis patients, Mumbai, India.
doi:10.1371/journal.pone.0110461.g002

Council of Medical Research (ICMR), New Delhi, India. Informed consent was obtained from all study participants.

Study design

This was a cross-sectional survey among HIV-infected adult and paediatric patients attending public and public-private ART clinics in the greater metropolitan Mumbai area. All patients with presumptive pulmonary or extra-pulmonary TB were assessed with smear microscopy and conventional liquid culture. All M. tuberculosis isolates underwent drug susceptibility testing (DST) for first- and second-line anti-TB drugs.

Sample size

The desired sample size was determined separately for new and previously treated culture positive TB cases. Previous tuberculosis treatment was defined as any anti-tuberculosis treatment reported by the patient. Assuming a prevalence of MDR-TB of 3% among new cases and 17% among retreated cases, based on a DST survey conducted in Gujarat [12], a sample size of 123 confirmed new cases and 110 confirmed retreatment cases was sought in order to estimate the prevalence of MDR-TB, with 95% confidence intervals having a margin of error of 3% for new cases and 7% for retreated cases respectively.

Study setting and study population

The study was carried out in five Mumbai District AIDS Control Society (MDACS) ART Centres [1) KEM Hospital, 2) SION Hospital, 3) SION Centre of Excellence in Paediatric HIV Care, 4) Godrej Hospital, Vikhroli and 5) Larsen & Toubro Hospital, Andheri] as well as in two Maharashtra State AIDS Control Society (MSACS) ART Centres [1) Thane Civil Hospital and 2) Navi Mumbai (Vashi) Municipal Corporation Hospital].

All HIV-infected adult and paediatric patients enrolled in the ART centres were potentially eligible to be enrolled in the study, if they had presumptive pulmonary or extra-pulmonary TB based on symptom screening, regardless of the time they were enrolled in the centres or whether they were on ART or not at the time of the

Table 2. Resistance profile (first and second-line) for all drug-resistant tuberculosis patients, Mumbai, India.

Resistance profile (culture-based DST)	Total TB Patients (N = 68), n (%)	New TB patients (N = 29), n (%)	Previously treated TB patients (N = 39), n (%)
H-mono	11 (16.2)	7 (24.1)	4 (10.3)
R-mono	-	-	-
Ofx-mono	1 (1.5)	1 (3.4)	-
Ofx Mfx	3 (4.4)	2 (6.9)	1 (2.6)
HE	7 (10.3)	6 (20.7)	1 (2.6)
HE Eto Ofx Mfx	1 (1.5)	-	1 (2.6)
HR	10 (14.7)	4 (13.8)	6 (15.4)
HRE	6 (8.8)	2 (6.9)	4 (10.3)
HR Eto	5 (7.4)	-	5 (12.8)
HR E Eto	5 (7.4)	2 (6.9)	3 (7.7)
HR Ofx Mfx E	2 (2.9)	2 (6.9)	-
HR Ofx Mfx Eto	1 (1.5)	1 (3.4)	-
HR Ofx Mfx E Lin	1 (1.5)	-	1 (2.6)
HR Ofx Mfx E Eto	8 (11.8)	1 (3.4)	7 (17.9)
HR Ofx Mfx E Eto PAS	2 (2.9)	-	2 (5.1)
HR Ofx Mfx Km Eto	1 (1.5)	-	1 (2.6)
HR Ofx Mfx Km E Eto	2 (2.9)	1 (3.4)	1 (2.6)
HR Ofx Mfx Km Cm E Eto	1 (1.5)	-	1 (2.6)
HR Ofx Mfx Km Cm E Eto PAS	1 (1.5)	-	1 (2.6)

H-isoniazid, R-rifampicin, E-ethambutol, Eto-ethionamide, Km-kanamycin, Cm-capreomycin, Ofx-ofloxacin, Mfx-Moxifloxacin, Lin- Linezolid, PAS- para-aminosalicylic acid.
doi:10.1371/journal.pone.0110461.t002

study. Patients on TB treatment at the time of the study were excluded.

Recruitment and sampling procedure

All HIV-infected ART center attendees were screened by an MSF-employed nurse during the study period. Patients with presumptive TB were investigated using a standard diagnostic algorithm recommended by the World Health Organization [13] that included TB culture and DST. The nurse explained in detail the objectives of the study to the patient and/or caregiver and obtained the signature or thumbprint of the patient if consent was given to participate. When pulmonary TB was presumed, two sputum specimens were collected on the same day, one hour apart, at each study site/hospital laboratory. When extra-pulmonary TB (EPTB) was presumed, biological specimens (fine needle aspirates, pleural fluid, cerebrospinal fluid, etc) were obtained from extra-pulmonary sites. All specimens were transferred to Hinduja Hospital Microbiology Laboratory in Mumbai for culture and first- and second-line DST.

Conventional microscopy with Ziehl-Neelsen (ZN) staining for acid-fast bacilli and further sputum decontamination was performed using the N-acetyl-L-cysteine and sodium hydroxide method. Concentrated sediment was inoculated in one liquid culture tube for testing using the Mycobacterial growth indicator tube (MGIT 960) method. Positive cultures underwent microscopy with ZN staining to confirm cord formation, and speciation with MPT 64 antigen detection by Immunochromatography was carried out to confirm *M. tuberculosis* complex. Specimens fulfilling the above criteria underwent further testing with phenotypic DST using the MGIT System for the following drugs: isoniazid, rifampicin, ethambutol, ofloxacin, moxifloxacin, kanamycin, capreomycin, PAS, ethionamide, clofazimine and linezolid. Non-tuberculous Mycobacteria (NTM) speciation was done by molecular methods using Reverse Line Blot Hybridisation. Hinduja laboratory is quality controlled and has been accredited for first-line DST by the WHO Supranational Reference Laboratory in Bangalore and the College of American Pathologists. The laboratory was also accredited by the TB programme for second-line DST in December 2013; prior to this date, if a strain was suspected to have resistance to one or more second-line anti-TB drugs, it was sent to the National Tuberculosis Institute Laboratory in Bangalore for confirmation.

Multidrug-resistant tuberculosis (MDR-TB) was defined as resistance to both isoniazid and rifampicin; pre-XDR-TB was defined as MDR-TB with additional resistance to either a fluoroquinolone or a second-line injectable agent; and extensively drug-resistant tuberculosis (XDR-TB) was defined as MDR-TB with additional resistance to both a fluoroquinolone and an injectable agent. Extremely drug-resistant tuberculosis (XXDR-TB) was defined as XDR-TB with additional resistance to any group IV and/or group V TB drugs (PAS, ethionamide, clofazimine, linezolid) [13].

Management of those diagnosed with DR-TB

All patients diagnosed with MDR- or XDR-TB were managed in accordance with the national DR-TB treatment guidelines [14], while those with pre-XDR-TB were offered individualized treatment with 4 drugs likely to be effective.

Data collection and analysis

Demographics, clinical and laboratory data, antiretroviral treatment (yes/no) and duration on ART, as well as data on

Table 3. Demographic and clinical factors associated with culture-positive tuberculosis in HIV-infected patients, Mumbai, India.

Explanatory Variable	Patients with tuberculosis (N = 202), n (%)	Patients without tuberculosis (N = 1522), n (%)	Chi-square/t-test (p-value)	aPR[a] (95% CI)
Age [years, median (IQR)]	38.0 (32.0–43.3)	35.0 (22.0–44.0)	8.9 (<0.01)	0.99 (0.99–1.00)
Sex of patients				
Male	138 (13.1)	915 (86.9)	5.0 (0.02)	1.01 (0.99–1.03)
Female	64 (9.5)	607 (90.5)		
Family income per month[+] (in Rupees)				
Less than 5000	96 (10.9)	788 (89.1)	0.2 (0.65)	
5000 and above	66 (11.6)	502 (88.4)		
ART status				
Pre-ART	72 (21.3)	266 (78.7)	37.3 (<0.01)	1.07 (1.04–1.10)
On ART	130 (9.4)	1256 (90.6)		
CD4 count, last visit* (in cells/μl)				
Less than 200	58 (22.5)	200 (77.5)	34.7 (<0.01)	1.08 (1.05–1.11)
200 and above	127 (9.6)	1197 (90.4)		
ART duration** [months, median (IQR)]	19.3 (5.2–35.7)	27.1 (11.3–47.9)	9.9 (<0.01)	
Previous episode of TB				
Yes	88 (9.4)	845 (90.6)	10.3 (<0.01)	1.01 (0.99–1.03)
No	114 (14.4)	677 (85.6)		

ART: Antiretroviral treatment, IQR: Inter-quartile range, CI; Confidence Intervals [+]Patients with recorded family income, N = 1452* Patients with available information on CD4, last visit, N = 1582** Patients on ART with available information about ART initiation date, N = 1370[a] aPR; adjusted Prevalence Ratios (calculated by Poisson regression using multiple imputation for CD4 missing data).

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previous TB treatment were doubly-entered into an EpiData database (Version 3.1, EpiData Association, Odense, Denmark), validated and analyzed.

To identify factors associated with TB and DR-TB, univariate and multivariate analyses were performed using Poisson and binary logistic regression models. Factors significant (p = 0.05) on univariate analysis were entered into the multivariate logistic regression models. Factors were coded as categorical variables and missing values for CD4 cell counts were imputed using a multiple imputation method. Transgender individuals (all were male to female) were grouped with biological males in the models. All factors were entered as a block into multivariate logistic regression models. Data analysis, including multivariate logistic regression models, was conducted with SPSS Version 20.0. Armonk, NY: IBM Corp. Released 2011).

Results

Screening for presumptive TB was carried-out during 14,135 patient visits at seven ART centers in metropolitan Mumbai between March 2013 and January 2014 (Figure 1). Individual patients might have been screened more than once during the study period. A total of 1741 HIV-infected patients with presumptive tuberculosis (TB) were identified. All of them consented to participate in the study and were investigated for drug-resistant TB. The sputum specimens of 17 patients were found insufficient for laboratory investigations and had to be excluded. Thus, 1724 (99%) of the eligible patients were included in the study.

Patient characteristics

The median age of the 1724 patients was 35 (Inter-quartile range, IQR: 24–44) years (Table 1) and the majority (60%) were male. A large proportion (53%) of patients had an average family income between 3500 and 7000 Indian National Rupees (equivalent to 60–120 USD) per month. Most of the patients (98%) had pulmonary TB. Among the entire study cohort, 80% were on ART during the study period and the majority (52%) had CD4 cell counts lower than 500 cells/μL at their last visit to an ART center. The median duration of exposure to ART prior to enrollment in the study was 26 months (IQR: 10.7–47.5). More than half (933/1724) of the presumptive TB patients had had at least one episode of active TB disease in the past.

Culture-positive and drug-resistant tuberculosis

All of the 1724 patients with presumptive TB included in the study (Figure 1) underwent smear, culture and drug susceptibility testing (DST). Of these, 72 (4.2%; 95% Confidence Intervals (CI): 3.3–5.2) patients had smear-positive TB while 202 (11.7%; 95% CI: 10.3–13.3) patients had culture-positive TB. Eleven TB

Table 4. Demographic and clinical factors associated with drug-resistant tuberculosis in HIV-infected tuberculosis patients, Mumbai, India.

Explanatory Variable	Patients with drug-resistant tuberculosis (N = 68), n (%)	Patients without drug-resistant tuberculosis (N = 134), n (%)	Chi-square/t-test (p-value)	aOR[a] (95% CI)
Age [years, median (IQR)]	35.5 (28.5–42.8)	38.0 (33.8–44.0)	2.15 (0.14)	0.98 (0.96–1.01)
Sex of patients				
Male	46 (33.3)	92 (66.7)	0.02 (0.88)	0.95 (0.49–1.82)
Female	22 (34.4)	42 (65.6)		
Family income per month[+] (in Rupees)				
Less than 5000	36 (37.5)	60 (62.5)	1.8 (0.17)	
5000 and above	18 (27.3)	48 (72.7)		
ART status				
Pre-ART	22 (30.6)	50 (69.4)	0.48 (0.49)	0.96 (0.49–1.90)
On ART	46 (35.4)	84 (64.6)		
CD4 count, last visit* (in cells/μl)				
Less than 200	19 (32.8)	39 (67.2)	0.22 (0.88)	0.96 (0.48–1.93)
200 and above	43 (33.9)	84 (66.1)		
ART duration** [months, median (IQR)]	19.3 (5.7–31.3)	19.2 (3.2–43.7)		
Previous episode of TB				
Yes	39 (44.3)	49 (55.7)	7.93 (<0.01)	2.31 (1.24–4.30)
No	29 (25.4)	85 (74.6)		

ART: Antiretroviral treatment, CI; Confidence Intervals [+]Patients with recorded family income, N = 162* Patients with available information about CD4 count, last visit N = 185** Patients on ART with available information about ART initiation date, N = 126[a] aOR; adjusted Odds ratios (calculated by binary logistic regression using multiple imputation for CD4 missing data).

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patients were smear-positive but culture negative and 141 patients were culture-positive but smear-negative. Those patients having a history of TB had a higher rate of smear-positivity (4.4% versus 3.9%), but lower culture-positivity rate (9.4% versus 14.4%) as compared to patients without TB history (Figure 2).

Among all culture-positive TB patients, 68 or 33.7% (95% CI: 27.4–40.4) had some form of drug-resistant TB. A high proportion of MDR-TB and pre-XDR-TB, 38% (26/68) and 21% (14/68) respectively, was observed amongst drug-resistant TB patients. Table 2 shows the detailed resistance patterns of all patients with DR-TB.

Of the newly diagnosed (114/791) and previously treated (88/933) culture-positive TB patients, 25.4% (95% CI: 18.1–34.0) and 44.3% (95% CI: 34.2–54.8) patients had drug-resistant TB. The proportion of patients with multidrug-resistant TB and more advanced TB resistance profiles was higher (36% versus 11%) in previously treated patients compared to newly diagnosed TB patients.

Children and extra-pulmonary tuberculosis patients

In the study, 283 children aged less than 15 years were investigated. The median (IQR) age of these children was 11 (8–13) years, just over half of them were male (56%), and sixty-eight percent were on ART. Of the 283 children investigated, 5% (15/283) had culture-positive TB, of whom seven (46.7%, 7/15) had drug-resistant TB; one had polydrug-resistant TB, two had MDR-TB and four had pre-XDR-TB.

Among 1724 HIV-infected patients investigated during the study period, 36 patients had presumptive extra-pulmonary TB (EPTB). The median (IQR) age of these patients was 45 (40–47) years and three-quarters of them were male (27/36, 75%). Among the 36 investigated presumptive EPTB patients, 14% (5/36) patients had culture-positive TB. Of these, 40% (2/5) patients had DR-TB: one had INH mono-resistant TB while another had pre-XDR-TB.

Factors associated with culture-confirmed TB, DR-TB and MDR-TB

The demographic and clinical factors were assessed for association with culture-confirmed TB, DR-TB and MDR-TB. The univariate and bivariate analyses found age, ART status, CD4 count at last visit and previous episode of TB significantly related to culture-positive TB (Table 3). A multivariate Poisson regression model showed that older age, pre-ART status (i.e. not yet on ART), CD4 count less than 200 cells/μL at the last visit and a previous episode of TB were associated with culture-positive TB. None of the factors other than previous history of TB were associated with drug-resistant TB (Table 4) and multi-drug resistant TB (Table 5) in bivariate and multivariate binary logistic regression models.

Table 5. Demographic and clinical factors associated with multidrug-resistant tuberculosis in HIV-infected tuberculosis patients, Mumbai, India.

Explanatory Variable	Patients with multidrug-resistant tuberculosis (N = 45), n (%)	Patients with drug-susceptible tuberculosis (N = 134), n (%)	Chi-square/t-test (p-value)	aOR[a] (95% CI)
Age [years, median (IQR)]	38.0 (30.0–42.5)	38.0 (33.8–44.0)	1.85 (0.18)	0.98 (0.95–1.01)
Sex of patients				
Male	30 (24.6)	92 (75.4)	0.06 (0.80)	0.83 (3.8–1.83)
Female	15 (26.3)	42 (73.7)		
Family income per month[+] (in Rupees)				
Less than 5000	21 (25.9)	60 (74.1)	1.03 (0.31)	
5000 and above	11 (18.6)	48 (81.4)		
ART status				
Pre-ART	12 (19.4)	50 (80.6)	1.69 (0.19)	0.85 (0.37–1.96)
On ART	33 (28.2)	84 (71.8)		
CD4 count, last visit* (in cells/μl)				
Less than 200	13 (25.0)	39 (75.0)	0.00 (1.00)	1.02 (0.43–2.41)
200 and above	28 (25.0)	84 (75.0)		
ART duration** [months, median (IQR)]	19.7 (5.7–34.8)	19.2 (3.2–43.7)	0.02 (0.88)	
Previous episode of TB				
Yes	32 (39.5)	49 (60.5)	16.2 (<0.01)	4.16 (1.93–8.95)
No	13 (13.2)	85 (86.7)		

ART: Antiretroviral treatment, CI; Confidence Intervals [+]Patients with recorded family income, N = 140* Patients with available information about CD4 count, last visit N = 164** Patients on ART with available information about ART initiation date, N = 126[a]aOR; adjusted Odds ratios (calculated by binary logistic regression using multiple imputation for CD4 missing data).

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Discussion

To our knowledge this is the first DR-TB survey carried out among HIV clinic attendees in India. This study shows that, among HIV-infected children and adults in Mumbai, the burden of drug-resistant tuberculosis is extremely high: almost one in four new TB cases and one in two of those previously treated for TB have a drug-resistant strain. Of just as great concern, a large proportion of these strains was resistant to one or more second-line tuberculosis drugs, especially fluoroquinolones.

The overall rate of culture positivity amongst presumptive TB cases was surprisingly low (11.7%). We hypothesize that this was due neither to limitations in laboratory techniques nor the presence of NTM disease, but instead to the broad inclusion criteria that required a person attending a study site to have just one of four possible TB symptoms as recommended by WHO [13]; a person with 'current cough', for example, who was otherwise stable was eligible for enrolment. Another possible contributor to the low rate of TB culture positivity was the relatively large number of poor quality specimens (e.g. consisting of saliva) despite active instruction being given by a dedicated study nurse at each site. In any case, this finding warrants further investigation.

Even though the overall yield of TB was small in the pediatric cohort as well, it remains significant that almost half of the children with TB were infected with drug-resistant strains, most commonly pre-XDR-TB. Since bacteriological confirmation of DR-TB is more challenging in young children than in adults, as they cannot expectorate sputum and are more likely to have paucibacillary and extra-pulmonary TB, we hypothesize that the burden of TB and DR-TB is likely to be underestimated among children in this study, similar to what has been found in a recent meta-analysis [15]. With less than 2% of all study participants having specimens taken from extrapulmonary sites, it is almost certain that EPTB is being underdiagnosed as well in this cohort. A separate analysis found no significant association between EPTB and DR-TB in children or adults.

Our statistical models revealed no significant associations between most demographic and clinical factors and the risk of DR-TB and MDR-TB. We believe that these findings are important for their lack of associations; it seems that most TB/HIV co-infected patients attending ART centers in Mumbai are at risk for DR-TB. Although the relatively small sample size limits the power of our analyses and calls for cautious interpretation, the lack of associations suggests that all those infected with HIV and presumed to have active TB be tested for drug-resistant strains.

Given the high population density in Mumbai, in which a large proportion of the population lives in slums under extreme poverty, the very high TB prevalence and the relatively high HIV burden reported in greater metropolitan Mumbai, these data are unlikely to be representative of a country as vast and diverse as India. Nevertheless the living conditions in Mumbai and common practices in the public and private health sectors (as for example the prescribing of inappropriate regimens and over-the-counter availability of fluoroquinolones and other drugs with anti-TB properties) are similar to those of other large metropolitan centres in the country, so these data could very well represent the DR-TB situation in such cities as New Delhi, Kolkata and Chennai.

While it may not be possible to generalise our estimates for the entire country or even for HIV-uninfected populations, they serve to highlight the overall magnitude of the DR-TB epidemic in Mumbai, which is not unknown [16,17]. A high prevalence of MDR-TB strains (11–68%) was reported in tertiary health facilities as early as 1991, followed by further documentation in 2006 [18–20], including information on the magnitude of the epidemic in children [21]. A study by D'Souza et al in 2009 [18] documented high levels of multiple drug resistance (both MDR and poly-drug resistance) amongst previously untreated cases in urban parts of Mumbai. In 2011 Udwadia et al reported a cases-series of totally-drug resistant TB (a term that has not officially been endorsed by WHO) in Mumbai, which captured the attention of local and international media [22,23]. However to-date such findings are often overlooked and their importance minimized as representing only selected populations, laboratory or tertiary care settings and small case-series. Our study confirms that there is more than one epidemic ongoing in Mumbai and reinforces the urgent need to accurately measure the overall prevalence and incidence of DR-TB around the country in order to define appropriate interventions. Studies in selected populations such as this complement the overall estimates and can help in directing resources and prioritizing interventions targeted at the most vulnerable groups.

This survey is subject to the usual limitations in survey design and data collection. There is likely to be a tendency for patients to not report previous treatment either because they do not remember (recall limitation) or, on purpose, to avoid going through a long course of treatment that includes daily injectable medication and is known among patients for debilitating side effects [24]. Such bias could have led to an overestimate of DR-TB among new cases and an underestimate among retreatment cases. However, most HIV-infected patients attending ART clinics are usually aware of tuberculosis and have been counseled and screened for TB on several occasions, so recall limitation is rather unlikely.

The majority of HIV-infected patients attending public and public-private ART centers in the city are likely to access the public national TB programme for TB diagnosis and treatment. However many still seek care from private practitioners or may switch between the public and private sectors. The contribution to DR-TB levels from suboptimal treatment regimens prescribed in the unregulated Indian private health sector has been well documented [25–27]. Cox et al in 2007 have shown that even under well-established DOTS programmes in areas with high levels of drug resistance, high levels of amplification of drug resistance are to be expected [28].

The high level of resistance to three or more first-line anti-TB drugs and to fluoroquinolones has been previously described by others [29]. The proportion of previously untreated cases in our study that were resistant to more than three drugs, especially isoniazid, rifampicin and a fluoroquinolone, was particularly alarming and highlights two major issues in the management of TB in the setting of HIV/ART clinics. Firstly, it points to the scenario of nosocomial transmission of TB and DR-TB. Those attending an ART clinic at least once a month are more likely to be exposed to susceptible and resistant strains of *M. tuberculosis* than the general population. Given that the ART centers in Mumbai are usually extremely busy, constantly crowded and that they often lack adequate TB infection control interventions, this scenario is not unlikely. Instead of hypothesizing that most cases of DR-TB are due to non-adherence among patients on treatment, exogenous infection or re-infection should first be considered [30,31]. Secondly, considering the high levels of resistance to second-line TB drugs and especially fluoroquinolones in this population, it is reasonable to assume that patients with presumptive TB may actually have pre-XDR-TB or even XDR-TB. This statement implies a huge investment in laboratory capacity in an already constrained public sector in Mumbai in order to screen all TB patients at the outset for strains that are resistant to fluoroquinolones and anti-TB injectables Nevertheless, we believe that it is a reasonable investment to make if the epidemic of DR-TB is to be controlled in the city in the future. Conversely, if DST is only offered afterward to those failing their TB treatment regimen, a large proportion of DR-TB cases will be missed due to the high risk of mortality among HIV-positive patients with untreated DR-TB [32].

There is an ongoing plan to systematically offer molecular TB diagnosis (mainly using Xpert MTB/RIF, also known as GeneXpert) to all HIV-infected patients in Mumbai and elsewhere in the country. While this is a giant leap forward, since GeneXpert can rapidly detect MTB and rifampicin resistance within 2 hours, we are concerned that 'scale up' of DR-TB diagnosis using this particular diagnostic may lead to suboptimal practices, since a diagnosis of rifampicin resistance alone and/or assumption that it represents a diagnosis of MDR-TB, may mask a diagnosis of pre-XDR or XDR-TB (or worse); the risks then associated with giving a suboptimal treatment regimen are significant both in terms of morbidity and mortality for the patient, as well as amplification of resistance and subsequent community transmission of resistant strains. While GeneXpert is an excellent and efficient diagnostic tool for MTB and screening test for DR-TB, in settings like Mumbai it is essential that it be complemented by culture and DST involving first- and second-line anti-TB drugs. The national programme has recently changed the policy to account for this risk, starting with HIV-infected patients in Mumbai and Maharashtra.

Our initial study protocol included fingerprinting studies using spoligotyping, which we had to abandon due to the high cost. Cox et al have in the past found a strong association between the Beijing genotype and amplification in situations of preexisting resistance in a central Asian setting [33]. Similarly, the proportion of the Beijing genotype was reported to be 35% in the urban Mumbai population studied by Almeida et al [34]. We need fingerprinting studies to establish how often nosocomial transmission occurs and to guide TB infection control interventions. Another area of research that is urgently needed relates to chemoprophylaxis for child contacts of DR-TB cases in Mumbai; preventative regimens that have shown to be effective in other settings are unlikely to prevent development of active disease in many children in Mumbai due to the high baseline rate of fluoroquinolone resistance [35].

Conclusion

Our findings strongly suggest that there is an ongoing DR-TB epidemic among people living with HIV and attending ART centers in Mumbai, which requires urgent, innovative and feasible models of care that allow for rapid and accurate detection and treatment of as many DR-TB patients as possible. Ideally all patients with presumptive TB attending any ART center in Mumbai, or settings with similar drug resistance patterns, should be screened with a rapid molecular diagnostic followed by DST to first- and second-line anti-TB drugs, including for fluoroquinolones, so that the correct diagnosis is made as early as possible and followed by prompt treatment initiation with an appropriate individualized regimen. The high rate of DR-TB amongst new TB patients also highlights the need for better TB infection control measures in order to prevent ongoing transmission of DR-TB in the community and health facilities, especially those attended by vulnerable populations, such as those living with HIV.

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Author Contributions

Conceived and designed the experiments: PI. Performed the experiments: CP CR MM AS. Analyzed the data: PI MD. Contributed reagents/materials/analysis tools: AMVK MK AB BA MM MP AK BBR AD LR PS KSS. Contributed to the writing of the manuscript: PI MD PS. Critically reviewed several versions of the manuscript: AMVK MK AB BA MM MP AK BBR AD LR PS KSS CP AS.

References

1. World Health Organization (WHO) (2013), Global tuberculosis report 2013. WHO Press, Geneva, WHO/HTM/TB/2013.11.
2. Department of AIDS Control (2013), National AIDS Control Organization, Annual Report 2012–2013, Ministry of Health & Family Welfare, Government of India.
3. Paramasivan CN, Venkataraman P (2004) Drug resistance in tuberculosis in India. Indian J Med Res; 120: 377–386.
4. Deivanayagam CN, Rajasekaran S, Venkatesan R, Mahilmaran A, Ahmed PR, et al. (2002) Prevalence of acquired MDR TB and HIV co-infection. Indian J Chest Dis Allied Sci 44: 237–242.
5. Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C (2005) The impact of HIV/AIDS on the control of tuberculosis in India. Proc Natl Acad Sci U S A 102: 9619–9624.
6. Swaminathan S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekeran S (2005) Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis 9: 896–900.
7. Maniar JK, Kanuth RR, Mandalia S, Shah K, Maniar A (2006) HIV and tuberculosis: partners in crime. Indian J Dermatol Venereol Leprol 72: 276–82.
8. Pereira M, Tripathy S, Inamdar V, Ramesh K, Bhavsar M, et al. (2005) Drug resistance pattern of Mycobacterium tuberculosis in seropositive and seronegative HIV-TB patients in Pune, India. Indian J Med Res 121: 235–239.
9. Sethi S, Mewara A, Dhatwalia SK, Singh H, Yadav R, et al. (2013) Prevalence of multidrug resistance in *Mycobacterium tuberculosis* isolates from HIV seropositive and seronegative patients with pulmonary tuberculosis in north India. BMC Infect Dis 1471–2334/13/137.
10. Menon S, Dharmshale S, Chande C, Gohil A, Lilani S, et al. (2012) Drug resistance profiles of *Mycobacterium tuberculosis* isolates to first line anti-tuberculous drugs: a five years study. Lung India 29: 227–231.
11. Kumar P, Balooni V, Sharma BK, Kapil V, Sachdeva KS, et al. (2014) High degree of multi-drug resistance and hetero-resistance in pulmonary TB patients from Punjab state of India. Tuberculosis (Edinb) 94(1): 73–80.
12. Ramachandran R, Nalini S, Chandrasekar V, Dave PV, Sanghvi AS, et al. (2009) Surveillance of drug-resistant tuberculosis in the state of Gujarat, India Int J Tuberc Lung Dis 13(9): 1154–1160.
13. World Health Organization (2011) Guidelines for the programmatic management of drug-resistant tuberculosis. 2011 update. Geneva, Switzerland: WHO.
14. Central TB Division (2012) Programmatic Management for Drug-resistant Tuberculosis guidelines-May version, Directorate General of Heath Services, Ministry of Health and Family Welfare. Available: http://www.tbcindia.nic.in/pdfs/Guidelines%20for%20PMDT%20in%20India%20-%20May%202012.pdf. Accessed 2014 May 5.
15. Jenkins HE, Tolman AW, Yuen CM, Parr JB, Keshavjee S, et al. (2014) Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. Lancet 383(9928): 1572–1579.
16. Almeida D, Rodrigues C, Udwadia ZF, Lalvani A, Gothi GD, et al. (2003) Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. Clin Infect Dis 36: e152–4.
17. Singh S, Sankar MM, Gopinath K (2007) High rate of extensively drug-resistant tuberculosis in Indian AIDS patients. AIDS 21(17): 2345–7.
18. D'souza DT, Mistry NF, Vira TS, Dholakia Y, Hoffner S, et al. (2009) High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. BMC Public Health 29: 9–211.
19. Rodrigues C, Shenai S, Sadani M, Thakkar P, Sodha A, et al. (2006) Multi drug-resistant tuberculosis in Mumbai: it's only getting worse. Int J Tuberc Lung Dis 10(12): 1421–1422.
20. Chowgule RV, D Lina (1998) Pattern of secondary acquired drug resistance to antituberculosis drugs in Mumbai, India 1991–1995. Ind J Chest Dis Allied Sciences 40: 23–31.
21. Karande S, Bavdekar SB (2002) Children and multidrug-resistant tuberculosis in Mumbai (Bombay), India. Emerg Infect Dis 8(11): 1360–1361.
22. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C (2011) Totally Drug-Resistant Tuberculosis in India. Clin Infect Dis. doi:10.1093/cid/cir889.
23. TIME Magazine (2013). Contagion; Why drug-resistant tuberculosis threatens us all. March 4, 2013.
24. Isaakidis P, Rangan S, Pradhan A, Ladomirska J, Reid T, et al. (2013) 'I cry every day': experiences of patients co-infected with HIV and multidrug-resistant tuberculosis. Trop Med & Int Health 18(9): 1128–1133.
25. Uplekar M, Juvekar S, Morankar S, Rangan S, Nunn P (1998) Tuberculosis patients and practitioners in private clinics in India. Int J Tuberc Lung Dis 2(4): 324–329.
26. Bhargava A, Pinto L, Pai M (2011) Mismanagement of tuberculosis in India: Causes, consequences, and the way forward. Hypothesis 9(1): e7.
27. Udwadia ZF, Pinto LM, Uplekar MW (2010) Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? PLoS One 5: e12023.
28. Cox HS, Niemann S, Ismailov G, Doshetov D, Orozco JD, et al. (2007) Risk of acquired drug resistance during short-course directly observed treatment of tuberculosis in an area with high levels of drug resistance. Clin Infect Dis 441421–1427.
29. Agrawal D, Udwadia ZF, Rodriguez C, Mehta A (2009) Increasing incidence of fluoroquinolone-resistant Mycobacterium tuberculosis in Mumbai, India. Int J Tuberc Lung Dis 13(1): 79–83.
30. Andrews JR, Gandhi NR, Moodley P, Shah NS, Bohlken L, et al. (2008) Exogenous reinfection as a cause of multidrug-resistant and extensively drug-resistant tuberculosis in rural South Africa. JID 198: 1582–1589.
31. March F, Garriga X, Rodriguez P, Moreno C, Garrigo M, et al. (1997) Acquired drug resistance in Mycobacterium tuberculosis isolates recovered from compliant patients with human immunodeficiency virus-associated tuberculosis. Clin Infect Dis 25: 1044–1047.
32. Gandhi NR, Shah NS, Andrews JR, Vella V, Moll AP, et al. (2010) HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. Am J Respir Crit Care Med 181: 80–86.
33. Cox HS, Kubica T, Doshetov D, Kebede Y, Rüsch-Gerdess S, et al. (2005). The Beijing genotype and drug resistant tuberculosis in the Aral Sea region of Central Asia. Respiratory research, 6(1): 134.
34. Almeida D, Rodrigues C, Ashavaid TF, Lalvani A, Udwadia ZF, et al. (2005). High incidence of the Beijing genotype among multidrug-resistant isolates of Mycobacterium tuberculosis in a tertiary care center in Mumbai, India. CID, 40(6): 881–886.
35. Seddon JA, Hesseling AC, Finlayson H, Fielding K, Cox H, et al. (2013) Preventive therapy for child contacts of multidrug-resistant tuberculosis: a prospective cohort study. CIDcit655.

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Prevalence of Multidrug Resistance Tuberculosis in Presumptive Multidrug Resistant Tuberculosis Cases Attending Tertiary Care Center

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ABSTRACT :

Background-The emergence of resistance to drugs used to treat tuberculosis (TB), and particularly multidrug-resistant TB (MDR-TB), has become a significant public health problem in a number of countries and an obstacle to effective TB control. In our study we tried to comprehend the prevalence of MDR TB in new cases and retreatment cases in suspected MDR cases in the department of pulmonary medicine MGM medical college and hospital Aurangabad, India.

Methods- A prospective observational study was conducted between January 2015 and March 2016 at department of pulmonary medicine. all the sputum samples were sent to Government medical college Aurangabad where they were examined for AFB by direct microscopy after homogenization and concentration by Petroffs method and staining by Ziehl-Neelsen method. The specimens were subjected culture and sensitivity for M.Tuberculosis. Culture was performed on LJ Media and sensitivity was done for Rifampicin and Isoniazid .On the bases of Sensitivity patients were labeled as MDR/Mono resistance or Non MDR.

Results- A total of 181 MDR suspects selected for the study .Patients were divided into 2 groups New cases and Retreatment cases which shows the prevalence of MDR is high in retreatment cases around 15.85 % when compared to New cases which is around 11.36 %. Mono resistance to INH was found to be 3.17%.

Conclusion- Our study was conducted in a small number but which clearly states the levels of MDR in new cases was high when compared to the WHO global estimate 2014 of MDR in new and previously treated cases .This show there is a almost need to improve our diagnostic modalities and good treatment plans to reduce the prevalence of MDR both new and retreatment cases. Subjecting new cases for first line drug sensitivity testing should be implemented. Our study emphasize the need of first line drug sensitivity testing in all the new cases of Tuberculosis.

Key words- MDR , XDR, PMDT ,WHO , DMC,TB

INTRODUCTION : Multidrug-resistant TB (MDR-TB), has become a significant public health problem in a number of countries and an obstacle to effective TB control. In India, the available information from the several drug resistance surveillance studies conducted in the past suggest that the rate of MDR-TB is relatively low in India. However this translates into a large absolute number of cases and as yet the management of patients with MDR-TB is inadequate. It is well known that poor treatment practices breed drug resistance. Areas with a poor TB control tend to have higher rates of drug resistant TB. It has been acknowledged that good

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treatment is a pre-requisite to the prevention of emergence of resistance. Prevention of emergence of MDR-TB in the community is more imperative rather than its treatment. It is impossible to tackle the problem of drug-resistant[1]

Drug-resistant TB has microbial, clinical, and programmatic causes. From a microbiological perspective, the resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. An inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB. [1] However it should be stressed that MDR-TB is a man-made phenomenon – poor treatment, poor drugs and poor adherence lead to the development of MDR-TB. [1] According to WHO Globally, an estimated 3.3% (95% CI: 2.2–4.4%) of new cases and 20% (95%CI: 14–27%) of previously treated cases have MDR-TB. In 2014, there were an estimated 480 000 (range: 360 000–600 000) new cases of MDR-TB worldwide, and approximately 190 000 (range: 120 000–260 000) deaths from MDR-TB. Among patients with pulmonary TB who were notified in 2014, an estimated 300 000 (range: 220 000–370 000) had MDR-TB. More than half of these patients were in India, China and the Russian Federation**.** [2]

In present study, we have estimated the prevalence of MDR-TB (defined as resistance to Rifampicin and Isoniazid with or without resistance to other drugs) in MDR- suspect patients attending the department of pulmonary medicine MGM Medical college and hospital Aurangabad, Maharashtra . These MDR-suspect patients (according to Guidelines on Programmatic Management of Drug Resistant TB (PMDT) in India may 2012) include any TB patient who fails an RNTCP category I or III treatment regimen, any RNTCP category II patient who is sputum smear positive at the end of the third month of treatment or later and close contacts of MDR-TB patients who are found to have smear positive pulmonary TB disease.

MATERIALS AND METHODS:

This study was a prospective observational study that involved all MDR-suspects attending the department of pulmonary medicine MGM Medical College and hospital Aurangabad, Maharashtra from January 2015 –March 2016.

DATA AND SPECIMEN COLLECTION-

Detailed history taken from patient. Patients were carefully inquired about their symptoms such as fever, cough, expectoration, chest pain, breathlessness, loss of appetite and loss of weight. Past history of anti tubercular drug intake was taken. Routine hematological investigations were requested for each patient including complete blood count, random blood sugar, liver function tests, kidney function tests, Elisa for HIV I & II and urine for routine-microscopy. A standard X-ray chest PA view was ordered for every patient.

Sputum for acid fast bacilli, smear microscopy, culture and drug susceptibility tests were performed at Department of Microbiology, Government Medical College Aurangabad. All sputa samples were first homogenized and concentrated by Petroff's method (modified) in GMC. Drug susceptibility testing was carried out by 'Minimal Inhibitory Concentration' method. Standard reference strain 'H37Rv' was tested additionally for comparability and precision Pt were divided into two groups.

Group A which included new cases of pulmonary tuberculosis [who never had tuberculosis in the past and was never exposed to any of the ATT drugs] who's sputum was positive after completion of 2 mouths of ATT which include $2H_3\ R_3Z_3E_3$.

Group B which include all treatment failure, relapse and treatment default cases [who had been exposed to anti tubercular drugs in the past] of cat I and cat II under RNTCP India who's sputum turned out to be positive for acid fast bacilli again.

INCLUSION CRITERIA –new cases of pulmonary tuberculosis who's sputum was

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positive after completion of 2 months of ATT All treatment failure, relapse and treatment default cases who had been exposed to ATT earlier and turned sputum positive for acid fast bacilli.

EXCLUSION CRITERIA-smear negative pulmonary tuberculosis MDR Treatment failure: MDR Treatment default: patients Still on MDR treatment:

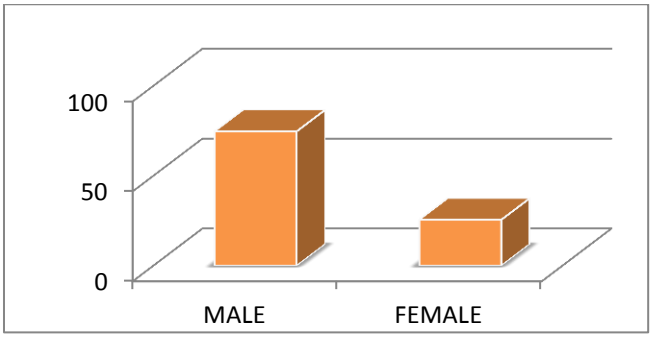
DRUG SUSCEPTIBILITY TESTING

Early morning sputum samples from patients were processed by the modified Petroff's method [3], stained by Ziehl-Neelsen Carbol Fuchsin, microscopically examined and cultured on Lowenstein-Jensen slopes (Himedia, India) as well as in Dubos broth (Himedia, India). Culture-negative or contaminated samples were excluded from the analysis. Biochemical tests for niacin and catalase production were performed to confirm the identity of *Mycobacterium tuberculosis*. Drug susceptibility testing of the samples was performed by the radiorespirometric Buddemeyer technique (a manual modification of the Bactec 460 technique) [4,5]. Briefly, samples were inoculated into Dubos broth containing ^{14}C Palmitic acid (Board of Radiation and Isotope Technology, India). Vials were set up in triplicate each containing 0.5×10^6/ml of Acid Fast Bacilli (AFBs) in absence (positive control) as well as presence of drugs (µgs/ml): Isoniazid (H – 0.1), Rifampicin (R – 2), Pyrazinamide (Z – 100) and Ethambutol (E – 2.5). Negative controls consisted of medium without acid fast bacilli (AFBs) as well as with heat killed AFBs. A 1:100 dilution of the positive control was also maintained. Readings were obtained daily until the eighth day in counts per minute (cpm) on a Wallac 1409 DSA liquid scintillation counter. Growth indices (GI) were calculated for the drug containing vials and the 1:100 positive control. Difference in growth indices (ΔGI), identical to that applied in the Bactec 460 method, calculated over consecutive days was used to determine susceptibility. The value of the mean ΔGI in the triplicate drug containing vials was compared to that for 1:100 control for the same day. If ΔGI was less in the drug containing vials than the 1:100 control, the bacteria were considered susceptible; if more, they were considered resistant [6,7].

Multidrug resistance (MDR) was defined as resistance to at least H and R. Other cases were categorized as follows: **Drug sensitive** – absence of resistance to any of the drugs, **monoresistance** – resistance to only 1 drug and **polyresistance** – resistance to at least two or more drugs excluding the HR combination.

RESULTS- A total of 184 MDR suspects patients selected for the study, out of which 58 patients were excluded from this study for various reasons. 126 patients were subjected for further evaluation in our study ,which included 94 male [74.60%] and 32 female [25.40%] patients .Patents were divided into 2 groups, Group A – [New Cases]Which had 44 patients from which 5 patients were MDR and 4 patients were detected with mono resistance to INH .The prevalence of MDR in New cases is around 11.36% and Group B – [Relapse/Retreatment]Which had 82 patients from which 13 patients were MDR which shows prevalence of MDR in Retreatment cases is 15.85 % and Out of 126 patients 3 patients were HIV Positive [2.38%].

SEX	FREQUENCY	PERCENT
MALE	94	74.60 %
FEMALE	32	25.40 %
TOTAL	126	100%



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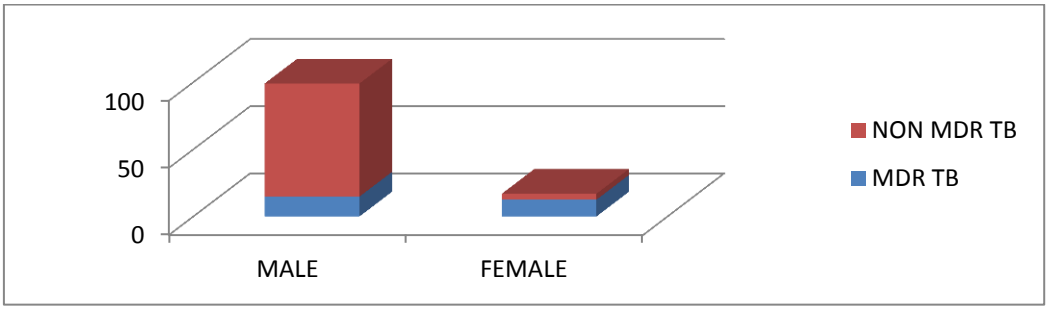
SEX	MDR TB	NON MDR TB	TOTAL
MALE	14 [14.89%]	80 [84.10%]	94 [100%]
FEMALE	4 [12.50%]	28 [87.50%]	32 [100%]
TOTAL	18 [14.28%]	108 [85.72%]	126 [100%]



GROUPS	A-NEW CASES	B- RELAPSE	TOTAL
MDR TB	05 [11.36%]	13 [15.85%]	18 [14.28%]
NON MDR TB	39 [88.64%]	69 [84.15%]	108 [85.72%]
TOTAL	44 [100%]	82 [100%]	126 [100%]

DISCUSSION

Drug-resistant TB poses a major threat to control of TB worldwide. By the end of 2014, data on anti-TB drug resistance were available for 153 countries, accounting for more than 95% of the world's population and estimated TB cases. Eighty of these countries have continuous surveillance systems, while the others rely on epidemiological surveys.

Extensively drug-resistant TB (XDR-TB) has been reported by 105 countries. On average, an estimated 9.7% (95% CI: 7.4–12%) of people with MDR-TB have XDR-TB.

There was major progress in coverage of drug susceptibility testing (DST) between 2013 and 2014. Worldwide, 12% of new bacteriologically-confirmed TB cases and 58% of previously treated TB patients were tested for drug resistance in 2014, up from 8.5% and 17% respectively in 2013 (representing proportional increases of 43% and 223%, respectively). Coverage was highest in the European Region (97% of new cases). In the South-East Asia and Western Pacific regions combined, two-thirds of previously treated cases underwent testing

Globally in 2014, 123 000 patients with MDR - TB or rifampicin resistant tuberculosis (RR-TB) were notified, of whom about 75% lived in the European Region, India, South Africa or China.

According to PMDT may 2012 the prevalence of MDR-TB in India to be about 3% in new cases and 12-17% in re-treatment cases. Study conducted at department of pulmonary medicine MGM hospital and medical college Aurangabad Maharashtra shows prevalence of MDR-TB in new cases is 10.52% and 16.25% in re-treatment cases

CONCLUSION

Our study shows that the prevalence of MDR TB is more in retreatment cases when compared to new cases, it should be emphasized that the prevalence of MDR TB in New cases is higher when compared to the values stated under PMDT

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2012 and WHO annual TB report 2015 .This is a threat to TB control Program in India [RNTCP] MDR TB diagnostic facility and surveillance activity should be expanded. Our study emphasize the need of first line drug sensitivity testing in all the new cases of Tuberculosis should be implemented to control, reduce the prevalence and improve the outcome of MDR TB treatment.

REFERENCES-

1. **Revised National Tuberculosis Control Program Guidelines on Programmatic Management of Drug Resistant TB (PMDT) in India**-May 2012
2. World health organization Global Tuberculosis report 2015
3. Baker JF, Silverton RE: **Routine bacteriological examination of specimens.** In*Introduction to medical laboratory technology*. 5th edition. Butterworths, London; 1978:528-530.
4. Shah DH, Devdhar MN, Ganatra RD, Narkar AA, Buddemeyer EU: **A rapid radiometric method for detection of *M. tuberculosis*: Optimization of experimental conditions.** *Int J Nucl Med Biol* 1984, **11**(3/4)**:**283-286. PubMed Abstract | Publisher Full Text
5. Shah DH, Devdhar MN, Ganatra RD, Kale PN, Virdi SS, Deshmukh MD: **Modified rapid radiometric method for detection of *Mycobacterium tuberculosis* from sputum samples.** *Int J Nucl Med Biol* 1985, **12**(4)**:**333-335. PubMed Abstract | Publisher Full Text
6. National Committee on Clinical and Laboratory Standards: *Susceptibility testing of Mycobacteria, Nocardia and other aerobic Actinomycetes. Tentative standard.* 1st edition. M24-T2, NCCLS; 2000. Mistry NF, Iyer A, D'souza DTB, Taylor GM, Young D, Antia N: **Spoligotyping of *Mycobacterium tuberculosis* isolates from multiple drug resistant tuberculosis patients from Bombay. India.** *J Clin Microbiol* 2002, **40**(7)**:**2677-2680. PubMed Abstract | Publisher Full Text | PubMed Central Full Text
7. **High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India** Desiree TB D'souza**1,** Nerges F Mistry**1***, Tina S Vira**1,** Yatin Dholakia**1,** Sven Hoffner**2,**Geoffrey Pasvol**3,** Mark Nicol**4 and** Robert J Wilkinson**345** ***BMC Public Health* 2009, 9:211 doi:10.1186/1471-2458-9-211**
8. Prevalence of Multi Drug Resistant Tuberculosis among Presumptive Multi Drug Resistant Tuberculosis Cases in Amhara National Regional State, Ethiopia -Daniel Mekonnen Nigus[1*], Wondemagegn Mulu Lingerew[1], Bayeh Abera Beyene[1], Aschalew Admassu Tamiru[2], Martha Tibebu Lemma[1]and Mulat Yimer Melaku[1] - **J Mycobac Dis 4:152. doi:10.4172/2161-1068.1000152**
9. PREVALENCE OF MULTIDRUG RESISTANT (MDR) TUBERCULOSIS IN MDRSUSPECT PATIENTS & ASSESSMENT OF VARIOUS REASONS FOR DEVELOPING DRUG RESISTANCE *Devesh Pratap Singh1 , Santosh Kumar2 , Gajendra Vikram Singh2 , Rajesh Kumar Gupta2 , D. S. Chauhan3 and Amir Mohammad4 --Ind. J. Sci. Res. and Tech. 2015 3(3):26-30/Singh et al ISSN:-2321-9262
10. TBC India: **TB India 2007: RNTCP Status report. New Delhi.** [http://www.tbcindia.org]*webcite* Central TB division, Directorate General of Health Services, Ministry of Health and Family welfare; Government of India; 2007.
11. Mondal R, Jain A: **Extensively drug-resistant *Mycobacterium tuberculosis*, India.**

DOI: 10.18535/ijmsci/v3i9.6

Emerg Infect Dis 2007, **13:**1429-1430. PubMed Abstract | Publisher Full Text

12. Bhargava A, Jain Y: **The revised national tuberculosis control programme in India: Time for revision of treatment regimens and rapid up scaling of DOTS-Plus initiative.** *Natl Med J Ind* 2008, **21:**27-31.

13. TBC India: **TB India 2008: RNTCP Status report.** [http://www.tbcindia.org] *webcite* New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare; Government of India; 2008

14. Udwadia ZF, Jain S, Rodrigues C, Mehta A: **XDR tuberculosis in India: what's in a name?** *Lancet ID* 2007, **7:**441-442. Publisher Full Text

DOI: 10.18535/ijmsci/v3i9.6

Role of Gene Xpert in Early Diagnosis and Treatment of Tuberculosis

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Abstract

Global Tuberculosis control efforts have been severely hampered due to lack of diagnostic tests that are accurate, simple to use and can be applied at the point of clinical care. This has been further compounded by the wide spread inability to test for drug resistance. The Xpert MTB/RIF assay is a rapid molecular assay that can be used close to the point of care by

operators with minimal technical expertise. In this study, 100 Sputum samples were obtained from the Tuberculosis (TB) suspected patients in the MGM Hospital and sent to the Central Research Laboratory (CRL), Department of Microbiology, MGM Medical College, Navi Mumbai, India from January 2016 to June 2016. The patients were examined clinically as per standard criteria and the sputum samples were then processed by three methods mainly includes Smear microscopy for Acid fast bacilli staining (AFB), Tuberculosis bacterial culture by Mycobacterium Growth Indicator Tube (MGIT), and Xpert MTB/RIF test platform (Gene Xpert, Cepheid), which integrates sample processing and Polymerase Chain Reaction (PCR) in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection. The Xpert MTB/RIF cartridge was then inserted into the Gene Xpert device, which provides results within 2 h. The sensitivity of Acid Fast Bacilli Smear Microscopy (AFB), Gene Xpert were found 55, 96% respectively and specificities were 96.67 and 96.67% respectively considering culture (MGIT) as a Gold standard. Gene Xpert assay was able to detect more Tuberculosis cases than Acid Fast Bacilli Smear microscopy alone. This technique though expensive is much quicker as compared to the conventional methods and is more sensitive and specific. This method could offer a new approach for accurate tuberculosis diagnosis, especially in remote regions of the world where culture-based diagnostic systems are not available and needs a specific environment for Sample processing and Growth of the Tuberculosis Bacteria.

Keywords

Tuberculosis Gene Xpert MTB/RIF AFB Culture MGIT PCR

References

1. Boehme CC et al (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005–1015
CrossRef (https://doi.org/10.1056/NEJMoa0907847)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Rapid%20molecular%20detection%20of%20tuberculosis%20and%20rifampin%20resistance&author=CC.%20Boehme&journal=N%20Engl%20J%20Med&volume=363&pages=1005-1015&publication_year=2010)

2. Boehme CC et al (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377(9776):505–1495
CrossRef (https://doi.org/10.1016/S0140-6736(11)60438-8)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Feasibility%2C%20diagnostic%20accuracy%2C%20and%20effectiveness%20of%20decentralised%20use%20of%20the%20Xpert%20MTB%2FRIF%20test%20for%20diagnosis%20of%20tuberculosis%20and%20multidrug%20resistance%3A%20a%20multicentre%20implementation%20study&author=CC.%20Boehme&journal=Lancet&volume=377&issue=9776&pages=505-1495&publication_year=2011)

3. Helb D et al (2010) Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48:229–237
CrossRef (https://doi.org/10.1128/JCM.01463-09)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Rapid%20detection%20of%20mycobacterium%20tuberculosis%20and%20rifampin%20resistance%20by%20use%20of%20on-demand%2C%20near-patient%20technology&author=D.%20Helb&journal=J%20Clin%20Microbiol&volume=48&pages=229-237&publication_year=2010)

4. Teran R, de Waard JH (2015) Recent advances in the laboratory diagnosis of tuberculosis. J Int Fed Clin Chem Lab Med 26(4):295–309
Google Scholar (http://scholar.google.com/scholar_lookup?title=Recent%20advances%20in%20the%20laboratory%20diagnosis%20of%20tuberculosis&author=R.%20Teran&author=JH.%20Waard&journal=J%20Int%20Fed%20Clin%20Chem%20Lab%20Med&volume=26&issue=4&pages=295-309&publication_year=2015)

5. Marlowe EM et al (2011) Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol 49:1621–1623
CrossRef (https://doi.org/10.1128/JCM.02214-10)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Evaluation%20of%20the%20Cepheid%20Xpert%20MTB%2FRIF%20assay%20for%20direct%20detection%20of%20mycobacterium%20tuberculosis%20complex%20in%20respiratory%20specimens&author=EM.%20Marlowe&journal=J%20Clin%20Microbiol&volume=49&pages=1621-1623&publication_year=2011)

6. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM Jr (2003) Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect Dis 3:633–643
CrossRef (https://doi.org/10.1016/S1473-3099(03)00772-2)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Diagnostic%20accuracy%20of%20nucleic%20acid%20amplification%20tests%20for%20tuberculous%20meningitis%3A%20a%20systematic%20review%20and%20meta-analysis&author=M.%20Pai&

author=LL.%20Flores&author=N.%20Pai&author=A.%20Hubbard&author=LW.%20Riley&author=JM.%20Colford&journal=Lancet%20Infect%20Dis&volume=3&pages=633-643&publication_year=2003)

7. Ho J, Marais BJ, Gilbert GL, Ralph AP (2013) Diagnosing tuberculous meningitis—have we made any progress? Trop Med Int Health 18:783–793
CrossRef (https://doi.org/10.1111/tmi.12099)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Diagnosing%20tuberculous%20meningitis%E2%80%94have%20we%20made%20any%20progress%3F&author=J.%20Ho&author=BJ.%20Marais&author=GL.%20Gilbert&author=AP.%20Ralph&journal=Trop%20Med%20Int%20Health&volume=18&pages=783-793&publication_year=2013)
8. Ioannidis P, Papaventsis D et al (2011) Cepheid GeneXpert MTB/RIF assay for *Mycobacterium tuberculosis* detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. J Clin Microbiol 49:3068–3070
CrossRef (https://doi.org/10.1128/JCM.00718-11)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Cepheid%20GeneXpert%20MTB%2FRIF%20assay%20for%20Mycobacterium%20tuberculosis%20detection%20and%20rifampin%20resistance%20identification%20in%20patients%20with%20substantial%20clinical%20indications%20of%20tuberculosis%20and%20smear-negative%20microscopy%20results&author=P.%20Ioannidis&author=D.%20Papaventsis&journal=J%20Clin%20Microbiol&volume=49&pages=3068-3070&publication_year=2011)
9. Blakemore R et al (2010) Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Cin Microbiol 48:2495–2501
CrossRef (https://doi.org/10.1128/JCM.00128-10)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Evaluation%20of%20the%20analytical%20performance%20of%20the%20Xpert%20MTB%2FRIF%20assay&author=R.%20Blakemore&journal=J%20Cin%20Microbiol&volume=48&pages=2495-2501&publication_year=2010)
10. Lawn SD, Nicol MP (2011) Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Fut Microbiol 6:1067–1082
CrossRef (https://doi.org/10.2217/fmb.11.84)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Xpert%C2%AE%20MTB%2FRIF%20assay%3A%20development%2C%20evaluation%20and%20implementation%20of%20a%20new%20rapid%20molecular%20diagnostic%20for%20tuberculosis%20and%20rifampicin%20resistance&author=SD.%20Lawn&author=MP.%20Nicol&journal=Fut%20Microbiol&volume=6&pages=1067-1082&publication_year=2011)
11. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E (2011) Rapid molecular detection of extra pulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbio 49:1202–1205

CrossRef (https://doi.org/10.1128/JCM.02268-10)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Rapid%20molecular%20detection%20of%20extra%20pulmonary%20tuberculosis%20by%20the%20automated%20GeneXpert%20MTB%2FRIF%20system&author=D.%20Hillemann&author=S.%20R%C3%BCsch-Gerdes&author=C.%20Boehme&author=E.%20Richter&journal=J%20Clin%20Microbio&volume=49&pages=1202-1205&publication_year=2011)

12. Batz HG, Cooke GS, Reid SD (2011) Towards lab-free tuberculosis diagnosis. Treatment Action Group, the TB/HIV Working Group of the Stop TB Partnership, Imperial College, and the MSF Access Campaig, pp 1–34
Google Scholar (https://scholar.google.com/scholar?q=Batz%20HG%2C%20Cooke%20GS%2C%20Reid%20SD%20%282011%29%20Towards%20lab-free%20tuberculosis%20diagnosis.%20Treatment%20Action%20Group%2C%20the%20TB%2FHIV%20Working%20Group%20of%20the%20Stop%20TB%20Partnership%2C%20Imperial%20College%2C%20and%20the%20MSF%20Access%20Campaig%2C%20pp%201%E2%80%9334)
13. Zeka AN, Tasbakan S, Cavusoglu C (2011) Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol 49:4138–4141
CrossRef (https://doi.org/10.1128/JCM.05434-11)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Evaluation%20of%20the%20GeneXpert%20MTB%2FRIF%20assay%20for%20rapid%20diagnosis%20of%20tuberculosis%20and%20detection%20of%20rifampin%20resistance%20in%20pulmonary%20and%20extrapulmonary%20specimens&author=AN.%20Zeka&author=S.%20Tasbakan&author=C.%20Cavusoglu&journal=J%20Clin%20Microbiol&volume=49&pages=4138-4141&publication_year=2011)
14. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C (2011) Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol 49:2540–2545
CrossRef (https://doi.org/10.1128/JCM.02319-10)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Xpert%20MTB%2FRIF%3A%20a%20new%20pillar%20in%20diagnosis%20of%20extrapulmonary%20tuberculosis%3F&author=V.%20Vadwai&author=C.%20Boehme&author=P.%20Nabeta&author=A.%20Shetty&author=D.%20Alland&author=C.%20Rodrigues&journal=J%20Clin%20Microbiol&volume=49&pages=2540-2545&publication_year=2011)
15. Van Rie A, Menezes C, Scott L (2011) High yield, sensitivity and specificity of Xpert MTB/RIF for *M. tuberculosis* detection in fine needle aspirates from HIV-infected TB suspects; conference paper: on retroviruses and opportunistic infections, Boston, MA, USA
Google Scholar (https://scholar.google.com/scholar?q=Van%20Rie%20A%2C%20Menezes%20C%2C%20Scott%20L%20%282011%29%20High%20yield%2C%20sensitivity%20and%20specificity%20of%20Xpert%20MTB%2FRIF%20for%20M.%20tuberculosis%20de

tection%20in%20fine%20needle%20aspirates%20from%20HIV-infected%20TB%20suspects%3B%20conference%20paper%3A%20on%20retroviruses%20and%20opportunistic%20infections%2C%20Boston%2C%20MA%2C%20USA)

16. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C (2011) Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol 49:2540–2545
CrossRef (https://doi.org/10.1128/JCM.02319-10)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Xpert%20MTB%2FRIF%3A%20a%20new%20pillar%20in%20diagnosis%20of%20extrapulmonary%20tuberculosis%3F&author=V.%20Vadwai&author=C.%20Boehme&author=P.%20Nabeta&author=A.%20Shetty&author=D.%20Alland&author=C.%20Rodrigues&journal=J%20Clin%20Microbiol&volume=49&pages=2540-2545&publication_year=2011)
17. Lawn SD, Nicol MP (2011) Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Fut Microbiol 6:1067–1082
CrossRef (https://doi.org/10.2217/fmb.11.84)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Xpert%C2%AE%20MTB%2FRIF%20assay%3A%20development%2C%20evaluation%20and%20implementation%20of%20a%20new%20rapid%20molecular%20diagnostic%20for%20tuberculosis%20and%20rifampicin%20resistance&author=SD.%20Lawn&author=MP.%20Nicol&journal=Fut%20Microbiol&volume=6&pages=1067-1082&publication_year=2011)

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A PROFILE OF TUBERCULOSIS CASES AMONG HIV POSITIVE PATIENTS IN NAVI MUMBAI.

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ABSTRACT

The purpose of the study was to find the prevalence of extra pulmonary and pulmonary TB in the area of Navi Mumbai 113 adult TB patients having confirmed HIV sero-positivity were screened for the current study. The diagnosis of TB was based on clinical impression and relevant investigations including CBC with ESR, Mantoux test, Chest X-ray, USG Abdomen and Pelvis, sputum smear AFB examination in case of pulmonary symptoms, Fluid analysis and studies Bac-T Alert methods, Fine needle aspiration cyto (FNAC), Real-time PCR (Light cycler Roche 480) and CT scan. Prevalence of tuberculosis in HIV -positive patients in this study was (43.3%). Extra pulmonary TB (61%) was found to be more common than pulmonary TB (39%). There is a high prevalence of extra pulmonary TB in HIV patients in this area of Navi Mumbai. The study also inferred that patients with relatively high CD4 counts should be essentially screened for tuberculosis.

Keywords: Extra pulmonary TB, HIV, Real time PCR, CD4 counts

INTRODUCTION

Mycobacterium tuberculosis (MTB) and Human Immunodeficiency virus / Acquired Immunodeficiency deficiency remains the most common infectious disease in resource limited countries. Mycobacterium tuberculosis – HIV co infection causes therapeutic and diagnostic challenges and in turn poses immense pressure on health care agencies in such countries where the populations of coinfected individuals are ever increasing. At least one-third of the 33.3 million people living with HIV worldwide is infected with TB. Persons co-infected with TB and HIV are 20-30 times more likely to develop active TB disease than persons without HIV[1]. In patients dually infected with Mycobacterium tuberculosis and HIV, both the organisms together will accelerate the deterioration of the immune system resulting in early death if untreated. Diagnosis of TB in HIV infected patients may be delayed because of atypical clinical presentations and involvement of inaccessible sites and low sputum smear positivity [2]. In individuals co- infected with HIV and tuberculosis, the lifetime risk of developing tuberculosis is 50%-70% as compared to a 10%

risk in HIV negative individuals [3,4,5]. Thus, because of the very frequent association of tuberculosis and HIV, it has become necessary to look for tuberculosis in HIV infected individuals and vice versa. In this study, we describe a profile of a case series of patients with TB/HIV co-infection, in order to obtain a better picture of the clinical profile of these patients. With the continuing emergence of MDR, XDR and XXDR tuberculosis creating havoc. The current study aims at screening HIV infected patients for co-infection with tuberculosis and to analyse the profile of the TB cases in HIV infected patients attending a tertiary care centre in Navi Mumbai.

METHDOLOGY

113 adult TB patients having confirmed HIV sero-positivity either attending OPD or hospitalized in MGM Medical College and Hospital, Navi Mumbai were included in this retrospective analysis from January 2011 to December 2011. Appropriate ethical clearance was taken from the institutional ethical research clearance committee. The HIV status of the patients was known by an initial screening with HIV Tridot (J. Mitra. And Co., India) further, if found positive, were confirmed by HIV ELISA (J. Mitra, India). Apart from clinical manifestations and history of contact with TB patients, the diagnosis of TB was based on clinical impression and relevant investigations including CBC with ESR, Mantoux test, Chest X-ray, USG Abdomen and Pelvis, sputum smear AFB examination in case of pulmonary symptoms, Fluid analysis and studies Bac-T Alert methods (Becton Dickenson,India), Fine needle aspiration cyto (FNAC), Real-time PCR (Light cycler Roche 480) and CT scan. The RT PCR was performed using IVD approved diagnostic kits obtained from Professional Biotech Ltd (Gudgaon, Haryana, India) Other suggestive investigations were also carried out as when required to establish the diagnosis of TB. The Inclusive criteria for patients data in this study were adult TB patients (>12 years old) diagnosed according to the WHO criteria and with confirmed positive HIV status. Patients with other concomitant non-TB active infections such as fungal or protozoal infection were excluded from this study. All analyses were performed using the Statistical Package for Social Sciences (SPSS version 17.0) by using Chi-square test with 95% confidence level. Values of $P \leq 0.05$ were considered to be statistically significant.

RESULTS AND OBSERVATIONS

Demographic details of the patients:

Of the 920 patients treated for TB in the year 2011 at the tertiary care centre were HIV co infected. There were 30 males and 19 females. Majority of the patients were in the age group of 35 – 59 (69.8%). It is common for patients to be diagnosed HIV-1 positive after developing TB, as shown in our study, 20% of patients confirmed their HIV-1 status after TB was diagnosed.

TABLE 1
TB POSITIVES AMONG SEROPOSITIVE PATIENTS

Total no. of HIV infecected patients	Total no. of TB patients	Males (TB & HIV co-infection)	Females (TB & HIV co-infection)
113	49 (43%)	30 (60%)	19 (40%)

Table 2
DISTRIBUTION OF PULMONARY AND EXTRA PULMONARY TB CASES

Total no. of TB patiens	Pulmonary TB cases	Extra Pulmonary TB cases
49	19 (39%)	30 (61%)

Table 3
DISTRIBUTION OF EXTRA PULMONARY CASES FROM DIFFERENT SAMPLES

Extra pulmonary TB cases	Cervical TB Lymphadenopathy	Pleural effusion	Abdominal TB	CNS TB	Miliary TB
30	14 (47%)	6 (20%)	4 (13%)	3 (10%)	3 (10%)

Table 4
CORRELATION BETWEEN POSITIVITY OF DIFFERENT TESTS

Total no. of Pulmonary TB patients	Sputum smear AFB positive cases	Bac- T Alert	PCR
19	6	19	19

Table 5
CORRELATION OF NO. OF CASES WITH CD4 COUNTS

CD4 counts	HIV cases (113)	No. of TB cases (49)	No. of Pulmonary TB cases (19)	No. of EPTB cases (30)
>500	37	04 (11%)	1	3
450-499	18	04 (22%)	2	2
350-449	31	16 (52%)	6	10
250-349	20	18 (90%)	6	12
<249	07	07 (100%)	4	3

DISCUSSION

In the present study, only pulmonary TB (PTB) was seen in 19 (39%) patients, while only EPTB was seen in 30 (61%) patients (three- military, 14- lymph node, three- CNS, 10- abdominal) which is similar to the study conducted by Sharma et al [6]. Out of 19 TB patients having pulmonary involvement sputum smear AFB was positive only in 6 (31.5%). Out of 6 sputum smear positive patients, 3 cases (50%) had 1+ positivity; one (16%) had 2+ positivity; one (16%) had 3+ positivity while scanty bacilli were seen in one (16%). Rest of 13 sputum negative cases, were positive by Bact-T Alert culture for Mycobacterium tuberculosis. Mantoux test was done in 113 patients and its positive results are 65. Among EPTB, cervical lymphadenopathy was seem in 14 (47%) patients followed by Pleural effusion 6 (20%), and abdominal TB4 (13%) patients. Other forms included CNS TB 3 (10%). Disseminated TB was seen in 3 (10%) patients. Dharmshale *et.al* found 47.5% incidence of EPTB among HIV positive patients [7]. Giri *et.al* found the prevalence of pulmonary tuberculosis among HIV positive patients to be 17% [8]. Patel *et.al* reported that 40% had only pulmonary TB (PTB), 46% had pulmonary and extra-pulmonary TB (EPTB), 10% had only EPTB and 4% had multisystemic EPTB [9]. Sandgren *et. al* in his descriptive analysis of extrapulmonary TB in EU/EEA countries reported that Extrapulmonary TB accounted for 19.3% of all notified cases [10]. Kingkaew *et. al* in his study of 769 patients, reported pulmonary TB only in 461 (60%), both pulmonary and extrapulmonary TB in 78 (10%), extrapulmonary TB at one site in 223 (29%), and extrapulmonary TB at more than one site in seven (1%) patients[11].

CONCLUSION

Prevalence of tuberculosis in HIV-positive patients in this study was (43.3%). Extra pulmonary TB (61%) was found to be more common than pulmonary TB (39%), which is usually the commonest in HIV negative individuals. Also among extrapulmonary TB cervical lymph node involvement was more common (47%). This study also showed that CD4 counts of greater than or equal to 500 were also positive (11%) for tuberculosis. So, even patients with relatively high CD4 counts should be essentially screened for tuberculosis.

REFERENCES

1. www.who.int/tb/challenges/hiv/factssheet_hivtb_2011 (Accessed 19.09.2013)
2. Deodhar L, Gogate. HIV Status of Culture positive tuberculosis cases: a study of 542 cases. Indian J Med Microbiol 1998; 16:84-85.
3. Havlir DV, Barnes PF. Tuberculosis in patients with human immunodefiency virus

infection. New England Journal of Medicine (1999); 340(5):367-73.
4. Zumla A. Impact of HIV infection on tuberculosis, Postgraduate medical Journal 2000; 70:259-68.
5. Perriens JH. Pulmonary tuberculosis in HIV-infected patients in Zaire. A controlled trial of treatment for either 6 or 12 months. New England Journal of Medicine 1995; 332(12):779-86.
6. Sharma SK. Extra Pulmonary Tuberculosis, Indian J Med Res 2004;120:316-353.
7. Dharmshale SN, Bharadwaj RS, Gohil AH, Chowdhary AS. "Extra-Pulmonary Tuberculosis in HIV & non HIV patients in a tertiary care Hospital, Mumbai." Indian Journal of Basic & Applied Medical Research 2012; 3(1):205-208.
8. Giri PA, Deshpande JD, Phalke DB. Prevalence of Pulmonary Tuberculosis Among HIV Positive Patients Attending Antiretroviral Therapy Clinic. N Am J Med Sci. Jun 2013; 5(6): 367–370.
9. Patel AK, Thakrar SJ, Ghanchi FD. Clinical and laboratory profile of patients with TB/HIV coinfection: A case series of 50 patients. Lung India. 2011; 28(2): 93–96.
10. Sandgren A, Hollo V, Werf M J. EXTRAPULMONARY TUBERCULOSIS IN THE EUROPEAN UNION AND EUROPEAN ECONOMIC AREA, 2002 TO 2011. Eurosurveillance March 2013; 18(12): 21.
11. Kingkaew N et al. HIV-associated extrapulmonary tuberculosis in Thailand: epidemiology and risk factors for death. International Journal of Infectious Diseases November 2009; 13(6):722-729.

Review Article :

Diagnosis and Management of Tuberculosis in Children

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Abstract:

The global annual incidence of tuberculosis is 9.6 million to which India contributes 2.2million cases. Continued detection of childhood TB to the tune of half a million cases per year indicates ongoing transmission at a higher rate in India, coupled with problems of malnutrition, HIV, poverty, illiteracy, lack of awareness, poor detection rate, noncompliance, apathy, so on and so forth. TB in children is often extra-pulmonary unlike adults. The subtle symptoms and signs, variable presentations make it difficult to diagnose at grass root level in the community due to lack of experience among general practitioners, besides poor facilities for early detection and treatment. The whole gamut of childhood tuberculosis – clinical, diagnostic and therapeutic aspects has been updated in this review.

Key words:

Pediatric tuberculosis, Latent TB, Extra-pulmonary TB, Mantoux test, Gastric aspirate, AFB, GeneXpert, AKT, HIV

Introduction:

Confronted with an alarming situation, the WHO declared TB as global emergency (1993).1/3rd of world's population are infected with My.TB, with an annual burden of 5, 30,000 children which is 6% of the global burden. 74,000 children died from TB in 2012, excluding HIV. TB in children always points to the recent transmission, not controlled as yet. Diagnosis of one child TB indicates 10 times adult cases in community – a tip of the iceberg. Under-5 children are the most vulnerable group. Most conventional tests are of low specificity and low positive predictive value. Still we have inadequate commitments towards the problem, wanting in services, poor detection and management commitments and an over-reliance on BCG, a false sense of security. Epidemiological investigations are of prime importance to establish at risk children as clinical diagnosis often delayed, compounded with late attention to symptoms in children. Low sensitivity of microscopy, slower process of culture and sensitivity, non-specific shadows in chest X-Ray and imprecise tuberculin skin testing compound the problem [1].

Symptoms:

Fever, loss of appetite, weight loss, night sweats, cachexia are known features of wasting diseases like tuberculosis. Diagnostic criteria for Pediatric TB: Specimen (Sputum / Gastric / Nasopharyngeal aspirate) positive for AFB or culture; or 2 or more of the following: (a) Contact history, (b) Cough for more than 2 weeks, (c) Weight loss more than 5% within last 3 months, (d) Reactive Mantaux (Mx), (e) Radiographic finding compatible with TB and (f) response to AKT (Indicated by improvement in weight by >10% in 2 months and decrease in symptoms). The triad of positive Mx, suggestive CxR, and history of contact are most predictive [2]. Specific focus on paediatric TB diagnosis should include all attempts to isolate AFB from GA / sputum / BAL / NPA. The Tuberculin Skin Testing (Mantoux test) to be done using 2 TU and considered positive with induration 10 mm or more after 72 hours. There is no role for sero-diagnosis as well as non-validated in-house PCR.

Bacteriology:

In one study, Induced sputum [3] among under 12 children with 3% saline nebulisation, 8 were found AFB positive out of 29. To obtain gastric aspirate, a feeding tube is left in situ overnight; gastric contents aspirated in morning while child still asleep without disturbing him/her. Yield 75% AFB positivity. Other body fluids,aspirates: Yield less than 50%. BAL in 36 children: 43%. ZN stain: AFB +Ve confirmatory, Culture takes 6-8 weeks, BACTEC MGIT960, fluorescence sequencing 3-14 days whereas GeneXpert on same day with sensitivity report [4].

X'Ray:

Variety of pictures are seen. One study revealed mediastinal lymphadenopathy (27%),adenopathy with consolidation (23%); consolidation with collapse and segmental hyperinflation (17%); miliary TB (11%); cavitary lesions (10%) and pleural effusion (12%).Primary TB as initial infection commonly seen in children. Also more of collapse and consolidation is seen in children. Re-activation or secondary TB is usually seen in adults.

Fig. 1: Primary complex. Heavy hilar and para-tracheal shadows, prominent B-V markings extending towards periphery

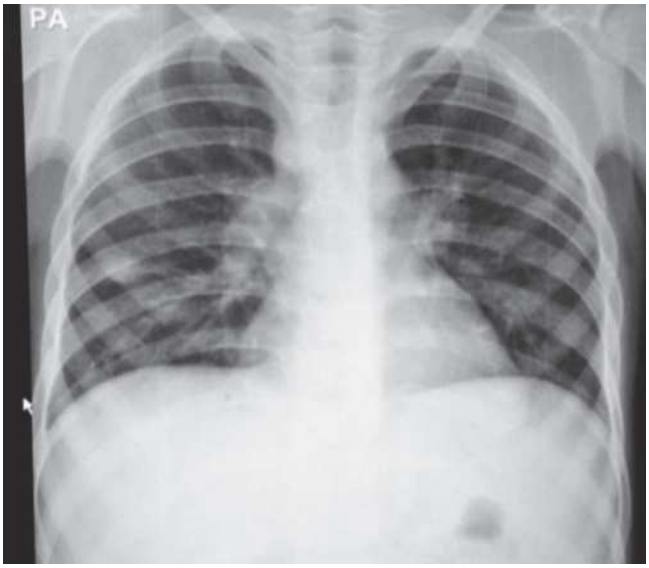
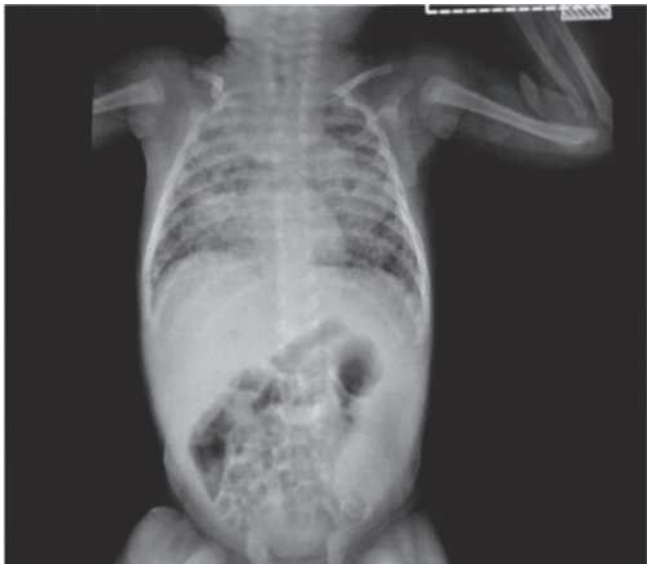


Fig. 2: Miliary & disseminated TB in one month old infant. Lung parenchyma show multiple modularity and interstitial infiltrations. Hepatosplenomegaly, Dilatation of bowel loops evident. Mother open case of pulmonary TB



Extra-pulmonary TB:

Tuberculosis can involve any system. In children, extrapulmonary TB constitutes to the tune of 60 to 80 percent. The scenario is fast changing with advent of HIV/AIDS. The presenting symptomatology would vary depending upon the system involved.

Abdominal TB:

Besides constitutional symptoms, presenting symptoms are variable, often non-specific. While weight loss, nausea, vomiting, diarrhoea predominate in paediatric series, cases of intestinal obstruction, acute pain abdomen mostly reported to surgical facilities [5].

Symptoms	Number	Percentage
Fever	8	18
Weight loss	38	83
Pain in abdomen	42	92
Nausea /Vomiting	10	22
Bowel disturbances	34	75
Constipation /Obstipation	16	35
Diarrhoea	4	9
Constipation alt diarrhoea	14	31
Fullness after food	12	26
Abdomonal distention	12	26
Barborygmi	10	22

Fig. 3: Ulcero-hypertrophic circumferencial lesions on colonoscopy

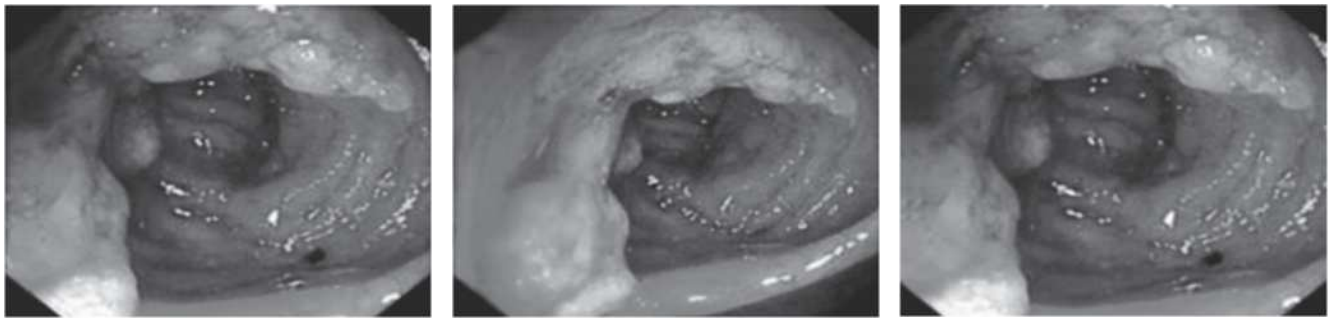
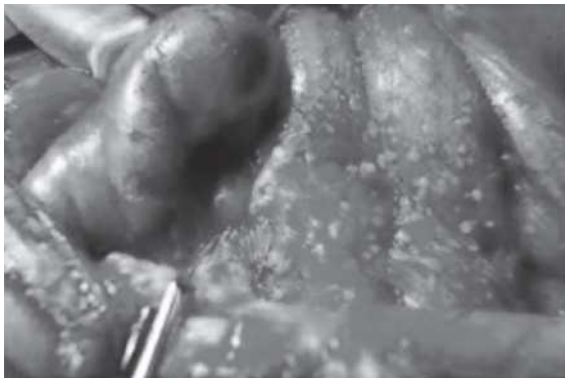


Fig. 4: Multiple tubercles studded on the surface of visceral peritoneum covering bowel loops.



Tuberculous Meningitis:

Symptoms:

Headache, Anorexia, Nausea, Vomiting, Restlessness, irritability, altered sensorium, Fever, myalgia, tachypnoea, tachycardia, Photo-phobia, neck-rigidity, Stupor, coma,seizures, back-pain.

Seizures: (Focal or generalized)

Due to cerebritis, tuberculoma, meningitis, hydrocephalus, infarction, vasculitis, electrolyte imbalance. Seizure which are difficult to control, often associated with bad prognosis.

Signs:

A. Signs of Meningeal irritation: Nuchal rigidity, Kerning's sign (Flexion of hip at 900 with subsequent pain on extension of leg), Brudzinski sign (Involuntary flexion of knees, Hip follows flexion of Neck while supine). These signs may not be evident in infants.

B. Signs of increased ICT and hydrocephalus: Irritability, headache, vomiting, bulging A.F., widening of cranial sutures, sunset sign, hypertension and bradycardia, apnoea or hyperventilation, stupor, coma, papilledma suggest chronic process, SOL e.g. - brain abscess / sub-dural effusion. Optic atrophy may be encountered.

C. Cranial Nerve Involvement (III, IV, VI, VII): Due to focal inflammation, vasculitis and raised ICT (False localizing signs).

D. Focal neurological deficit

E. Miscellaneous manifestations: Altered Mental Status and decreased sensorium due to raised ICT, hypoxia, lethargy, irritability, stupor, and coma are bad prognosis factors.

Photophobia, cranial nerve involvement, bulging fontanels, separation of sutures and UMN signs are common.

CSF:

- Pressure increased
- Cloudy, cob-web formation
- Cells increased, usually > 10 - 500 / HPF (Lymphocytes)
- Proteins > 40 mg % (Significantly high, can be 100mg% to 5,000 mg%)
- Sugar < 40 mg % (Range 36% - 56%)
- Gram Stain, C /S, AFB, GeneXpert

CT / MRI:

May reveal ring enhanced lesions suggestive of tuberculoma, ventricular dilatations, basal exudates, vasculitis, cerebritis and infarction.

Latent tuberculosis [7]:

After inhalation, most children remain asymptomatic, do not develop active disease, but LTBI; Mx+ve. In-vitro tests measure IFN-y response to T-cell stimulation by My TB Antigens: Protein 10, Antigen target-6.

Untreated infants with LTBI have 40% chance of developing active tuberculosis.

At risk for active TB who deserve treatment are required to be identified on merit for treatment. The risk factors of LTB for progression to active TB are:

1. Age < 5 years, infected recently (< 2 most vulnerable). Have higher risk for progression.
2. Risk of progression decrease through childhood until adulthood when increases again.
3. Infants and children likely to have life threatening TBM, disseminated disease
4. Children have more years at risk to develop disease than adults.
5. Adolescents and young adults, immune compromised, HIV, Malnutrition, associated CRF, diabetes, Silicosis and Mx conversion within last 1-2 years.

Tuberculin test:

My. TB PPD, 1, 2, 5 TU; intradermal,

marked. Read after 72 hrs for induration (mm) measured horizontally.

Interpretation:

- 5 mm +ve in recent contact / HIV / abn CxR
- 10 mm in infants / drug addicts / health workers
- 15 mm in all other, even without any risk factor.

Same interpretation if has had BCG.

BCG test is no longer used for diagnosis of TB. In-vitro assessment of gamma-interferon production or testing for CMI may replace Mx test in future.

IGRA:

Preferred in BCG recipients and children aged more than 5 years:

1. T-SPOT.TB measures number of lymphocyte / monocytes producing IFN-y
2. QuantiFERON-TB measures whole blood IFN-y. Do not contain Agn of My. Bovis & My. Avium from Environment. Hence Higher specificity as compared to Mx TST.
3. Only one patient encounter Vs. twice with Mx (Mx preferred in < 5 year olds)

Indications for undertaking advanced tests:

1. for diagnosis of PTB: Xpert MTB/RIF should be used as an initial test for suspected cases and MDR TB with HIV (Depending on resources).
2. for diagnosis of extra-PTB: Xpert MTB/RIF is preferred to conventional microscopy and CSF culture in suspected TBM.
3. Xpert/RIF are considered as an alternative test for non-respiratory specimens.
4. Lymph-node biopsy, FNAC and BAL for AFB resorted to for tissue diagnosis.

Rational approach in cases of children:

1. Liquid BACTEC-MGIT960 C/S and molecular based My.TB NAAT for early diagnosis
2. Combined AFB + Culture, clinical exam; algorithm based approach reliable.
3. High index of suspicion in the appropriate clinical setting is the key

Certain important definitions [8] on treatment out-come must be understood:

1. Cured: Bact +ve at initiation, proved to be smear/ culture - ve in last month of treatment
2. Treatment completed: Completed treatment but My.TB positivity report in last month N/A
3. Treatment failure: Positive for My. TB for 5 months on therapy.
4. Lost to follow-up: Did not receive ATT or has interruption for 2 months or more.
5. Drug resistant (Mono): To at least 1st line drug
6. Poly-resistance: To > 2 drugs other than INH, RIF
7. MDR TB: Resistant to INH and RIF
8. XDR TB: Resistant to any one of fluoroquinolone, one 2nd line injectable and INH, RIF
9. Primary drug resistance: Resistance in patient who never took ATT in past
10. Acquired resistance: in view of previous treatment, may be infection with resistant TB

Drug therapy [1, 9] according to Revised Categories (2013):

I. New case (Smear +ve / -ve PTB / extra-pulmonary): = 6 months therapy

Intensive phase: 2 H3R3Z3E3 = 2 months

Maintenance phase: 4 H3R3 = 4 months

II. Re-treatment (Relapse, fail to respond or lost to follow-up) for 8 months

Intensive phase: 2 H3R3Z3E3S3 + 1 H3R3Z3E = 3 months

Maintenance phase: 5 H3R3E3 = 5 months

Daily dosing is preferred in paediatric practice which is also being tried in 5 different states under RNTCP. Intermittent therapy in maintenance phase for HIV uninfected under DOTS (Supervised) is reasonable. However, daily dosing advocated for initial 2 weeks (5).

Supplimentation of 5 to 10 mg Pyridoxine has been recommended by WHO [10], although there is no such recommendation under RNTCP within DOTS.

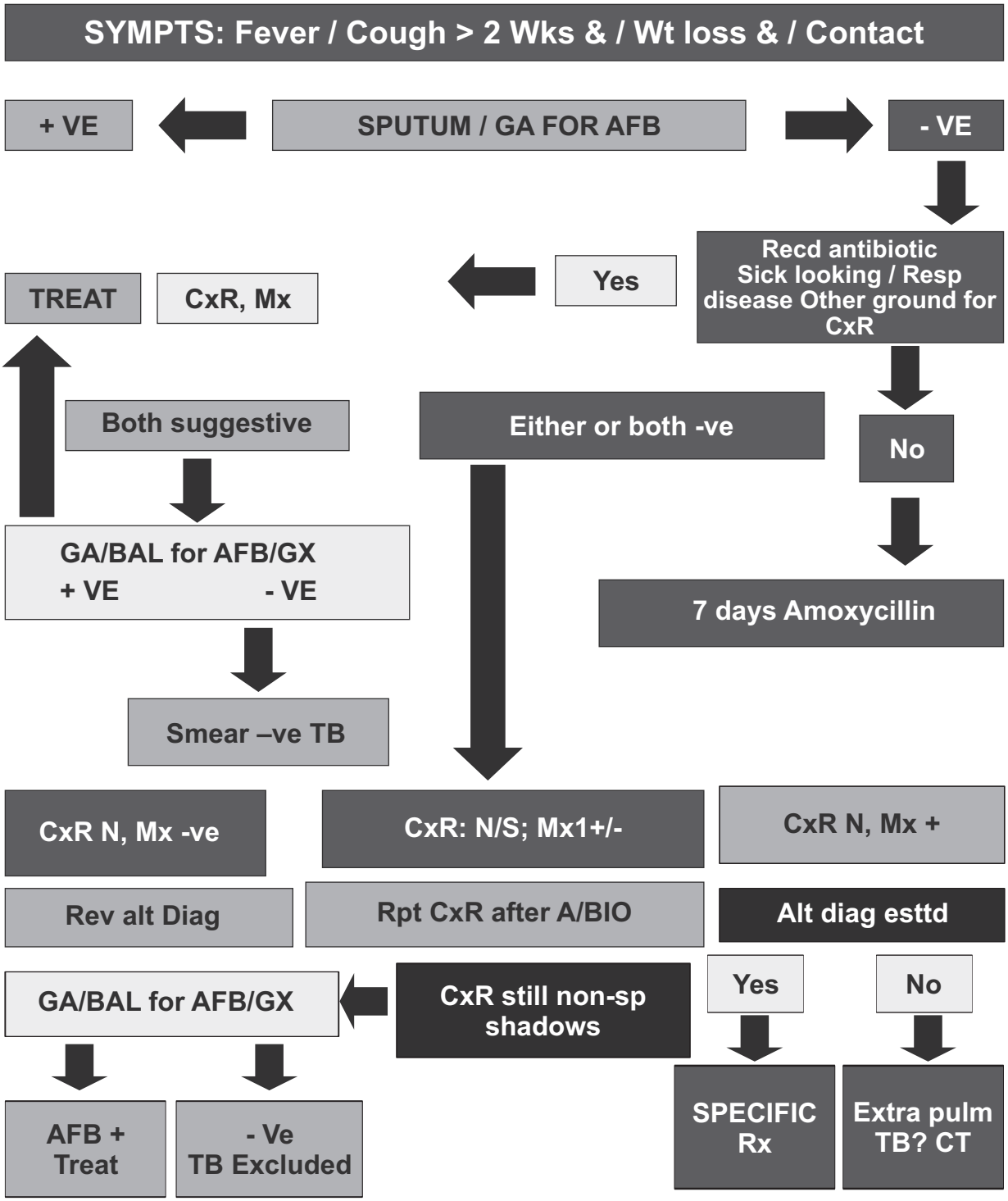
Table 2: First line Anti-TB drugs and their doses for children

Drugs	**Dose** (National Guideline)	**Dose** (WHO Guideline)
INH	10 mg/kg	10(10-15)mg/kg
RIFAMPICIN	10-12 mg/kg	15(10-20)mg/kg
ETHAMBUTOL	20-25mg/kg	25(15-25)mg/kg
PYRAZINAMIDE	30-35 mg/kg	35(30-40)mg/kg
STREPTOMYCIN	15 mg/kg	15-25 mg/kg

Difficulties in treating Paediatric TB cases under DOTS:

1. DOTS regimen is not preferred for seriously sick children, TBM, HIV, intestinal TB, hepatitis where supervised daily dosing in hospital is desirable. WHO recommends daily regimen in areas of high HIV prevalence (If more than 5% among adults; and more than 1% among pregnant ladies).
2. If there are persistent symptoms and signs, extend the intensive phase by 1 month and maintenance by 3 months. Also in disseminated TB, CNS, LN, bone TB.
3. Conventionally, the Clinicians' Choice have been: For TBM & LN: 1 Year and for Bone TB: 1 ½ years.
4. Studies on short-term chemotherapy were originally conducted for pulmonary tuberculosis among adults. Subsequently the results were extrapolated to extra-pulmonary forms and children.
5. Very few studies are available on children with long-term follow-up, especially on extrapulmonary TB.
6. There are certain other genuine difficulties peculiar to children in DOTS. TB in children is generally pauci-bacillary in nature with a higher proportion as extra-pulmonary TB.
7. Training of doctors and staff in specimen collection from children remains much needed.
8. Pediatric formulations, particularly liquid preparations and appropriate dosage forms (Being resolved) are not available under DOTS.
9. Regarding duration of therapy, particularly extra-pulmonary types, STC is not preferred.
10. Notification, treatment with due attention, sincerity and seriousness by family as well as by practitioners with standard regimen are important.

FIG. 7. ALGORHYTHM FOR DIAGNOSIS AND MANAGEMENT OF TUBERCULOSIS IN CHILDREN



Chemoprophylaxis:

Serves 2 goals: reduce reservoir, protect young from active disease.

INH prophylaxis - 9 months reduce risk by 90% (6 months by 26-90%; 3, 6,12 months by 31,63, 93%) (Cochrane DB syst rev.2000(2) Z:CD001363). RNTCP recommends INH 10mg / Kg OD for 6 months (For 9 months in US). Recently, a 12 dose combination therapy of Rifapentine + INH over 3 months duration has been tried [11].

Indications:

Symptomatic children below 6 months, babies with sputum +ve contacts (Irrespective of Mx); First rule out active TB.

All HIV +ve who were exposed to sputum +ve TB case; or having Mx > 5mm

Mx +ve children on immunosuppression and Neonate born to sputum AFB +ve mother.

Managing Drug induced hepatitis [12]:

Indicated by appetite loss, nausea, vomiting, icterus. May result in acute liver failure (ALF)

in 2-39% cases. Rule out other causes of ALF. Out of 5, three drugs (INH, RIF, RBN,

PZM) induce liver enzymes. ALT > 1.5 times rise is usual; Upto 3 times of upper limit of normal range doesn't warrant discontinuing AKT. Withheld only if ALT rise > 5 times of ULN. In severe disease (TBM, miliary): SM, OFL, EMB. LFT every 3rd day. If ALT < 3 timesof ULN, restart AKT. Start Rifampicin at lowdose, increase after 3 days. Add INH, dose subsequently hiked. S2E2H2 + E10H10 if RIF induced hepatotoxicity. E9R9Z9 in case INH induced hepatotoxicity. H9R9 if PZM not used in initial induction phase.

Newer Anti-TB drugs:

Most are in Ph-III / IV clinical trials. Expected to be more potent, reduce duration, inhibit new targets to be suitable for MDR TB; must be compatible with existing AKT and ART drugs, having no antagonism. Exploring newer uses of existing anti-micobials: Fluoroquinolones, Rifamycins, Riminophenazines, Clofazimines, meropenem / imipenem plus Clavunate combinations, Oxazolidinones (PA 824), SQ 109, Sutezolid (PNU 100480).

Approved for use: (Both included in WHO essential drug list, 2015)

1. Bedaquiniline (Diarylquinoline derivative). Being provided by RNTCP to XDR TB on close monitoring and on limited trial basis. 2. Dalamanid (OPC 67683)

Vaccines:

At present, BCG is the only vaccine available for prevention of TB. However, the existing data indicates that it prevents only 5% of the vaccinated individuals from TB associated fatalities. Though BCG is the most widely administered of all vaccines, TB still continues to scourge human life. It was found to be protective against the meningeal TB in young children. Nevertheless, its efficacy in preventing adult pulmonary TB which is responsible for the major burden of morbidity and mortality varies dramatically. In carefully conducted studies throughout the world, BCG efficacy varies from 0% in Chinglepet, India to 80% in the UK. Of late, several vaccines are under trial. They are for pre-exposure and postexposure varieties. Recombinant BCG (rBCG), live attenuated, hybrid sub-unit, protein adjuvented, vector delivery dependent and booster vaccines are under active clinical trial.

Conclusion:

After Small-pox and polio, the world aims at eradicating tuberculosis by 2035. It is a herculean task no doubt, although not impossible. It's success depends on intensive and sustained case detection, besides cent percent drug compliance at all levels to stamp outthe reservoir of infection. Chest physicians usually manage TB patients. Their availability is scarce. Physicians and general practitioners usually avoid handling TB cases for obvious reasons, taking excuses like lack of

training and logistics. The childhood TB, mostly extrapulmonary and LTB, are handled by pediatricians. Their number and presence are very limited in rural India, looking at 42% of our population being children. Hence intensive campaign is required not only to train all our medical students, interns and general practitioners, but also initiate a mass movement at the grass root level, the way we could involve our school and college teachers, students, village panchayats, Government officials, Armed Forces in case of Polio for ensuring success. Training modules need to be developed on priority for expeditious implementation on voluntary and PPP mode inaddition to concerted efforts at Government establishments and all medical colleges. Otherwise the 'Stop TB by 2035' is likely to remain as a distant dream.

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References:

1. Kumar A et al. IAP National guideline for pediatr TB. Indian Pediatr, 2013Mar; 50:301-6.
2. Gie R. Diagnostic atlas if intra-thoracic TB in children: A guide for low income countries. Internatl Union AgainstTuberculosis and Lung Diseases, 2003
3. Shata AM, Coulter JB, Parry CM et al. Sputum induction for diagnosis of tuberculosis. Arch Dis Child,1996;74(6):1-7.
4. Tortoli E et al. Use of BACTEC MGIT960 for recovery of My TB from clinical specimens. Journal of Clinical Microbiology, 1999;37 :3578-82
5. Sharma SK et al. Abdominal tuberculosis. Indian journal of Medical Research, 2004
6. Faten Tinsa et al. Abdominal tuberculosis. JPGN _ Volume 50, Number 6, June 2010
7. Menzes D, Al Jahdali H, Al Otaibi B. Recent developments in treatment of latent tuberculosis infection. Ind J Med Res. 2011 Mar;133:257-66.
8. WHO. Definitions and reporting frame-work for tuberculosis, 2013 Revision. http;//apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf.
9. WHO. Guidance for national TB programs on management of tuberculosis in children. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Restrive&db=PubMed&dopt = Citation_uis=24999516.214.
10. Pellock JM, Howell J, Kendig EL et al. Pyridoxine deficiency in children treated with Isoniazid. Chest. 1985. May;87(5):658-61.
11. Villarino ME et al. Treatment for preventing tuberculosis in children and adults: A randomised clinical trial of a 3 month, 12 dose regimen of Rifapentine and Isoniazid. JAMAPediatr. 1015 Jan, 2012.
12. Kishore PV et al. Drug induced hepatitis with anti-TB chemotherapy. Challendes and difficulties in treatment. KU Med J, 2007 Apr-Jun;5(2):256-60.

Original Research Paper Medicine

Role of Sputum Induction in Diagnosis of Pulmonary Tuberculosis

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ABSTRACT

To study the role of sputum induction by 3% hypertonic saline and sputum samples subjected to ZN staining method.110 cases having clinico-radiological features of pulmonary tuberculosis with sputum smear negative for AFB were studied. Total of 110 cases were studied. The present study revealed that sputum induction is safe, simple and cost effective method. It also helps in obtaining adequate amount of quality sputum in patients who are smear negative on spontaneously produced sputum or are unable to produce sputum.

KEYWORDS Sputum Induction (SI) , 3% Hypertonic saline , Pulmonary tuberculosis , Zeihl Neelsen staining.

Introduction -

Despite the advent of new tests for the diagnosis of tuberculosis with greater sensitivity than smear microscopy of appropriate sputum samples, about 20-30% of TB patients will not have microbiologic confirmation. This figure may be much higher in children and patients with extra-pulmonary TB or PL-HIV. Although it is recommended that any sample from a suspected TB patient that is initially negative by a rapid diagnostic test be cultured for TB growth and confirmed diagnosis, there will be a group of patients that have TB but without microbiologic confirmation.

Overall 1/3rd of tuberculosis still remains undiagnosed. Rapid and precise diagnosis will reduce the risk of nosocomial &community transmission of TB. Direct sputum smear microcopy remains a fundamental tool of diagnosis, but may be negative up to 50% case of active pulmonary TB[1]. Alternative methods of obtaining sputum specimens are frequently needed in those patients with radiological suspicion of TB who are unable to expectorate or are smear negative. The methods include – sputum induction (SI), bronchoalveolar lavage (BAL) and gastric washings (GW) specimens [2]. These methods have their own advantages and disadvantages and diagnostic yield vary so also safety and tolerability, and feasibility in different set-ups.

GW is reported to reveal the organism only in 25-50% of children with active TB, when a set of three samples are subjected to both microscopy and culture [3]. Bronchoscopy is an invasive procedure, only available in the large hospitals, needs experts for performance, costly and may not be feasible if the large numbers of patients are to be tested. SI is a cheap and non-invasive alternative with a diagnostic yield "same if not better" than bronchoscope[4]. SI with hypertonic saline has been used in the diagnosis of various respiratory disorders. The present study was performed to evaluate the use of SI in establishing the diagnosis in patients with suspected pulmonary TB, who are unable to produce adequate sputum or are found negative on smear examinations.

Aims and objectives –

To study the role of sputum induction in determining the diagnostic yield of the test in diagnosis of pulmonary tuberculosis.

To detect the sputum positive cases in patients who have clinic-radiological evidence of pulmonary tuberculosis.

Methodology -

110 patients with clinical and radiological features suggestive of pulmonary tuberculosis both on Outpatient & Inpatient basis in Mahatma Gandhi Mission Institute Of Health Sciences, Kamothe, Navi Mumbai were involved in the study . Patients aged 18-80 years with features of pulmonary tuberculosis and having spontaneously produced 2 sputum smear negative samples and not able to expectorate or having dry cough/ scanty sputum were included.

Inclusion Criteria:-

Patients between the age group 18-80 years with

- Persistent cough for 3 weeks or more, with or without one or more of the following symptoms are suggestive of pulmonary tuberculosis. [20]
- Weight loss,tiredness,fever particularly with rise of temperature in the evening , night sweats, chest pain, loss of appetite, coughing up of blood and presence of dry cough or scanty sputum radiological pulmonary tuberculosis.

Pattients in whom cough induction was considered hazardous like those with Haemoptysis .,Acute respiratory distress. Unstable cardiovascular status, (arrhythmias, angina).Thoracic, abdominal or cerebral aneurysms.Hypoxia (SaO2 less than 90 % on room air). FEV1 less than 1.0 Litre.Pneumothorax and pulmonary emboli.Fractured ribs or other chest trauma.Recent eye surgery.

Following informed consent, in a well ventilated room reservoir device of nebulizer was filled with 20ml of 3 % hypertonic saline and subjects were asked to inhale mist until 2 ml of sputum is obtained or for a maximum of 20 to 30 minutes .The patient could expectorate the quality sputum within 2 hours. If the patient requires assistance, the health professional wore a TB respiratory protection device (N95 mask) before entering the room and remove it after leaving the room. The

procedure was stopped when: The patient has produced 1-2 ml of sputum for each specimen or15 minutes of nebulization was reached or the patient complained of dyspnea, chest tightness or wheeze.

The sample selected for the study belong to different age groups and it includes males and females as per the following distributions in Table 1, 2 & 3

Table 1: Gender wise frequency distribution of the sample selected.

Gender	Number	Percent
Male	73	66.4
Female	37	33.6
Total	110	100.0

Males in the study are 73 (66.4 %) and females are 37 (33.4 %).The distribution is depicted in the following figure.

Table 2: Age Wise Frequency Distribution of the sample.

Age Group	Number	Percent
18-30	31	28.2
31-40	26	23.6
41-50	18	16.4
51-60	12	10.9
61-70	16	14.5
71-80	7	6.4
TOTAL	110	100.0

Total number of cases in the study is 110,among which 57 (51.81 %) cases were in the age group of 18-40 years ,29 (26.36 %) cases were in the age group of 41-60 years ,24 (21.74 %) were in the age group of 61 -80 years.

RESULTS -

Microbiology results-In all 110 patients suspected to have pulmonary tuberculosis on clinical & radiological basis underwent sputum induction (330 samples). Nearly 2/3rd patients [71 out of 110] had productive cough & remaining 39 had dry cough in whom induced sputum was the only sample available for microscopy. Sputum induction did not yield any sample in 6 patients.

1st Sample positive – 63

2nd Sample Positive – 86

3rd Sample Positive – 87

All Sample Negative – 94

Only 1 Patient was Smear Positive and Culture Negative and 22 patients were Smear & Culture Negative.

Twenty three patients were categorized as 'Probable TB ie patients with symptoms suggestive of TB without microbiological confirmation (sputum smear microscopy, culture and molecular diagnosis), but with but strong clinical and other evidence (e.g. X-ray) may be diagnosed as "Probable TB".ALL 23 had satisfactory clinicoradiological response to empirical antiTB therapy.

From the above data it is clear that there are no positive cases identified through the two trials of testing the spontaneous sputum and in the testing after induction of the same patient, the number of negative cases is substantially reduced to 47 from 110 first, then to 24 and finally in the third testing the number of negative cases is 23.

Table: 3 – Distribution According to Respiratory Pathology

Sr. No	Respiratory Involvements	No. of Patients	Percentage
1	Cavitatory lesion	21	19.09
2	Pleural effusion	10	9.09
3	Consolidation	79	71.81
	Total	110	100.00

Of the 110 study population,23 cases were sputum negative for acid fast bacilli after Ziehl Nelson staining and 11 patients were started on empirical anti TB drugs due to the very clear radiological clues and clinical manifestation ,and 12 were asked to follow up after 15 days if the symptoms persists, of the 8 patients who returned 6 were started on empirical anti tuberculosis drugs and 2 were asked to come back for review after 1 month if the respiratory symptoms persists, when the 4 patients returned for review they were started on empirical anti TB drugs

Discussion -

SI has performed well both in resource-poor and resource-rich countries [5,6]. In these studies, SI provided adequate samples for diagnosis &was cost-effective and about 29-33% patients were smear positive on SI samples. Its usefulness has also been confirmed in children[7] & extrapulmonary tuberculosis[8].However, few studies in developed countries showed that SI added little to overall diagnosis and was deemed costly [9].However , Mc Williams et al [10] et al found that (2002) the culture yield of Fiberoptic Bronchoscopy was only 52 percent and the cost was three times that of doing three sputum induction studies..The authors preferred strategy was to employ SI followed by FOB only in those patients who were negative on sputum induction but had features of pulmonary tuberculosis on chest radiograph. This approach is more practical taking into consideration the cost of missing the diagnosis. Naseem (2006) [11] et al in their comparative study of diagnostic yield of AFB with sputum induction to spontaneous sputum examination in suspected pulmonary tuberculosis found out that in expectorating patients, AFB smear and culture positivity results remain comparable with spontaneous and induced sputum sampling .Sputum induction improves the diagnostic yield for AFB in patients unable to expectorate adequate sputum sample. **Biswas [2]et al --118 (2013).Overall, 32% of patients were** found positive on smear examination after sputum induction. Out of 58 patients who were smear negative with spontaneous adequate sputum, 21 (34 %) were found positive on induced sputum culture examination.SI culture was successful in confirmation of diagnosis in 14 (33 %) out of 42 patients with no / adequate sputum. The sensitivity of smear and culture of induced sputum in diagnosis of pulmonary TB were 80 % and 87 % respectively.

In our study, out of 110 patients undergoing SI, 71 had cough with inadequate sputum while 39 patients had adequate sputum. SI successful in 95 % of patients who could expectorate adequate sputum similar to Gupta et al [12].Overall, 74 .54 % of patients were found positive on smear examination after SI.

Conclusion:

The present study shows that sputum induction is safe, simple and cost effective method of obtaining adequate amount of quality sputum in patients, who are smear negative on spontaneously produced sputum or are unable to produced sputum. The number of negative cases substantially reduced from 110 to &47 to 24 and finally in the third testing the number of negative cases were 23. From this it is clear that after induction it is possible to identify more cases.

Our study is not without limitations. Patients suffering from extrapulmonary tuberculosis &HIV were not assessed. Children were not part of our study .Microbiological samples are more difficult in these groups. Study was carried out in a tertiary-care cantre.The smear yield could have been higher if LED microscopy was used which was introduced

later in our institute.Larger studies are needed to assess its utility in different settings so as to include it in RNTCP.

References

1. American Thoracic Society and Centers for Disease Control and Prevention. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med. 2002;161:1376–95. [PubMed]
2. Biswas S,Das A, Sinha A, Das SK,Bairagya TD. The role of induced sputum in the diagnosis of pulmonary tuberculosis. Lung India 2013;30:199-202
3. Geneva: World Health Organization; 2006. World Health Organization. Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children; p. 371. [PubMed]
4. Anderson C, Inhaber N, Menzies D. Comparison of sputum induction with fiber-optic bronchoscopy in the diagnosis of tuberculosis. Am J Respir Crit Care Med.1995;152:1570–4.[PubMed]
5. Daly JF, Brown DS, Lincoln EM, Wilkins VN . Endobronchial Tuberculosis in children. Dis Chest (1952); 22-35.
6. Citron KM, Thomas GO . ocular toxicity from Ethambutol. Thorax (1986); 41:737.
7. Winter RJD , Banks RA , Collins CMP , Hoffbrand BI , Rifampicin induced light chain proteinuria with renal failure. Thorax (1984); 39: 952.
8. Smith LS , Schillaci RF , Sarlin RF . Endobronchial Tuberculosis. Serial fiberoptic bronchoscopy and natural history . Chest (1987); 85 : 107.
9. Schoch OD, Rieder P, Tueller C, Altpeter E, Zellweger JP, Rieder HL, et al. Diagnostic yield of sputum, induced sputum, and bronchoscopy after radiologic tuberculosis screening. Am J Respir Crit Care Med.2007;175:80–6. [PubMed]
10. McWilliams T,Wells AU, Harrison AC, Lindstrom S, Cameron RJ, Foskin E. induced sputum and Bronchoscopy in the diagnosis of pulmonary tuberculosis. Thorax.2002 Dec;57(12):1010-4
11. Muhammad Atiq-ur-rehman,Arshad Naseem,Tassawar Hussain. Comparison of Diagnostic Yield of AFB With Sputum Induction To Spontaneous Sputum Examination in Suspected Pulmonary Tuberculosis. Journal of the College of Physicians and Surgeons Pakistan 2009,Vol.19(8):506-509
12. Gupta KB, Garg S. Sputum Induction – A useful tool in diagnosis of respiratory diseases.Lung India 2006;23:82-6

Rapid Detection of Mycobacterium Tuberculosis Complex with IS6110 Marker Based on Real Time PCR High Resolution Melting Analysis

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Original Research Article

Rapid Detection of Mycobacterium Tuberculosis Complex with IS6110 Marker Based on Real Time PCR High Resolution Melting Analysis

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ABSTRACT

Mycobacterium tuberculosis *(MTB)* is a major agent of infection for human tuberculosis (TB) worldwide. *Although T.B. treated by a combination of six-monthly course of antibiotics, the rise in prevalence of drug-resistance TB (MDR-TB, XDR-TB) makes the it global health problem.* Molecular methods have a significant role to play in MTB and drug resistance TB. *Recently, high-resolution melting (HRM) Real time PCR analysis was used for amplicon genotyping and mutation scanning.* The present study aims to develop a diagnostic method for MTB detection based on HRM analysis that does not require labeled oligonucleotides. Polymerase Chain Reaction was performed for MTB detection with IS6110 as a target gene. Derivative melting curves of the IS 6110 specific target amplified duplexes were characteristic of the genotype of MTB and non tubercular clinical isolates. On fluorescence analysis, Light cycler 480 gene scanning software analyzes and distinguishes presence or absence of MTB depending on the shape of the curve. We collected 100 suspected clinical isolates, including 10 non tuberculosis cases. These samples were analyzed for acid-fast bacilli (AFB), culture & Real Time PCR. The sensitivity and specificity of detecting MTB by HRM Real Time PCR was 98.51% and 93.94% respectively. This technique requires the unlabeled primers specific to the target sequence and a DNA specific intercalating dye which can prove a promising method for detection of Mycobacterium tuberculosis.

Keywords: *Mycobacterium tuberculosis*, HRM, RT PCR, IS6110.

INTRODUCTION

Tuberculosis is known to cause significant mortality all over the world. World Health Organization has recognized India as a serious hot-spot region for tuberculosis and as the leading cause of death. [(1,2)] Detection of MTB in clinical isolate is important for the definitive diagnosis of tuberculosis. Microscopic examination needs a comparatively detectable amount of bacterium in specimens. The gold standard method of identifying mycobacteria is through mycobacterial culture. Though culture based detection is a sensitive method; a period of 4 to 8 weeks is necessary for definite diagnosis. [(3)] The development of diagnostic tests that are rapid, sensitive and specific for recognition of the causative agent of tuberculosis is essential to successfully control the disease. Conventional MTB PCR, targeting IS6110, TRC4, or hsp60 genes sequences have been extensively evaluated as an alternative for rapid diagnosis of tuberculosis. [(4)] To demonstrate the diagnostic potential of real-time PCR for detecting MTB in sputum specimens, we

performed AFB staining, Culture and real-time MTB PCR assays using 100 Clinical Isolates. The sensitivity and specificity of these methods were calculated using mycobacterial culture results as the reference point. [5] High-resolution melting (HRM) curve study is a novel, accurate and simple technique for analyzing the genotype of *Mycobacterium tuberculosis* without the need for specific probes. The dye LC Green, SYTO9, or Eva Green saturates amplified DNA, SYBR green dye, during identical melting curve analysis. [6-9] HRM curve analysis generates a difference plot curve, which analyzes nucleic acid sequences with high accuracy. Application of genotyping by HRM curve analysis has been followed for detection of much drug resistance case. [10] The aim of the study described here was to develop a useful molecular technique for the detection of M. *tuberculosis* in an accurate, fast and cost effective manner. [6-9] HRM dye could successfully detect IS6110 amplified product; a signature for MTB and at the same time did not inhibit or adversely affect PCR. In this article, we showed the usefulness of applications of HRM dye in MTB detection that involve high-resolution melting curve analysis of HRM RT PCR for detection of MTB. We also demonstrate the fluorescence normalization. Difference plot analysis was distinguished by shape of the curve and/or specific position as a useful method of differentiating MTB and non-MTB cases.

MATERIALS AND METHODS

Specimens: Clinical samples were obtained from patients with strong clinical symptoms of TB including clinical response to antitubercular treatment referred from different clinics like jay Clinic & MGM Hospital, Kamothe, MGM Central Research Laboratory. In this study, 90 sputum samples were obtained from adult pulmonary TB patients. 10 sputum samples were obtained from nontuberculous individuals (chronic asthmatics, chain smokers) initially screened by (-)ve AFB smear examination and chest X-ray were used as negative controls. Samples were processed immediately after collection. Samples were decontaminated and concentrated by modified Petroff's method. [11,12] Briefly, the samples were decontaminated by mixing 4% NaOH with an equal volume of sputum sample, mixed and placed in water bath (37°C) for 1 hour with intermittent shaking. The sample was then centrifuged at 3500 *g* for 30 minutes, the pellet was resuspended in 1 ml of 1× phosphate buffered saline (PBS). To inactivate the bacteria, the samples were kept at 56°C for 30 minutes in dry bath. From the pellet obtained, smears were prepared for AFB staining & other part of inactivation of samples was confirmed by culturing in LJ medium slant which were incubated at 37°C for 4-6 weeks and the inactivation found adequate. The inactivated sample was stored at -20°C until DNA extraction. DNA extraction was performed using QIAamp blood DNA extraction kit (Qiagen, Hilden, Germany) as per manufacturer's instructions. [13,14]

DNA preparation: Processed sputum samples were used for DNA for positive control was obtained from solid cultures of a *M. tuberculosis* reference strain (H37RV) and *M. Bovis, M. kansasii* laboratory-identified strain grown on Lowenstein Jensen (LJ) agar medium. DNA extraction was performed using QIAamp blood DNA extraction kit (Qiagen, Hilden, Germany) as per manufacturer's instructions. [13,14] DNA extraction was carried as described above.

Primers and PCR conditions: The primer sequences used are IS-F 5'-CCTGCGAGCGTAGGCGTCGG-3' and IS-R 5'-CTCGTCCAGCGCCGCTTCGG-3'.

Given Primer set was previously described for MTB detection. [15] Two primer sets, IS–F & IS-R and were used to amplify123 bp (65%GC). Genome DNA (5 ug) was added to 25 ul of a reaction mixture

containing 5 uM primers each of *IS-F & IS-R* and ingredients of the Light Cycler® 480 High Resolution Melting Master Mix. The PCR was performed on a LightCycler®480 Instrument II, 96-well. The Positive control for each reaction consisted of two tubes of DNA of *M. tuberculosis* H37Rv. The PCR cycles were run according to the following conditions: for amplification of the 123bp fragment of*IS6110*, 1 cycle of 95°C for 10 min and 45 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s; final elongation at 72°C for 7 min, and finally cooling to room temperature. Prior to HRM analysis, the products were heated to 95^0C for 1 min and then cooled to 40^0C for 1min. HRM was performed from 60 to 95^0C, rising at 1^0C/s with 25 acquisitions per degree. Light Cycler® 480 Gene Scanning Software was used for HRM curve analysis. The melting curves were normalized and temperature shifted to allow samples to be directly compared. Difference plots were generated by selecting a negative control/Positive control, converting the melting profile to a horizontal line and normalizing the melting profiles of the other samples against this sample. Significant differences in fluorescence from the horizontal baseline were indicative of nontubercular Isolates. Differences were judged as significant if the amplified DNA isolates fell outside the range of variation seen in the wild-type samples. The software analyzes the difference in the shape of the melting curve for a sample from the shape of the melting curve for the control strain (M. tuberculosis H37Rv) to detect sequence variants and generates a difference plot curve, which helps cluster samples into groups that have similar melting curves .The operator was blinded to the phenotype and genotype MTB Clinical Isolates data.

Statistical analysis: The data obtained were statistically analyzed for diagnostic values (Sensitivity, Specificity, positive predictive value, negative predictive value).The sensitivity, specificity, and the diagnostic odds ratio of different detection methods were calculated by MedCalc®. The performances of the HRM *IS6110* Real-Time PCR, Culture and AFB smear were compared statistically.

RESULTS

For optimization of PCR amplification, we used MTB H37Rv & *M. bovis* BCG genomic DNA as a template. For HRM Master Mix annealing temperature of 60^0C of stopped formation of primer dimers and improved the sensitivity of amplification to as much 100 copies of genomes. 5ug/ml DNA concentration was used in each reaction mixture. In that we tested a range of MgCl2 concentrations for each Master Mix (range of 2 to 5 mM tested in 0.5 mM increments) and finally decided on using 3 mM for all reactions. A primer concentration of 5 pmol per 20ul reaction mixture (5uM) (2.5 to 25 pmol tested), an elongation time of 40 s (20 to 60 s tested), an annealing time of 30 s (5to 50 s tested), and an annealing temperature of 60°C (58 to 62°C tested) allowed detection.

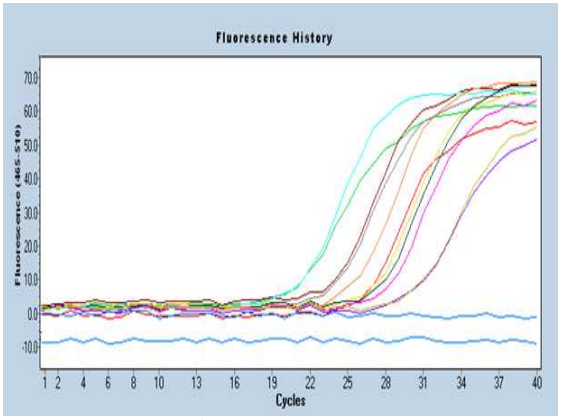

Figure 1-Detection of *IS6110* gene using HRM technique in LC480II. Amplification curves indicate positive control with a number of positive *IS6110* gene detection samples. Negative control did not show any amplification curve.

High-resolution melting curve analysis (HRM) is a recently developed technique for rapid and efficient screening of mutations in nucleic acid samples. This post- PCR method requires only the usual unlabeled primers and a ds DNA binding dye which detects subtle genetic

alterations in PCR- amplified samples based on their strand dissociation behavior. For further confirmation, samples were analyzed by 10% Poly-Acrylamide Gel Electrophoresis (PAGE) followed by silver staining of gel after run using standard methods.

For the HRM master mix kit, detection of only a single product, as analyzed by Tm, was evident. The Tm values for HRM Roche Kit were 88.5+/-0.5^0C (Fig.2-A). This Tm value corresponds to the IS6110 gene product (123bp) as determined by melt curve analysis (Figure 2A). Analysis of PCR products of IS6110 by PAGE demonstrated that HRM master mix for IS6110 gene targets were being amplified (Figure 3).The peaks showing the melting curve profiles suggested that only the IS6110 gene was being amplified for most of the reaction conditions tested.

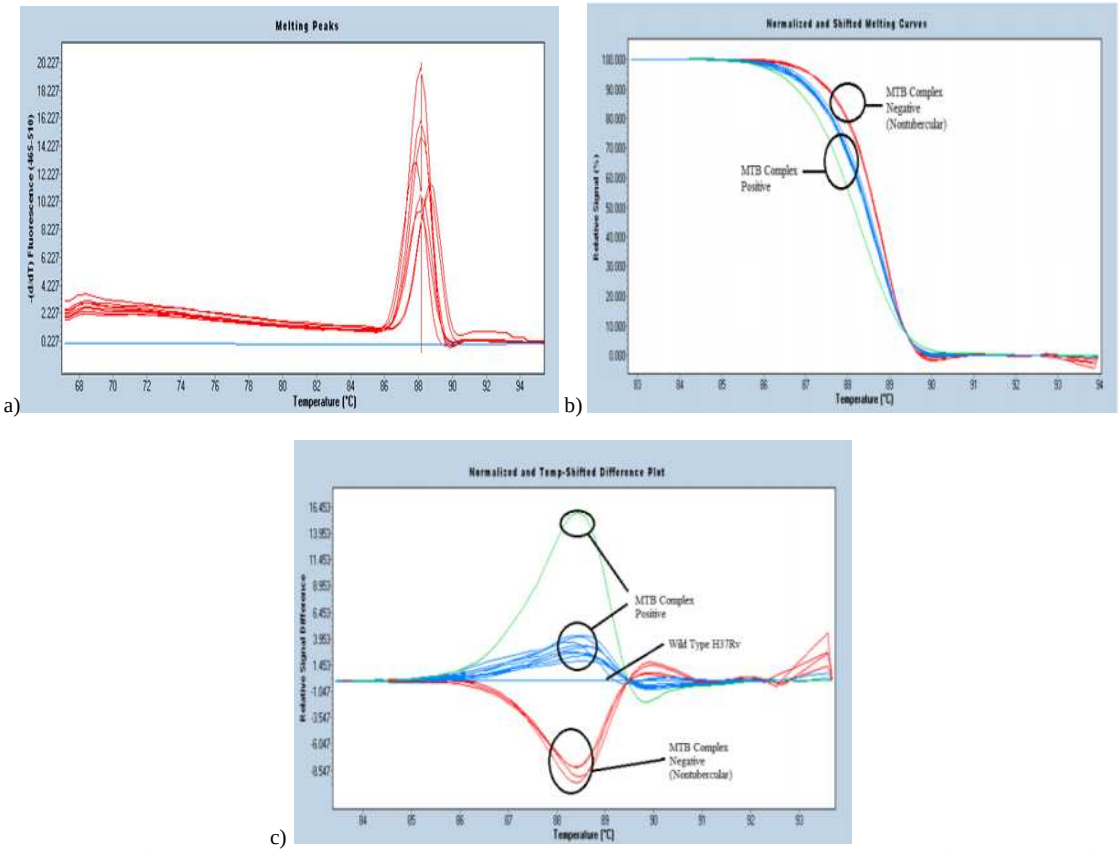

Figure 2-Detection of TB Positive *IS6110* gene by using HRM analysis in LC480II. The alternation in the shape of melting curves from the wildtype indicates the nontubercular isolates Amplification.2a)-Melting Peaks, 2b) Normalized Curve, 2c) Difference Plot Curves.

LightCycler® 480 Gene Scanning Software has module to process raw melting curve data to form Normalization Curve & a difference plot. The melting curve data at first glance does not appear to contain adequate information for detection. However, simple normalization of fluorescence before and after melting transition allows visual dissemination between MTB and non-tubercular Isolates (Figure 2B). The difference plot gives clearer representation of MTB and non-tubercular isolates. After normalization, the non-tubercular is easily distinguishable based on its temperature shifting and difference plot analysis. Automatic clustering algorithm could be devised for detection of MTB, simple visual clustering on difference plot appears simple & accurate (Figure 2C). Some cases Non-tubercular isolates showed amplification somewhere in range of Tm of desired gene sequence .This non specific amplification of undesired gene product is distinguished by differentiation curve. This differentiation between MTB & non-

tubercular isolates is concordant with Culture & AFB results. When HRM RT PCR amplified product was electrophoresis on PAGE, it was observed that few negative sample also resulted in an amplification product of 123bp. On primary observation based on amplification and melting curve, it is unable to distinguish between positive & negative samples as these isolates fall in the same melting peak area.

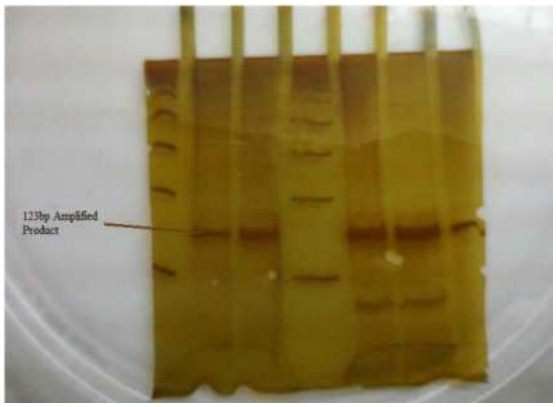

Figure 3- HRM RT PCR amplification of 123 bp region in IS*6110* targeting PCR for *M. tuberculosis* Complex Lanes 1&4: molecular weight marker; Lanes 2, 3, 7: positive for 123 bp MTB Positive; Lanes 5 and 6: MTB Suspected Samples Showing Amplification but in Difference Plot Showing Non tubercular Isolates.

Table 1-Comparison of sensitivity of PCR test with other Conventional Test

Conventional Tests	Total Clinical Isolates	PCR Sensitivity		
		+ve	-ve	Total Sensitivity
ZN positive	52	51	1	98.07%
ZN negative	48	17	31	35.41%
LJ positive	67	66	1	98.50%
LJ negative	33	2	31	0.6%
ZN positive LJ positive	52	49	3	94.23%
ZN negative LJ positive	16	16	0	100%
ZN negative LJ negative	32	11	21	34.37%

Table2: Summary of Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) results from different *Mycobacterium tuberculosis* detection methods

Method →			**Method to be compared with**			
			Culture		**ZN Smear**	
	Result →		(+)	(-)	(+)	(-)
	↓	**Total(n)** →	67	33	52	48
IS6110 HRM	(+)	68	66	2	51	17
	(-)	32	1	31	1	31
				95% Confidence level		95% Confidence level
Sensitivity			98.51%	91.96% to 99.96%	98.08%	89.74% to 99.95%
Specificity			93.94 %	79.77% to 99.26%	64.58 %	49.46% to 77.84%
Positive Predictive Value(PPV)			97.06%	89.78% to 99.64%	75.00%	63.02% to 84.71%
Negative Predictive Value(NPV)			96.88 %	83.78% to 99.92%	96.88 %	83.78% to 99.92%
Disease Prevalence			67.00%	56.88% to 76.08%	52.00%	41.78% to 62.10%
AFB Smear	(+)	52	51	1		
	(-)	48	26	22		
				95% Confidence level		
Sensitivity			66.23%	54.55% to 76.62%		
Specificity			95.65 %	78.05% to 99.89%		
Positive Predictive Value(PPV)			98.08%	89.74% to 99.95%		
Negative Predictive Value(NPV)			45.83 %	31.37% to 60.83%		
Disease Prevalence			77.00%	67.51% to 84.83%		

We examined about 90 clinical isolates of MTB based on their clinical findings and other investigations performed at the time of diagnosis and 10 healthy non tubercular patients were included in this study. This study shows that 52 samples were positive for AFB, 67 were positive for culture and 68 were positive by HRM RT PCR. We evaluated the sensitivity & specificity of HRM PCR with two most widely used conventional method i.e. AFB staining & Culture. The highest PCR Sensitivity was found with culture about 98.5%, the sensitivity of PCR for smear positive isolates was 98% as summarized in table 1 & 2.

DISCUSSION

In India, mostly the diagnosis of tuberculosis is based primarily on clinical features, demonstration of acid fast bacilli and culturing of *Mycobacterium tuberculosis*. The direct AFB smear is cost effective; however, suffers from low sensitivity and specificity. [16]

Mycobacterial culture supposed to be a gold commonplace technique for detection of TB; however it takes a minimum of a pair of weeks, even with the recently introduced liquid culture system. [13] Nucleic acid amplification technique to detect Mycobacterium tuberculosis in clinical specimens is progressively used as a tool for TB identification.

Application of molecular techniques for routine diagnosis in country like India depends on varied factors like high value and trained personnel to perform the test & time to complete assay. [17-20] The Real Time LC480 instrument is a commercially available system designed to decrease the time of PCR by monitoring the intensity of amplification of the target sequences by use of fluorescent dyes. This technology is a significant get through in PCR amplification and target gene detection compared to conventional detection methods, and its benefits for clinical assays have been reported. [8, 21-24]

In this study, PCR was performed by amplification of IS6110 is a insertion sequence, that belongs to the IS3 family and was found in MTB Complex members. Most strains carry 2910 to 15 copies, which are present in a wide variety of chromosomal sites. [25-29] Previous studies have documented increased TB positive cases using the IS*6110* target in Pulmonary Samples. Negi *et al.* analyzed various targets precise for *M. tuberculosis* and reported the highest PCR positivity rates for pulmonary (90%) when using IS6110; their results further confirmed the poor sensitivity of smear microscopy for pulmonary (49%). The specificity, sensitivity and speed of PCR test in diagnosis of *M. tuberculosis detection* shown during this study ought to encourage to employ this technique in routine diagnosing of TB method in routine diagnosis of TB. [30-33]

With the use of HRM RT PCR test, we were able to detect *M. tuberculosis complex* in 98.51% smear positive samples found to be positive by the culture methods. HRM RT PCR test detected *M. tuberculosis complex* in less than one day, compared to 3.5-4 weeks required for detection by conventional (LJ) medium. In few samples, HRM RT PCR results were negative but AFB smear examination and culture reported positive. This could be due to PCR Inhibition & low bacterial load. Clinical isolates where PCR were positive, AFB smear and culture were negative may be due to occurrence of dead mycobacteria within the samples. The lower specificity rate during this study is also because of the use of complex genomic DNA (instead of plasmids) or to the high number of amplicons (+1 Sputum Sample) that we analyzed to screen the clinical isolates.

CONCLUSION

We conclude from our information that high-resolution melting analysis is as sensitive as alternative normally used pre-screening molecular methods. The key benefit of high resolution melting is no post-PCR processing demand, creating it a few manual work techniques whereas rising its cost-effectiveness, simple use, and high throughput. In summary, we are here with a fast and reliable *M. tuberculosis complex* detection strategy by high-resolution melting analysis. By introducing this method, our detection time for the *M. tuberculosis complex* has been reduced considerably (one third compared to Conventional PCR MTB detection) owing to the relatively low cost of the consumables (no need of fluorescence-labeled primers or special polymers) and the lower workload compared with other MTB Detection techniques. Thus proves to be a highly cost

efficient technology. We conclude that HRM analysis is a fast, economical, sensitive methodology, simple enough to be readily implemented in a diagnostic laboratory.

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REFERENCES

1. Falkinham 3rd JO. Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev. 1996; 9(2):177.
2. Gamboa F, Fernandez G, Padilla E et al. Comparative evaluation of initial and new versions of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for direct detection of Mycobacterium tuberculosis in respiratory and nonrespiratory specimens. J Clin Microbiol. 1998; 36(3):684-689.
3. Jonas V, Alden MJ, Curry JI, Kamisango K et al. Detection and identification of Mycobacterium tuberculosis directly from sputum sediments by amplification of rRNA. J Clin Microbiol. 1993 1; 31(9):2410-2416.
4. Montenegro SH, Gilman RH, Sheen P, Cama R et al. Improved detection of Mycobacterium tuberculosis in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. Clin Infect Dis 2003; 36(1):16-23.
5. Drobniewski F, Nikolayevskyy V, Maxeiner H et al. Rapid diagnostics of tuberculosis and drug resistance in the industrialized world: clinical and public health benefits and barriers to implementation. BMC Med. 2013; 11(1):190.
6. Herrmann MG, Durtschi JD, Bromley LK et al. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. Clin. Chem. 2006; 52(3):494-503.
7. Montgomery J, Wittwer CT, Palais R, Zhou L. Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. Nat. Protoc. 2007; 2(1):59-66.
8. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP. Continuous fluorescence monitoring of rapid cycle DNA amplification. Biotechniques. 1997; 22(1):130-139.
9. Ririe KM, Rasmussen RP, Wittwer CT. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal. Biochem. 1997; 245(2):154-160.
10. Choi GE, Lee SM, Yi J, Hwang SH, Kim HH, Lee EY, Cho EH, Kim JH, Kim HJ, Chang CL. High-resolution melting curve analysis for rapid detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis clinical isolates. J Clin Microbiol. 2010; 48(11):3893-3898.
11. Della-Latta, P. Mycobacteriology and antimycobacterial susceptibility testing. Clinical microbiology procedures handbook *In* H. D. Isenberg (ed.), 2nd ed.,vol. 2.Washington, DC. ASM Press 2004.p. 7111-7883
12. Perkins, M. D. New diagnostic tools for tuberculosis. Int. J. Tuberc. Lung. Dis. 2000.4**:**182-188.
13. Kent PT, Kubica GP, Division of laboratory training and consultation. Laboratory Program Office. US department of health and human services PHS 1985 CDC Atlanta Georgia. 30333.
14. Buckingham L. Flaws Nucleic Acid Amplification Molecular diagnostics: fundamentals, methods and clinical applications. FA Davis; 2011.p. 122-149.
15. Eisenach KD, Cave MD, Bates JH, Crawford JT. Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. J Infect Dis. 1990; 161(5): 977-981.
16. Nolte FS, Metchock B. Mycobacterium. Manual of Clinical

Microbiology (6th edn). Trends in Microbiology. Washington, DC. ASMPress. 1995; 3(11).p.400-437.
17. Araj GF, Talhouk RS, Itani LY, Jaber W, Jamaleddine GW. Comparative performance of PCR-based assay versus microscopy and culture for the direct detection of Mycobacterium tuberculosis in clinical respiratory specimens in Lebanon. Int. J. Tuberc. Lung Dis. 2000; 4(9):877-881.
18. Piersimoni C, Scarparo C. Relevance of commercial amplification methods for direct detection of Mycobacterium tuberculosis complex in clinical samples. J. Clin. Microbiol. 2003; 41(12):5355-5365.
19. Pounder JI, Aldous WK, Woods GL. Comparison of real-time polymerase chain reaction using the Smart Cycler and the Gen-Probe amplified Mycobacterium tuberculosis direct test for detection of M. tuberculosis complex in clinical specimens. Diagn. Micrbiol. Infect. Dis. 2006; 54(3):217-222.
20. Espy MJ, Uhl JR, Mitchell PS, Thorvilson JN, Svien KA, Wold AD, Smith TF. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. 2000; 38(2):795-799.
21. Espy MJ, Ross TK, Teo R, Svien KA, Wold AD, Uhl JR, Smith TF. Evaluation of Light Cycler PCR for implementation of laboratory diagnosis of herpes simplex virus infections. J. Clin. Microbiol. 2000; 38(8):3116-3118.
22. Espy MJ, Teo R, Ross TK, Svien KA, Wold AD, Uhl JR, Smith TF. Diagnosis of varicella-zoster virus infections in the clinical laboratory by Light Cycler PCR. J. Clin. Microbiol. 2000; 38(9):3187-3189.
23. Espy MJ, Uhl JR, Mitchell PS, Thorvilson JN, Svien KA, Wold AD, Smith TF. Diagnosis of herpes simplex virus infections in the clinical laboratory by Light Cycler PCR. J. Clin. Microbiol. 2000; 38(2):795-799.
24. Schaade L, Kockelkorn P, Ritter K, Kleines M. Detection of cytomegalovirus DNA in human specimens by LightCycler PCR. . J. Clin. Microbiol. 2000; 38(11):4006-4009.
25. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S. Diagnostic potential of IS6110, 38kDa, 65kDa and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. I. Indian J Med Microbiol. 2007; 25(1):43.
26. Rattan A. PCR for diagnosis of tuberculosis: where are we now? Indian J Tuberc I. 2014; 47(2):79-82.
27. Kesarwani RC, Pandey A, Misra A, Singh AK. Polymerase chain reaction (PCR): Its comparison with conventional techniques for diagnosis of extra-pulmonary tubercular diseases. Indian J Surg 2004; 66:84-88.
28. Clarridge J3, Shawar RM, Shinnick TM, Plikaytis BB. Large-scale use of polymerase chain reaction for detection of Mycobacterium tuberculosis in a routine mycobacteriology laboratory. J Clin Microbiol. 1993; 31(8):2049-56.
29. Sekar B, Selvaraj L, Alexis A, Ravi S, Arunagiri K, Rathinavel L. The utility of IS6110 sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of extra-pulmonary tuberculosis. Indian J Med Microbiol. 2008; 26(4):352.
30. Bloemberg GV, Voit A, Ritter C, Deggim V, Böttger EC. Evaluation of Cobas TaqMan MTB for direct detection of the Mycobacterium tuberculosis complex in comparison with Cobas Amplicor MTB. J Clin Microbiol .2013; 51(7):2112-2117.
31. Thierry D, Cave MD, Eisenach KD, Crawford JT, Bates JH, Gicquel B, Guesdon JL. IS6110, an IS-like element of Mycobacterium tuberculosis complex. Nucleic Acids Res. 1990; 18(1):188.
32. Kim JH, Kim YJ, Ki CS, Kim JY, Lee NY. Evaluation of Cobas TaqMan MTB PCR for detection of Mycobacterium tuberculosis. J Clin Microbiol. 2011; 49(1):173-176.

33. Lim JS, Kim JW, Sohn YH, Kim JY. Multicenter evaluation of Seegene Anyplex TB PCR for the detection of Mycobacterium tuberculosis in respiratory specimens. J. Microbiol. Biotechnol. 2014; 24(7):1004-1007.

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Validation of in House PCR Using IS6110 for Detection of *M. tuberculosis* and Its Comparison with ZN Staining, Cultures and RT PCR Kit Methods

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Abstract

was highly specific for the *M. tuberculosis* complex. PCR was positive in all 51 smear and culture-positive samples, in 17 of 17 smear-negative and culture-positive samples, 8 of 132 smear and culture negative samples. The overall sensitivity and specificity were 84 and 94%. Rapid detection of MTB using commercial diagnostic systems is limited by their costs and/or their requirement of specific equipment. The Genosens's MTB Complex/MOTT Qualitative Real Time PCR assay kit for direct detection of *M. tuberculosis* complex in pulmonary tuberculosis samples have been used in MGM CRL on routine basis and also compatible with Light Cycle 480 Real time PCR Instrument. Conventional polymerase chain reaction has a much higher sensitivity than microscopy and can facilitate therapeutic decisions for those with suspected pulmonary tuberculosis. Thus, IS6110 as a PCR target was found to be very useful for rapid diagnosis of *M. tuberculosis* infection and start of antituberculous chemotherapy.

Keywords

Mycobacterium tuberculosis Conventional PCR Diagnosis IS6110

References

1. Jaggarajamma K, Sudha G, Chandrasekaran V, Nirupa C, Thomas A, Santha T, Narayanan PR (2007) Reasons for non-compliance among patients treated under Revised National Tuberculosis Control Programme (RNTCP), Tiruvallur district, South India. Ind J Tub 54(3):130
Google Scholar (http://scholar.google.com/scholar_lookup?title=Reasons%20for%20non-compliance%20among%20patients%20treated%20under%20Revised%20National%20Tuberculosis%20Control%20Programme%20%28RNTCP%29%2C%20Tiruvallur%20district%2C%20South%20India&author=K.%20Jaggarajamma&author=G.%20Sudha&author=V.%20Chandrasekaran&author=C.%20Nirupa&author=A.%20Thomas&author=T.%20Santha&author=PR.%20Narayanan&journal=Ind%20J%20Tub&volume=54&issue=3&pages=130&publication_year=2007)

%29%20Comparison%20of%20culture%20and%20acid-fast%20bacilli%20stain%20to%20PCR%20for%20detection%20of%20Mycobacterium%20tuberculosisin%20clinical%20samples.%20Mol%20cell%20probes%2012%284%29%3A207%E2%80%93211)

3. Nolte FS, Metchock B, McGowan JE, Edwards A, Okwumabua O, Thurmond C, Shinnick T (1993) Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J Clin Microbiol 31(7):1777–1782
Google Scholar (http://scholar.google.com/scholar_lookup?title=Direct%20detection%20of%20Mycobacterium%20tuberculosis%20in%20sputum%20by%20polymerase%20chain%20reaction%20and%20DNA%20hybridization&author=FS.%20Nolte&author=B.%20Metchock&author=JE.%20McGowan&author=A.%20Edwards&author=O.%20Okwumabua&author=C.%20Thurmond&author=T.%20Shinnick&journal=J%20Clin%20Microbiol&volume=31&issue=7&pages=1777-1782&publication_year=1993)
4. Kocagöz T, Yilmaz E, Ozkara S, Kocagöz S, Hayran M, Sachedeva M, Chambers HF (1993) Detection of *Mycobacterium tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. J Clin Microbiol 31(6):1435–1438
Google Scholar (http://scholar.google.com/scholar_lookup?title=Detection%20of%20Mycobacterium%20tuberculosis%20in%20sputum%20samples%20by%20polymerase%20chain%20reaction%20using%20a%20simplified%20procedure&author=T.%20Kocag%C3%B6z&author=E.%20Yilmaz&author=S.%20Ozkara&author=S.%20Kocag%C3%B6z&author=M.%20Hayran&author=M.%20Sachedeva&author=HF.%20Chambers&journal=J%20Clin%20Microbiol&volume=31&issue=6&pages=1435-1438&publication_year=1993)
5. García-Quintanilla A, Garcia L, Tudó G, Navarro M, González J, de Anta MTJ (2000) Single-tube balanced heminested PCR for detecting *Mycobacterium tuberculosis* in smear-negative samples. J Clin Microbiol 38(3):1166–1169
Google Scholar (http://scholar.google.com/scholar_lookup?title=Single-tube%20balanced%20heminested%20PCR%20for%20detecting%20Mycobacterium%20tuberculosis%20in%20smear-negative%20samples&author=A.%20Garc%C3%ADa-Quintanilla&author=L.%20Garcia&author=G.%20Tud%C3%B3&author=M.%20Navarro&author=J.%20Gonz%C3%A1lez&author=MTJ.%20Anta&journal=J%20Clin%20Microbiol&volume=38&issue=3&pages=1166-1169&publication_year=2000)
6. Gengvinij N, Pattanakitsakul SN, Chierakul N, Chaiprasert A (2001) Detection of *Mycobacterium tuberculosis* from sputum specimens using one-tube nested PCR
Google Scholar (https://scholar.google.com/scholar?q=Gengvinij%20N%2C%20Pattanakitsakul%20SN

method and the Gen-Probe amplified *Mycobacterium tuberculosis* direct test for detection of *Mycobacterium tuberculosis* in pulmonary and nonpulmonary specimens. J Clin Microbiol 42(9):4307–4309
CrossRef (https://doi.org/10.1128/JCM.42.9.4307-4309.2004)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Comparison%20of%20the%20real-time%20PCR%20method%20and%20the%20Gen-Probe%20amplified%20Mycobacterium%20tuberculosis%20direct%20test%20for%20detection%20of%20Mycobacterium%20tuberculosis%20in%20pulmonary%20and%20nonpulmonary%20specimens&author=N.%20Lema%C3%AEtre&author=S.%20Armand&author=A.%20Vach%C3%A9e&author=O.%20Capilliez&author=C.%20Dumoulin&author=RJ.%20Courcol&journal=J%20Clin%20Microbiol&volume=42&issue=9&pages=4307-4309&publication_year=2004)

8. Takahashi T, Nakayama T (2006) Novel technique of quantitative nested real-time PCR assay for *Mycobacterium tuberculosis* DNA. J Clin Microbiol 44(3):1029–1039
CrossRef (https://doi.org/10.1128/JCM.44.3.1029-1039.2006)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Novel%20technique%20of%20quantitative%20nested%20real-time%20PCR%20assay%20for%20Mycobacterium%20tuberculosis%20DNA&author=T.%20Takahashi&author=T.%20Nakayama&journal=J%20Clin%20Microbiol&volume=44&issue=3&pages=1029-1039&publication_year=2006)
9. De Beenhouwer H, Lhiang Z, Jannes GA, Mijs W, Machtelinckx L, Rossau R, Portaels F (1995) Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tuber Lung Dis 76(5):425–430
CrossRef (https://doi.org/10.1016/0962-8479(95)90009-8)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Rapid%20detection%20of%20rifampicin%20resistance%20in%20sputum%20and%20biopsy%20specimens%20from%20tuberculosis%20patients%20by%20PCR%20and%20line%20probe%20assay&author=H.%20Beenhouwer&author=Z.%20Lhiang&author=GA.%20Jannes&author=W.%20Mijs&author=L.%20Machtelinckx&author=R.%20Rossau&author=F.%20Portaels&journal=Tuber%20Lung%20Dis&volume=76&issue=5&pages=425-430&publication_year=1995)
10. Della LP (2004) Mycobacteriology and mycobacterial susceptibility tests. In Henry D (ed) Clinical microbiology procedures handbook, 2 edn. ASM Press
Google Scholar (https://scholar.google.com/scholar?q=Della%20LP%20%282004%29%20Mycobacteriology%20and%20mycobacterial%20susceptibility%20tests.%20In%20Henry%20D

Google Scholar (http://scholar.google.com/scholar_lookup?title=Drug-susceptibility%20testing%20in%20tuberculosis%3A%20methods%20and%20reliability%20of%20results&author=SJ.%20Kim&journal=Eur%20Respir%20J&volume=25&issue=3&pages=564-569&publication_year=2005)

12. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S (2007) Diagnostic potential of IS6110, 38 kDa, 65 kDa and 85B sequence-based polymerase chain reaction in the diagnosis of *Mycobacterium tuberculosis* in clinical samples. Indian J Med Microbiol 25(1):43
CrossRef (https://doi.org/10.4103/0255-0857.31061)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Diagnostic%20potential%20of%20IS6110%2C%2038%C2%A0kDa%2C%2065%C2%A0kDa%20and%2085B%20sequence-based%20polymerase%20chain%20reaction%20in%20the%20diagnosis%20of%20Mycobacterium%20tuberculosis%20in%20clinical%20samples&author=SS.%20Negi&author=R.%20Anand&author=ST.%20Pasha&author=S.%20Gupta&author=SF.%20Basir&author=S.%20Khare&author=S.%20Lal&journal=Indian%20J%20Med%20Microbiol&volume=25&issue=1&pages=43&publication_year=2007)
13. Shawar RM, El-Zaatari FA, Nataraj A, Clarridge JE (1993) Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridization methods. J Clin Microbiol 31(1):61–65
Google Scholar (http://scholar.google.com/scholar_lookup?title=Detection%20of%20Mycobacterium%20tuberculosis%20in%20clinical%20samples%20by%20two-step%20polymerase%20chain%20reaction%20and%20nonisotopic%20hybridization%20methods&author=RM.%20Shawar&author=FA.%20El-Zaatari&author=A.%20Nataraj&author=JE.%20Clarridge&journal=J%20Clin%20Microbiol&volume=31&issue=1&pages=61-65&publication_year=1993)
14. Eisenach KD, Sifford MD, Cave MD, Bates JH, Crawford JT (1991) Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am Rev Respir Dis 144(5):1160–1163
CrossRef (https://doi.org/10.1164/ajrccm/144.5.1160)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Detection%20of%20Mycobacterium%20tuberculosis%20in%20sputum%20samples%20using%20a%20polymerase%20chain%20reaction&author=KD.%20Eisenach&author=MD.%20Sifford&author=MD.%20Cave&author=JH.%20Bates&author=JT.%20Crawford&journal=Am%20Rev%20Respir%20Dis&volume=144&issue=5&pages=1160-1163&publication_year=1991)
15. Negi SS, Khan SF, Gupta S, Pasha ST, Khare S, Lal S (2005) Comparison of the conventional diagnostic modalities, bactec culture and polymerase chain reaction test for diagnosis of tuberculosis. Indian J Med Microbiol 23(1):29
CrossRef (https://doi.org/10.4103/0255-0857.13869)

author=S.%20Khare&author=S.%20Lal&journal=Indian%20J%20Med%20Microbiol&volume=23&issue=1&pages=29&publication_year=2005)

16. Banavaliker JN, Bhalotra B, Sharma DC, Goel MK, Khandekar PS, Bose M (1998) Identification of *Mycobacterium tuberculosis* by polymerase chain reaction in clinical specimens. Indian J Tuberc 45:15–18
Google Scholar (http://scholar.google.com/scholar_lookup?title=Identification%20of%20Mycobacterium%20tuberculosis%20by%20polymerase%20chain%20reaction%20in%20clinical%20specimens&author=JN.%20Banavaliker&author=B.%20Bhalotra&author=DC.%20Sharma&author=MK.%20Goel&author=PS.%20Khandekar&author=M.%20Bose&journal=Indian%20J%20Tuberc&volume=45&pages=15-18&publication_year=1998)

17. Bechnoosh A, Lieberman JM, Duke MB (1997) Stutman comparison of quantitative polymerase chain reaction, therapy for pulmonary tuberculosis. Diag Microb Infect Dis 29(2):9–73
Google Scholar (http://scholar.google.com/scholar_lookup?title=Stutman%20comparison%20of%20quantitative%20polymerase%20chain%20reaction%2C%20therapy%20for%20pulmonary%20tuberculosis&author=A.%20Bechnoosh&author=JM.%20Lieberman&author=MB.%20Duke&journal=Diag%20Microb%20Infect%20Dis&volume=29&issue=2&pages=9-73&publication_year=1997)

18. D'Amato RF, Hochstein LH, Colaninno PM, Scardamaglia M, Kim K, Mastellone AJ, Miller A (1996) Application of the Roche Amplicor, *Mycobacterium tuberculosis* (PCR) test to specimens other than respiratory secretions. Diagn Microbiol Infect Dis 24(1):15–17
CrossRef (https://doi.org/10.1016/0732-8893(95)00256-1)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Application%20of%20the%20Roche%20Amplicor%2C%20Mycobacterium%20tuberculosis%20%28PCR%29%20test%20to%20specimens%20other%20than%20respiratory%20secretions&author=RF.%20D%E2%80%99Amato&author=LH.%20Hochstein&author=PM.%20Colaninno&author=M.%20Scardamaglia&author=K.%20Kim&author=AJ.%20Mastellone&author=A.%20Miller&journal=Diagn%20Microbiol%20Infect%20Dis&volume=24&issue=1&pages=15-17&publication_year=1996)

19. Singh KK, Muralidhar M, Kumar A, Chattopadhyaya TK, Kapila K, Singh MK, Tyagi JS (2000) Comparison of in house polymerase chain reaction with conventional techniques for the detection of *Mycobacterium tuberculosis* DNA in granulomatous lymphadenopathy. J Clin Pathol 53(5):355–361
CrossRef (https://doi.org/10.1136/jcp.53.5.355)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Comparison%20of%20in%20house%20polymerase%20chain%20reaction%20with%20con

publication_year=2000)

20. Rimek D, Tyagi S, Kappe R (2002) Performance of an IS6110-based PCR assay and the COBAS AMPLICOR MTB PCR system for detection of *Mycobacterium tuberculosis* complex DNA in human lymph node samples. J Clin Microbiol 40(8):3089–3092
CrossRef (https://doi.org/10.1128/JCM.40.8.3089-3092.2002)
Google Scholar (http://scholar.google.com/scholar_lookup?title=Performance%20of%20an%20IS6110-based%20PCR%20assay%20and%20the%20COBAS%20AMPLICOR%20MTB%20PCR%20system%20for%20detection%20of%20Mycobacterium%20tuberculosis%20complex%20DNA%20in%20human%20lymph%20node%20samples&author=D.%20Rimek&author=S.%20Tyagi&author=R.%20Kappe&journal=J%20Clin%20Microbiol&volume=40&issue=8&pages=3089-3092&publication_year=2002)

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Alarming Levels of Drug-Resistant Tuberculosis in HIV-Infected Patients in Metropolitan Mumbai, India

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Abstract

Background: Drug-resistant tuberculosis (DR-TB) is a looming threat to tuberculosis control in India. However, no countrywide prevalence data are available. The burden of DR-TB in HIV-co-infected patients is likewise unknown. Undiagnosed and untreated DR-TB among HIV-infected patients is a major cause of mortality and morbidity. We aimed to assess the prevalence of DR-TB (defined as resistance to any anti-TB drug) in patients attending public antiretroviral treatment (ART) centers in greater metropolitan Mumbai, India.

Methods: A cross-sectional survey was conducted among adults and children ART-center attendees. Smear microscopy, culture and drug-susceptibility-testing (DST) against all first and second-line TB-drugs using phenotypic liquid culture (MGIT) were conducted on all presumptive tuberculosis patients. Analyses were performed to determine DR-TB prevalence and resistance patterns separately for new and previously treated, culture-positive TB-cases.

Results: Between March 2013 and January 2014, ART-center attendees were screened during 14135 visits, of whom 1724 had presumptive TB. Of 1724 attendees, 72 (4%) were smear-positive and 202 (12%) had a positive culture for *Mycobacterium tuberculosis*. Overall DR-TB was diagnosed in 68 (34%, 95% CI: 27%–40%) TB-patients. The proportions of DR-TB were 25% (29/114) and 44% (39/88) among new and previously treated cases respectively. The patterns of DR-TB were: 21% mono-resistant, 12% poly-resistant, 38% multidrug-resistant (MDR-TB), 21% pre-extensively-drug-resistant (MDR-TB plus resistance to either a fluoroquinolone or second-line injectable), 6% extensively drug-resistant (XDR-TB) and 2% extremely drug-resistant TB (XDR-TB plus resistance to any group-IV/V drug). Only previous history of TB was significantly associated with the diagnosis of DR-TB in multivariate models.

Conclusion: The burden of DR-TB among HIV-infected patients attending public ART-centers in Mumbai was alarmingly high, likely representing ongoing transmission in the community and health facilities. These data highlight the need to promptly diagnose drug-resistance among all HIV-infected patients by systematically offering access to first and second-line DST to all patients with 'presumptive TB' rather than 'presumptive DR-TB' and tailor the treatment regimen based on the resistance patterns.

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Introduction

India is a high burden country for tuberculosis (TB) and multidrug-resistant TB (MDR-TB). The World Health Organization has estimated that India accounted for 26% of the total number of TB cases worldwide in 2012, with 2.2% and 15% of the new and retreatment cases respectively being caused by multidrug-resistant strains [1]. Further, India is home to approximately 2.4 million people living with HIV [2] and considered to have a high burden on account of the large absolute numbers of people living with HIV in the country.

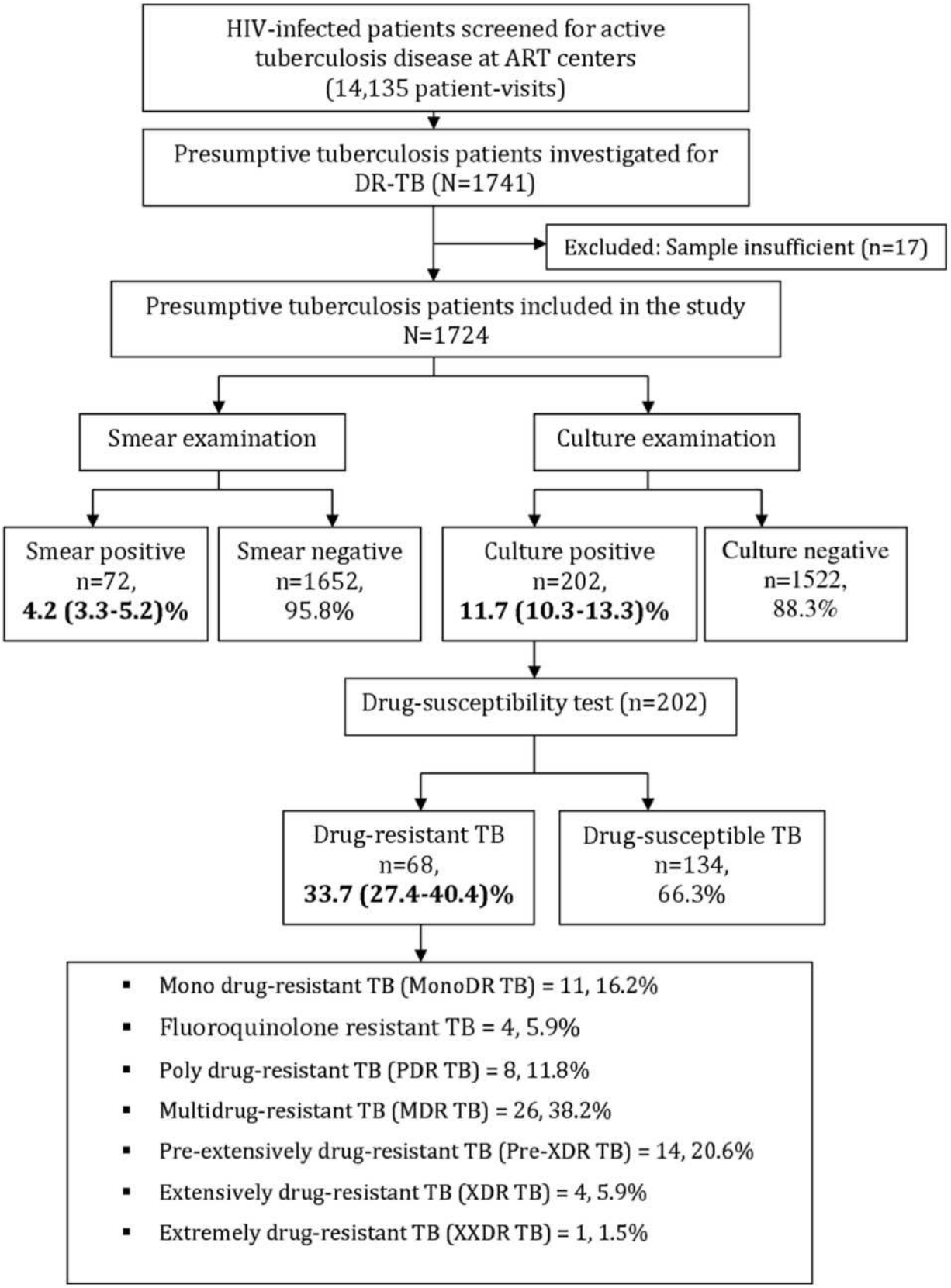

Figure 1. Drug-resistant tuberculosis among HIV-infected patients with presumptive tuberculosis, Mumbai, India.
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The dual burden of HIV and TB/DR-TB in India is significantly high with a combined rate of 5.2%, ranging from 0.4% to 28.8% in various studies, with increasing trends noted in states having a higher burden of HIV infection [3–7]. However, nation-wide studies do not exist and previous studies have occurred mainly in hospitals and tertiary care centres [2,6–11]. A crude estimate from these studies suggests that 2500–3000 HIV-infected persons develop MDR-TB annually in India.

Country-wide or state-wide drug resistance surveys (DRS) aim to estimate the DR-TB burden at the country or state level. While this approach is scientifically and operationally acceptable, it may mask significant and important variance in the magnitude of the epidemic in different localities, communities or specific populations. For India, a vast country with an enormous burden of TB and a relatively large burden of HIV in absolute numbers, this statement seems to hold true; from an overcrowded impoverished slum in Mumbai to a small isolated village in the Northern Eastern Provinces of the country, one can assume that several different epidemics may exist. A description of such local epidemics is necessary so as to complement the country-wide prevalence estimate. While there is an urgent need for a nationally representative, country-wide DRS in India, specific studies to identify pockets of extremely high DR-TB prevalence or extensive drug resistance patterns are equally needed in order to advocate for and implement effective control strategies.

The overall aim of this study was to assess the burden of drug-susceptible and drug-resistant tuberculosis among HIV-infected patients attending antiretroviral treatment (ART) centers in the metropolitan area of Mumbai. The specific objectives were a) to determine the proportion of HIV-infected patients with DR-TB among those attending public ART centers, b) to describe drug susceptibility patterns among *Mycobacterium tuberculosis* isolates from this population, and c) to identify factors associated with TB and drug-resistant TB among HIV patients. We aimed to contribute to the evidence base that informs policies and practices and help to estimate the resources needed to control the epidemic in this specific group, as well as the community.

Methods

Ethics

The study was approved by the Institutional Ethics Committee of Grant Medical College and Sir J.J. Group of Hospitals (Mumbai, India), the Ethics Review Board of Médecins Sans Frontières (Geneva, Switzerland) and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (Paris, France). The study protocol was approved by the Indian

Table 1. Demographic and clinical characteristics of HIV-infected patients with presumptive TB, Mumbai, India.

Characteristics	HIV-infected patients with presumptive TB (N = 1724)
	n (%)
Age [years, median (IQR)]	35.0 (24.3–44.0)
Sex of patients	
Male	1042 (60.4)
Female	671 (38.9)
Transgender	11 (0.6)
Family income per month (in Rupees)	
Less than 3500	88 (5.1)
3500–6999	910 (52.8)
7000 and above	454 (26.3)
Patient did not disclose	272 (15.6)
TB site	
Pulmonary	1688 (97.9)
Extra-pulmonary	36 (2.1)
ART status	
On ART	1386 (80.4)
Pre-ART	338 (19.6)
CD4 count, last visit (in cells/μl)	
Less than 200	258 (15.0)
200–349	351 (20.4)
350–499	289 (16.8)
500 and above	684 (39.7)
No information	142 (8.2)
ART duration* [months, median (IQR)]	26.0 (10.7–47.5)
Previous episode of TB	
Yes	933 (54.1)
No	791 (45.9)

ART: Antiretroviral treatment* Patients on ART with available information about ART initiation date, N = 1370.
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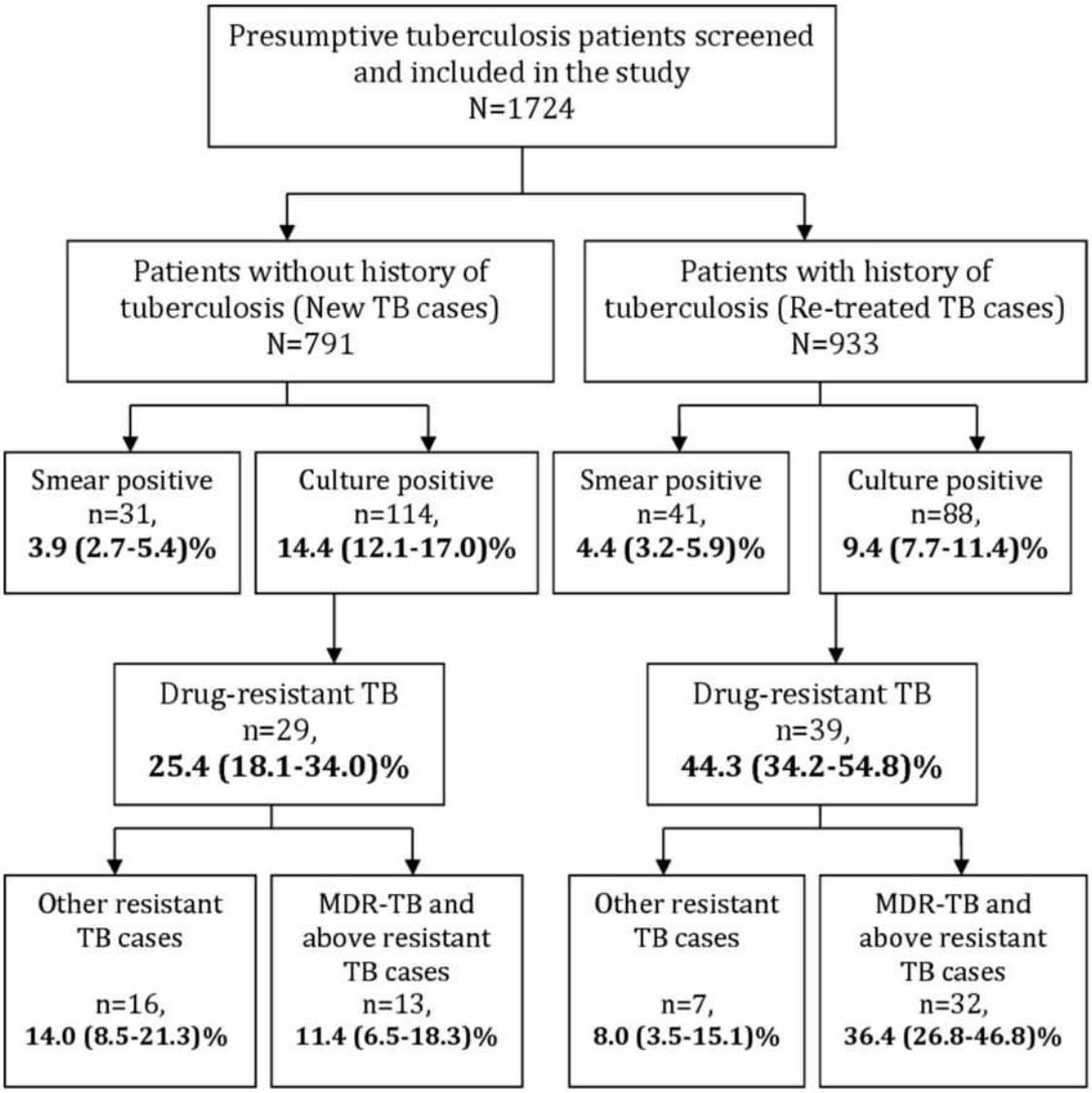

Figure 2. Distribution of Drug-resistant tuberculosis among HIV-infected (new and previously treated) with presumptive tuberculosis patients, Mumbai, India.
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Council of Medical Research (ICMR), New Delhi, India. Informed consent was obtained from all study participants.

Study design

This was a cross-sectional survey among HIV-infected adult and paediatric patients attending public and public-private ART clinics in the greater metropolitan Mumbai area. All patients with presumptive pulmonary or extra-pulmonary TB were assessed with smear microscopy and conventional liquid culture. All M. tuberculosis isolates underwent drug susceptibility testing (DST) for first- and second-line anti-TB drugs.

Sample size

The desired sample size was determined separately for new and previously treated culture positive TB cases. Previous tuberculosis treatment was defined as any anti-tuberculosis treatment reported by the patient. Assuming a prevalence of MDR-TB of 3% among new cases and 17% among retreated cases, based on a DST survey conducted in Gujarat [12], a sample size of 123 confirmed new cases and 110 confirmed retreatment cases was sought in order to estimate the prevalence of MDR-TB, with 95% confidence intervals having a margin of error of 3% for new cases and 7% for retreated cases respectively.

Study setting and study population

The study was carried out in five Mumbai District AIDS Control Society (MDACS) ART Centres [1) KEM Hospital, 2) SION Hospital, 3) SION Centre of Excellence in Paediatric HIV Care, 4) Godrej Hospital, Vikhroli and 5) Larsen & Toubro Hospital, Andheri] as well as in two Maharashtra State AIDS Control Society (MSACS) ART Centres [1) Thane Civil Hospital and 2) Navi Mumbai (Vashi) Municipal Corporation Hospital].

All HIV-infected adult and paediatric patients enrolled in the ART centres were potentially eligible to be enrolled in the study, if they had presumptive pulmonary or extra-pulmonary TB based on symptom screening, regardless of the time they were enrolled in the centres or whether they were on ART or not at the time of the

Table 2. Resistance profile (first and second-line) for all drug-resistant tuberculosis patients, Mumbai, India.

Resistance profile (culture-based DST)	Total TB Patients (N = 68), n (%)	New TB patients (N = 29), n (%)	Previously treated TB patients (N = 39), n (%)
H-mono	11 (16.2)	7 (24.1)	4 (10.3)
R-mono	-	-	-
Ofx-mono	1 (1.5)	1 (3.4)	-
Ofx Mfx	3 (4.4)	2 (6.9)	1 (2.6)
HE	7 (10.3)	6 (20.7)	1 (2.6)
HE Eto Ofx Mfx	1 (1.5)	-	1 (2.6)
HR	10 (14.7)	4 (13.8)	6 (15.4)
HRE	6 (8.8)	2 (6.9)	4 (10.3)
HR Eto	5 (7.4)	-	5 (12.8)
HR E Eto	5 (7.4)	2 (6.9)	3 (7.7)
HR Ofx Mfx E	2 (2.9)	2 (6.9)	-
HR Ofx Mfx Eto	1 (1.5)	1 (3.4)	-
HR Ofx Mfx E Lin	1 (1.5)	-	1 (2.6)
HR Ofx Mfx E Eto	8 (11.8)	1 (3.4)	7 (17.9)
HR Ofx Mfx E Eto PAS	2 (2.9)	-	2 (5.1)
HR Ofx Mfx Km Eto	1 (1.5)	-	1 (2.6)
HR Ofx Mfx Km E Eto	2 (2.9)	1 (3.4)	1 (2.6)
HR Ofx Mfx Km Cm E Eto	1 (1.5)	-	1 (2.6)
HR Ofx Mfx Km Cm E Eto PAS	1 (1.5)	-	1 (2.6)

H-isoniazid, R-rifampicin, E-ethambutol, Eto-ethionamide, Km-kanamycin, Cm-capreomycin, Ofx-ofloxacin, Mfx-Moxifloxacin, Lin- Linezolid, PAS- para-aminosalicylic acid.
doi:10.1371/journal.pone.0110461.t002

study. Patients on TB treatment at the time of the study were excluded.

Recruitment and sampling procedure

All HIV-infected ART center attendees were screened by an MSF-employed nurse during the study period. Patients with presumptive TB were investigated using a standard diagnostic algorithm recommended by the World Health Organization [13] that included TB culture and DST. The nurse explained in detail the objectives of the study to the patient and/or caregiver and obtained the signature or thumbprint of the patient if consent was given to participate. When pulmonary TB was presumed, two sputum specimens were collected on the same day, one hour apart, at each study site/hospital laboratory. When extra-pulmonary TB (EPTB) was presumed, biological specimens (fine needle aspirates, pleural fluid, cerebrospinal fluid, etc) were obtained from extra-pulmonary sites. All specimens were transferred to Hinduja Hospital Microbiology Laboratory in Mumbai for culture and first- and second-line DST.

Conventional microscopy with Ziehl-Neelsen (ZN) staining for acid-fast bacilli and further sputum decontamination was performed using the N-acetyl-L-cysteine and sodium hydroxide method. Concentrated sediment was inoculated in one liquid culture tube for testing using the Mycobacterial growth indicator tube (MGIT 960) method. Positive cultures underwent microscopy with ZN staining to confirm cord formation, and speciation with MPT 64 antigen detection by Immunochromatography was carried out to confirm *M. tuberculosis* complex. Specimens fulfilling the above criteria underwent further testing with phenotypic DST using the MGIT System for the following drugs: isoniazid, rifampicin, ethambutol, ofloxacin, moxifloxacin, kanamycin, capreomycin, PAS, ethionamide, clofazimine and linezolid. Non-tuberculous Mycobacteria (NTM) speciation was done by molecular methods using Reverse Line Blot Hybridisation. Hinduja laboratory is quality controlled and has been accredited for first-line DST by the WHO Supranational Reference Laboratory in Bangalore and the College of American Pathologists. The laboratory was also accredited by the TB programme for second-line DST in December 2013; prior to this date, if a strain was suspected to have resistance to one or more second-line anti-TB drugs, it was sent to the National Tuberculosis Institute Laboratory in Bangalore for confirmation.

Multidrug-resistant tuberculosis (MDR-TB) was defined as resistance to both isoniazid and rifampicin; pre-XDR-TB was defined as MDR-TB with additional resistance to either a fluoroquinolone or a second-line injectable agent; and extensively drug-resistant tuberculosis (XDR-TB) was defined as MDR-TB with additional resistance to both a fluoroquinolone and an injectable agent. Extremely drug-resistant tuberculosis (XXDR-TB) was defined as XDR-TB with additional resistance to any group IV and/or group V TB drugs (PAS, ethionamide, clofazimine, linezolid) [13].

Management of those diagnosed with DR-TB

All patients diagnosed with MDR- or XDR-TB were managed in accordance with the national DR-TB treatment guidelines [14], while those with pre-XDR-TB were offered individualized treatment with 4 drugs likely to be effective.

Data collection and analysis

Demographics, clinical and laboratory data, antiretroviral treatment (yes/no) and duration on ART, as well as data on

Table 3. Demographic and clinical factors associated with culture-positive tuberculosis in HIV-infected patients, Mumbai, India.

Explanatory Variable	Patients with tuberculosis (N = 202), n (%)	Patients without tuberculosis (N = 1522), n (%)	Chi-square/t-test (p-value)	aPR[a] (95% CI)
Age [years, median (IQR)]	38.0 (32.0–43.3)	35.0 (22.0–44.0)	8.9 (<0.01)	0.99 (0.99–1.00)
Sex of patients				
Male	138 (13.1)	915 (86.9)	5.0 (0.02)	1.01 (0.99–1.03)
Female	64 (9.5)	607 (90.5)		
Family income per month[+] (in Rupees)				
Less than 5000	96 (10.9)	788 (89.1)	0.2 (0.65)	
5000 and above	66 (11.6)	502 (88.4)		
ART status				
Pre-ART	72 (21.3)	266 (78.7)	37.3 (<0.01)	1.07 (1.04–1.10)
On ART	130 (9.4)	1256 (90.6)		
CD4 count, last visit* (in cells/μl)				
Less than 200	58 (22.5)	200 (77.5)	34.7 (<0.01)	1.08 (1.05–1.11)
200 and above	127 (9.6)	1197 (90.4)		
ART duration** [months, median (IQR)]	19.3 (5.2–35.7)	27.1 (11.3–47.9)	9.9 (<0.01)	
Previous episode of TB				
Yes	88 (9.4)	845 (90.6)	10.3 (<0.01)	1.01 (0.99–1.03)
No	114 (14.4)	677 (85.6)		

ART: Antiretroviral treatment, IQR: Inter-quartile range, CI; Confidence Intervals [+]Patients with recorded family income, N = 1452* Patients with available information on CD4, last visit, N = 1582** Patients on ART with available information about ART initiation date, N = 1370[a] aPR; adjusted Prevalence Ratios (calculated by Poisson regression using multiple imputation for CD4 missing data).

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previous TB treatment were doubly-entered into an EpiData database (Version 3.1, EpiData Association, Odense, Denmark), validated and analyzed.

To identify factors associated with TB and DR-TB, univariate and multivariate analyses were performed using Poisson and binary logistic regression models. Factors significant (p = 0.05) on univariate analysis were entered into the multivariate logistic regression models. Factors were coded as categorical variables and missing values for CD4 cell counts were imputed using a multiple imputation method. Transgender individuals (all were male to female) were grouped with biological males in the models. All factors were entered as a block into multivariate logistic regression models. Data analysis, including multivariate logistic regression models, was conducted with SPSS Version 20.0. Armonk, NY: IBM Corp. Released 2011).

Results

Screening for presumptive TB was carried-out during 14,135 patient visits at seven ART centers in metropolitan Mumbai between March 2013 and January 2014 (Figure 1). Individual patients might have been screened more than once during the study period. A total of 1741 HIV-infected patients with presumptive tuberculosis (TB) were identified. All of them consented to participate in the study and were investigated for drug-resistant TB. The sputum specimens of 17 patients were found insufficient for laboratory investigations and had to be excluded. Thus, 1724 (99%) of the eligible patients were included in the study.

Patient characteristics

The median age of the 1724 patients was 35 (Inter-quartile range, IQR: 24–44) years (Table 1) and the majority (60%) were male. A large proportion (53%) of patients had an average family income between 3500 and 7000 Indian National Rupees (equivalent to 60–120 USD) per month. Most of the patients (98%) had pulmonary TB. Among the entire study cohort, 80% were on ART during the study period and the majority (52%) had CD4 cell counts lower than 500 cells/μL at their last visit to an ART center. The median duration of exposure to ART prior to enrollment in the study was 26 months (IQR: 10.7–47.5). More than half (933/1724) of the presumptive TB patients had had at least one episode of active TB disease in the past.

Culture-positive and drug-resistant tuberculosis

All of the 1724 patients with presumptive TB included in the study (Figure 1) underwent smear, culture and drug susceptibility testing (DST). Of these, 72 (4.2%; 95% Confidence Intervals (CI): 3.3–5.2) patients had smear-positive TB while 202 (11.7%; 95% CI: 10.3–13.3) patients had culture-positive TB. Eleven TB

Table 4. Demographic and clinical factors associated with drug-resistant tuberculosis in HIV-infected tuberculosis patients, Mumbai, India.

Explanatory Variable	Patients with drug-resistant tuberculosis (N = 68), n (%)	Patients without drug-resistant tuberculosis (N = 134), n (%)	Chi-square/t-test (p-value)	aOR[a] (95% CI)
Age [years, median (IQR)]	35.5 (28.5–42.8)	38.0 (33.8–44.0)	2.15 (0.14)	0.98 (0.96–1.01)
Sex of patients				
Male	46 (33.3)	92 (66.7)	0.02 (0.88)	0.95 (0.49–1.82)
Female	22 (34.4)	42 (65.6)		
Family income per month[+] (in Rupees)				
Less than 5000	36 (37.5)	60 (62.5)	1.8 (0.17)	
5000 and above	18 (27.3)	48 (72.7)		
ART status				
Pre-ART	22 (30.6)	50 (69.4)	0.48 (0.49)	0.96 (0.49–1.90)
On ART	46 (35.4)	84 (64.6)		
CD4 count, last visit* (in cells/μl)				
Less than 200	19 (32.8)	39 (67.2)	0.22 (0.88)	0.96 (0.48–1.93)
200 and above	43 (33.9)	84 (66.1)		
ART duration** [months, median (IQR)]	19.3 (5.7–31.3)	19.2 (3.2–43.7)		
Previous episode of TB				
Yes	39 (44.3)	49 (55.7)	7.93 (<0.01)	2.31 (1.24–4.30)
No	29 (25.4)	85 (74.6)		

ART: Antiretroviral treatment, CI; Confidence Intervals [+]Patients with recorded family income, N = 162* Patients with available information about CD4 count, last visit N = 185** Patients on ART with available information about ART initiation date, N = 126[a] aOR; adjusted Odds ratios (calculated by binary logistic regression using multiple imputation for CD4 missing data).

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patients were smear-positive but culture negative and 141 patients were culture-positive but smear-negative. Those patients having a history of TB had a higher rate of smear-positivity (4.4% versus 3.9%), but lower culture-positivity rate (9.4% versus 14.4%) as compared to patients without TB history (Figure 2).

Among all culture-positive TB patients, 68 or 33.7% (95% CI: 27.4–40.4) had some form of drug-resistant TB. A high proportion of MDR-TB and pre-XDR-TB, 38% (26/68) and 21% (14/68) respectively, was observed amongst drug-resistant TB patients. Table 2 shows the detailed resistance patterns of all patients with DR-TB.

Of the newly diagnosed (114/791) and previously treated (88/933) culture-positive TB patients, 25.4% (95% CI: 18.1–34.0) and 44.3% (95% CI: 34.2–54.8) patients had drug-resistant TB. The proportion of patients with multidrug-resistant TB and more advanced TB resistance profiles was higher (36% versus 11%) in previously treated patients compared to newly diagnosed TB patients.

Children and extra-pulmonary tuberculosis patients

In the study, 283 children aged less than 15 years were investigated. The median (IQR) age of these children was 11 (8–13) years, just over half of them were male (56%), and sixty-eight percent were on ART. Of the 283 children investigated, 5% (15/283) had culture-positive TB, of whom seven (46.7%, 7/15) had drug-resistant TB; one had polydrug-resistant TB, two had MDR-TB and four had pre-XDR-TB.

Among 1724 HIV-infected patients investigated during the study period, 36 patients had presumptive extra-pulmonary TB (EPTB). The median (IQR) age of these patients was 45 (40–47) years and three-quarters of them were male (27/36, 75%). Among the 36 investigated presumptive EPTB patients, 14% (5/36) patients had culture-positive TB. Of these, 40% (2/5) patients had DR-TB: one had INH mono-resistant TB while another had pre-XDR-TB.

Factors associated with culture-confirmed TB, DR-TB and MDR-TB

The demographic and clinical factors were assessed for association with culture-confirmed TB, DR-TB and MDR-TB. The univariate and bivariate analyses found age, ART status, CD4 count at last visit and previous episode of TB significantly related to culture-positive TB (Table 3). A multivariate Poisson regression model showed that older age, pre-ART status (i.e. not yet on ART), CD4 count less than 200 cells/μL at the last visit and a previous episode of TB were associated with culture-positive TB. None of the factors other than previous history of TB were associated with drug-resistant TB (Table 4) and multi-drug resistant TB (Table 5) in bivariate and multivariate binary logistic regression models.

Table 5. Demographic and clinical factors associated with multidrug-resistant tuberculosis in HIV-infected tuberculosis patients, Mumbai, India.

Explanatory Variable	Patients with multidrug-resistant tuberculosis (N = 45), n (%)	Patients with drug-susceptible tuberculosis (N = 134), n (%)	Chi-square/t-test (p-value)	aOR[a] (95% CI)
Age [years, median (IQR)]	38.0 (30.0–42.5)	38.0 (33.8–44.0)	1.85 (0.18)	0.98 (0.95–1.01)
Sex of patients				
Male	30 (24.6)	92 (75.4)	0.06 (0.80)	0.83 (3.8–1.83)
Female	15 (26.3)	42 (73.7)		
Family income per month[+] (in Rupees)				
Less than 5000	21 (25.9)	60 (74.1)	1.03 (0.31)	
5000 and above	11 (18.6)	48 (81.4)		
ART status				
Pre-ART	12 (19.4)	50 (80.6)	1.69 (0.19)	0.85 (0.37–1.96)
On ART	33 (28.2)	84 (71.8)		
CD4 count, last visit* (in cells/μl)				
Less than 200	13 (25.0)	39 (75.0)	0.00 (1.00)	1.02 (0.43–2.41)
200 and above	28 (25.0)	84 (75.0)		
ART duration** [months, median (IQR)]	19.7 (5.7–34.8)	19.2 (3.2–43.7)	0.02 (0.88)	
Previous episode of TB				
Yes	32 (39.5)	49 (60.5)	16.2 (<0.01)	4.16 (1.93–8.95)
No	13 (13.2)	85 (86.7)		

ART: Antiretroviral treatment, CI; Confidence Intervals [+]Patients with recorded family income, N = 140* Patients with available information about CD4 count, last visit N = 164** Patients on ART with available information about ART initiation date, N = 126[a]aOR; adjusted Odds ratios (calculated by binary logistic regression using multiple imputation for CD4 missing data).

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Discussion

To our knowledge this is the first DR-TB survey carried out among HIV clinic attendees in India. This study shows that, among HIV-infected children and adults in Mumbai, the burden of drug-resistant tuberculosis is extremely high: almost one in four new TB cases and one in two of those previously treated for TB have a drug-resistant strain. Of just as great concern, a large proportion of these strains was resistant to one or more second-line tuberculosis drugs, especially fluoroquinolones.

The overall rate of culture positivity amongst presumptive TB cases was surprisingly low (11.7%). We hypothesize that this was due neither to limitations in laboratory techniques nor the presence of NTM disease, but instead to the broad inclusion criteria that required a person attending a study site to have just one of four possible TB symptoms as recommended by WHO [13]; a person with 'current cough', for example, who was otherwise stable was eligible for enrolment. Another possible contributor to the low rate of TB culture positivity was the relatively large number of poor quality specimens (e.g. consisting of saliva) despite active instruction being given by a dedicated study nurse at each site. In any case, this finding warrants further investigation.

Even though the overall yield of TB was small in the pediatric cohort as well, it remains significant that almost half of the children with TB were infected with drug-resistant strains, most commonly pre-XDR-TB. Since bacteriological confirmation of DR-TB is more challenging in young children than in adults, as they cannot expectorate sputum and are more likely to have paucibacillary and extra-pulmonary TB, we hypothesize that the burden of TB and DR-TB is likely to be underestimated among children in this study, similar to what has been found in a recent meta-analysis [15]. With less than 2% of all study participants having specimens taken from extrapulmonary sites, it is almost certain that EPTB is being underdiagnosed as well in this cohort. A separate analysis found no significant association between EPTB and DR-TB in children or adults.

Our statistical models revealed no significant associations between most demographic and clinical factors and the risk of DR-TB and MDR-TB. We believe that these findings are important for their lack of associations; it seems that most TB/HIV co-infected patients attending ART centers in Mumbai are at risk for DR-TB. Although the relatively small sample size limits the power of our analyses and calls for cautious interpretation, the lack of associations suggests that all those infected with HIV and presumed to have active TB be tested for drug-resistant strains.

Given the high population density in Mumbai, in which a large proportion of the population lives in slums under extreme poverty, the very high TB prevalence and the relatively high HIV burden reported in greater metropolitan Mumbai, these data are unlikely to be representative of a country as vast and diverse as India. Nevertheless the living conditions in Mumbai and common practices in the public and private health sectors (as for example the prescribing of inappropriate regimens and over-the-counter availability of fluoroquinolones and other drugs with anti-TB properties) are similar to those of other large metropolitan centres in the country, so these data could very well represent the DR-TB situation in such cities as New Delhi, Kolkata and Chennai.

While it may not be possible to generalise our estimates for the entire country or even for HIV-uninfected populations, they serve to highlight the overall magnitude of the DR-TB epidemic in Mumbai, which is not unknown [16,17]. A high prevalence of MDR-TB strains (11–68%) was reported in tertiary health facilities as early as 1991, followed by further documentation in 2006 [18–20], including information on the magnitude of the epidemic in children [21]. A study by D'Souza et al in 2009 [18] documented high levels of multiple drug resistance (both MDR and poly-drug resistance) amongst previously untreated cases in urban parts of Mumbai. In 2011 Udwadia et al reported a cases-series of totally-drug resistant TB (a term that has not officially been endorsed by WHO) in Mumbai, which captured the attention of local and international media [22,23]. However to-date such findings are often overlooked and their importance minimized as representing only selected populations, laboratory or tertiary care settings and small case-series. Our study confirms that there is more than one epidemic ongoing in Mumbai and reinforces the urgent need to accurately measure the overall prevalence and incidence of DR-TB around the country in order to define appropriate interventions. Studies in selected populations such as this complement the overall estimates and can help in directing resources and prioritizing interventions targeted at the most vulnerable groups.

This survey is subject to the usual limitations in survey design and data collection. There is likely to be a tendency for patients to not report previous treatment either because they do not remember (recall limitation) or, on purpose, to avoid going through a long course of treatment that includes daily injectable medication and is known among patients for debilitating side effects [24]. Such bias could have led to an overestimate of DR-TB among new cases and an underestimate among retreatment cases. However, most HIV-infected patients attending ART clinics are usually aware of tuberculosis and have been counseled and screened for TB on several occasions, so recall limitation is rather unlikely.

The majority of HIV-infected patients attending public and public-private ART centers in the city are likely to access the public national TB programme for TB diagnosis and treatment. However many still seek care from private practitioners or may switch between the public and private sectors. The contribution to DR-TB levels from suboptimal treatment regimens prescribed in the unregulated Indian private health sector has been well documented [25–27]. Cox et al in 2007 have shown that even under well-established DOTS programmes in areas with high levels of drug resistance, high levels of amplification of drug resistance are to be expected [28].

The high level of resistance to three or more first-line anti-TB drugs and to fluoroquinolones has been previously described by others [29]. The proportion of previously untreated cases in our study that were resistant to more than three drugs, especially isoniazid, rifampicin and a fluoroquinolone, was particularly alarming and highlights two major issues in the management of TB in the setting of HIV/ART clinics. Firstly, it points to the scenario of nosocomial transmission of TB and DR-TB. Those attending an ART clinic at least once a month are more likely to be exposed to susceptible and resistant strains of *M. tuberculosis* than the general population. Given that the ART centers in Mumbai are usually extremely busy, constantly crowded and that they often lack adequate TB infection control interventions, this scenario is not unlikely. Instead of hypothesizing that most cases of DR-TB are due to non-adherence among patients on treatment, exogenous infection or re-infection should first be considered [30,31]. Secondly, considering the high levels of resistance to second-line TB drugs and especially fluoroquinolones in this population, it is reasonable to assume that patients with presumptive TB may actually have pre-XDR-TB or even XDR-TB. This statement implies a huge investment in laboratory capacity in an already constrained public sector in Mumbai in order to screen all TB patients at the outset for strains that are resistant to fluoroquinolones and anti-TB injectables Nevertheless, we believe that it is a reasonable investment to make if the epidemic of DR-TB is to be controlled in the city in the future. Conversely, if DST is only offered afterward to those failing their TB treatment regimen, a large proportion of DR-TB cases will be missed due to the high risk of mortality among HIV-positive patients with untreated DR-TB [32].

There is an ongoing plan to systematically offer molecular TB diagnosis (mainly using Xpert MTB/RIF, also known as GeneXpert) to all HIV-infected patients in Mumbai and elsewhere in the country. While this is a giant leap forward, since GeneXpert can rapidly detect MTB and rifampicin resistance within 2 hours, we are concerned that 'scale up' of DR-TB diagnosis using this particular diagnostic may lead to suboptimal practices, since a diagnosis of rifampicin resistance alone and/or assumption that it represents a diagnosis of MDR-TB, may mask a diagnosis of pre-XDR or XDR-TB (or worse); the risks then associated with giving a suboptimal treatment regimen are significant both in terms of morbidity and mortality for the patient, as well as amplification of resistance and subsequent community transmission of resistant strains. While GeneXpert is an excellent and efficient diagnostic tool for MTB and screening test for DR-TB, in settings like Mumbai it is essential that it be complemented by culture and DST involving first- and second-line anti-TB drugs. The national programme has recently changed the policy to account for this risk, starting with HIV-infected patients in Mumbai and Maharashtra.

Our initial study protocol included fingerprinting studies using spoligotyping, which we had to abandon due to the high cost. Cox et al have in the past found a strong association between the Beijing genotype and amplification in situations of preexisting resistance in a central Asian setting [33]. Similarly, the proportion of the Beijing genotype was reported to be 35% in the urban Mumbai population studied by Almeida et al [34]. We need fingerprinting studies to establish how often nosocomial transmission occurs and to guide TB infection control interventions. Another area of research that is urgently needed relates to chemoprophylaxis for child contacts of DR-TB cases in Mumbai; preventative regimens that have shown to be effective in other settings are unlikely to prevent development of active disease in many children in Mumbai due to the high baseline rate of fluoroquinolone resistance [35].

Conclusion

Our findings strongly suggest that there is an ongoing DR-TB epidemic among people living with HIV and attending ART centers in Mumbai, which requires urgent, innovative and feasible models of care that allow for rapid and accurate detection and treatment of as many DR-TB patients as possible. Ideally all patients with presumptive TB attending any ART center in Mumbai, or settings with similar drug resistance patterns, should be screened with a rapid molecular diagnostic followed by DST to first- and second-line anti-TB drugs, including for fluoroquinolones, so that the correct diagnosis is made as early as possible and followed by prompt treatment initiation with an appropriate individualized regimen. The high rate of DR-TB amongst new TB patients also highlights the need for better TB infection control measures in order to prevent ongoing transmission of DR-TB in the community and health facilities, especially those attended by vulnerable populations, such as those living with HIV.

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Author Contributions

Conceived and designed the experiments: PI. Performed the experiments: CP CR MM AS. Analyzed the data: PI MD. Contributed reagents/materials/analysis tools: AMVK MK AB BA MM MP AK BBR AD LR PS KSS. Contributed to the writing of the manuscript: PI MD PS. Critically reviewed several versions of the manuscript: AMVK MK AB BA MM MP AK BBR AD LR PS KSS CP AS.

References

1. World Health Organization (WHO) (2013), Global tuberculosis report 2013. WHO Press, Geneva, WHO/HTM/TB/2013.11.
2. Department of AIDS Control (2013), National AIDS Control Organization, Annual Report 2012–2013, Ministry of Health & Family Welfare, Government of India.
3. Paramasivan CN, Venkataraman P (2004) Drug resistance in tuberculosis in India. Indian J Med Res; 120: 377–386.
4. Deivanayagam CN, Rajasekaran S, Venkatesan R, Mahilmaran A, Ahmed PR, et al. (2002) Prevalence of acquired MDR TB and HIV co-infection. Indian J Chest Dis Allied Sci 44: 237–242.
5. Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C (2005) The impact of HIV/AIDS on the control of tuberculosis in India. Proc Natl Acad Sci U S A 102: 9619–9624.
6. Swaminathan S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekeran S (2005) Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis 9: 896–900.
7. Maniar JK, Kanuth RR, Mandalia S, Shah K, Maniar A (2006) HIV and tuberculosis: partners in crime. Indian J Dermatol Venereol Leprol 72: 276–82.
8. Pereira M, Tripathy S, Inamdar V, Ramesh K, Bhavsar M, et al. (2005) Drug resistance pattern of Mycobacterium tuberculosis in seropositive and seronegative HIV-TB patients in Pune, India. Indian J Med Res 121: 235–239.
9. Sethi S, Mewara A, Dhatwalia SK, Singh H, Yadav R, et al. (2013) Prevalence of multidrug resistance in *Mycobacterium tuberculosis* isolates from HIV seropositive and seronegative patients with pulmonary tuberculosis in north India. BMC Infect Dis 1471–2334/13/137.
10. Menon S, Dharmshale S, Chande C, Gohil A, Lilani S, et al. (2012) Drug resistance profiles of *Mycobacterium tuberculosis* isolates to first line anti-tuberculous drugs: a five years study. Lung India 29: 227–231.
11. Kumar P, Balooni V, Sharma BK, Kapil V, Sachdeva KS, et al. (2014) High degree of multi-drug resistance and hetero-resistance in pulmonary TB patients from Punjab state of India. Tuberculosis (Edinb) 94(1): 73–80.
12. Ramachandran R, Nalini S, Chandrasekar V, Dave PV, Sanghvi AS, et al. (2009) Surveillance of drug-resistant tuberculosis in the state of Gujarat, India Int J Tuberc Lung Dis 13(9): 1154–1160.
13. World Health Organization (2011) Guidelines for the programmatic management of drug-resistant tuberculosis. 2011 update. Geneva, Switzerland: WHO.
14. Central TB Division (2012) Programmatic Management for Drug-resistant Tuberculosis guidelines-May version, Directorate General of Heath Services, Ministry of Health and Family Welfare. Available: http://www.tbcindia.nic.in/pdfs/Guidelines%20for%20PMDT%20in%20India%20-%20May%202012.pdf. Accessed 2014 May 5.
15. Jenkins HE, Tolman AW, Yuen CM, Parr JB, Keshavjee S, et al. (2014) Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. Lancet 383(9928): 1572–1579.
16. Almeida D, Rodrigues C, Udwadia ZF, Lalvani A, Gothi GD, et al. (2003) Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. Clin Infect Dis 36: e152–4.
17. Singh S, Sankar MM, Gopinath K (2007) High rate of extensively drug-resistant tuberculosis in Indian AIDS patients. AIDS 21(17): 2345–7.
18. D'souza DT, Mistry NF, Vira TS, Dholakia Y, Hoffner S, et al. (2009) High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. BMC Public Health 29: 9–211.
19. Rodrigues C, Shenai S, Sadani M, Thakkar P, Sodha A, et al. (2006) Multi drug-resistant tuberculosis in Mumbai: it's only getting worse. Int J Tuberc Lung Dis 10(12): 1421–1422.
20. Chowgule RV, D Lina (1998) Pattern of secondary acquired drug resistance to antituberculosis drugs in Mumbai, India 1991–1995. Ind J Chest Dis Allied Sciences 40: 23–31.
21. Karande S, Bavdekar SB (2002) Children and multidrug-resistant tuberculosis in Mumbai (Bombay), India. Emerg Infect Dis 8(11): 1360–1361.
22. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C (2011) Totally Drug-Resistant Tuberculosis in India. Clin Infect Dis. doi:10.1093/cid/cir889.
23. TIME Magazine (2013). Contagion; Why drug-resistant tuberculosis threatens us all. March 4, 2013.
24. Isaakidis P, Rangan S, Pradhan A, Ladomirska J, Reid T, et al. (2013) 'I cry every day': experiences of patients co-infected with HIV and multidrug-resistant tuberculosis. Trop Med & Int Health 18(9): 1128–1133.
25. Uplekar M, Juvekar S, Morankar S, Rangan S, Nunn P (1998) Tuberculosis patients and practitioners in private clinics in India. Int J Tuberc Lung Dis 2(4): 324–329.
26. Bhargava A, Pinto L, Pai M (2011) Mismanagement of tuberculosis in India: Causes, consequences, and the way forward. Hypothesis 9(1): e7.
27. Udwadia ZF, Pinto LM, Uplekar MW (2010) Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? PLoS One 5: e12023.
28. Cox HS, Niemann S, Ismailov G, Doshetov D, Orozco JD, et al. (2007) Risk of acquired drug resistance during short-course directly observed treatment of tuberculosis in an area with high levels of drug resistance. Clin Infect Dis 441421–1427.
29. Agrawal D, Udwadia ZF, Rodriguez C, Mehta A (2009) Increasing incidence of fluoroquinolone-resistant Mycobacterium tuberculosis in Mumbai, India. Int J Tuberc Lung Dis 13(1): 79–83.
30. Andrews JR, Gandhi NR, Moodley P, Shah NS, Bohlken L, et al. (2008) Exogenous reinfection as a cause of multidrug-resistant and extensively drug-resistant tuberculosis in rural South Africa. JID 198: 1582–1589.
31. March F, Garriga X, Rodriguez P, Moreno C, Garrigo M, et al. (1997) Acquired drug resistance in Mycobacterium tuberculosis isolates recovered from compliant patients with human immunodeficiency virus-associated tuberculosis. Clin Infect Dis 25: 1044–1047.
32. Gandhi NR, Shah NS, Andrews JR, Vella V, Moll AP, et al. (2010) HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. Am J Respir Crit Care Med 181: 80–86.
33. Cox HS, Kubica T, Doshetov D, Kebede Y, Rüsch-Gerdess S, et al. (2005). The Beijing genotype and drug resistant tuberculosis in the Aral Sea region of Central Asia. Respiratory research, 6(1): 134.
34. Almeida D, Rodrigues C, Ashavaid TF, Lalvani A, Udwadia ZF, et al. (2005). High incidence of the Beijing genotype among multidrug-resistant isolates of Mycobacterium tuberculosis in a tertiary care center in Mumbai, India. CID, 40(6): 881–886.
35. Seddon JA, Hesseling AC, Finlayson H, Fielding K, Cox H, et al. (2013) Preventive therapy for child contacts of multidrug-resistant tuberculosis: a prospective cohort study. CIDcit655.

ORIGINAL RESEARCH PAPER

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TO STUDY THE DIAGNOSTIC ACCURACY OF ACID FAST BACILLI SMEAR FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS.

Microbiology

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ABSTRACT

To study the diagnostic accuracy of Acid fast bacilli smear with the gold standard TB culture by MGIT method.

Materials and Methods: 547 patients were screened for tuberculosis based on criteria for Tuberculosis like cough for more than 2 weeks, fever, loss of weight and loss of appetite, haemoptysis.

Observations & Results: Out 547 patients screened for tuberculosis, 237 patients were positive for tuberculosis by MGIT culture method and only 100 patients were positive by AFB smear method. The sensitivity was 42.19%, specificity was 99.03%, and the diagnostic accuracy was 74.41%.

Conclusion: The sensitivity of acid fast bacilli smear is low with high specificity. There is a need for a inexpensive and better tool for rapid diagnosis of tuberculosis.

KEYWORDS

Tuberculosis, Ziehl Neelson staining, AFB smear, TB MGIT culture

Introduction: The disease Tuberculosis is major public health problem. As per WHO about 1.3 million people died of the tuberculosis in 2014.[1] There is a requirement of rapid, accurate diagnosis for timely start of treatment.

Materials and Methods: Our study was observational prospective study. The study was cleared by Institutional ethics and research committee. About 547 patients were screened for tuberculosis based on clinical features suggestive of Pulmonary Tuberculosis. Patients' consent was taken and accordingly sputum samples were collected in Falcons tube as per RNTCP protocol.

From each patient's sputum sample, One part was processed for Bactec MGIT 960 system for mycobacterium tuberculosis culture whereas the other part of sputum was processed for acid fast bacilli smear (Ziehl Neelson staining method) as per standard guidelines mentioned in RNTCP (Revised national Tuberculosis Programme).

N o Of AFB seen	Report	Fields
1-2/300 fields	Doubtful	1-2/300 field
1-9 /100fields	1+	1-9/100 field
1-9/10 field	2+	1-9/10 field
1-9/field	3+	1-9/field
>9/field	4+	>9/field

Observations and Results:

Out of 547 eligible TB suspected patients, 230 positive by Gold standard Tb Culture were included as positive for active pulmonary TB and entered into the analysis of data.

TABLE 1: Gender wise distribution of TB Suspected Patients

Gender	TB Suspected Patients	(%)
Male	334	61.0
Female	213	39.0
Total	547	100

Out of 547 patients screened for Tb, 334 (61%) were males and 213(39%) were females.

Table2: Diagnostic accuracy of Smear AFB in comparison with TB Culture as Gold standard

	TB Culture Positive	TB Culture Negative	Total
Smear AFB Positive	100	3	103
Smear AFB Negative	137	307	444
Total	237	310	547

Statistic	Value (%)	95%CI
Sensitivity	42.19	35.83 to 48.76
Specificity	99.03	97.20 to 99.80
PPV	97.09	91.46 to 99.05
NPV	69.14	66.76 to
Diagnostic accuracy	74.41	70.53 to 78.01

Our results show that5 Smear AFB microscopy had a sensitivity of 42.19% and specificity of 99.03%. The diagnostic accuracy was 74.41%

Fig 1. Acid fast bacilli in ZN stained smear microscopy

Fig 2: Biosafety cabinet for TB sample processing

Discussion:

Our study evaluates smear for AFB against Gold standard TB MGIT culture results by MGIT culture come earlier than conventional LJ culture methods.[2,3]

Comparative evaluation of Sensitivity, PPV & NPV of Smear AFB microscopy in following studies.[4,5,6]

Study	Sensitivity	Specificty	PPV	NPV
Pierrrae et al[4]	25	95.8	45.5	90.1
Dewald et al[5]	41	98.6	94.1	75.8
Kanwal et al[6]	39	100	100	11.86
Our study	42	99	97.03	69.14

After comparing with Gold standard MGIT TB culture, sensitivity, specificity, PPV & NPV of Smear AFB microscopy were 42%, 99%,

97.03%, 69.14% respectively, which is similar to other studies as shown in the table above.

Smear AFB microscopy is the most practical and fast method for screening & diagnosis of PTB. There has to be approx 10^4 tubercle bacilli per ml of sputum to be seen positive in AFB smear microscopy.[7] AFB Microscopy has good specificity at 99% & diagnostic accuracy of 74.41%. Still, a negative smear should be interpreted with caution and does not rule out the possibility of Pulmonary TB.

Conclusion: The smear AFB microscopy commonly used in most laboratories because of shorter time and low risk of infection to Lab personnel. Though it has lesser sensitivity, specificity is good. In order to accurately diagnose pulmonary tubetrculosis, a culture should be always requested concommitanlty with AFB smear as negative smear does not rule out active tuberculosis and not all positive AFB smears are M. tuberculosis, but could be atypical mycobacteria.

References:

1. World Health Organisation. Global tuberculosis report 2014 Geneva: WHO; 2014.
2. Sun JR, Lee SY, Perng CL, Lu JJ. Detecting Mycobacterium tuberculosis in Bactec MGIT 960 cultures by in house IS6110- based PCR assay in routine clinical practice. J Formos Med Assoc. 2009;108:119–25.
3. Zhao P, Fang F, Yu Q, Guo J, Zhang JH, Qu J, et al. Evaluation of Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to first-line drugs in China. PLoS One. 2014; 9:e996594.
4. Pierre Le, Palud P, Cattoir V, Malbruny B, Magnier R, Campbell K, Oulkhouir Y, et al. Retrospective observational study of diagnostic accuracy of the Xpert® MTB/RIF assay on fiberoptic bronchoscopy sampling for early diagnosis of smear-negative or sputum-scarce patients with suspected tuberculosis. BMC Pulm Med. 2014; 14(1):137.
5. Barnard DA, Irusen EM, Bruwer JW, Plekker D, Whitelaw AC, Deetlefs JD, et al. Koegelenberg The utility of Xpert MTB/RIF performed on bronchial washings obtained in patients with suspected pulmonary tuberculosis in a high prevalence setting. BMC Pulm Med. 2015; 15:103.
6. Khalil KF, Butt T. Diagnostic yield of bronchoalveolar lavage gene xpert in smear-negative and sputum-scarce pulmonary tuberculosis. Journal of the College of Physicians and Surgeons Pakistan. 2015; 25(2):115–18.
7. Contreras A, et al.Pulmonary infection with NTM. AM Rev Resp Dis 1988;137:142-152

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Original Research Article

Effectiveness of CBNAAT in the diagnosis of extrapulmonary tuberculosis

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ABSTRACT

Background: Tuberculosis is still a major health problem worldwide. It is estimated that about one-third of the world's population is infected with mycobacterium tuberculosis. While pulmonary tuberculosis is most common presentation; extrapulmonary tuberculosis is also an important clinical problem. CBNAAT is cartridge based nucleic acid amplification test with a well-established role in the diagnosis of pulmonary tuberculosis (PTB). We determined the effectiveness of CBNAAT in the diagnosis of extrapulmonary tuberculosis (EPTB) cases in comparison to AFB smear.
Methods: Retrospective study of suspected extrapulmonary tuberculosis patients in a tertiary care centre of the study area was conducted. The study period was from January 2017 to July 2018. Data of 166 consecutive suspected extrapulmonary tuberculosis patients was retrieved. Effectiveness of CBNAAT in the diagnosis of EPTB was assessed as compared to that of AFB smear.
Results: Samples collected from 166 suspected EPTB patients were subjected to AFB smear and CBNAAT. Samples collected included lymph node, pus, pleural fluid, tissue, CSF, gastric lavage, cystic fluid, peritoneal fluid, ascitic fluid, colonic fluid, synovial fluid, urine. In AFB smear results, 17 cases were positive for TB bacilli and 149 were negative for the same. In CBNAAT results, 25 cases were positive for TB bacilli and 141 cases were negative. In comparative analysis, 8 cases were AFB smear negative but CBNAAT positive.
Conclusions: CBNAAT is a useful tool in the diagnosis of EPTB cases because of its simplicity and rapid turnaround time. CBNAAT is more effective as compared to AFB smear in the diagnosis of EPTB cases.

Keywords: CBNAAT, Extrapulmonary tuberculosis, Pleural effusion, Tuberculosis

INTRODUCTION

Tuberculosis is still a major health problem worldwide. It is estimated that about one-third of the world's population is infected with mycobacterium tuberculosis.

While pulmonary tuberculosis is most common presentation; extrapulmonary tuberculosis is also an important clinical problem. Worldwide, extrapulmonary tuberculosis (EPTB) accounts for ~25% of all TB cases.[1]

Tuberculosis is one of the dreaded diseases which accounts for 9.6 million cases globally as per the WHO Global TB Report 2015. Among these cases India contributes to 2.2 million incidence cases. It has not only high morbidity but also the mortality is high with 0.22 million deaths in India in 2015.[2] The prevalence of tuberculosis was estimated to be 10.5 million. In India alone 1.8 million new cases of TB arise annually.[3,4] It is estimated that about 40% of the Indian population is infected with TB bacteria.

To reduce the incidence and prevalence, India introduced National Tuberculosis Control Programme (NTP) in 1962, followed by Revised National Tuberculosis Control Programme (RNTCP) 1993-1996 and with Directly Observed Treatment Short-Course chemotherapy (DOTS) strategy in 1997. WHO released STOP TB STRATEGY in 2006. India adopted it in 2007. There are continuous efforts made to decrease the incidence and prevalence of tuberculosis, continuous change in the strategies under RNTCP are made. Further there was adoption of Goals of NSP with a vision of TB Free India in 12th five-year plan in (2012-17). The current adoption of END TB STRATEGY has a vision of WORLD FREE OF TB.[5]

TB affecting other sites-known as extra-pulmonary TB, is rarely smear-positive; it is generally accepted that the contagious potential of this form is negligible, and it has, therefore, never been a priority in the campaigns undertaken by national TB control programs.[6,7] Lymph nodes are the most common site of involvement followed by pleural effusion and virtually every site of the body can be affected.[8]

Extrapulmonary tuberculosis forms a significant proportion of the total TB cases and is a major health problem in both developing and developed countries. Diagnosing EPTB is challenging due to its varied clinical presentations and paucibacillary nature of the disease.[9] AFB smear hasn't proved to be much useful in diagnosing EPTB. CBNAAT is cartridge-based nucleic acid amplification test which detects the presence of TB bacilli and tests for resistance to Rifampicin also. CBNAAT is likely to revolutionize the diagnosis and treatment of EPTB, as it is a very cost-effective and rapid test. Hence, we performed a retrospective analysis to assess the effectiveness of CBNAAT for diagnosing EPTB as compared to that of AFB smear. Our study aimed to define the role of CBNAAT in clinical decision-making in suspected EPTB cases.

In this study, all patients suspected to have EPTB are evaluated by means of AFB smear and CBNAAT. Comparative analysis of the results of AFB smear and CBNAAT is done.

METHODS

The study is a retrospective descriptive study conducted at MGM Hospital Aurangabad after obtaining permission of the institutional ethical committee. The study used data from January 2017 to July 2018.

All the patients who were suspected to have extrapulmonary tuberculosis and are above 18 years of age were included in the study. Patients suspected to have or those who already have pulmonary tuberculosis and are below 18 years of age were excluded from the study.

The study population consisted of all the suspected extrapulmonary tuberculosis patients visiting the OPD of MGM Hospital Aurangabad and who were willing for AFB smear and CBNAAT investigations.

To fulfil the objectives of research, samples from all the patients suspected to have extrapulmonary tuberculosis were collected and subjected to AFB smear and CBNAAT. CBNAAT is cartridge-based nucleic acid amplification test which detects the presence of TB bacilli and tests for resistance to Rifampicin also. It is simple, rapid, cost effective and doesn't require technical expertise. It can diagnose TB within 2hours and gives accurate results due to use of disposable closed cartridges preventing cross contamination. In settings where resources are limited for facilities like culture DST, CBNAAT is extremely useful, simple and reliable test.

Samples collected included lymph node, pus, pleural fluid, tissue, CSF, gastric lavage, cystic fluid, peritoneal fluid, ascitic fluid, colonic fluid, synovial fluid, urine. The results of both the AFB smear and CBNAAT of the samples of all the suspected extrapulmonary tuberculosis patients were compared in the study. Reports were retrieved from MGM Hospital Aurangabad. This laboratory is accredited by RNTCP for AFB smear and CBNAAT testing of samples taken both pulmonary and extrapulmonary tuberculosis patients.

It was a record-based study so consent of the patients for inclusion criteria was not taken into consideration. Confidentiality of the patients was maintained.

RESULTS

Total of 166 patients were included in the study. All the patients who were suspected to have extrapulmonary tuberculosis and are above 18 years of age were included in the study. Patients suspected to have or those who already have pulmonary tuberculosis and are below 18 years of age were excluded from the study. Samples collected included lymph node, pus, pleural fluid, tissue, CSF, gastric lavage, cystic fluid, peritoneal fluid, ascitic fluid, colonic fluid, synovial fluid, urine. Samples from all the patients suspected to have extrapulmonary tuberculosis were collected and subjected to AFB smear and CBNAAT.

Among 166 suspected extrapulmonary tuberculosis cases, 17 samples were AFB smear positive and 149 were negative for AFB smear. Figure 1 shows the distribution of patients according to diagnosis based on AFB smear.

In CBNAAT results, 25 out of 166 suspected extrapulmonary tuberculosis cases were positive for TB bacilli and 141 were negative for the same. Figure 2 shows the distribution of patients according to diagnosis based on CBNAAT.

When the results of the 166 samples collected form suspected extrapulmonary tuberculosis patients subjected to AFB smear and CBNAAT were compared, 8 cases

turned out to be negative for TB bacilli in AFB smear whereas the same samples positive for TB bacilli in CBNAAT i.e. 8 cases were falsely reported as negative for TB bacilli in AFB smear. As the suspected extrapulmonary tuberculosis samples included many samples other than pleural fluid and lymph node, the overall samples turning out to be positive for TB bacilli in both AFB smear and CBNAAT is less. It would have been higher if only pleural fluid and lymph node samples were included in the samples used for the study.

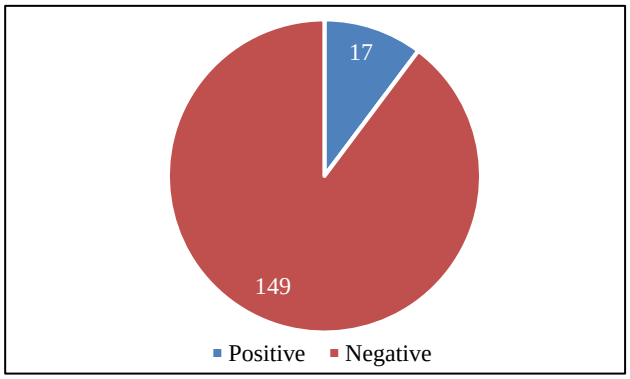

Figure 1: Distribution of patients according to diagnosis based on AFB smear.

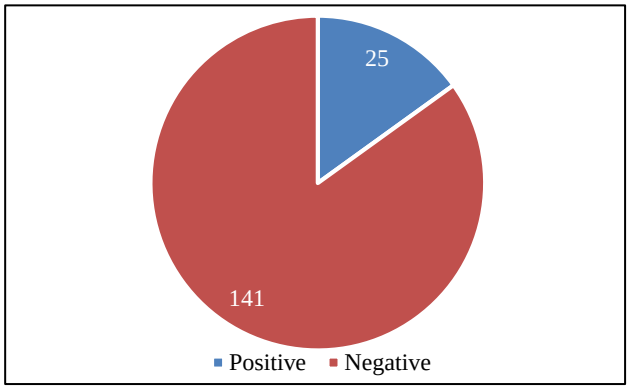

Figure 2: Distribution of patients according to diagnosis based on CBNAAT.

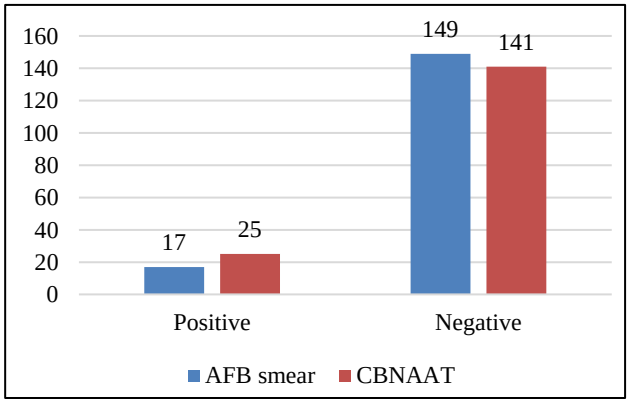

Figure 3: Distribution of patients according to diagnosis. A comparison between AFB smear and CBNAAT results.

Figure 3 shows the distribution of patients according to diagnosis, a comparison between AFB smear and CBNAAT results. The above comparison of the results of AFB smear and CBNAAT examination of various samples of suspected extrapulmonary tuberculosis patients proves that CBNAAT is much better alternative to AFB smear in the diagnosis of extrapulmonary tuberculosis.

DISCUSSION

EPTB contributes to a significant burden of mortality and morbidity due to its complex and subclinical presentations, leading to a delay in diagnosis. The conventional methods such as culture DST are time consuming and require trained laboratory personnel.

CBNAAT is a semi-quantitative nested nucleic acid amplification test based on molecular detection of mutated gene. It is simple, rapid, cost effective and doesn't require technical expertise. It can be carried out in automated manner including bacterial lysis, nucleic acid extraction, and amplification and amplicon detection. It can diagnose TB within 2hours and gives accurate results due to use of disposable closed cartridges preventing cross contamination.[10] In settings where resources are limited for facilities like culture DST, CBNAAT is extremely useful, simple and reliable test. It also has a significant role to play in the diagnosis of extrapulmonary tuberculosis. Its potential in EPTB detection has been underutilized due to lack of awareness regarding the same. Hence, we conducted the study to determine effectiveness of this rapid and logistically simplified test in the diagnosis of EPTB.

The prevalence of EPTB is showing a rising trend. The heterogenous clinical presentations, paucibacillary nature and difficulty in obtaining specimens (often requiring invasive procedures) make the diagnosis of EPTB, a challenging task and hence the requirement for a rapid, simplified and cost effective diagnostic tool arises.[11] This is where CBNAAT plays an important role leading to early initiation of appropriate therapy, improved treatment outcomes, minimizing morbidity and mortality.

According to a metaanalysis findings of Denkinger et al, sensitivity among pleural fluid was 46% and sensitivity among lymph node specimens was 83%.[12] Another meta-analysis finding of Penz et al, suggested sensitivity among pleural fluid was 37% and lymph node specimen was 87%.[13] A recently published study by Sharma et al, regarding the utility of GeneXpert in diagnosing EPTB has shown an overall sensitivity of 71% and PPV ranging from 98 to 100%.[14] But direct comparisons could not be drawn with our study. In this study, we found that the results of CBNAAT were better when compared to AFB smear for EPTB samples. Although the overall positive cases of EPTB diagnosed through these tests were less as the suspected extrapulmonary tuberculosis samples included many

samples other than pleural fluid and lymph node, it is however consistent with other studies of EPTB in the literature.[12-14] It would have been higher if only pleural fluid and lymph node samples were included in the samples used for the study.

This findings suggest that CBNAAT plays a major and important role in the diagnosis of EPTB, particularly in places with high burden and limited availability of resources. CBNAAT could be the best aid for physicians in diagnosing EPTB if more awareness is brought among them regarding its utility. Our study highlighted that CBNAAT can be a faster alternative to time taking methods like culture DST and at the same time a more efficient alternative to other rapid methods like AFB smear examination in the diagnosis of EPTB. With our study, we conclude that CBNAAT is more effective as compared to AFB smear in the diagnosis of EPTB cases and CBNAAT should be routinely utilized for rapid diagnosis of EPTB along with other conventional methods like AFB smear examination and culture DST for better overall results in the diagnosis of EPTB.

CONCLUSION

CBNAAT is a useful tool in the diagnosis of EPTB cases because of its simplicity and rapid turnaround time. CBNAAT is more effective as compared to AFB smear in the diagnosis of EPTB cases and CBNAAT should be routinely utilized for rapid diagnosis of EPTB along with other conventional methods like AFB smear examination and culture DST for better overall results in the diagnosis of EPTB. As the suspected extrapulmonary tuberculosis samples included many samples other than pleural fluid and lymph node, the overall samples turning out to be positive for TB bacilli in both AFB smear and CBNAAT is less. It would have been higher if only pleural fluid and lymph node samples were included in the samples used for the study.

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REFERENCES

1. World Health Organization. Global tuberculosis report 2012. Geneva, WHO, 2012. Available at: http://www.who.int/tb/publications/global_report/gtbr12_main.pdf.
2. Fundamental of tuberculosis. Global tuberculosis institute. Available at: globaltb.njms.rutgers.edu/downloads/products/Mantoux.../FundamentalsofTB.ppt. Accessed on 12 December 2017.
3. World Health Organization. Tuberculosis Fact Sheet. Fact Sheet No. 104. Available at: http://www.who.int/mediacenter/factsheets/fs104/en/print.html. Accessed on 15 February 2009.
4. Steinbrook R. Tuberculosis and HIV in India. New Engl J Med. 2007;356:1198-9.
5. Park K. Park's Textbook of Preventive and Social Medicine. 24th ed. Jabalpur: M/s Banarsidas Bhanot Publishers;2017.
6. World Health Organization. Tuberculosis programme: Framework for effective tuberculosis control. Geneva, Switzerland: WHO;1994:179.
7. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Ind J Med Res. 2004;120(4):31653.
8. Gupta S, Shenoy VP, Bairy I, Srinivasa H, Mukhopadhyay C. Diabetes mellitus and HIV as co-morbidities in tuberculosis patients of rural south India. J Infec Pub Heal. 2011 Aug 1;4(3):140-4.
9. Maurya AK, Kant S, Nag VL, Kushwaha RA, Dhole TN. Trends of anti-tuberculosis drug resistance pattern in new cases and previously treated cases of extrapulmonary tuberculosis cases in referral hospitals in northern India. J Postgrad Med. 2012;58(3):185-9.
10. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampicin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010 Jan;48(1):229-37.
11. Chaudhary A, Barve K, Desai U, Joshi J. Utility of GeneXpert in diagnosis of multidrug-resistant extrapulmonary tuberculosis. Inter J Recent Surg Med Sci. 2017;3(2):85-7.
12. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2014;44(2):435-46.
13. Penz E, Boffa J, Roberts DJ, Fisher D, Cooper R, Ronksley PE, James MT. Diagnostic accuracy of the Xpert MTB/RIF assay for extra-pulmonary tuberculosis: a meta-analysis. Int J Tuber Lung Dis. 2015 Mar;19(3):278-84.
14. Sharma SK, Kohli M, Chaubey J, Yadav RN, Sharma R, Singh BK, et al. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. Eur Respir J. 2014 Oct;44(4):1090-3.

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Clinical Case Report

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Rare case of pulmonary lymphomatoid granulomatosis in conjunction with tuberculosis

A case report

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Abstract

Rationale: Lymphomatoid granulomatosis is a very rare Epstein-Barr virus-driven lymphoproliferative disease. This disease has high mortality owing to its low incidence in conjunction with nonspecific presentations, which contribute to delays in diagnosis.

Patient: An 87-year-old male had a week-long history of intermittent fever and general weakness. A chest radiograph showed multifocal patchy consolidations with nodular lesions.

Diagnoses: Open lung biopsy using video-assisted thoracic surgery resulted in a diagnosis of grade III lymphomatoid granulomatosis. Three days after surgery, *Mycobacterium tuberculosis* complex was identified from the culture of sputum samples collected at admission.

Intervention and outcomes: Antituberculous treatment was commenced first. However, after 34 days of antituberculosis medication, the patient died owing to aggravated lymphomatoid granulomatosis.

Lessons: This case highlights the fact that rare diseases should also be considered in differential diagnosis, particularly with a common presentation such as multiple lung nodules. Furthermore, a diagnosis of pulmonary lymphomatoid granulomatosis was made after open lung biopsy. To our knowledge, this is the first case of lymphomatoid granulomatosis coexisting with active tuberculosis in the Republic of Korea, where tuberculosis is endemic.

Abbreviations: AFB = acid-fast bacilli, ANA = antinuclear antibodies, anti-HCV = antihepatitis C antibody, BAL = bronchoalveolar lavage, CRP = C-reactive protein, CT = computed tomography, EBER = Epstein-Barr virus-encoded small RNA, EBV = Epstein-Barr virus, ECOG = Eastern Cooperative Oncology Group, ESR = erythrocyte sedimentation rate, GMS = Grocott–Gomori's methenamine silver, HBsAg = hepatitis B surface antigen, HIV = human immunodeficiency virus, hpf = high-power field, IFN-γ = interferon gamma, LDH = lactate dehydrogenase, LYG = lymphomatoid granulomatosis, PAS = periodic acid–Schiff, PCNB = percutaneous core needle biopsy, PLG = pulmonary lymphomatoid granulomatosis, Th1 = type 1 helper T lymphocyte, VATS = video-assisted thoracic surgery, WBC = white blood cell, WHO = World Health Organization.

Keywords: Epstein-Barr virus, pulmonary lymphomatoid granulomatosis, pulmonary tuberculosis

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Y.W.H. drafted the manuscript. Y.K.Y. coordinated and revised the manuscript. C.H.K. was responsible for the pathological review. Y.P., J.W.S., M.J.K., Y.W.H. and Y.K.Y. were responsible for patient treatment. All authors read and approved the final manuscript.

The case report was approved by the institutional review board of Korea University Anam Hospital (No. AN17098–001). Informed consent was obtained from the patient's son for publication of this case report and the associated images.

The authors declare no conflicts of interest.

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1. Introduction

Pulmonary lymphomatoid granulomatosis (PLG), which has been defined as a clinicopathological entity is a rare Epstein–Barr virus (EBV)-driven lymphoproliferative pulmonary disorder. It involves an angiocentric and angiodestructive process that affects the lung, via the invasion of bilateral nodular infiltrates composed of EBV-driven B-cells that lack true granulomatous features, and subsequent destruction of blood vessels.[1,2] Clinical manifestation suggestive of an atypical lymphoma and pulmonary vasculitis represents an overlap syndrome between angiitis and lymphoma. Thus, various pathogenetic conditions can be comprised in autoimmunity, infection, and malignancy.[3,4]

Lymphomatoid granulomatosis is usually observed as primary lesions in the lung; however, nonspecific clinical features of PLG are similar to those of more common pulmonary disorders, including tuberculosis, histoplasmosis, Wegener's granulomatosis, Churg-Strauss syndrome, sarcoidosis, cryptogenic organizing pneumonia, and malignancy.[5] Its low incidence combined with manifestations that overlap with other diseases results in difficulty diagnosing PLG. Other common sites of extranodal involvement include kidney (40%–50%), skin (25%–50%), central (25%–50%) or peripheral (15%–20%) nervous system, liver (10%), spleen (10%), and lymph nodes (<10%).[6]

A complex relationship exists between lymphomatoid granulomatosis and functioning of the host's immune system.[6] Most patients have been diagnosed in conjunction with autoimmune diseases, chronic hepatitis infections, postorgan transplantation and postintensive therapy for malignancy.[7–9] Treatment options of these patients includes corticosteroids, anti-CD20 monoclonal antibodies, interferon-α-2b and combination chemotherapy, but PLG has extremely poor prognosis.

Herein, we report a case of PLG in conjunction with active tuberculosis. To our knowledge, this manifestation has not been previously described.

2. Case report

The patient was an 87-year-old male, nonsmoker, with a known history of pulmonary tuberculosis 14 years previously and complete recovery from prostate cancer 10 years earlier, who was also diagnosed with type 2 diabetes mellitus, hypertension, and hypothyroidism. Previous diagnosis of pulmonary tuberculosis was confirmed based on a positive culture for *Mycobacterium tuberculosis*. He was treated with a standard four-drug therapy for drug-susceptible tuberculosis. However, a negative sputum culture for *M. tuberculosis* was not confirmed during the 6-month treatment, owing to improvement of his respiratory symptoms. After recovery, the patient was subsequently rehospitalized for treatment of prostate cancer. Although the patient lived in South Korea, which has a high prevalence of tuberculosis, he did not report of any memorable exposure to patients with active tuberculosis.

He was regularly taking the following medications: metformin and saxagliptin for diabetes, levothyroxine sodium hydrate for hypothyroidism, and ramipril for hypertension.

The patient presented to our hospital with a week-long history of intermittent fever and general weakness. On admission, he had a cough, poor oral intake, and experienced dyspnea after walking for a few minutes on level ground. General physical examination revealed bilateral crackles and rales on both lung fields. There was no evidence of cardiac murmur, lymphadenopathy, hepatosplenomegaly, or skin lesion. No Osler nodes, Janeway lesions or splinter hemorrhages were observed.

In the initial laboratory results, the patient's complete blood counts were as follows: hemoglobin 9.9 g/dL (normal limits 12~16 g/dL), white blood cell (WBC) count 9500/μL (normal limits 4,500~11,000/μL), and platelet count 376,000/μL (normal limits 150,000~400,000/μL). WBC differential count with neutrophilia was 7837/μL (82.5%, normal limits 45%~75%). Erythrocyte sedimentation rate (ESR) of 57 mm/hr (normal limits 0~20 mm/hr) and C-reactive protein (CRP) of 57.4 mg/L (normal limits 0~5 mg/L) were mildly elevated; procalcitonin was 0.14 ng/mL (normal limits 0~ 0.05 ng/mL). A chest radiograph and computed tomography (CT) scan showed multifocal patchy consolidations with nodular lesions of variable sizes and irregular margins combined with right pleural effusion, but there was no hilar or mediastinal lymphadenopathy (Fig. 1 A and B).

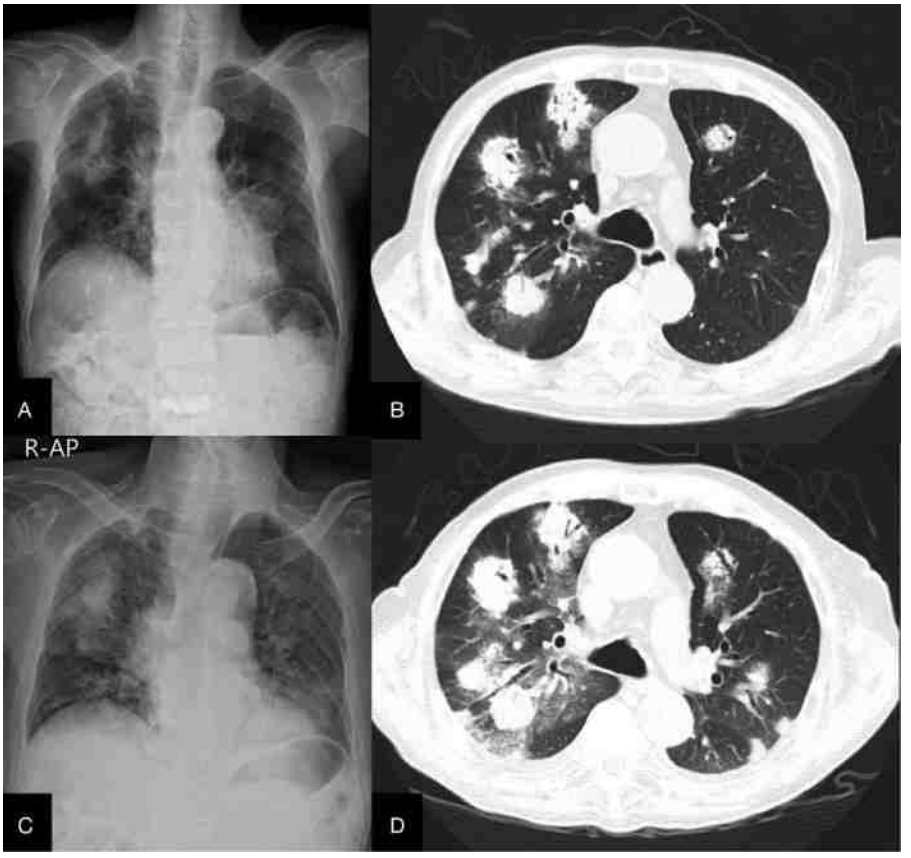

Figure 1. (A) Initial chest radiograph showing multiple nodular lesions in both lung fields. (B) Chest computed tomography (CT) scans revealing multifocal consolidations with ground-glass opacity and right pleural effusion. (C and D) Follow-up chest radiograph and chest CT scans demonstrating interval aggravation of multiple nodular opacities.

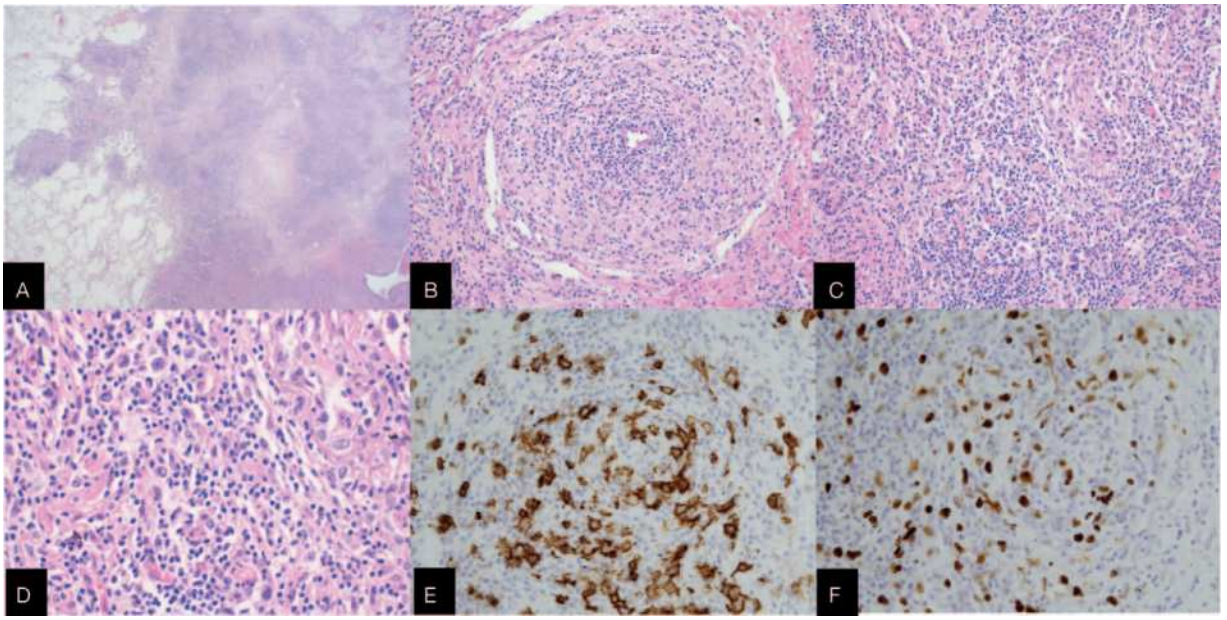

Figure 2. A, A well-circumscribed mass destroying lung parenchyma, composed of proliferated lymphoid cells (H & E stain, × 40). B, Histologic appearance shows transmural infiltration of atypical lymphoid cells around small- and medium-sized vessel walls (H & E stain, × 200). C, Most infiltrated cells are scattered, large atypical lymphoid cells with small lymphocytes, plasma cells, and histiocytes (H & E stain, × 200). D, Some atypical lymphoid cells are binucleated, similar to Reed–Sternberg cells (H & E stain, × 400). E, Immunohistochemical staining shows scattered CD20-positive large atypical B cells (H & E stain, × 400). F, These large B cells are positive for EBV by in situ hybridization (H & E stain, × 400).

Early differential diagnosis of fever and lung nodules included tuberculosis, pulmonary septic emboli, fungal infection, vasculitis, and malignancy. Transthoracic echocardiography demonstrated no evidence of infective endocarditis. Analysis of pleural effusion showed a WBC count of 340/mm^3 with 94% lymphocytes, 4% neutrophils, and 2% monocytes, protein of 1.9 g/dL, lactate dehydrogenase (LDH) of 231 U/L, and pH of 8.0. Polymerase chain reaction for *M. tuberculosis* and cytology for malignancy were all negative. Fiber optic bronchoscopy with bronchoalveolar lavage (BAL) was inconclusive. All blood culture using the BacT/ALERT 3D Microbial Detection System (bioMérieux, Inc., Durham, NC) and sputum cultures using Vancomycin-Bacitracin-Clindamycin agar, MacConkey agar, and blood agar plates were negative for bacterial or fungal growth. Respiratory specimens, including BAL fluid, taken for acid fast bacillus (AFB) staining on more than three occasions were negative. A viral work-up for hepatitis B surface antigen (HBsAg), antihepatitis C antibody (anti-HCV) and human immunodeficiency virus (HIV), respectively; an autoimmune disease work-up for antinuclear antibodies (ANA) and a fungal work-up for *Aspergillus* antigen were also negative.

Under the clinical diagnosis of bacterial pneumonia, cefepime (2 g, twice daily) and intravenous teicoplanin (400 mg, once daily) were administered as empirical antibiotic therapy. However, after 2 weeks of antimicrobial treatment, follow-up chest radiograph and chest CT scans showed numerous aggravated nodular densities with cavities (Fig. 1 C and D), and his Eastern Cooperative Oncology Group (ECOG) scale of performance status deteriorated from grade II to grade IV. Thus, amphotericin B deoxycholate was prescribed concurrently based on a suspicion for fungal pneumonia.

Percutaneous core needle biopsy (PCNB) of the left lung was performed to differentiate between the suspected diagnoses of fungal pneumonia and malignancy. However, histological examination revealed only nonspecific findings of interstitial chronic inflammation with fibrosis and focal necrosis. After maintaining the combination antimicrobial therapy for 28 days, multifocal patchy consolidations with nodular lesions on chest CT scan showed no further improvement. Accordingly, open lung biopsy using video-assisted thoracic surgery (VATS) was conducted for wedge resection of the right lung (middle and lower lobes). The histopathology showed grade III lymphomatoid granulomatosis, composed of polymorphous infiltrate with large atypical and small lymphoid cells showing angiocentricity with fibroblastic stroma (Fig. 2).

The large atypical cells and small lymphocytes were positive for CD20 and CD3, respectively. The atypical lymphoid cells were positive for Epstein-Barr virus-encoded small RNA (EBER) with $>$ 50/high-power field (hpf). Grocott–Gomori's methenamine silver (GMS) and periodic acid–Schiff (PAS) stains revealed no fungal organisms. The AFB stain was negative and there was no granulomatous lesion consistent with mycobacterial infection on our biopsy specimen.

On the 30th day of hospitalization, a therapeutic plan for PLG was carefully established with intensive CHOP and rituximab; however, the initiation of chemotherapy was delayed because of general weakness of the patient. Subsequently, *M. tuberculosis* complex, which is susceptible to all antituberculous drugs, was identified on the 36th day of hospitalization from the culture of sputum samples collected at admission. Therefore, anticancer treatment was deferred until after antituberculous treatment. After 34 days of antituberculosis medication, the patient showed a consciousness deterioration and became completely disabled. The patient's subsequent death was attributed to have resulted because of the disease progression of PLG.

3. Discussion

PLG is a rare disease entity in the differential diagnosis of multiple pulmonary nodules. The rareness of PLG together with its

nonspecific clinical manifestations and radiological findings make its diagnosis difficult. PLG is even harder to cure because of the lack of an established treatment strategy. In our patient, the presentation of confounding features that were suggestive of pulmonary septic emboli, fungal pneumonia or lung abscess, and no prominent aggravation of pulmonary nodules during antimicrobial therapy for Gram-positive and Gram-negative germs, together contributed to delay in diagnosis.

The definite diagnosis of PLG hinges on histopathology, with mixed mononuclear cell infiltrate containing several CD20-positive large B-cells in a background of CD3-positive small lymphocytes. These findings are often accompanied by plasma cells and histiocytes, which together replace the lung parenchyma and cause vascular infiltration, as in our case. Multiple lung nodules radiologically with necrosis of the cellular infiltrate and positive EBER in situ hybridization were useful supportive findings. However, there was no skin or nervous system involvement, defined as optional manifestations.

As shown in our case, although the lung is the primary site of involvement, sputum cytology, transthoracic needle aspiration, and PCNB did not support PLG diagnosis. To obtain adequately sized lung tissue samples for evaluation, VATS or open thoracotomy should be performed at an early stage of diagnosis. Similar to our case, several previously reported cases of PLG were confirmed by open lung biopsy because transbronchial or percutaneous needle biopsy were inconclusive.[10–12]

PLG with diverse synonyms including angiocentric immunoproliferative lesion and angiocentric lymphoma, is currently classified as part of a spectrum of angiocentric and immunoproliferative lesions, composed of lymphoreticular cells lacking true granulomatous features.[13] This case was initially thought to be of T-cell phenotype, but recent papers have shown that PLG is an EBV-positive B-cell proliferation associated with an exuberant T-cell response.[1,14] This unusual disease is adversely affected by the uncommon complication of intercurrent tuberculosis.[15,16] The type 1 helper T lymphocyte (Th1) response, capable of synthesizing interferon gamma (IFN-γ) and other cytokines, contains *M. tuberculosis* in a latent state without active replication. Alteration of the Th1 cell response in PLG might lead to an impaired immune response that most likely promotes the progression from latent tuberculosis infection to its active form.[17]

PLG complicated with tuberculosis is an extremely rare and challenging condition owing to confusion in differential diagnosis, and it is usually incompatible with treatment for both diseases. In this case, commencing immune-suppressing chemotherapy in a much debilitated patient could aggravate the clinical severity of primary infections and the risk of drug toxicity. Thus, we had no choice but to defer chemotherapy and start antituberculosis therapy alone.

To prioritize risks and justify the treatment strategy, a formal staging system for the diagnosis of PLG would be valuable. The World Health Organization (WHO) recommends that lymphomatoid granulomatosis (LYG) be classified as grade I, grade II, or grade III, according to the number of EBV-positive large B-cells. Grade 1 is a finding of < 5 EBV-positive cells per hpf, and grade 3 is > 50 EBV-positive cells per hpf. However, there is considerable variation in EBV-positive cell counts between specimens.[13] Therefore, the treatment strategy is generally established comprehensively, based on the presence and severity of symptoms, the extent of extrapulmonary involvement, the histopathologic grade of the lesion and underlying diseases. Our case was categorized as grade 3, according to the WHO grading system, which corresponds to high-grade PLG

Options in the management of patients with lower-grade PLG includes treating the cause of immune dysfunction and observation for regression. Patients with higher-grade PLG require immediate therapy similar to aggressive lymphoma, including corticosteroids, anti-CD20 monoclonal antibodies, interferon α-2b, anticancer chemotherapy, radiotherapy and hematopoietic stem cell transplantation.[7,13] However, no standard treatment has yet been established. The disease is aggressive in most patients, with median survival of 2 years; the 5-year mortality is 60% to 90%.[18] Additional clinical data for development of a formal staging system and therapeutic plan for PLG should be collected, to improve the prognosis.

In conclusion, though uncommon, the possibility of PLG should be considered with a high degree of suspicion in differential diagnosis of lung nodules. In addition, if PLG is suspected, invasive investigations such as open lung biopsy should be performed, to reach an early diagnosis. Another implication of this report is that in patients from countries with high incidence of tuberculosis, it should be determined whether PLG is accompanied by tuberculosis.

References

[1] Nicholson AG, Wotherspoon AC, Diss TC, et al. Lymphomatoid granulomatosis: evidence that some cases represent Epstein-Barr virus-associated B-cell lymphoma. Histopathology 1996;29:317–24.

[2] Liebow AA, Carrington CR, Friedman PJ. Lymphomatoid granulomatosis. Hum Pathol 1972;3:457–558.

[3] Jaffe ES, Wilson WH. Lymphomatoid granulomatosis: pathogenesis, pathology and clinical implications. Cancer Surv 1997;30:233–48.

[4] Pisani RJ, DeRemee RA. Clinical implications of the histopathologic diagnosis of pulmonary lymphomatoid granulomatosis. Mayo Clin Proc 1990;65:151–63.

[5] Poletti V, Ravaglia C, Tomassetti S, et al. Lymphoproliferative lung disorders: clinicopathological aspects. Eur Respir Rev 2013;22:427–36.

[6] Roschewski M, Wilson WH. Lymphomatoid granulomatosis. Cancer J 2012;18:469–74.

[7] Katzenstein AL, Carrington CB, Liebow AA. Lymphomatoid granulomatosis: a clinicopathologic study of 152 cases. Cancer 1979;43:360–73.

[8] Kwon EJ, Katz KA, Draft KS, et al. Posttransplantation lymphoproliferative disease with features of lymphomatoid granulomatosis in a lung transplant patient. J Am Acad Dermatol 2006;54:657–63.

[9] Moertel CL, Carlson-Green B, Watterson J, et al. Lymphomatoid granulomatosis after childhood acute lymphoblastic leukemia: report of effective therapy. Pediatrics 2001;107:E82.

[10] Oosting-Lenstra SF, van Marwijk Kooy M. Failure of CHOP with rituximab for lymphomatoid granulomatosis. Neth J Med 2007;65:442–7.

[11] Connors W, Griffiths C, Patel J, et al. Lymphomatoid granulomatosis associated with azathioprine therapy in Crohn disease. BMC Gastroenterol 2014;14:127.

[12] Lad D, Malhotra P, Maskey D, et al. Pyrexia, lung nodules, granulomas: pulmonary lymphomatoid granulomatosis. Indian J Hematol Blood Transfus 2014;30:418–21.

[13] Colby TV. Current histological diagnosis of lymphomatoid granulomatosis. Mod Pathol 2012;25:S39–42.

[14] McNiff JM, Cooper D, Howe G, et al. Lymphomatoid granulomatosis of the skin and lung. An angiocentric T-cell-rich B-cell lymphoproliferative disorder. Arch Dermatol 1996;132:1464–70.

[15] Damjanov I, Duraković Z, Radonić M. Lymphomatoid granulomatosis of the lung associated with active tuberculosis. Z Erkr Atmungsorgane 1975;143:56–60.

[16] Morioka A. A case of lymphomatoid granulomatosis complicated with pulmonary tuberculosis. Japanese J Chest Dis 2012;71:700–7.

[17] Anibarro L, Pena A. Tuberculosis in patients with haematological malignancies. Mediterr J Hematol Infect Dis 2014;6:e2014026.

[18] Gitelson E, Al-Saleem T, Smith MR. Review: lymphomatoid granulomatosis: challenges in diagnosis and treatment. Clin Adv Hematol Oncol 2009;7:68–70.

NIJP

Original Research

Assessment of children in contact with sputum positive adult patients with respect to TB disease and latency

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Abstract:

Introduction: Tuberculosis (TB) contacts are people who have close contact with patients with infectious TB. TB contacts should be investigated systematically and actively for TB infection and disease. Such interventions are called 'tuberculosis contact investigations'. They contribute to early identification of active TB, thus decreasing its severity and reducing transmission of Mycobacterium tuberculosis to others, and identification of latent TB infection (LTBI), to allow preventive measures. The present study was undertaken to study epidemiological,socio-economic ,clinical factors in contact children of sputum positive cases. During study ,investigations were also carried out to differentiate stages of tubercular infection ,latency and disease.

Aims & Objectives: 1. To identify children in contact with adult index case and to assess contact investigation according to WHO guidelines. 2. Evaluate socioeconomic and Clinical parameters 3. Investigate as per guidelines and perform IGRA. 4. To find proportion of infection, disease and latency in contact children.

Materials and Methods Study Design: It was a prospective observational study, conducted in MGM Medical College and Hospital which is a tertiary care referral unit in Navi Mumbai, Raigad District of Maharashtra.

Study Period: The study was performed over a period from April 2015 to October 2016.

Sample Size: (80) SS = Z 2 x P x (1-P) 2 Z: value (1.96 for 95% CI) P: % of picking choice C: confidence interval.

Sampling Technique: *Selection of index cases*: An index case was defined as a newly pulmonary tuberculosis case in DOTS OPD (confirmed by 3 consecutive sputum smears positive for acid-fast bacilli and/or positive culture).Selection of contacts: Children contacts were defined as a family members or living with the index case in the same house before the starting of tuberculosis treatment of index case. They were recruited into study, and consent was obtained from the parent/ representatives to undertake the study.

Methodology: This was a prospective observational study consisting of 80 contact children, of smear positive PTB. The parents were interviewed using a standard questionnaire to obtain demographic and socio-economic information of the household and the health condition of the children. After the interview, children were examined by a clinician as well as nutritional assessment was done according to WHO protocol and necessary laboratory tests (x-ray, induced sputum and gastric lavage) were done to rule out active TB. All children were given a TST and 3 ml of blood was obtained for laboratory tests for IGRA.

Conclusion: Active tuberculosis (TB) and TB Infection is common among household contacts of index cases in India, especially among young children. Although all children with household exposure have a high risk of contracting the infection and disease, specific risk factors include severity of disease in cases and the intensity of exposure of the child.

Present study found that IGRAs is a good diagnostic tool for the diagnosis of latent tuberculosis infection as well as active disease.

Keywords: Active tuberculosis, Latent tuberculosis, IGRA, TST, Mantaux test

Introduction:

Tuberculosis (TB) contacts are people who have close contact with patients with infectious TB. TB contacts should be investigated systematically and actively for TB infection and disease. Such interventions are called 'tuberculosis contact investigations'. They contribute to early identification of active TB, thus decreasing its severity and reducing transmission of Mycobacterium tuberculosis to others, and identification of latent TB infection (LTBI), to allow preventive measures.

Contacts are commonly investigated in high-income countries with low TB burdens and in settings in which a TB elimination policy is implemented, in order to identify persons with early active TB or who have recently been infected. People identified as infected are then treated for LTBI with isoniazid for at least 6 months (usually 9 months) or with shorter combination regimens including isoniazid and rifampicin.

TB contact investigations are rarely and inconsistently carried out in resource-limited settings. In most low and middle-income countries, it is included in the national policy to control and prevent TB. However, in the vast majority of countries, it is either not undertaken or is implemented on the basis of no or poor standards, because of the absence of clear definitions of index cases, contacts and procedures. Furthermore, the health personnel who should be involved are usually not clearly identified.

Information on the contribution of routine contact investigations to early TB case detection is scarce in these countries or is non-standardized, thus precluding an assessment of its impact on reducing transmission.

Many studies in countries with a high TB incidence have shown that the prevalence may reach 5% or more among contacts, particularly among household members. Other data suggest that contact investigations could be particularly useful for identifying childhood TB.

WHO estimates show that worldwide highly infectious smear-positive pulmonary TB develops in over 4 million people annually. If we assume that each of these patients has at least three close contacts, such as in their household, and that the prevalence of active TB among the close contacts is 2.5%, the number of early TB cases that could be identified among close contacts is at least 300 000 per year.

Early identification means a better chance of cure and especially a reduction in further transmission. Furthermore, contact investigation allows identification of people who are latently infected and at high risk for active TB, who can be treated preventively.[1,2,3]

The present study was undertaken to study epidemiological, socio-economic ,clinical factors in contact children of sputum positive cases. During study, investigations also carried out to differentiate stages of tubercular infection, latency and disease.

Aims & Objectives:

1. To identify children in contact with adult index case and to assess contact investigation according to WHO guidelines.
2. Evaluate socioeconomic and Clinical parameters
3. Investigate as per guidelines and perform IGRA.
4. To find proportion of infection, disease and latency in contact children.

Materials and Methods Study Design:

It was a prospective observational study, conducted in MGM Medical College and Hospital which is a tertiary care referral unit in Navi Mumbai, Raigad District of Maharashtra.

Study Period: The study was performed over a period from April 2015 to October 2016.

Sample Size: (80) SS = Z 2 x P x (1-P) 2 Z: value (1.96 for 95% CI) P: % of picking choice C: confidence interval.

Sampling Technique:

Selection of index cases: An index case was defined as a newly pulmonary tuberculosis case in DOTS OPD (confirmed by 3 consecutive sputum smears positive for acid-fast bacilli and/or positive culture).

Selection of contacts: Children contacts were defined as a family members or living with the index case in the same house before the starting of tuberculosis treatment of index case. They were recruited into study, and consent was obtained from the parent/representatives to undertake the study.

Inclusion Criteria*:* Contact children from 6 months to 15 yr.

Exclusion Criteria: Children with immunodeficiency.

Methodology: This was a prospective observational study consisting of 80 contact children, of smear positive PTB. The parents were interviewed using a standard questionnaire to obtain demographic and socio-economic information of the household and the health condition of the children. After the interview, children were examined by a clinician as well as nutritional assessment was done according to WHO Protocol and necessary laboratory tests (x-ray, induced sputum and gastric lavage) were done to rule out active TB. All children were given a TST and 3 ml of blood was obtained for laboratory tests for IGRA.

Tuberculin Skin Test: All the children received 2 TU of PPD RT 23 with Tween 80 injected on the volar aspect of arm using the Mantoux method on the day of the initial visit. TST readings were obtained using the palpation method where horizontal diameter of the induration to the long axis of the arm was measured (WHO, 2006b) at 48-72 hours by trained laboratory technicians and results were classified according to the degree of indurations as negative (<5 mm), intermediate (5 to 9 mm) and positive (e" 10 mm).

IGRAs: The test was performed by drawing 1 ml of blood into one of each of the three manufacturer-precoated, heparinised tubes. Within 16 hours of blood collection, the tubes were incubated for another 16 to 24 hours at 37 °C. The plasma was harvested after centrifugation (tubes contain a gel plug that separates the plasma from the cells when centrifuged) and used to assess the concentration of IFN-α by ELISA test.

ELISA assay(3) - Immunocheck TB Platinum(IGRA) Make-Immunocheck

- 50 µl of working conjugate added to each well, then 50 µl of plasma or standard was added.

- The covered plate was shaked for 1 minute, and then incubated for 120 minutes at room temperature.

- The plate was washed 6 times with washing buffer and 100 µl substrate was added & incubated for 30 minutes at room temperature.

- 50µl stop solution was added and absorbance was read at 450 nm (620 ref) - IFN-α values (IU/ml) for TB-specific antigens were corrected for background by subtracting the value obtained for the respective negative control.

As recommended by the manufacturer, the cut-off value for a positive test was IFN-α e" 0.35 IU/ml.

Treatment: All active cases were treated with AKT according to RNTCP guidelines, rest children less than 6 years old with latent infection received INH chemoprophylaxis.

Results:

Most of the contacts were in between 5 to 10 years of age (57.5%). Rest 35% were <5 years and 7.5% were more than 10 years. Distribution of gender was done and out of total 80 contacts, 53.8% were males while 46.3% were females.

We had 43 index cases and 80 contact children related to them .The distribution of cases based on sputum grade out of 43 index cases 30.2% had +1 sputum positivity while 46.5% and 23.3% had +2 and +3 sputum positivity.

We assessed the relationship of index and contact cases. In most of the cases index case was either father (48.8%) or mother (18.8%).

We found in our study that BCG scar was absent in 18.8% contacts and was present in 81.3 %.The nutritional evaluation was based on WHO classification of malnutrition.57.5% were normal, 37.5% had chronic malnutrition and 2.5% had Acute and Acute on chronic malnutrition each.

Socio economic status was assessed by modified Kuppuswami scale. Most of the contacts were from lower (57.5%), middle (36.3%) and Upper (11.6%) socio-economic status respectively.

Out of 80 contact cases household size of </=4, 5-6 and > 6 was seen in 43.8%, 31.3% and 25% contacts respectively. Most of the children were asymptomatic; cough was present in (5.3%), weight loss (5%) and fever was seen in 2.5% cases. All the contacts underwent X ray examination of chest; however it is not advised according to RNTCP. Abnormal chest x-ray was seen 8.8% contacts.

Out of 80 contacts Mantoux test was positive in 13 (16.3%) contacts**.** Out of 80 contacts who were only MT (n=13) or had an abnormal x-ray (n=7) and both x-ray and MT positive (n=6) . Sputum or gastric lavage for AFB was done however we did not find sputum positive in any of the contacts suggesting they are non-infective.

Distribution of cases based on Interferon-gamma release assay (IGRAs) results was done and out of 80 contacts IGRA was positive in 34(42.5%) contacts.

The prevalence of active TB in contacts was in 7 (8.8%) contacts in our study

while latent TB was seen in 27 (33.8%) contacts. All the contacts of active TB were started on AKT as per RNTCP guidelines, while Isoniazid chemoprophylaxis was given in 41 (51.3%).

A significant association was seen between incidences of Tb in contacts with the grade of sputum positivity of cases ($p<0.05$). The incidence of TB in sputum grade 1 and 2 and 3 was 7.7%, 2.6% and 25%.

In the present study there was no association seen between presence of BCG scar and incidence of TB in contacts (p-0.311), socio-economic status and incidence of TB in contacts (p-0.281) household size and incidence of TB in contacts (p-0.794). In our study there was no association seen between number of rooms and incidence of TB in contacts (p-0.141).

A significant association was seen between incidences of Tb in contacts with malnutrition ($p<0.01$). The incidence of TB was 14.7% in children with malnutrition as compared to 4.3% in those with normal nutritional status.

In our study IGRA was positive in all the cases of active (n=7) and latent tuberculosis (n=27). Out of 80 contact children Abnormal chest x-ray findings was seen in all active TB cases (n=7). Out of 80 contacts Mantoux positivity (> 10 mm) was seen in 85.7% cases with active TB while it was positive in 25.9% cases with latent TB. A significant association was seen between IGRA and MT results ($p<0.05$). All the IGRA negative cases were negative on MT too while 38.2% cases with positive IGRA results were MT positive. The sensitivity and specificity of IGRA compared to MT in our study was 100% and 68.6% respectively.

MT was positive in 85.7% cases with active TB (6/7) and 25.9% cases with latent TB (7/27) while IGRA was positive in all cases of active/ latent disease.

Discussion :

The present study was thus conducted to find the incidence of Tuberculosis Infection and active disease in children in contact with adult index case.

Incidence of TB Infection & Disease

The prevalence of active TB in contacts was 8.8% in present study while latent TB was seen in 33.8% contacts.

Studies conducted in the 1960s and 1970s showed that household contacts of individual with TB had higher risk of infection than individuals in the general population[4,5]. This was confirmed in several recent studies conducted among children in New York City[6], Botswana[7], and Brazil[8] in which contact with an individual with TB came out as the strongest risk factor for TB infection.

Guwatudde D et al.[9], Among the 1,206 household contacts, 76 secondary cases (6%) of tuberculosis were identified. The risk for secondary tuberculosis was greater among young children than adults (10% vs. 1.9%). In another study by Gessner et al. [10], Infection developed in 25% of the children and progressed to active disease in 9.6%. In another study by Songpol Tornee et al[11] prevalence of tuberculosis infection among household contacts to be 47.80%. In another study by Hiral H Shah et[12] 90(30%) out of 300 children were positive for the latent infection. In a similar study by Seddon et al.[13], 4.7% children were classified as infected and 14.7% had TB disease. In a study by Kinikar et al.[14], in 15 (30%) of the 50 pediatric index cases, the household contained known TB contacts, 14 (86%) of whom were adults. Singh et al. 36, in their study observed the prevalence of tuberculosis infection and disease as 33.8% in children with household contacts of sputum positive cases.

Risk Factors:

In present study a significant association was seen between incidences of Tb in contacts with malnutrition ($p<0.01$). The incidence of TB was 14.7% in children with malnutrition as 58 compared to 4.3% in those with normal nutritional status. However no association was seen between SES, household size and no. of rooms with incidence of TB in contacts ($p>0.05$).

Seventy-eight percent of children have received a BCG vaccination according to NFHS-3 55. In our study we found that BCG scar was present in 81.3 percent contacts while BCG was not given in 18.8 percent.

It is now universally accepted that the risk of acquiring TB is directly proportional to the number of bacilli to which a subject is exposed[15]. In the first place, it depends on the characteristics of the source case: contagiousness is generally limited to subjects with lung disease, and is greater among the patients with bacilleferous forms (i.e. those with positive microscopic test results), in whom the estimated transmission rate is about 35% as against the 17% observed among those with non-bacilleferous forms[16]. Similarly, it is important to evaluate the time spent in an enclosed space with the source case.

The effect of the combination of these two variables has been clearly shown in studies of the contacts arising during air flights[17]. Living together gives rise to the greatest exposure to TB: this has been documented in studies such as that of Singh et al. who evaluated the prevalence of TB in children living with adults with active TB and found a significant difference between those living with adults with microscopic positive or negative expectorate (respectively 68.4 and 31.6%)[18].

A recent meta-analysis[19] has shown that contact with expectorate positive TB patients is a factor indicating a similar risk of infection in both high income (odds ratio [OR] 3.3; 95% confidence interval [CI] 2.2–4.8) and low income countries (OR 3.3; 95% CI 2,2– 5.1). The risk of acquiring tuberculous infection is particularly high in children who live with expectorate-positive adults (relative risk [RR] 6.78; 95% CI 3.51–13.10) or adults with cavitating lesions revealed by chest X-ray (RR 2.45; 95% CI 1.60–3.76), or in those who have close contacts with drug users (RR 1.81; 95% CI 1.03–3.19)(20,21). Children whose families include women with TB are exposed to an even higher risk

Table 1: epidemiological and socioeconomic factors (N=80)

Criteria of study	Percentage	Association With TB
Age <5 years 5 to 10 years >10 years	35 57.5 7.5	Not Significant
Sex males females	53.8 46.2	Not Significant
Sputum positivity +1 +2 +3	 30.2 46.5 23.3	Significant (p,0.05) 7.71 12.6 25
Relation with child Father Mother Other	 48.8 18.8 30.4	 Not Significant
BCG scar Absent Present	18.8 81.2	Not Significant
Nutrition Normal Chronic Acute Acute on chronic	57.5 37.5 2.5 2.5	Significant
Socio economic status Lower Middle Upper	57.5 36.3 11.7	Not Significant
Household size </=4 5-6 > 6	43.8 31.2 25	Not Significant

(RR 1.34; 95% CI 1.34–3.14), probably because their contacts are more frequent than in the case of male relatives.

One case-control study carried out in Thailand found that the risk of developing the disease was high in children having any kind of contact with TB patients (very close: OR 85.67; 95% CI 33–647.79; p < 0.001; close: OR 31.11; 95%CI 4.18–255.94; p = 0.001; not close: OR 32.70; 95% CI 4.18–255.94; p < 0.001).(22) In present study too, we observed a significant association between incidences of TB in contacts with the grade of sputum positivity of cases (p<0.05). The incidence of TB in sputum grade 1 and 2 and 3 was 7.7%, 2.6% and 25%.

Living arrangements and housing conditions play an important role: a case-control study carried out in Bangladesh (23)found that co-dwellers were protected against transmission if there were < 2 people per bedroom (OR 0.29; 95% CI 1.79–6.03; p < 0.0001), if the kitchen was separated from the bedroom (OR 0.35; 95% CI 0.2–0.62; p = 0,001), and if the home was adequately ventilated (OR 0.25; 95% CI 0.13–0.49; p < 0.0001). The risk of transmission is also affected by overcrowding and

the economic conditions of the family (OR 1.35; 95% CI 1.06–1.72; p < 0.017). 4 as well as by an inadequate supply of food (OR 1.52; 95% CI 1.15–2.02; p < 0.003) .(24)

Diagnosis:

In present study Interferon-gamma release assay (IGRAs) was positive in 34 (42.5%) cases, out of which X-ray was abnormal in 7 cases (8.8%) showing presence of active disease. All the IGRA negative cases were also negative on TST too while only 13 out of 34 (38.2%) cases with positive IGRA results were also TST positive. Thus TST was positive in 85.7% cases with active TB (6/7) and 25.9% cases with latent TB (27/47).

A large number of studies have evaluated the efficacy of IGRAs in diagnosing LTBI (25-29). An IGRA is recommended by WHO, for subjects who have been vaccinated with BCG in order to confirm /exclude the presence of TB in subjects with a positive TST 21.

It is therefore recommended that these tests should not be used in individuals suspected of active pulmonary or extra-pulmonary TB, irrespective of their HIV status. This recommendation also applies to paediatric TB based on the generalisation of data from adults."[30]

Management:

The overall prevalence of active TB in contacts was 8.8% in present study while latent TB was seen in 33.8% contacts. All the cases of active TB were started on AKT as per RNTCP guidelines, while isoniazid chemoprophylaxis was given for LTBI cases .The rationale underlying the treatment of LTBI is based on the possibilityof eliminating dormant bacilli, thus reducing their activation and the development of active disease.

A Cochrane review has shown that treating LTBI with isoniazid reduces the risk of pulmonary and extra-pulmonary TB and the related deaths, but there does not seem to be any significant difference in the efficacy of 6- and 9-month treatment.[31]

Table:2 clinical and investigation related factors

	Percentage	Association
Symptom asymptomatic cough weight loss fever	 87.2 5.35 2.5	Not Significant
X ray examination Abnormal Normal	 8.89 1.2	Not Significant
Mantoux test Positive Negative Mantoux test Positive and Xray positive	16.3 83.7 8.7	 Significant
Sputum or gastric lavage for AFB Positive Negative	 NIL	 Not Significant
IGRA Positive Negative	42.5 57.5	Significant
Active TB Latent TB	8.7 33.8	Significant

Conclusion:

Active tuberculosis (TB) and TB Infection is common among household contacts of index cases in India, especially among young children. We assessed 43 contacts an d 80 children in contact with them. A significant association was seen between incidences of Tb in contacts with malnutrition ($p<0.01$). The incidence of TB was 14.7% in children with malnutrition as compared to 4.3% in those with normal nutritional status.

In our study IGRA was positive in all the cases of active (n=7) and latent tuberculosis (n=27). Out of 80 contact children .Abnormal chest x-ray findings was seen in all active TB cases (n=7). Out of 80 contacts Mantoux positivity (> 10 mm) was seen in 85.7% cases with active TB while it was positive in 25.9% cases with latent TB. A significant association was seen between IGRA and MT results ($p<0.05$). All the IGRA negative cases were negative on MT too while 38.2% cases with positive IGRA results were MT positive. The sensitivity and specificity of IGRA compared to MT in our study was 100% and 68.6% respectively.

MT was positive in 85.7% cases with active TB (6/7) and 25.9% cases with latent TB (7/27) while IGRA was positive in all cases of active/ latent disease.

Declarations:

Contribution of authors - Rakesh Thamke- concept, strategy; Vijay kamale - supervision and revision of manuscript, guidance, Ragi Rajan - data collection,statistical analysis, initial manuscript,

Conflict of interest - Nil

Funding source and its role in the study - No

References:

1) Recommendations for investigating contacts of persons with infectious tuberculosis in low- and middle-income countries..World Health Organization. ISBN 978 92 4 150449 2 (NLM classification: WF 205)

2) Global Tuberculosis Report 2018 ISBN 978-92-4-156564-6 © World Health Organization 2018

3) IGRA ASSAY- Immunocheck TB Platinum(IGRA) Make-Immunocheck product information sheet

4) Andersen S, Geser A. The distribution of tuberculous infection among households in African communities. Bull World Health Organ.1960;22 :39– 60

5) Grzybowski S, Barnett GD, Styblo K. Contacts of cases of active pulmonary tuberculosis. Bull Int Union Tuberc Lung Dis.1975;5 :90– 106.

6) Saiman L, San Gabriel P, Schulte J, Pimentel Vargas M, Kenyon T, Onorato I. Risk factors for latent tuberculosis infection among children in New York City. Pediatrics.2001;107:999–1003.

7) Lockman S, Tappero JW, Kenyon TA, Rumisha D, Huebner RE, Binkin NJ. Tuberculin reactivity in a paediatric population with high BCG vaccination coverage. Int J Tuberc Lung Dis.1999;1 :23– 30.

8) Almeida LM, Barbieri MA, Da Paixao AC, Cueva LE. Use of purified protein derivative to assess the risk of infection in children in close contact with adults with tuberculosis in a population with high Calmette-Guerin bacillus coverage.Pediatr Infect Dis J.2001;20 :1061–1065

9) Guwatudde D, Nakakeeto M, Jones-Lopez EC, Maganda A, Chiunda A, Mugerwa RD, Ellner JJ, Bukenya G, Whalen CC. Tuberculosis in household contacts of infectious cases in Kampala, Uganda. American Journal of epidemiology. 2003 Nov 1;158(9):887-98.

10) Gessner BD, Weiss NS, Nolan CM. Risk factors for pediatric tuberculosis infection and disease after household exposure to adult index cases in Alaska. The Journal of Pediatrics. 1998 Mar 31;132(3):509-13.

11) Songpol Tornee1, Jaranit Kaewkungwal1 risk factors for tuberculosis infection among household contacts in bangkok, thailand Vol 35 No. 2 June 2004

12) Hiral H Shah1, Halak J Vasavada1 study of transmission of tuberculous infection among children in contact with parents having tuberculosis Volume 3%Issue 3%July – Sept 2013

13) Seddon JA, Hesseling AC, Godfrey-Faussett P, Fielding K, Schaaf HS. Risk factors for infection and disease in child contacts of multidrug-resistant tuberculosis: a cross-sectional study. BMC infectious diseases. 2013 Aug 26;13(1):1.

14) Kinikar A, Adhav PS, Kamble S, Sahoo P, Koli H, Kanade S, Mave V, Suryavanshi N, Gupte N, Gupta A, Mathad J. Source Case Investigation for Children with TB Disease in Pune, India. Tuberculosis research and treatment. 2014;18(2):832-836.

15) Marais BJ, Gie RP, Schaarf HS, Hesseling AC, Obihara CC, Nelson LJ, et al. The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis 2004;8:278–85.

16) Erikens CGM, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, Haas W, et al. Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J 2010;36:925–49.

17) Driver CR, Valway SE, Morgan WM, Onorato IM, Castro KG. Transmission of Mycobacterium tuberculosis associated with air travel. JAMA 1994;272:1031–5.

18) Singh M, Mynak ML, Kumar L, Mathew JL, Jindal SK. Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis. Arch Dis Child 2005;90:624–8

19) Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Resp J 2013;41:140–56.

20) Nguyen TH, Odermatt P, Slesak G, Barennes H. Risk of latent tuberculosis infection in children living in households with tuberculosis patients: a cross sectional survey in remote northern Lao People's Democratic Republic. BMC Infect Dis 2009;9:96.

21) Stout JE, Saharia KK, Nageswaran S, Ahmed A, Hamilton CD. Racial and ethnic disparities in pediatric tuberculosis in North Carolina. Arch Pediatr Adolesc Med 2006;160:631–7.

22) Tipayamongkholgul M, Podhipak A, Chearskul S, Sunakorn P. Factors associated with the development of tuberculosis in BCG immunized children. Southeast Asian J Trop Med Public Health 2005;36:145–50.

23) Karim MR, Rahman MA, Mamun SA, Alam MA, Akhter S. What cannot be measured cannot be done; risk factors for childhood tuberculosis: a case control study. Bangladesh Med Res Counc Bull 2012;38:27–32.

24) Tornee S, Kaewkungwal J, Fungladda W, Silachamroon U, Akarasewi P, Sunakorn P. The association between environmental factors and tuberculosis infection among household contacts. Southeast Asian J Trop Med Public Health 2005;36:221–4.

25) Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007; 146:340.

26) Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008; 149:177.

27) Van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g

assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLoS One 2009; 4:e8517.

28) Chang KC, Leung CC. Systematic review of interferon-gamma release assays in tuberculosis: focus on likelihood ratios. Thorax 2010; 65:271.

29) Magdorf K, Detjen AK. Proposed management of childhood tuberculosis in lowincidence countries. Eur J Pediatr 2008;167:927–38.

30) Use of tuberculosis interferon-gamma release assays (IGRAs) in low and middle income countries: Policy Statement, World Health Organization, Geneva 2011. Commercial serodiagnostic tests for diagnosis of tuberculosis: Policy Statement. Geneva, World Health Organization 2011.

31) Ongoing trials for treating TB infection include Short and ultra-short course treatment Treatment of MDR TB infectionTechnical Consultation on the Programmatic Management of Latent Tuberculosis Infection, Seoul, Republic of Korea, 31 August – 1 September 2017 WHO

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ORIGINAL RESEARCH PAPER

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A RETROSPECTIVE STUDY ON DIFFERENT ANATOMICAL SITE INVOLVEMENT OF EXTRA-PULMONARY TUBERCULOSIS PATIENTS ATTENDING VARIOUS OPD IN TERTIARY CARE CENTER

General Medicine

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ABSTRACT

Tuberculosis remains a major global public health problem with one-third of the world's population being infected with the Mycobacterium Tuberculosis. The burden of tuberculosis in India is the highest accounting for one fifth (21%) of the global incidence. Tuberculosis infection of any part of body other than lung parenchyma is defined as extra-pulmonary tuberculosis. In this study of 796 EPTB patients over 3 years, prevalence of EPTB was found to be higher in females than in males. involvement of pleural cavity and lymph nodes were commonest manifestation among the 20-39 yrs age group. In the youngest age group (< 9 years), lymph node TB was the most frequently observed, (n-26/62). In contrast, in the adolescent and in geriatric age group (>60 yrs.) the most common type of EPTB was pleural cavity. Whereas in the remaining adult population (20-39, 40-60 yrs.), the most common types was pleural cavity and lymph-nodes

KEYWORDS

INTRODUCTION:

Tuberculosis (TB) remains a major global public health problem with one-third of the world's population being infected with the Mycobacterium Tuberculosis [1]. Recently identified TDR (total drug resistant) tuberculosis is biggest threat for human being. According to WHO 6.1 million TB cases were reported in 2013,out of which 5.7 million were people newly diagnosed and another 0.4 million were already on treatment. [2].The burden of tuberculosis (TB) in India is the highest accounting for one fifth (21%) of the global incidence. [3]

Tuberculosis infection of any part of body other than lung parenchyma is defined as extra-pulmonary tuberculosis [EPTB].Diagnosis of EPTB is done as per RNTCP guidelines which is based on one culture-positive specimen from the extra-pulmonary site; or histological evidence; or strong clinical evidence consistent with active EPTB disease followed by a medical officer's decision to treat with a full course of anti-TB therapy under DOTS[4].The timely detection & accurate diagnosis of any form of EPTB is necessary for the proper treatment of EPTB[5].Atypical presentation, lack of diagnostic resources for procurement of tissue or fluid for diagnosis from inaccessible sites and a poor yield of conventional diagnostic methods lead to considerable delay in making the diagnosis or diagnosis is even missed. Furthermore guidelines regarding diagnosis of EPTB are not covered by RNTCP but all patients are given treatment as per DOTS regimen. Although there is a rising trend in EPTB in recent decade, still EPTB has never been a priority in the campaigns undertaken by Revised National TB Control Programme (RNTCP) for its control [4,6,7]

It was found that the percentage of patients with EPTB was more in tertiary care centres of India, ranging from 30% to 53% [4].This implies that, tertiary care centres such as medical colleges, caters a large and varied type of population and provides an excellent place for economical and advanced diagnostic facilities for early diagnosis and treatment of EPTB cases, backed up by research facilities.

MATERIALAND METHODS:

AIM: To categorised EPTB patients according to anatomical site involvement.

STUDY DESIGN: This is a retrospective, descriptive,record-based study of diagnosed patients of EPTB of all age groups.

STUDY AREA: The study was conducted in the MGM Medical College & Hospital a tertiary care centre, so patients from nearby villages and adjoining districts were referred for diagnosis and treatment.

STUDY PERIOD: Data for this study has been obtained from 1st April 2014 to 30th april 2017. The population includes all patients attending various OPD of Hospital who were suspected for extra pulmonary tuberculosis infection during the study period.

SOURCE OF INFORMATION: For this study data has been obtained from Patient Record Sheets of Hospital, Lab register, treatment Cards or Referral Registers of RNTCP and utilized for analysis.

INCLUSION CRITERIA:

All patients suspected of tuberculosis attending OPD of various departments of MGM Medical College and Hospital, aurangabad.

EPTB - a patient with active tuberculosis of any part of body other than lung parenchyma.

EXCLUSION CRITERIA:

Patients with PTB.
Patients of EPTB with PTB.

METHOD:

The diagnosis of Pulmonary and Extra pulmonary Tuberculosis cases were established, following the RNTCP programme guidelines, which required one culture positive specimen from an extra-pulmonary site or histological evidence or strong clinical evidence consistent with active EPTB followed by concerned Medical Officer's decision to treat with a full course of anti-TB therapy. Whenever needed, investigative procedures such as X-Ray, FNAC, Pleural fluid aspiration, ultrasonography, computed tomography, MRI were performed for diagnosis and specimen collection. The specimen was then subjected to a culture or histopathology for evidence of TB. After diagnosis of EPTB, patients were registered at DOTS Centre, whereas patients belonging to other villages or districts were referred to DOTS centres of their respective area. Data analysis has been done using Microsoft Office Excel 2010 and expressed in percentages. At the first step, all the records pertaining to EPTB cases diagnosed during the study period were collected and analysed. Total 796 cases diagnosed as EPTB were included in the study.

Table no 1:Demographic characteristics of EPTB CASES

Age group (in years)	Sex distribution			
	Male (%)		Female(%)	
0-9 years	13	1.63%	14	1.75%
10-19 years	50	6.28%	66	8.29%
20-39 years	191	23.99%	234	29.39%
40-60 years	104	13.06%	71	8.91%
>60 years	32	4.02%	21	2.63%
Total	390	48.99%	406	51.00%

Table No-01 shows that women and men each accounted for

approximated half of the cases. Out of 796 EPTB patients, 390 (48.99%) were males, 406 (51.00%) were females. Among the 5 age groups studied, the age group of 20-39 years had the highest proportion of EPTB 425 (53.39%) both in males and females which is the economically productive population of society. Next most affected was 40-60 years age group (n-175, 21.98%). geriatric age group has 6.65% contribution (n-53 ,>60 years old). The lowest proportion (n-27, 3.39%%) was observed in the paediatric age group (0-9 years old).

TABLE NO 02: FREQUENCY DISTRIBUTION OF DIFFERENT SITES OF EPTB

SR. NO	SITE OF TUBERCULOSIS	TOTAL NO. OF CASES (%)	
1	PLEURAL CAVITY	293	36.80%
2	LYMPH NODES AND PERIPHERAL LYMPHATICS	259	32.53%
3	ABDOMEN	90	11.30%
4	BONES & JOINTS	72	9.04%
5	GENITOURINARY	7	0.87%
6	SKIN AND MUSCLE	32	4.02%
7	OTHER	43	5.40%

Table No-02 shows that maximum number of cases belongs to pleural cavity (36.80%), lymph nodes and lymphatic is second most common site with 32.53%. Rest of the cases were found in decreasing order in abdomen 11.30%, bones & joints 9.04%,other 5.40%, skin & muscles 4.02% and, Genitourinary tuberculosis 0.87%.

TABLE NO-03: AGE, SEX AND SITE SPECIFIC DISTRIBUTION OF EPTB

SR. NO	SITE OF EPTB	AGE GROUP (IN YEARS)																	
		0-9			10-19			20-39			40-60			>60			TOTAL		
		M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
1	PLEURAL CAVITY	3	1	4	19	16	35	94	62	156	53	24	77	15	6	21	184	109	293
2	LYMPHAT- ICS	3	10	13	11	26	37	43	96	139	22	21	43	4	8	12	83	176	259
3	ABDOMEN	1	1	2	7	12	19	25	29	54	7	5	12	2	1	3	42	48	90
4	BONES& JOINTS	2	0	2	3	4	7	13	17	30	12	11	23	7	3	10	37	35	72
5	SKIN& MUSCLE	0	1	1	6	0	6	8	10	18	4	3	7	0	0	0	18	14	32
6	GENITO- URINARY	0	0	0	0	0	0	0	3	3	1	2	3	0	1	1	1	6	7
7	OTHERS	3	0	3	4	4	8	9	11	20	5	2	7	4	1	5	25	18	43
	TOTAL	12	13	25	50	62	112	192	228	420	104	68	172	32	20	52	390	406	796

Table No. 03 shows that involvement of pleural cavity and lymph nodes were the commonest manifestation among the 20-39 yrs age group (n-293, 36.80% & n-259, 32.53% respectively). In the youngest age group (< 9 years), lymph node TB was the most frequently observed, (n-26/62). In contrast, in the adolescent and in geriatric age group (>60 yrs.) the most common type of EPTB was pleural cavity. Whereas in the remaining adult population (20-39, 40-60 yrs.), the most common types was pleural cavity (n-156, 19.59%), lymph-nodes (n-139, 17.46%) and abdomen (n-54, 6.78%).

DISCUSSION:

Globally, women were found to be more at risk of developing EPTB [7,8,9]. Prevalence of EPTB was found to be higher in female than male (51.00% Vs 48.99%).

Although EPTB cases were found in all age groups but majority of cases (53.39%) belonged to the age group of 20-39 years, which constitutes of young adult and working individuals. This is the reproductive and working group in both males and females and is economically productive population. Our study shows that young adult is itself an independent risk factor for EPTB. The possible explanation of this may be because of reactivation and spread of TB from primary infection from the lungs to extra-pulmonary sites, delayed diagnosis of primary tuberculosis because of lack of time and decreased immunity to due life style changes and improper nutrition.

Although lymph node tuberculosis being the most common site, in our study the most common form of EPTB is pleural cavity. Rest of the EPTB cases distributed in decreasing order of sites were abdomen, bones & joints, skin & muscles and genitourinary tuberculosis, which is similar to studies from India and world [10,11,12,13].

The difference in the occurrence of EPTB by site in different age groups and sexes shows the difference in predilection to involve one site over the other depending on the host factors such as immunity status. Tubercular lymphadenitis is more frequent in female whereas tubercular pleural effusion is more common in male. Genitourinary tuberculosis was most common in young females (20-39yrs).

Our study had several limitations .The main limitation of the study is that the being a retrospective, hospital-based study, the findings cannot be generalized to the community, but it gives valuable information regarding trend of EPTB cases.

CONCLUSION:

Extra pulmonary tuberculosis remains a significant health problem in developing countries. In conclusion, our study expands the knowledge regarding the epidemiology of EPTB. The frequency of EPTB in this study was higher with the highest proportion in pleural cavity. Moreover, being female patient was at higher rate of positivity for EPTB than male. Young adults between age 20-39 yrs, and associated diabetes mellitus were significant risk factors for patient being EPTB positive. Based on the above conclusions the following recommendations are forwarded:

Newer diagnostic tests like molecular characterization, PCR etc which are sensitive and specific and easy to use for early detection and confirmation of diagnosis of EPTB, should be made available through government programmes in rural resource-poor settings.

Large scale, community based studies and well-defined programme-specified protocols for education and prevention of EPTB are needed for decreasing its burden as it is a curable disease.

Young adult males of 20-39 yrs. is the target population, who should be examined and investigated thoroughly to rule out EPTB, so that burden of EPTB cases on society decreases and hence improve the nation's economy.

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FUNDING: none

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Reference

1. "Tuberculosis Fact sheet N°104". World Health Organization. November 2010. Retrieved 26 July 2011. http://www.who.int/mediacentre/factsheets/fs104/en/
2. Global Tuberculosis Control 2014, WHO, Geneva, 2014.www.who.int/tb / publications/global_report/en/
3. District- wise performance of RNTCP .TB India 2011.114–5. http://www.tbcindia.org ISBN 81-902652-5-3.
4. Prakasha SR, Suresh G, D'sa IP, Shetty SS, Kumar SG. Mapping the Pattern and Trends of Extrapulmonary Tuberculosis. J Glob Infect Dis. 2013 Apr;5(2):54-9. doi: 10.4103/0974-777X.112277. [PubMed]
5. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res.2004; 120:316–53. Indian J Med Res. 2004 Oct;120(4):316-53.PMID:15520485. [PubMed]
6. Narain JP, Lo YR. Epidemiology of HIV-TB in Asia. Indian J Med Res. 2004 Oct;120(4):277-89. [PubMed]
7. Yang Z, Kong Y, Wilson F, Foxman B, Fowler AH, Marrs CF, Cave MD, Bates JH. Identification of risk factors for extrapulmonary tuberculosis. Clin Infect Dis. 2004 Jan 15;38(2):199-205. Epub 2003 Dec 19. doi: 10.1086/380644.
8. Chandir S, Hussain H, Salahuddin N, Amir M, Ali F, Lotia I: Extrapulmonary tuberculosis: a retrospective review of 194 cases at a tertiary care hospital in Karachi, Pakistan.J Pak Med Assoc 2010 Feb;60(2):105-9.
9. Peto HM, Pratt RH, Harrington TA, et al. Epidemiology of extrapulmonary tuberculosis in the United States,1993-2006.Clin Infect Dis. 2009 Nov 1;49(9):1350-7. doi: 10.1086/605559. [PubMed].
10. H.Yang, S.K. Field, D.A. Fisher, R.L. Cowie. Tuberculosis in Calgary, Canada, 1995-2002: site of disease and drug susceptibility. Int J Tuberc Lung Dis;2005; 9 (3): 288-293.
11. Arora VK, Gupta R. Trends of extra-pulmonary tuberculosis under Revised National Tuberculosis Control Programme: A study from South Delhi. Indian J Tuberc. 2006;53:77–83.
12. Rai DK, Bisht RS, Sikarwar V, Upadhyay SK. Clinicoepidemiological trend of

tuberculosis in garhwal region.IOSR Journal of Pharmacy. 2012. 2(5) : 39-43.
13. Xinyu Zhang, Aase B. Andersen, TroelsLillebaek, ZazaKamper-Jørgensen, VibekeØstergaard Thomsen, Karin Ladefoged, Carl F. Marrs, Lixin Zhang, Zhenhua YangEffect of Sex, Age, and Race on the Clinical Presentation of Tuberculosis: A 15-Year Population-Based Study. Am J Trop Med Hyg. 2011 Aug;85(2):285-90. doi: 10.4269/ajtmh.2011.10-0630.

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Levels of microRNA miR-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy.

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Author information

Abstract

Identification of blood biomarkers that can be useful for predicting Mycobacterium tuberculosis (M.TB) infection, effect of therapy and Multi Drug Resistant (MDR) TB infected individuals is clinically useful for combating tuberculosis epidemic. In this study, we have evaluated the levels of selected miRNAs in serum of TB and MDR TB patients. In addition, we have studied their levels in serum of patients post-therapy. The levels of 4-miRNAs (miR-16, miR-29a, miR-125b and miR-155) were measured in 30 newly diagnosed TB patients, 19 Multi Drug Resistant (MDR) TB patients, 10 patients who completed TB therapy and were TB negative. 30 healthy individuals were recruited as controls. The levels of the miRNAs were estimated by qRT-PCR. Of the four miRNAs studied, the levels of miR-16 were significantly elevated and miR-155 were significantly reduced in serum of TB patients as compared to uninfected controls. The Receiver Operating Characteristic (ROC) curve of miR-16 and miR-155 exhibited a significant distinguishing efficiency with an AUC value of 1 (95% CI, 1 to 1) and 0.967 (95% CI, 0.92-1.04) respectively. Following the therapy, the levels of miR-16 and miR-155 returned to those observed in healthy subjects. In patients with MDR TB, miR-155 was lower as compared to healthy controls and TB treated group but higher as compared to TB naïve patients. miR-16 levels were lowest in serum of MDR TB patients compared to TB naïve, TB treated group and healthy controls. In conclusion, miR-16 and miR-155 in serum may act as surrogate biomarker for studying TB infection, progression of therapy and MDR TB.

KEYWORDS: Biomarker; Drug resistance; Serum miRNA; Therapy; Tuberculosis; microRNA

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Research Article

Assessment of oxidative stress in serum of pulmonary tuberculosis patients

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ABSTRACT

Background: Tuberculosis (TB) remains a human health issue and often deadly infectious disease in low-middle income nations. In TB, oxidative stress is a result of tissue inflammation, poor dietary intake of micronutrients due to illness, free radical burst from activated macrophages. This study was conducted prospectively to evaluate the oxidative stress in TB.
Methods: The study included 30 newly diagnosed TB positive patients and 30 healthy individuals. Pro-oxidant markers like the thiobarbituric acid reactive species (TBARS) and nitric oxide were studied from serum. Antioxidant parameter like serum total-SH was also assessed.
Results: Levels of pro-oxidants were significantly increased whereas antioxidant defense markers were significantly impaired in the TB group. Nitric oxide and TBARS were increased ($p<0.0001$) where glutathione was decreased ($p<0.0001$) in TB population compared to healthy controls.
Conclusions: Marked oxidative stress were seen in the TB population as compared to the healthy cohort. The role of antioxidant therapy may therefore be evaluated in the management of TB.

Keywords: Tuberculosis, Oxidative stress, TBARS, Nitric oxide, GSH

INTRODUCTION

Mycobacterium tuberculosis (MTB), the responsible agent of tuberculosis (TB), remains a issue of high morbidity and mortality worldwide. TB causes 1.9 million deaths annually among a pool of infected individuals close to 2 billion people.[1] TB occurs because of dysregulation of the immune system and/or poor immune response against the infection. Innate immune response critically acts against MTB infection. MTB is recognized intracellular bacteria that replicates and grow within macrophages. MTB can stimulate activated macrophages to produce reactive oxygen species (ROS), which is an important part of host defense against mycobacterium.[2] Oxidative stress has been implicated as a significant contributor to the development and prognosis of TB.[3,4] Oxidative stress parameters are intricately involved in the homeostasis of the immune system. Imbalance in oxidative levels results in cellular damage due to the oxidation of amino acid residues on protein.[5]

Critical immunological functions like inflammation are regulated by reduced glutathione (GSH).[6] Decreased in glutathione levels indicates the potential of oxidative damage to erythrocyte and erythrocyte membrane of pulmonary TB patients.[7] In HIV infected individuals, the risk of developing TB is high due to decreased level of

GSH which affects the capacity of monocyte to kill MTB.[8] Biomarkers of lipid oxidation such as thiobarbituric reactive substances (TBARs), NO among others have been studied as indicators of oxidative stress. Oxidative stress also has been shown to be associated with TB infection through activation of phagocytes by mycobacteria which may further contribute to immunosuppression.[9] Both nitrogen intermediates and oxygen radicals may also play an important role in the suppression of the infection through mycobacterial killing. Nitric oxide is also an important mediator of immune homeostasis.[10] Oxidative stress increases susceptibility of MTB to isoniazid, suggesting importance of oxidative stress in physiology.[11]

The present study was conducted to assess the levels of these oxidative parameters can be useful in predicting and diagnosis markers need to be evaluated. The levels of three important markers of oxidative stress in patients suffering from TB were estimated.

METHODS

The study was approved by the Research and Review Committee (RRC) and Institutional Ethical Committee (IEC) of the MGM Institute of Health Science, Kamothe, Navi Mumbai vide Certificate No. MGM/HIS/RS/2014/112 dated: 11.08.2014 Written informed consent was obtained from each participants prior to sample collection.

Study groups

The study population consists of 30 newly diagnosed TB positive patients and 30 Healthy individuals were included in the study. Patients belonging to both sex i.e. male and female of age of 16 years and above included. AFB staining was used for the diagnosis and confirmation of TB infection. Informed consent of the patients was taken before participation in the study.

Blood collection

5 milliliters of whole blood from TB positive patients and healthy volunteers were collected in plain vacuum tubes and was used immediately for the determination. The serum was separated from plain vacuum tube, aliquoted and stored at -20°C and used for the following assays.

Determination of NO (Nitric oxide)

1% Sulfanilamide Solution in 5% o-phosphoric acid and 0.1 % N-(1-Naphthyl) ethylene diamine dihydrochloride solution was allowed to equilibrate to room temperature. 50µl of standard and 50µl of serum was added. To this then, 50µl of the 1% sulfanilamide solution was added. 50µl of 0.1% NED Solution was added. A purple/magenta color of Azo-compound will begin to form immediately. The absorbance was taken at 520 nm.[12]

Estimation of TBARS

Lipid peroxides were estimated by measurement of thio barbituric acid reactive substances (TBARS) by the method of Brown and Kelly.[13] The pink chromogen produced by the reaction of Thiobarbituric acid with TBARS, a secondary product of lipid peroxidation was measured at 532 nm. Results was estimated as nmole/mL.

Estimation of reduced glutathione

Serum Total-SH was determined with slight modification by method described by Ellman and Sedlak and Lindsay.[14,15] Concentration of SH groups was measured colorimetrically with modified Ellman method in blood serum. To 445µl of PBS buffer (pH) 7.4, 25 µl of 2 mM of dithionitrobenzoic acid (DTNB) and 50 µl of standard or sample were added. Tubes were centrifuged at 15,000 rpm for 10 minutes and absorbance was measured at 412 nm against blank with DTNB.

Statistical analysis

Each result was expressed as Mean±SEM. The statistical significance of the data was determined by t-test. Statistical analysis was done using Graph Pad Prism 7 Software.

RESULTS

Demographic characteristics of the TB Patient

The demographic characteristics of the participants are showed in Table 1. A total of 60 participants were included in this study. These include newly diagnosed TB infected subjects and healthy volunteers. Average age from the TB group and healthy control was 32.46 and 28.71 years.

Table 1: Demographic characteristic of TB patient and healthy control.

Characteristic	Group	
	Control	TB (Naïve)
Number of participants	30	30
Age (Year)	28.71±6.34	32.46±12.03
Height (ft)	5.4±0.38	5.3±0.37
Weight (Kg)	56.85±11.82	49.52±8.52
Male:Female (%)	43:57	85:15

Levels of pro-oxidants in TB group

The levels of TBARS and NO were elevated in the TB as compared to healthy population (Figure 1). The mean serum TBARS levels were found to be 8.32 nmole/mL in TB population whereas 1.17 nmole/mL in healthy population. This difference was statistically significant. The mean serum NO levels were found to be 35.96 uM in TB population whereas 14.77 uM in healthy controls. The

levels of NO showed significantly increase while compared to healthy controls (Table 2).

Levels of antioxidants in TB group

Levels of GSH in TB infected population were estimated. The levels of GSH were decreased in the TB as compared to healthy population (Figure 2). The mean serum GSH levels were found to be 258.8 uM in TB population whereas 556.5 uM in healthy controls. The levels showed significant increase while compared to healthy population (Table 2).

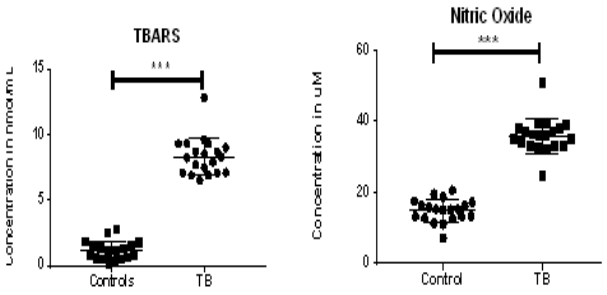

Figure 1: Levels of TBARS and nitric oxide in TB group and healthy control; (*P<0.0001).**

Table 2: Biochemical parameters for pro-oxidants and antioxidants (*p<0.0001).**

Parameters			Control	TB
Pro-oxidants	**TBARS** (nmole/mL)	Mean±SEM	1.175±0.1573	8.323±0.3178
		P value	***	
	NO (uM)	Mean±SEM	14.77±0.7106	35.96±1.09
		P value	***	
Antioxidants	**GSH** (uM)	Mean±SEM	556.5±26.2	258.8±8.795
		P value	***	

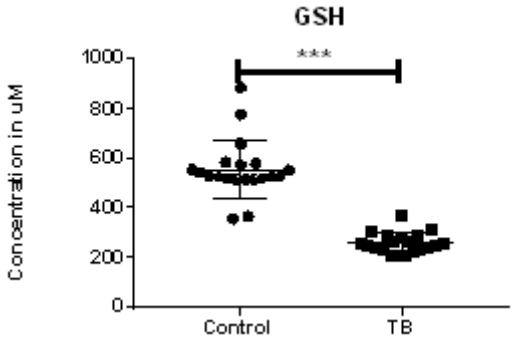

Figure 2: Levels of GSH in the study population; (*p<0.0001).**

DISCUSSION

Oxidative stress results from an imbalance between the generation of reactive oxygen and protective mechanisms. Free radicals, the main causes of oxidative stress, may react with variety of biomolecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue. The oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases. Reduced glutathione (GSH) is the most prevalent non-protein thiol in animal cells. GSH levels have an impact on many immune functions, including activation of lymphocytes. Consequently, it was postulated that GSH deficiency could lead to the progression of immune dysfunction, a hallmark of TB.[16,17]

In the present study levels of GSH were found to be significant decreased in serum of TB infected individuals compared to healthy controls. Various studies showed that GSH play important role in TB.[8,18,19] Venketaraman V et al showed that H37Rv grown in vitro is sensitive to glutathione (GSH) at physiological concentrations. Data showed that GSH at a 5 mM concentration is bacteriostatic to TB strain H37Rv, suggesting the possibility that the presence of a high concentration of GSH may result in an imbalance in a bacterium containing an alternative thiol for regulating reduction or oxidation activity.[18] In another study, blood cultures from human immunodeficiency virus infected subjects treated with N-acetyl cysteine, which is a precursor of glutathione, caused improved control of intracellular MTB infection.[8] Examination of the effects of GSH in improving the ability of neutrophils to control intracellular MTB infection was evaluated. Findings indicated that increasing intracellular levels of GSH with a liposomal formulation of GSH (L-GSH) resulted in reduction in the levels of free radicals and increased acidification of MTB containing phagosomes leading to the inhibition in the growth of MTB.[19] Decreased GSH levels have been shown to activate NFκB, leading to a series of downstream signal transduction events that facilitate TB survival and growth. The low GSH levels in serum of TB infected patients may be a survival mechanism that pathogen employs.

TBARS are a product of lipid peroxidation and an important marker of oxidative stress. In this study, levels of TBARS exhibited a significant increased in TB population in comparison with healthy controls. Rashmi Kulkarni R et al conducted a study of serum malondialdehyde (MDA) and TNF-α in TB patients. TNF α and MDA levels in serum were significantly increased in pulmonary TB patients as compared to those of controls.[20] Madebo T et al also studied the lipid peroxidation products in untreated TB patients in Ethiopia. Data showed that serum MDA concentrations, were significantly higher in patients with TB than in healthy Ethiopian control subjects.[21] Kandukuri RE et al suggested that MDA level are increased indicating progression of TB. They also concluded that in addition to serum adenosine deaminase (ADA) levels, estimation of MDA are useful biochemical parameters to assess whether the TB in progression.[22]

Nitric oxide is an important molecule to study the oxidative stress markers in the bacterial infections. The nitric oxide serves as a pro-oxidant molecule. In the present study, NO levels were significantly raised in TB population as compared to healthy control ($p<0.0001$). Pearl JE et al demonstrated that the level of nitric oxide within the lesion site can dramatically impact the local protective and immune-pathological response by reducing accumulation of specific subsets of activated effector cells and by altering the potency of the lymphocytes with regard to accumulation within the lesion and cytokine production.[23] Jonna Idh et al showed correlation between resistance to first-line anti-TB drugs and reduced NO susceptibility in clinical strains of MTB.[24]

In summary, the results of the present study demonstrated that TB (naïve) infected patient, there is increase oxidative stress with concomitant reduction in antioxidative machinery. Since oxidative environment is crucial for survival of the MTB, it appears that MTB altered the host physiology biasing towards pro-oxidative environment. Such an adaptation of the host by the bacteria would be beneficial for the survival and proliferation of the pathogen. Since high oxidative stress is also favorable for the other pathogen like HIV, it is possible that modulation of the host oxidative stress machinery might further aid in developing co-infections. It will be of interest to determine the levels of oxidative stress molecules in patients with and without co-infection to understand the role of oxidative stress in TB pathophysiology.

CONCLUSION

Although preliminary, present data strongly suggest occurrence of oxidative stress upon TB infection. Wherever the levels of these oxidative parameters can be useful in predicting and diagnosis markers need to be evaluated. Our data also suggest the possible use of antioxidant therapy for treatment of TB.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bulatovic VM, Wengenack NL, Uhl JR, Hall L, Roberts GD, Iii FRC, et al. Oxidative Stress Increases Susceptibility of Mycobacterium tuberculosis to Isoniazid. Antimicrob Agents Chemother. 2002;46(9):2765-71.
2. Cooper AM, Segal BH, Frank AA, Holland SM, Orme IM, Orme IANM. Transient Loss of Resistance to Pulmonary Tuberculosis in p47 phox − / − Mice Transient Loss of Resistance to Pulmonary Tuberculosis. Infect Immun. 2000;68(3):1231-4.
3. Wiid I, Seaman T, Hoal EG, Benade AJ, Van Helden PD. Total Antioxidant Levels are Low During Active TB and Rise with Anti-tuberculosis Therapy. IUBMB Life. 2004;56(2):101-6.
4. Palanisamy GS, Kirk NM, Ackart DF, Shanley CA, Orme IM, Randall J. Evidence for Oxidative Stress and Defective Antioxidant Response in Guinea Pigs with Tuberculosis. Plos one. 2011;6(10).
5. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19.
6. Ghezzi P. Role of glutathione in immunity and inflammation in the lung. Int J Gen Med. 2011;4:105-13.
7. Vijayamalini MMS. Lipid peroxidation, vitamins C, E and reduced glutathione levels in patients with pulmonary tuberculosis. Cell Biochem Funct. 2004;22(1):19-22.
8. Venketaraman V, Rodgers T, Linares R, Reilly N, Swaminathan S, Hom D, et al. Glutathione and growth inhibition of Mycobacterium tuberculosis in healthy and HIV infected subjects. AIDS Res Ther. 2006;3:5.
9. Adebimpe WO, Faremi AO, Nassar SA. Effects of treatment on free radicals in patients with pulmonary tuberculosis in South Western Nigeria. Afr Health Sci. 2015;15(4):1256-61.
10. Pavanelli WR, Jerley J, Silva N. The Role of Nitric Oxide in Immune Response Against Trypanosoma Cruzi Infection. The Open Nitric Oxide Journal 2010;2:1-6.
11. Bulatovic VM, Wengenack NL, Uhl JR, Hall L, Roberts GD, Rusnak F. Oxidative Stress Increases Susceptibility of Mycobacterium tuberculosis to Isoniazid. Antimicrob Agents Chemother. 2002;46(9):2765-71.
12. Green LC, Wagner DA, Glogowski J, Skipper PL WJ et al. Analysis of nitrate, nitrite, and nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8.
13. Brown RK KF. Free Radicals: A Practical Approach. Oxford University Press. 1996. 119-131 p.

14. Sedlak JLR. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.
15. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7.
16. Venketaraman V, Millman AMS, Swaminathan S, Goetzb M, Lardizabalc AF, Homb DNDC. Glutathione levels and immune responses in tuberculosis patients. Microbial Pathogenesis. 2008. p. 255–61.
17. Allen M, Bailey C, Cahatol I, Dodge L, Yim J, Kassissa C, et al. Mechanisms of control of Mycobacterium tuberculosis by NK cells : role of glutathione. Front Immunol. 2015;6(October):1-9.
18. Venketaraman V, Dayaram YK, Talaue MT, Connell ND. Glutathione and nitrosoglutathione in macrophage defense against Mycobacterium tuberculosis. Infect Immun. 2005;73(3):1886-9.
19. Morris D, Nguyen T, Kim J, Kassissa C, Khurasany M, Luong J, et al. An elucidation of neutrophil functions against Mycobacterium tuberculosis infection. Clin Dev Immunol. 2013;2013:959650.
20. Kulkarni R, Deshpande A, Saxena R, Saxena K. A study of serum malondialdehyde and cytokine in tuberculosis patients. J Clin Diagnostic Res. 2013;7(10):2140-2.
21. Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid untreated tuberculosis patients in peroxidation products in Ethiopia. Am J Clin Nutr. 2003;78(1):117-22.
22. Kandukuri E, Sarma DVHS, Sushma P, Moulali D. Serum MDA (Malondialdehyde),hs-CRP and Adenosine Deaminase Levels in Pulmonary Tuberculosis Patient's. International Journal of Scientific and Research Publications. 2015;5(2):1-3.
23. Pearl JE, Torrado E, Tighe M, Fountain JJ, Solache A, Strutt T, et al. Nitric oxide inhibits the accumulation of CD4+CD44hiTbet+CD69lo T cells in mycobacterial infection. Eur J Immunol. 2012;42(12):3267-79.
24. Idh J, Mekonnen M, Abate E, Wedajo W, Werngren J, Ängeby K, et al. Resistance to first-line anti-TB drugs is associated with reduced nitric oxide susceptibility in Mycobacterium tuberculosis. PLoS One. 2012;7(6):3-8.

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Pancreatic Tuberculosis: A Case Report

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ABSTRACT

We report a case of pancreatic tuberculosis. A 30 year woman who presented with abdominal distention, low grade fever, night sweats and anorexia for two month. She recovered with anti tubercular therapy. Pancreatic tuberculosis, in particular is extremely rare, and only a few cases have been reported. Diagnosis of pancreatic TB has always been a challenge, but radiological investigations and image guided intervention helped in the diagnosis and prevention of unnecessary laparotomy.

Key Words: *Pancreatic tuberculosis, Fine needle aspiration cytology, Polymerase chain reaction*

INTRODUCTION

Tuberculosis is common in developing countries, but tuberculosis affecting intra-abdominal organs is relatively uncommon. The incidence varies from country to country. Pancreatic TB is an extremely rare entity.[1] In a classical study of 300 cases of abdominal TB carried out by Bansali,[2] not a single case of pancreatic tuberculosis was reported. In 1944, Auerbach reported that pancreas was affected in 4.7% of cases of military tuberculosis.[3] Tuberculosis being a curable disease, every effort should be made to arrive at an early and timely diagnosis so as to avoid unnecessary interventions.

CASE REPORT

A 30 year old woman presented with abdominal distention, fever, night sweats, anorexia & weight loss since one month. She had lost 14 kg over a period of two months. She had enjoyed a good past health and there was no history of pulmonary tuberculosis. On clinical examination, she was poorly built with a weight of 38 kg. She was conscious, oriented with a pulse rate of 76 beats/min, blood pressure of 120/80 mm Hg. Physical examination revealed ascites, but was otherwise normal. On admission, she was found to have diabetes. Her blood sugar level (random) was 400 mg/dl with no ketones in her urine. She was diagnosed as a case of newly detected diabetes mellitus and started on insulin therapy. Her hemoglobin level was 10.8 mg/dl, WBC 7800/cu.mm, liver enzymes and kidney function tests were normal. ESR was 65mm/hr at end of one hour. HlV and Mantoux test were found to be negative. USG abdomen & pelvis revealed gross ascites. Ascitic fluid analysis was not done. Her chest x ray was normal. During hospitalisation, she had intermittent episodes of hyperglycemia & hypoglycaemia for which insulin was adjusted as per her blood sugar levels. She was diagnosed as a case of newly detected diabetes mellitus with ascites and was discharged. She came to us and was admitted after 8 days for same complaints and uncontrolled sugar levels, and suspected of abdominal kochs with a suspicious of pancreatic tuberculosis. She had brittle diabetes with large excuration and falls of blood sugar levels. Chest x ray suggestive of left sided pleural effusion. Ultrasound of abdomen showed edematous pancreas with small hypoehoeic cystic lesion in the the head of pancreas, peripancreatic and mesenteric lymphadenopathy. The pancreatic duct was normal. CT abdomen did not reveal anything more than the USG. On history, she had mild pancreatic exocrine insufficiency of recent onset. Ascitic fluid analysis was done which revealed amylase of 800 lU/l, lipase of 1200 lU/l and ADA was 63 lU/l. Further fine needle aspiration cytology & Polymerase chain reaction (PCR) was performed, USG guided FNAC revealed caseous necrosis and PCR was positive. Both the reports confirmed the clinical suspicion of pancreatic tuberculosis. She was started on anti kochs therapy and within six weeks her symptoms got relieved. She gained weight of 12 kg within a period of 2 months. At the end of 6 week, her amylase and lipase levels fell and her resolving ascites, no fever, increased appetite and increased weight gain confirm rebust response to AKT therapy. Her blood sugar level fluctuations were reduced and blood sugar level stabilized at the end of 2 months. We treated her for exocrine pancreatic insufficiency & insulin deficiency syndrome. Patient was discharged and under constant follow up after six months of anti kochs therapy, after her release from treatment of T.B. She still suffers from mild pancreatic exocrine insufficiency syndrome. USG has been found with normal pancreas and abdomen. Her weight and appetite was improved. Blood sugar levels were under control. This case has been presented with a view to highlight the entity of pancreatic tuberculosis which is extremely rare.

DISCUSSION

Tuberculosis is a systemic disease with protean manifestations. Approximately 15% of cases involve extrapulmonary sites.[4] Since pancreatic tuberculosis is rare,[5] it poses a clinical dilemma,[6] as it does not commonly figure in the differential diagnosis of a pancreatic mass, or ascites.[7, 8]

Pancreas is biologically protected from infection by mycobacterium tuberculosis because of the presence of pancreatic enzymes, however when pathogen is able to overcome the resistance, the disease pattern can be varied. The exact way in which pancreas is involved is yet to be completely understood. But two main hypotheses have been proposed. One theory suggests that by hematogenous dissemination after pulmonary disease organisms reach pancreas.[9] Another theory proposes that pancreatic tuberculosis can be caused by direct spread from adjacent peripancreatic lymph nodes.[10]

The pancreas is rarely affected by tuberculosis. In 1944, Auerbach reported pancreatic involvement in 4.7% of biopsies in cases of miliary tuberculosis.[11] Between 1891 and 1961, Paraf et al. reported 11 cases of pancreatic involvement in necrosis of miliary tuberculosis, with 2.1% incidence of involvement of this organ. Between 1980 and 1998, 14 cases were reported in the literature,[12] the majority in young adults (mean age 33 years).

Possible mechanisms of involvement of the pancreas are as follows:

- The first possible way[13] is that tubercle bacilli reach the pancreas through haematogenous dissemination from an occult lesion in the lungs or abdomen.
- The second way[14] is that the route by direct spread from contiguous lymph nodes may be responsible for most of the cases with isolated pancreatic TB.
- The third possible way[15] is that dormant bacilli in an old tubercular lesion can re-activate in an immunosuppressive state.

Rana et al.[16] Isolated pancreatic tuberculosis mimicking focal pancreatitis and causing segmental hypertension. CECT revealed focal pancreatitis involving head and body of pancreas with splenic vein thrombosis. Cytological examination of image-guided aspiration revealed caseous material. Culture demonstrated growth of Mycobacterium tuberculosis.

Arora et al.[17] Patient presented with chronic abdominal pain and weight loss. CT revealed a well marginated cystic lesion in the head of pancreas with upstream biliary dilatation. Endoscopic ultrasound-guided fine needle aspiration biopsy (FNAB) showed epitheloid granulomas PCR based assay confirmed Mycobacterium tuberculosis.

Falkowski et al.[18] Patient presented with pancreatitis. CT showed multi cystic solid mass with slight contrast enhancement in area of pancreatic head, located in the branching of celiac trunk and adjacent to the portal vein. Endoscopic ultrasound-guided FNAC revealed necrotising granulomatous infection and numerous acid-fast bacilli on microscopy. PCR positive for Mycobacterium tuberculosis.

DM is now a recognized risk factor and common complication encountered among TB patients.[19, 20] Recent evidence suggests that diabetic patients have an increased tendency to develop TB due to impaired cell-mediated immunity, renal failure, micronutrient deficiency, and pulmonary microangiopathy.[21] Chronic infections like TB are associated with reactionary hyperglycemia which occurs due to increased production of counter-regulatory stress hormones like epinephrine, glucagon, cortisol, and growth hormone that act synergistically.[22]

Treatment of DM among patients with TB should involve the use of insulin or oral hypoglycaemic agents (OHAs) in order to achieve the optimal goals of therapy, that is, maintaining an HbA1c of <7%, random blood sugar level <180 mg/dl or a FBG level <120 mg/dl.[23] However, therapeutic doses of most glucose lowering drugs may need to be increased during the initial phases of TB treatment. This is because rifampicin induces an acute transient hyperglycemia due to its effect of augmenting intestinal absorption of glucose.[24] Due to its cytochrome P450 enzyme inducing properties, it also augments hepatic metabolism of most OHAs.[25]

The common presenting features are non-specific abdominal pain, fever, anorexia and weight loss.[26] Less common symptoms include iron deficiency anaemia, vomiting, obstructive jaundice, upper gastro-intestinal bleeding and portal hypertension.[27] Patients may or may not have had other forms of tuberculosis in the past. Clinical examination is usually non-contributory.[28] Ultrasonographic features include a diffusely enlarged pancreas with focal hypoechoic lesions or cystic lesions of the pancreas.[29] Associated findings include peripancreatic and mesenteric lympha-denopathy,[30, 31] bowel wall thickening (usually in the ileocaecal region), focal hepatic or splenic lesions and ascites.[32] CT scan most commonly reveals a mass lesion.[33, 34]

Therefore, to establish the diagnosis of pancreatic TB, histological, cytological as well as bacteriological confirmations are necessary. Ultra-sound-guided (USG) or CT-guided FNAC has been used to confirm the diagnosis and to prevent unnecessary laprotomies.[35-37.]

Ultrasound or CT-guided FNAC may provide the diagnosis, especially with the help of an expert cytologist experienced in the diagnosis of tuberculosis.[34]

The varied presentation and rare occurrence of pancreatic tuberculosis is the main reason for its diagnosis becoming difficult and a high degree of suspicion is necessary for a medical and pre or intraoperative diagnosis. USG or CT guided aspiration cytology may help in differentiating this from carcinoma, lymphoma, chronic pancreatitis or sarcoidosis.[38]

A recent diagnostic test is the polymerase chain reaction (PCR) based assay, which detects mycobacterium tuberculosis DNA in resected specimens. It is a highly specific assay and may give a positive result even when special staining techniques and cultures of these tissues are negative.[39]

The treatment of pancreatic tuberculosis comprises multi-drug anti-tuberculous

chemotherapy for between 6 and 12 months. Response to therapy is predictable and complete. These patients still need to be followed up carefully for subjective and objective response to therapy to rule out the rare possibility of tuberculosis coexisting with malignancy, especially in endemic areas.[40]

CONCLUSION

The diagnosis of pancreatic tuberculosis requires a high degree of suspicion and, although is a rare condition, should be considered as a differential diagnosis in patients with pancreatic lesions. Pancreatic TB may present as SOL pancreas, peripancreatic collection as pseudocyst, peripancreatic lymphadenopathy, ascites and portal hypertension. High index of suspicion, CT/ USG FNAC is extremely important to make the diagnosis of pancreatic TB. The majority of patients respond well to anti-tubercular chemotherapy and prognosis is good and surgical intervention can be overted in developing countries as like India.

REFERENCES

1. Kaushik N, Schoedel K, Mc Grath K. Isolated pancreatic tuberculosis of the pancreas diagnosed by endoscopic ultrasound – guided fine needle aspiration: a case report. Journal of the pancreas, 2006,7(2):205-10.
2. Bhansali SK. Abdominal tuberculosis. Am J Gastroenterol 1977;67:324–7.
3. Levine R, Tenner S, Steinberg W et al. Tuberculous abscess of the pancreas.Case report and review of the literature. Dig Dis Sci 1992; 37:1141-4.
4. Glassroth J, Robins AG, Snider DE Jr. Tuberculosis in the 1980s. N Engl J Med 1980;302:1441–50.
5. Ladas SD, Vaidakis E, Lariou C et al. Pancreatic tuberculosis in non-immunocompromised patients: reports of two cases and a literature review. Eur J Gastroenterol Hepatol 1998;10:973–6.
6. Lo SF, Ahchong AK, Tang CN, Yip AW. Pancreatic tuberculosis: case reports and review of the literature. J R Coll Surg Edinb 1998;43:65–8.
7. Brusko G, Melvin WS, Fromkes JJ, Ellison ES. Pancreatic tuberculosis. Am Surg 1995;61:513–5.
8. Jena GP, Manoharan GR, Mbete DL, Pillay SS. Tuberculous pancreatic abscess in HIV-positive patients. A report of three cases and a review of the literature. S Afr J Surg 1999;37:69–71.
9. Stambler JB, K Libanr M, Bliss CM, Lamont T Tuberculous abscess of pancreas Gastroenterology 1082; 82:922-925.
10. Stock K.P, Riemann JF, Stadler W, et al Tuberculosis of pancreas Endoscopy 1981; 13:178-180.
11. Paraf A, Menanger C, Texier J. La tuberculose du pancreas et la tuberculose des ganglions de l'etage superieur de l'abdomen. Rev Med Chir Mal Foie 1996;41:101–126.

12. Ahmad Z, Bhargava R, Pandey DK, Sharma DK. Pancreatic tuberculosis – a case report. Ind J Tuberc 2003;50:221–223.
13. Liu Q, He Z, Bie P. Solitary pancreatic tuberculous abscess mimicking pancreatic cystadenocarcinoma: a case report. BMC Gastroenterol. 2003 Jan;3:1.
14. Gupta MM, Wig JD, Suri AS. Tuberculosis of pancreas: a case report. Ind J Tub.1992;39:247.
15. Bakhshi GD, Shaikh AS, Borisa AD, Bhattu AS, Suryawanshi MM. Primary pancreatic tuberculosis: a case report.Bombay Hospital Journal. 2008;50(3).
16. Rana SS, Bhasin DK, Rao C, Singh K. Isolated pancreatic tuberculosis mimicking focal pancreatitis and causing segmental portal hypertension JOP. J Pancreas (Online). 2010 Jul; 11(4): 393–5.
17. Arora A, Mukund A, Garg H. Isolated pancreatic tuberculosis: a rare occurrence. Am J Trop Med Hyg. 2012 Jul;87(1):1-2.
18. Falkowski AL, Graber J, Haack HG, Tarr PE, Rasch H. Isolated pancreatic tuberculosis: a case report and radiological comparison with cystic pancreatic lesions. Radiology Case. 2013 Jan;7(1): 1–11.
19. Faurholt-Jepsen D, Range N, Praygod G, Jeremiah K, Faurholt-Jepsen M, Aabye MG, et al. Diabetes is a risk factor for pulmonary tuberculosis: A case-control study from Mwanza, Tanzania. PLUS One 2011;6:e24215.
20. Jeon C, Murray M. Diabetes mellitus increases the risk of active tuberculosis: A systematic review of 13 observational studies. PLUS Med 2008;5:1091-101.
21. Harriesa A, Billo N, Kapurc A. Links between diabetes mellitus and tuberculosis: Should we integrate screening and care? Trans R Soc Trop Med Hyg 2009;103:1-2.
22. Van-Cromphaut S, Vanhorebeek I, Vanden-Berghe G. Glucose metabolism and insulin resistance in sepsis. Curr Pharm Des 2008;14:1887-99.
23. Standards of Medical Care in Diabetes by the American Diabetes Association. Diabetes Care 2009;32:S13-61.
24. Takasu N, Yamada T, Miura H, Sakamoto S, Korenaga M, Nakajima K, et al. Rifampicin-induced early phase hyperglycemia in humans. Am Rev Respir Dis 1982;125:23-7.
25. Park JY, Kim KA, Park PW, Park CW, Shin JG. Effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide. Clin Pharmacol Ther 2003;74:334-40.
26. Wu CS, Wang SH, Kuo TT. Pancreatic tuberculosis mimicking pancreatic head carcinoma: a case report and review of the literature. Infection 1994;22:287–9.
27. Takhtani D, Gupta S, Suman K et al. Radiology of pancreatic tuberculosis: a

report of three cases. Am J Gastroenterol 1996;91:1832–4.

28. Coelho JC, Weiderkehr JC, Parolin MB, Balbi E, Nassif AE. Isolated tuberculosis of the pancreas after orthotopic liver transplantation. Liver Transplantation and Surgery 1999;5:153–5.
29. Fischer G, Splenger U, Neubrand M, Sauerbruch T.Isolated tuberculosis of the pancreas masquerading as a pancreatic mass. Am J Gastroenterol 1995;90:2227–30.
30. Morris DL, Wilkinson LS, al Mokhtar N. Case report: emphysematous tuberculous pancreatitis diagnosis by ultrasound and computed tomography. Clin Radiol 1993;48:286–7.
31. Desai DC, Swaroop VS, Mohandas KM et al. Tuberculosis of the pancreas: report of three cases Am J Gastroenterol 1991;86:761–3.
32. Zalev AH, Sacks JS, Warren RE. Pancreaticoduodenal tuberculosis simulating metastatic ovarian carcinoma. Can J Gastroenterol 1997;11:41–3.
33. Crowson MC, Perry M, Burden E. Tuberculosis of the pancreas: a rare cause of obstructive jaundice. Br J Surg 1984;71:239.
34. Fan ST, Yan KW, Lau WY, Wong KK. Tuberculosis of the pancreas: a rare cause of massive gastrointestinal bleeding. Br J Surg 1986;73:373.
35. Song TJ, Lee SS, Park do H, Lee TY, Lee SO, Seo DW, et al. Yield of EUS-guided FNA on the diagnosis of pancreatic/peri-pancreatic tuberculosis. Gastrointest Endosc. 2009 Mar;69(3 Pt 1):484–91.
36. Ahlawat SK. EUS-guided FNA diagnosis of pancreatic tuberculosis. Gastrointest Endosc. 2007 Mar;65(3):557–8.
37. Itaba S, Yoshinaga S, Nakamura K, Mizutani T, Honda K, Takayanagi R, et al. Endoscopic ultrasound-guided fine- needle aspiration for the diagnosis of peri-pancreatic tuberculous lymphadenitis. J Gastroenterol. 2007 Jan;42(1):83–6.
38. S.Varshney, CD Johnson Tuberculosis of pancreas Journal of postgraduate medicine 1995; 71:564-566.
39. Yokoyama T, Miyagawa S, Noike T, Shimada R, Kawasaki S. Isolated pancreatic tuberculosis. Hepatogastroenterology 1999;46:2011-14.
40. Jaber B, Gleckman R. Tuberculous pancreatic abscess as an initial AIDS-defining disorder in a patient infected with the human immunodeficiency virus: case report and review. Clin Infect Dis,1995;20:890–4.

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Research Paper

Study of Serum Adenosine Deaminase (ADA) Level in Diagnosis of Extrapulmonary and Smear Negative Tuberculosis

Biochemistry

KEYWORDS : Extrapulmonary TB, Pulmonary smear negative TB, Adenosine deaminase.

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ABSTRACT

Introduction: *Tuberculosis is a major cause of morbidity and mortality throughout the world. One-third of the world's population is infected with the TB, 5.2 million incident pulmonary TB patients notified globally in 2014. Only 3.0 million (58%) smear positive were bacteriologically confirmed, 42% of patients who were not bacteriologically confirmed were diagnosed clinically i.e. based on symptoms, chest X-Ray abnormalities or suggestive histologically and remains diagnostic challenge. In view of role of serum ADA in TB diagnosis this study was planned to investigate the diagnostic value of serum adenosine deaminase in diagnosis of extra-pulmonary negative tuberculosis (EPTB) and pulmonary smear negative tuberculosis (SNPTB), because rapid and accurate diagnosis is an important element of TB treatment and control.*

Objective: *To evaluate the diagnostic value and compare serum ADA activity in extra-pulmonary and pulmonary smear negative tuberculosis and to compare it with control.*

Method: *Total of 120 volunteers was enrolled in the study after obtaining informed written consent. They were divided into 3 groups. Group I includes 40 healthy Individuals; Group II 40 patients newly diagnosed with extra-pulmonary TB and Group III 40 patients newly diagnosed with pulmonary smear negative TB. Serum ADA was estimation by modified GIUSTI method.*

Result: Serum ADA levels were significantly increased ($p<0.001$) in extrapulmonary TB (27.81 ± 7.034U/L) and Pulmonary Smear Negative TB (35.12 ± 12.1 U/L) as compared to control (14.603 ± 4.69U/L) with cut-off value 20 U/L.

Conclusion: *Moreover our study being cost effective, highly reliable, reproducible, simple to perform and less time consuming; in addition it helps in rapid and accurate diagnosis of EPTB and pulmonary smear negative tuberculosis, it can be included in routine diagnosis and prognosis of tuberculosis.*

INTRODUCTION:

Tuberculosis (TB) is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years. [1] It is a major cause of morbidity and mortality throughout the world. One-third of the world's population is infected with the TB bacillus.[2, 3] The tuberculosis epidemic results in nearly two million deaths and nine million new cases per year, 95% in developing countries. In India 2.3 million cases are estimated to have occurred, accounting for approximately one fifth of the global incidence. The World Health Organization (WHO) 1990 report on the Global Burden of diseases ranked tuberculosis as the seventh most morbidity causing disease in the world, and expected it to continue in the same position up to 2020.[4]

Once infected active disease develops in about 10% of cases usually within 1-2 years after exposure from TB.[5]Some of the healthy subjects getcontracts tuberculosis every four seconds and one of them dies every 10 seconds.[6,] In 2006, about 1.4 million cases of tuberculosis were registered for treatment in India; 28.7% of them were new smear negative cases.[8]

The initial diagnostic approach to suspected cases of pulmonary tuberculosis is to demonstrate *Mycobacterium tuberculosis* in stained smears of expectorated sputum. In most of the tuberculosis centers, even after careful search, the bacteriological positive yield from sputum is around 16 to 50% and large portion remain negative in spite of clinical profile and radiological lesions being consistent with diagnosis of pulmonary tuberculosis.[9] However, 40 to 60% of patients with pulmonary disease and about 75% of patients with extra-pulmonary disease are smear negative, and in this situation even contemporary culture methods take several weeks to become positive.

There are different diagnostic methods but they have some limitations. [12,13,14,15] Pulmonary TB is usually diagnosed from clinical and radiological findings. The laboratory diagnosis is based on Ziehl-Neelsen (ZN) staining for acid-fast bacilli (AFB) and the growth of the causative organism Mycobacterium tuberculosis on Löwenstein-Jensen (LJ) culture, [16] which is the golden standard for TB diagnosis. However *Mycobacterium tuberculosis* grows very slowly, it can take up to six weeks to isolate it in culture. Determination of susceptibility to drugs can add another three to six weeks to process. Meanwhile the disease may progress and be transmitted to others when appropriate treatment is delayed and hospitalization increases. [17] ZN staining is rapid, easy to perform and inexpensive, but it lacks sensitivity which is already discussed. Problem arises when sputum smear result is repeatedly negative for acid fast bacilli. [18]

Polymerase Chain Reaction (PCR) is expensive and is not found to be more sensitive to pleural fluid. All these diagnostic tests including the newer Nucleic Acid Amplification, Interferon gamma and Lysozyme, either time-consuming, lack sensitivity or specificity or required technology is very intensive and expensive as well, thus limiting their usefulness and access especially in developing countries with insufficient resources.[19]

Adenosine deaminase activity (ADA) is also measured in diagnosis of TB and also in differential diagnosis of TB. Adenosine deaminase is an enzyme of purine catabolism; its activity has been found to be increased in various diseases such as tuber-

culosis, HIV, typhoid, infectious mononucleosis and certain malignancies especially those of hemopoietic origin. ADA assay in various body fluids had established its usefulness in the laboratory diagnosis of extrapulmonary TB (such as meningeal, pleural, peritoneal and pericardial TB) and SNPTB. [20]

By considering the importance of rapid and accurate diagnosis, in TB treatment and control, the present study was planned to investigate the diagnostic value of serum adenosine deaminase in diagnosis of EPTB and SNPTB and to compare it with control group.

AIM: To study serum adenosine deaminase (ADA) level in diagnosis of extrapulmonary and pulmonary smear negative tuberculosis.

OBJECTIVES: To evaluate the diagnostic value of serum ADA activity in extra-pulmonary and pulmonary smear negative tuberculosis and to compare serum ADA activity in study groups and control group.

MATERIAL & METHODS: The Institutional Ethics Committee approval was obtained before initiating the study. The study was conducted in the Departments of Biochemistry, Dept. of Respiratory Medicine, Dept. of Surgery, MGM Medical College & Hospital, Kamothe, Navi Mumbai. Present study is a prospective study completed over a period of 12 months i.e.from February 2014 to February 2015

The volunteers are grouped into following three groups:-
Group I: 40 healthy Individuals.
Group II: 40 patients newly diagnosed with extra-pulmonary TB (EPTB).
Group III: 40 patients newly diagnosed with pulmonary smear negative TB (SNPTB).
Inclusion Criteria: Following patients are included for the present study

Patients with Extra-pulmonary TB diagnosed by clinician.
Extra pulmonary TB patient with smear positive histopathologicaly.

Two sets (taken at least 2 weeks apart) of at least two sputum specimens negative for Acid Fast Bacilli and radiographic abnormalities consistent with pulmonary TB.

Exclusion criteria: Following patients will be excluded from the study -
No respiratory symptoms
Normal chest X-rays.
Liver diseases
HIV/AIDS
Gross congestive heart failure
Typhoid fever
Infectious mononucleosis
Gout / Rheumatoid arthritis
Skeletal muscle injury
Renal failure
Brucellosis.Bronchogenic carcinoma

Study Procedure: 2.0 ml of blood was collected by venepuncture. Serum was separated preserved at -60°C till the analysis. Serum ADA estimated by ADA kit (Kinetic Reaction), the modified ***GIUSTI*** method using semi auto analyzer.

RESULTS:

Table 1: Gender wise distribution of healthy individuals, extrapulmonary TB and pulmonary smear negative TB

Groups	Sex		Total
	Male	Female	
Healthy Individuals	32	8	40
Extra Pulmonary TB	12	28	40
Pulmonary Smear Negative TB	30	10	40
Total	74	46	120

Tables 2: Comparison of subjects according to weight (Kg) in healthy individuals, extra pulmonary TB and pulmonary smear negative TB.

Groups	N	Mean ± SD
Healthy Individuals	40	62.97 ± 9.78
Extra Pulmonary TB	40	47.1 ± 15.05**
Pulmonary Smear Negative TB	40	48.17 ± 10.01**

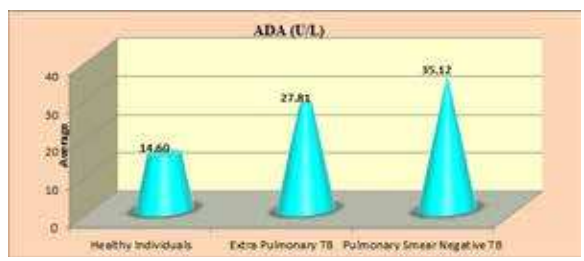
**p<0.001(highly statistically significant)

Table 3: Comparison of ADA level (U/L) of healthy individuals, extra Pulmonary TB and pulmonary Smear negative TB.

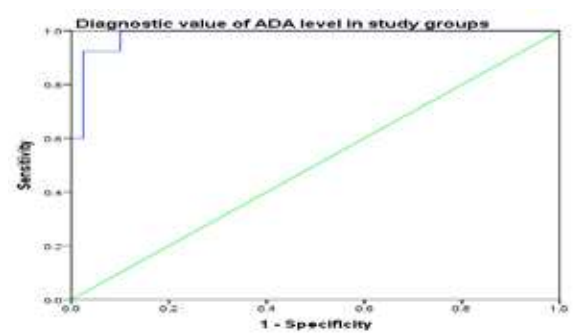
Groups		N	Mean ± SD
ADA (U/L)	Healthy Individuals	40	14.603 ± 4.69
	Extra Pulmonary TB	40	27.81 ± 7.034**
	Pulmonary Smear Negative TB	40	35.12 ± 12.09**

**p<0.001(highly statistically significant)

Graph 1: Comparison of ADA levels (U/L) in different groups such as healthy individuals, extrapulmonary TB and pulmonary smear negative TB.



Graph 2: ROC (Receiver operating characteristic) curve for serum ADA in TB diagnosis



DISCUSSION:
Tuberculosis (TB) is caused by Mycobacterium tuberculosis and is one of the most important infective causes of human mortality and morbidity worldwide. [21] India is the highest TB burden country with WHO statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB in India, out of a global incidence of 8.7 million cases. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB. [22]

The present study reveals that male subjects were more prone to pulmonary smear negative TB (4:1 male to female), whereas female were more prone to extrapulmonary TB (1:2.3 male to female) **Table 1.** Balasubramanian R *et al.*[23] reported same prevalence in their review i.e. Lymph node TB (LNTB) is the commonest form of EPTB. Peripheral LNTB have been in majority female, while pulmonary TB is more common in adult males.

The weight of control subjects was (62.97 ± 9.78 Kg), extrapulmonary subjects were (47.1 ± 15.05 Kg) and smear negative subjects was (48.17 ± 10.01 Kg). Our study shows that the study groups' patient weights is significantly declined as comparison of control group (p<0.001) but non-significant between study groups (p>0.05) **Table 2.**

Patients with Tuberculosis (TB) often suffer from severe weight loss, a symptom those are considered immuno-suppressive and a major factor of severity and disease outcome. Malnutrition is an important risk factor for TB, as cell-mediated immunity (CMI) is key host defense against TB and other factors such as socio-economic demographic characteristics, smoking and drinking habits. [23]

Serum ADA levels are compared in healthy subjects with extrapulmonary TB and pulmonary Smear negative TB. Serum ADA levels are significantly increased (p<0.001) in extrapulmonary TB (27.81 ± 7.034U/L) and Pulmonary Smear Negative TB (35.12 ± 12.1 U/L) as compared to healthy individual. The results are concurrent with Agarawal MK *et al.*[24], Rathod VS *et al.*[25], Verma M*et al.*[26],, Stevanovic G. *et al.*[27] and Sharma D *et al.* [28]

Significantly increased serum ADA activity (p<0.001) in pulmonary smear negative TB and EPTB compared to healthy controls is due to activation of cell mediated immunity. In tuberculosis there are increased numbers of T-lymphocytes and macrophages, which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes. In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T-cells, may cause a high serum ADA activity in patients with TB. [29]

Serum ADA **cut-off value** is assessed from receiver operating characteristic (**ROC) curve** for extra pulmonary TB and pulmonary smear negative TB. The cut-off value 20 U/L and taken as the best cut-off point. For this cut off value the sensitivity was 99 % and specificity 90% (p<0.001). The area under ROC Curve is 0.984 (95% CI-0.962-1.00). In present study none of the healthy individual showed ADA value above this limit and none of study subjects showed a lower value than 20 U/L. Our results are concurrent with Afrasiabian S *et al.*[30] and Stevanovic G. *et al.*[27]

ADA is the enzyme which is present in every cell. Highest concentration of ADA is seen in monocytes, macrophages and T-lymphocytes. These cells consist of ADA, 5-20 times more than B-cell and 10 times more than erythrocytes. Monocytes/macrophages are the main targets of mycobacterium tuberculosis, which multiply slowly inside the host cell. Then macrophages stimulate the cell-mediated immune response by releasing cytokines, which attract T-lymphocytes to the site. Defensive environment kill or limit the replication of pathogens and finally destroys the macrophages. Thus increased concentration of ADA may be found due to antigenic stimulation of phagocytic cell, its propagation, differentiation and macrophages destructions.

Elevated levels of ADA may depend on severity of TB, immune status and age. Limitation of present study is that increased serum ADA activity is also found in diseases of cell mediated immunity of unknown illness.

CONCLUSION:

Significant increased concentrations of serum adenosine deaminase in study groups as compared to control group, aids to use ADA as a screening test. However, larger sample size is required to confirm reference values for serum ADA and to assess its diagnostic utility. The serum concentration of ADA may be used as a surrogate marker for EPTB and pulmonary smear negative TB and the findings should be correlated with clinical presentation of disease. Moreover our study being cost effective, highly reliable, reproducible, simple to perform and less time consuming; in addition it helps in rapid and accurate diagnosis of EPTB and pulmonary smear negative tuberculosis, it can be included in routine diagnosis and prognosis of tuberculosis.

REFERENCE

1. Hirsh AE, Tsolaki AG, De Riemer K, Feldman MW, Small PM. Stable association between strains of Mycobacterium tuberculosis and their human host populations. Proc Natl Acad Sci USA. 2004;101:4871–6. 2. Farazi A, Moharamkhani A, Sofian M. Validity of serum Adenosine deaminase in diagnosis of tuberculosis, Pan African Medical Journal. 2013; 15:133. 3. Global Tuberculosis Control, A short update to the 2009 report, World health organization. "WHO/HTM/TB/2009.426". 4. Bachh AA, Gupta R, Haq I and Varudkar HG. Diagnosing sputum smear negative pulmonary tuberculosis: Does fibre optic bronchoscopy play a significant role? Lung India. 2010; 27(2): 58–62. 5. Nettleman MD, Geerdes H, Roy MC. The cost-effectiveness of preventing tuberculosis in physicians using tuberculin skin testing or a hypothetical vaccine. Arch Intern Med. 1997; 157(10):1121-7. 6. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: Estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA. 1999; 282:677–86. 7. Ministry of Health and Family Welfare, Directorate General of Health Services, Central TB Division. TB India: RNTCP status report. Chapter 7, Performance of RNTCP. 2007:71–106. 8. Ministry of Health and Family Welfare, Directorate General of Health Services, Central TB Division. TB India: RNTCP status report. Performance of RNTCP. 2007; Chapter 7:71–106. 9. Kulpati DD, Heera HS. Diagnosis of smear negative pulmonary tuberculosis by flexible fibreoptic bronchoscopy. Indian J Tuberc. 1986; 33:179–82. 10. Pottumarthy S, Wells VC, Morris AJ.A comparison of seven tests for serological diagnosis of tuberculosis. J ClinMicrobiol. 2000; 38(6):2227-31. 11. Adjei AA, Armah H, Duah OA, Adiku T, Hesse IF. Evaluation of a rapid serological chromatographic immunoassay for the diagnosis of pulmonary tuberculosis in Accra, Ghana. Jpn J Infect Dis. 2003; 56(4):161. 12. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: A systematic review. Lancet Infect Dis. 2006; 10:664-74. 13. Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics for developing countries. Lancet. 2006; 367(9514):942-3. 14. World Health Organization. Diagnostic and treatment delay in Tuberculosis. 2006; 21. 15. Storla DG, Y imer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health. 2008; 8:15. 16. Dimakou K, Hillas G and Bakakos P. Adenosine deaminase activity and its isoenzymes in the sputum of patients with pulmonary tuberculosis. The International Journal of Tuberculosis and Lung Disease. 2009; 13(6):744–748. 17. Lamsal M, Gautam N, Bhatta N, Majhi S, Baral N and Bhattacharya SK. Diagnostic utility of adenosine deaminase (ADA) activity in pleural fluid and serum of tuberculous and non-tuberculous respiratory disease patients. Southeast Asian J Trop Med Pub Health. 2007; 38 : 2 18. Agarwal MK, Nath J, Mukerji PK and Srivastava VML. A study of serum adenosine deaminase activity in sputum negative patients of pulmonary tuberculosis. Ind. L Tub. 1991; 38: 139. 19. Wallis RS, Pai M, Menzies Dick, Doherty TM, Walzl G, Perkins MD and Zumla A. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet 2010; 375: 1920–37. 20. Chander A and Shrestha CD. Diagnostic value of serum adenosine deaminase levels in sputum smear negative pulmonary tuberculosis patients in Nepalese population. Asian Pacific Journal of Tropical Biomedicine. 2012. 21. Maher D, Chalet P, Spinaci S and Harries. Treatment of tuberculosis: guidelines for national programmes, 2nd edition Geneva: WHO. 1997. 22. TB Facts, TB statistics for India.http://www.tbfacts.org/tbstatisticsindia.html 23. Vasantha M, Gopi PG and Subramani R. Weight gain in patients with tuberculosis treated under directly observed treatment short-course (dots); Indian Journal of Tuberculosis. 2009; 56: 5-9 24. Agarwal MK, Nath J, Mukerji PK and Srivastava VML. A study of serum adenosine deaminase activity in sputum negative patients of pulmonary tuberculosis. Ind. L Tub. 1991; 38: 139. 25. Rathod VS, Sunitha S and Huliraj N. Serum adenosine deaminase activity in pulmonary tuberculosis and other common respiratory diseases. International journal of basic medical science. 2014; 5: 5. 26. Verma M, Narang S, Moonat A and VermaAkshra. Study of adenosine deaminase activity in pulmonary tuberculosis and other common respiratory diseases. Indian Journal of Clinical Biochemistry. 2004; 19 (1) 129-131. 27. Stevanovic G, Pelemis M, Pavlovic M, Lavadinovic L, Dakic Z, Milosevic I and Milosevic B. Significance of adenosine deaminase serum concentrations in the diagnosis of extra-pulmonary tuberculosis. JIMAB. 2011; 17: 1. 28. Sharma D and dhimanP. Osteoarticular tuberculosis: in search of new biomarkers. European Federation of National Associations of Orthopaedics and Traumatology. 2015. 29. Baganha MF, Alice P, Lima MA, Gaspar EV, and Cordeiro AR. Serum and Pleural Adenosine Deaminase Correlation with Lymphocytic Populations. Chest. 1990; 97: 3. 30. Afrasiabian S, Mohsenpour B, Bagheri KH, Sigari N, and Aftabi K. Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis. J Res Med Sci. 2013; 18(3): 252–254.

Clinical Evaluation

Evaluation of Nested Polymerase Chain Reaction for the Diagnosis of Pulmonary Tuberculosis

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Abstract

Utility of Polymerase Chain Reaction (PCR) test for diagnosis of pulmonary tuberculosis was evaluated in 50 clinically diagnosed cases. All samples were also processed for microscopy by Ziehl-Neelsen (ZN) staining and culture on Lowenstein-Jensen medium (LJ). PCR was positive in all microscopy and culture positive specimens. There were 11 samples positive by PCR but failed to grow in culture. With respect to culture the sensitivity (SN), specificity (SP), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of PCR was 100%, 57%, 68% and 100% respectively. The values for PCR on final evaluation taking into consideration clinical, radiological, microbiological evidence and response to antitubercular treatment were SN-100%, SP-88%, PPV-94% & NPV-100% respectively. PCR detects *M. tuberculosis* complex with greater sensitivity and should be useful for rapid diagnosis of tuberculosis.

Keywords

mycobacterium tuberculosis, polymerase chain reaction, IS 6110, sensitivity, specificity

Introduction

Tuberculosis (TB) continues to be a health hazard world over in spite of relentless global efforts directed at containing the scourge. Conventional methods available for diagnosis namely tuberculin test, radiological examination, smear microscopy and culture have their own limitations. Sputum smear microscopy requires more than 10,000 organisms per ml be present in the sample and has low sensitivity[1-6]. Culture is more sensitive than microscopy and is still considered the "gold standard" being 100% specific but is time consuming (3 to 8 weeks)[1,3,5]. Great progress have been made in reducing the time required for detecting the growth of mycobacteria using various culture systems like BACTEC, MGIT, MB/BACT etc, still however, on an average 8 to 12 days are needed to detect the growth[2,7,8]. Another recent approach to culture of *M. tuberculosis* is TK medium (Salubris, Inc.MA USA) with average time to detection of two weeks as compared to four weeks on LJ medium. It promises to be a practical, low cost, simple test[9]. A new test being introduced by WHO in national tuberculosis control programs is Xpert MTB / RIF which provides sensitive detection of *M. tuberculosis* and rifampicin resistance detection in less than two hours. This is being implicated in a phased manner[10].

Recent advances in DNA amplification using Polymerase Chain Reaction (PCR) has allowed great progress to be

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made in the rapid and accurate diagnosis of infections due to organisms that are not cultivable by *in vitro* means, that require complex media or cell cultures and prolonged incubation times, or for which culture is too insensitive[11]. PCR for diagnosis of tuberculosis has been found to be useful in a number of studies[12,13,14,15]. This study reports the application of nested PCR for the diagnosis of pulmonary tuberculosis by amplification of IS6110, an IS-like element of *M. tuberculosis* complex. As the IS6110 sequence has been shown to be repetitively present in *M. tuberculosis* genome, it helps to increase the sensitivity of the test[12,16,17,18]. The nested format has been found to be useful by other studies[19,20]. This is the first comprehensive study comparing conventional methods with PCR for the diagnosis of tuberculosis from region of Marathwada of Maharashtra State, India.

Material and Methods

A total of 50 pulmonary specimens (sputum 32, pleural fluid 14, bronchoalveolar lavage (BAL) 2, intercostal drain (ICD) 2) were obtained from patients clinically suspected to have tuberculosis. The preliminary clinical diagnosis was made if the patient had fever, night sweats, persistent cough for more than 3 weeks, and weight loss, supported by radiological evidence of disease. Microscopic examination of the specimens was done by Ziehl-Nelsen staining and if negative staining was repeated on the specimens after concentration using 4% sodium hydroxide. The concentrated material was also used for culture on Lowenstein Jensen's medium in duplicate. The cultures were incubated at 37^0C for 8 weeks.

All isolates of acid fast bacilli on LJ medium were further identified to species level by colony characteristics including the speed of growth and pigmentation and a battery of biochemical tests (niacin, nitrate reduction, catalase and growth on medium containing p-nitrobenzoic).

PCR assay was carried out using Genei ™ Amplification Reagent Set MTB-25 for *M. tuberculosis*, manufactured by Bangalore Genei Bangalore, India. This test is based on the principle of single tube nested PCR method. The assay is a two-step sequential assay. In the first step in the IS region of *M. tuberculosis* complex DNA sequence, a 220 bp is amplified by specific external primers. In the second step, the nested primers are added to further amplify a 123 bp amplification product. In this test false positive reactions that may be caused by previous amplicon contamination are prevented by the use of uracil DNA glycolase (UDG) and dUTP instead of dTTP. DNA extraction was carried using Proteinase K according to the manufacturer's instructions. DNA extraction was done in a separate room. DNA amplification was carried out on the same day of receiving the sample.

DNA amplification :

A) First amplification :

Master Mix I was prepared, so that for each specimen the master mix I contained Amplification Premix I8.2 µl, Gene Hotstart Taq DNA Polymerase 0.33 µl and uracil DNA glycolase (UDG) 0.5 µl. To each 0.2ml PCR tube 9 µl of Master Mix-I was taken and 3 µl of extracted DNA was added. Positive and Negative controls were also included. DNA amplification was carried using the thermal cycler MJ Research using the calculated mode as suggested by the manufacturer. The first amplification profile was as follows : Step-1 was 22°C for 10 minutes and initial denaturation at 94°C for 5 minutes (No. of cycles -1). Step-2 was denaturation at 94°C for 30 second, annealing at 68°C for 1 minute and extension at 72°C for 1 minute (No. of cycles - 20). Step-3 was final extension at 72°C for 1 minute and storage at 4°C (No. of cycles – 1).

B) Nested or Second Amplification:

For each specimen Master Mix-II contained Amplification premix-II 14.7 µl and Genei Hot Start Taq DNA polymerase 0.33 µl. Fifteen µl of the Master Mix-II thus prepared was added to the same PCR tubes used in the first amplification. Nested Amplification was performed using the following profile : Step-1 was initial denaturation at 94°C for 5 minutes (No.of cycles-1). Step-2 was denaturation at 94°C for 30 seconds, annealing at 68°C for 30 seconds and extension at 72°C for 30 seconds (No.of cycles 30). Step-3 was final extension at 72°C for 7 minutes and storage at 4°C (No.of cycles 1).

Analysis of amplified product:

Analysis of the amplified product was done using submarine electrophoresis using 2.5% agarose gel containing 10 µl of 10 mg per ml ethidium bromide dye solution for 100 ml gel. The gel was visualized under UV Transilluminator. An amplification product of size 123 bp was indicative of infection with *M. tuberculosis* complex. The amplification product of internal control DNA was 340 bp (**Fig. 1**).

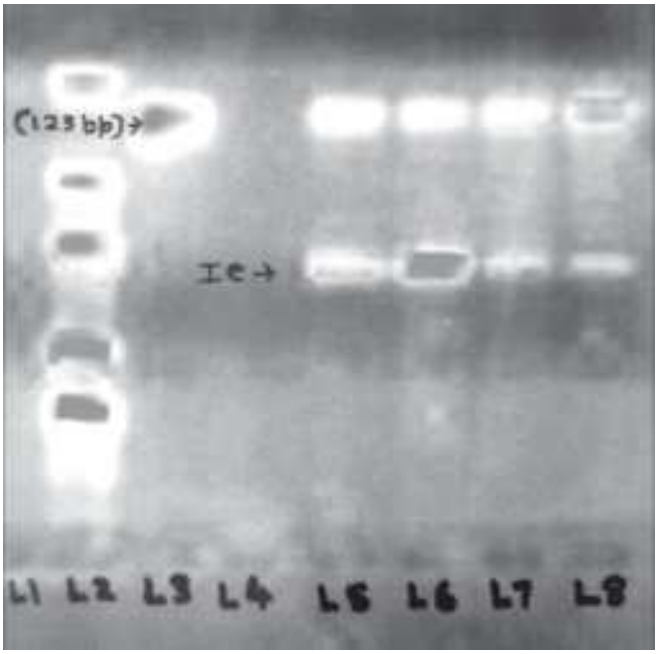

Fig. 1

Agarose gel electrophoresis analysis for DNA amplification products. Lanes : L1 empty, L2 Molecular weight marker, L3 positive control (123 bp), L4 negative control. L5, L6, L7, L8 specimen positive for *M.tuberculosis* complex (123 bp). IC: Internal control band (340 bp)

PCR was also carried out on MOTT (Mycobacteria Other Than Tubercle) strains namely *M.chelonei, M.szulgai, M.phlei, M.avium and M.intracellulare* and also *M. tuberculosis* H37Rv obtained from Department of Microbiology, MGIM's Sewagram, district Wardha, Maharashtra, India, to check the specificity of the primers.

Sensitivity, Specificity, Positive predictive value and Negative Predictive value were calculated as per Parks text book of Preventive and social medicine[21].

Results

We have analyzed 50 pulmonary specimens obtained from patients of suspected pulmonary tuberculosis. Ziehl-Neelsen staining for AFB was positive in 19 (38%) specimens and culture was positive in 24 (48%) of the specimens. All culture isolates were identified as *M. tuberculosis*. PCR gave a positive amplification result in 35 (70%) of the specimens.. PCR was positive in all smear and culture positive specimens. There were 16 (51.61%) specimens which were PCR positive but smear negative. PCR gave a positive amplification in 11 out of 26 culture negative specimens (42.30%) (**Table 1**). As culture is the gold standard, with respect to culture these 11 specimens would be false positive. Therefore with respect to culture

Table 1
Correlation of PCR with conventional methods

PCR (n=50)	Smear (n=50)		Culture (n=50)	
	Smear +ve (n=19)	Smear –ve (n=31)	Culture +ve (n=24)	Culture –ve (n=26)
Positive (n=35)	19 (100%)	16 (51.61%)	24 (100%)	11 (42.30%)
Negative (n=15)	00	15 (48.38%)	00	15 (57.69%)
N = number of specimens.				

Table 2
Results for microscopy, culture and PCR with respect to disease.

Test	Disease TB present (n=33)		Disease TB absent (n=17)	
	TP	FN	FP	TN
Microscopy	19	14	00	17
Culture	24	09	00	17
PCR	33	00	02	15
TP = True Positive (Disease present and test positive), FN = False Negative (Disease present and test negative), FP = False Positive (Disease absent and test positive), TN = True Negative (Disease absent and test negative)				

Table 3
Values after final evaluation

Test	SN (%)	SP (%)	PPV (%)	NPV (%)
Microscopy	57	100	100	54
Culture	72	100	100	65
PCR	100	88	94	100

Table 4
Specimen wise results for the three test's.

Specimen	Number	Smear +ve	Culture +ve	PCR +ve
Sputum	32	15	17	24*
Pleural fluid	14	2	5	8
BAL	2	1	1	1
ICD	2	1	1	2
Total	50	19	24	35

* Two false positives

the sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive values (NPV) for PCR are 100%, 57%, 68% and 100% respectively.

As the culture results in the present study was very low we studied each case on the parameters of clinical, microbiological, radiological evidence and response to anti-tubercular treatment to know whether the patients were diseased or not. We thought this to be a better way of evaluating the results. On this basis we found of the total 50 cases, 33 to be diseased and 17 not diseased (**Table 2**). Specimens from all the 33 diseased were positive by PCR. Microscopy culture detected 19 and 24 cases respectively. Of the specimens from the 17 non diseased patients, 15 were negative by PCR and 2 were positive (false positive sputum) specimens. For microscopy and culture there were no false positives. After knowing the number of diseased we did a final evaluation of results for the three tests. Accordingly for microscopy the SN, SP, PPV & NPV were 57%, 100%, 100% and 54% respectively. For culture the values were 72%, 100%, 100% and 65% respectively. And for PCR the resulting values were 100%, 88%, 94% and 100% respectively (**Table 3**). Maximum positivity for PCR was observed in ICD, followed by sputum, pleural fluid and BAL (**Table 4**). PCR gave a negative amplification result for all the MOTT strains tested and a positive result for *M. tuberculosis* H37Rv.

Discussion

In this study we explored the usefulness of PCR for diagnosis of Tuberculosis. Smear microscopy for acid fast bacilli is an important test for diagnosis of tuberculosis. In this study smear positivity was 38%. Whereas culture positivity in this study was 48% . There were no false positives for smear and culture ascertaining the usefulness of these tests. With respect to culture specificity of PCR was low (57%) as there were 11 specimens negative in culture but PCR positive. Of these 11, 2 specimens were false positive after final evaluation as these patients responded to routine antibiotic treatment. These were also negative in microscopy. The 2 false positive PCR may be because of contamination somewhere during sample processing. This must not be because of previous amplicon transfer as we had used uracil DNA glycolase (UDG) and dUTP instead of dTTP, which eliminates previous amplicon transfer[22,23]. This points towards the importance of good laboratory practices. Of the remaining nine culture negative specimens after final evaluation all proved to be diseased. Out of these, 3 were microscopy positive of which 2 had culture contamination and one was negative in culture. This culture negative specimen was from a patient who was already on anti-TB drugs. This is consistent with the fact that patients can still harbor mycobacteria long after culture for mycobacteria have become negative. This may suggest that DNA amplification method could detect mycobacteria that are unable to grow *in vitro*[24]. Of the remaining six specimens from patients proved to have disease, all were microscopy negative and culture negative or contaminated. PCR in the present study with respect to culture, as well as after final evaluation showed a 100% SN and NPV confirming the usefulness of the technique. After final evaluation PCR had a specificity of 88% and PPV of 94%. We found no difficulty in detecting *M.tuberculosis* in all pulmonary specimens using this target, suggesting it to be suitable for the diagnosis of tuberculosis . In this study PCR gave a negative amplification result with the MOTT bacteria indicating the specificity of the IS6110 target for *M.tuberculosis* complex as was found by other workers[5,23,25].

Conclusion

In conclusion PCR was found to be efficient in the diagnosis of pulmonary tuberculosis. It not only correlated well with conventional techniques but also proved to be useful in the diagnosis of tuberculosis in specimens, where conventional techniques failed. The method is fast with results available in 10-12 hours. At the same time the importance of microscopy and culture cannot be overlooked from the findings of the present study and their low cost and availability. Disadvantage of culture is delay in getting results. New media like TK might answer this delay. Disadvantage of PCR is that if MOTT is present in the specimen they will be missed by PCR using primers targeting the *M.tuberculosis* complex. To conclude we find PCR targeting IS6110 to be useful for the rapid diagnosis of tuberculosis. For good results trained and dedicated staff following good laboratory practices is a must.

References

1. Bhargava A., Jain A., Agarwal SK., — A comparison of liquid and solid culture media with radiometric system for detection of *Mycobacterium tuberculosis* in clinical specimens. *Ind J Tub*. **48** : 9-12, 2001.
2. Beige J., Lokiees J., Schaberg T., Finckh U., Fisher M., Mauch H., Lode H., Kohter B., Roff SA. — Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. *J Clin Microbiol*.; **33** : 90-95, 1995.
3. Peter Hermans WM., Anja Schuitema RJ., Dick Van Soolingen., Cees., Verstynen JH., Elisabeth Bick M., Jelle Thole ER., Arend Kolk HJ., Embden Janda. — Species detection of *M.tuberculosis* complex strains by polymerase chain reaction. *J Clin Microbiol*.; **28** : 1204-1213, 1990.
4. ULF Sjobring., Michel Meckleburg., Ase Bengard Anderson., Hakan Miorner. — Plolymerase chain reaction for detection of *Mycobacterium tuberculosis*. *J Clin Microbiol*.; **28** : 2200-2204, 1990.
5. Borun M., Sajduda A., Pawlowska I., McFadden JJ., Dziadek J. — Detection of *Mycobacterium tuberculosis* in clinical samples using insertion sequence IS 6110 and IS 990. *Tuberculosis*.; **81** : 271-278, 2001.
6. Beerbal., Sachin AS., Singh D., Gupta RK., Chauhan DS., Sharma VD., Katoch VM. —Application of polymerase chain reaction for detection of *M.tuberculosis* in sputum specimens. *Ind J Tub*.; **46** : 235-238, 1999.
7. Rodrigues C., Nukala R., Menon S., Hakimiyan A,. Mehta AP. — DNA amplification of IS 6110 in rapid detection of *Mycobacterium tuberculosis*. *Ind J Med Microbiol*. **15** : 167-171, 1997.
8. Koneman EW., Allen SD., Janda WM., Schreckenberger PC., Winn., Jr WC. — Color atlas and textbook of diagnostic microbiology : 5th ed. (Lippincott, Philadelphia, New York); 893-952, 1997.
9. Chaudhary M., Gupta S., Khare shashi., lal S. — Diagnosis of tuberculosis in an era of HIV pandemic: A review of current status and future prospects. *Ind J Med Microbiol*. **28**(4) : 281-289, 2010.
10. Rodrigues C. — Diagnostics of tuberculosis : Time to usher in a new era. *Ind J Med Microbiol* . **29**(1) :2-3, 2011.
11. Honore-Bouakline S., Vincensini JP., Giacuzzo V., Lagrange PH., Herrmann JL.— Rapid diagnosis of extrapulmonary tuberculosis by PCR. Impact of sample preparation and DNA extraction. *J Clin Microbiol* .; **41** : 2323-2329, 2003.
12. Negi SS., Anand R., Pasha ST., Gupta S., Basir SF., Khare S,. Lal S. — Diagnostic potential of IS6110, 38KDa and 85B sequence based polymerase chain reaction in the diagnosis of *Mycobacterium tuberculosis* in clinical samples. *Ind J Med Microbiol* .; **25** : 43-49, 2007.
13. Banavalikar JL., Bhalotra Bobby., Sharma DC., Goel Manoj K., Khandekar PS, Bose M. — Identification of *M.tuberculosis* by polymerase chain reaction in clinical specimens. *Ind J Tub*.; **45** : 15-18, 1998.
14. Parandaman Vijayalaxmi., Narayanan Shujatha., Narayanan PR. — Utility of polymerase chain reactions using two probes for diagnosis of tubercular pleuritis in comparison to conventional methods. *Ind J Med Res* .; **112** : 47-51, 2000.
15. Abe Chiyogi., Hirano Kazue., Wada Masako., Kazumi Yuko., Takahashi Mitsuyoshi., Fukasawa Yutaka.,

Yoshimura T., Miyagi C., Goto S. — Detection of *Mycobacterium tuberculosis* in clinical specimens by PCR and Gen Probe amplified *Mycobacterium tuberculosis* direct test. *J Clin Microbiol* .; **31** : 3270-3274, 1993.

16. Prasad R., Lath SK., Mukharji PK., Agarwal SK., Shrivastava R. — Clinical utility of polymerase chain reaction in patients of pulmonary tuberculosis. *Ind J Tub.*; **48** : 135-138, 2001.

17. Mauricio Morishi Ogusku and Julia Ignez Salem. — Analysis of different primers used in PCR method : Diagnosis of tuberculosis in the state of Amezonas, Brazil. *J Bras Pneumol*; **30**, 2004.

18. Chauhan DS., Sharma VD., Parashar Dipti., Chauhan Aradhana., Singh D., Das R., Singh SB *et al*. — Molecular typing of *M.tuberculosis* isolates from different parts of India based on IS 6110 element polymorphism using RFLP analysis. *Ind J Med Res.* **125** : 577-581, 2007.

19. Sankar S., Balakrishnan B., Nandagopal B., Thangaraju K.,— Comparison of two polymerase chain reactions targeting different genomic regions to detect *M.tuberculosis* in sputum. *Ind J Med Microbiol.*; **28**(4) : 303-307, 2010.

20. Nandagopal B., Sankar S., Lingesan K., Apuu KC., Sridharen G., Ak Gopinathan.— Evaluation of nested PCR targeting IS6110 of *M.tuberculosis* for the detection of the organisms in the leukocyte fraction of blood samples. *Ind J Med Microbiol.*; **28**(3) : 227-232, 2010.

21. JE Park. — Screening for disease. In : K Park, editor. Text book of preventive and social medicine. 20th ed. Banarsidas Banot, Jabalpur ,India ;. p. 123 – 130, 2009.

22. Ashok Ratan. — PCR for diagnosis of tuberculosis : where are we now? *Ind J Tub*. **47** : 79-82, 2000.

23. Kox LFF., Rhienthony D., Medo Miranda A., Udomasantisuk N., Ellis K., J Van Leeuwen., S Van Heusden. — A more reliable PCR for detection of *Mycobacterium tuberculosis* in clinical samples. *J Clin Microbiol*. **32** : 672-678, 1994.

24. Chia C Pao. Yen Benedict TS., Jinn-Bang You, Juehn-Shin Maa., Ellen H Fiss., Chau-Hsiung Chang. — Detection and identification of *Mycobacterium tuberculosis* by DNA amplification. *J Clin Microbiol.*; **28** : 1877-1880, 1990.

25. Pasricha Gunisha., HN Madhavan., U-Jayanthi., K Lily Therese. — Polymerase chain reaction using IS 6110 primer to detect *Mycobacterium tuberculosis* in clinical samples. *Ind J Pathol Microbiol*. **44** : 97-102, 2001.

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Original Research Article

Evaluation of Nested Polymerase Chain Reaction for the Diagnosis of Pulmonary and Extrapulmonary Tuberculosis

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ABSTRACT

Utility of Polymerase Chain Reaction (PCR) test for diagnosis of pulmonary and extrapulmonary specimens was evaluated in 91 clinically diagnosed cases of tuberculosis (50 pulmonary & 41 extrapulmonary). All samples were also processed for microscopy by Ziehl-Neelsen (ZN) staining and culture on Lowenstein-Jensen medium (LJ). PCR was positive in all microscopy and culture positive specimens. There were 11 samples positive by PCR but failed to grow in culture both in the pulmonary and extrapulmonary specimens. With respect to culture the sensitivity (SN), specificity (SP), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of PCR for the pulmonary specimens was 100%, 57%, 68% and 100% respectively. Whereas the values for EPS were SN- 100%, SP- 65%, PPV- 45% and NPV- 100%. The values for PCR on final evaluation taking into consideration clinical, radiological, microbiological evidence and response to antitubercular treatment were SN-100%, SP-88%, PPV-94% & NPV-100% for the PS and SN- 95%, SP- 100%, PPV- 100 & NPV- 95% for the EPS. PCR detects *M. tuberculosis* complex with greater sensitivity and should be useful for rapid diagnosis of tuberculosis.

Key words: *Mycobacterium tuberculosis*, Polymerase Chain Reaction, IS 6110, Sensitivity, Specificity.

INTRODUCTION

Tuberculosis (TB) is an increasing public health problem in developing countries. India is the highest TB burden country accounting for fifth of global incidence. Reported global annual incidence of TB case estimate is 9.4 million cases, out of which it is estimated that 1.8 million cases are from India. [1] Studies involving immunocompetent adults have revealed that Extrapulmonary Tuberculosis (EPTB) constitutes about 15 to 20 percent of all cases of tuberculosis. With global rise of Human Immunodeficiency Virus infection EPTB accounts for more than 50 percent of all cases of TB among HIV positive patients. [2] Conventional methods available for diagnosis namely tuberculin test, radiological examination, smear microscopy and culture have their own limitations. Sputum smear microscopy requires more than 10,000 organisms per ml be present in the sample and has low sensitivity. [3-6] In case of EPTB microscopy is still less sensitive. Culture is more sensitive than microscopy and is still considered the "gold

standard" being 100% specific. [3-5] But culture is time consuming requiring 3 to 8 weeks for growth to appear. [3,6,7] Great progress have been made in reducing the time required for detecting the growth of mycobacteria using various culture systems like BACTEC, MGIT, MB/BACT etc, still however, on an average 2 to 3 weeks are needed to detect the growth. [3,6,7] Another recent approach to culture of *M. tuberculosis* is TK medium (Salubris, Inc.MA USA) with average time to detection of two weeks as compared to four weeks on LJ medium. It promises to be a practical, low cost, simple test. [8] A new test being introduced by WHO in national tuberculosis control programs is Xpert MTB / RIF which provides sensitive detection of *M. tuberculosis* and rifampicin resistance detection in less than two hours. This is being implicated in a phased manner. [9]

Recent advances in DNA amplification using Polymerase Chain Reaction has allowed great progress to be made in the rapid and accurate diagnosis of infections due to organisms that are not cultivable by in vitro means, that require complex media or cell cultures and prolonged incubation times, or for which culture is too insensitive. [10] Nested PCR (nPCR) for diagnosis of tuberculosis has been found to be useful in a number of studies. [6,10-12] This study reports the application of nPCR for the diagnosis of tuberculosis by amplification of IS6110, an IS-like element of *M. tuberculosis* complex. As the IS6110 sequence has been shown to be repetitively present in *M. tuberculosis* genome, it helps to increase the sensitivity of the test. [6, 11,13-15] This study evaluates nPCR for diagnosis of pulmonary and EPTB along with culture and microscopy. This is the first comprehensive study comparing conventional methods with nPCR for the diagnosis of tuberculosis from region of Marathwada of Maharashtra State, India.

MATERIALS AND METHODS

Clinical specimens and conventional methods:

A total of 50 pulmonary specimens (sputum 32, pleural fluid 14, bronchoalveolar lavage (BAL) 2, intercostal drain (ICD) 2) and 41 extrapulmonary specimens (ascetic fluid11, pus 12, blood 5, tissue biopsy 4, aspirates 3, CSF 3) were obtained from patients clinically suspected to have tuberculosis. Microscopic examination of the specimens was done by Ziehl-Nelsen staining and if negative staining was repeated on the specimens after concentration and decontamination using 4% sodium hydroxide (Except: CSF, blood and tissue). The decontaminated material was also used for culture on Lowenstein Jensen's medium in duplicate. The cultures were incubated at 37^0C for 8 weeks. All isolates of acid fast bacilli on LJ medium were further identified to species level by colony characteristics including the speed of growth and pigmentation and a battery of biochemical tests (niacin, nitrate reduction, catalase and growth on medium containing p-nitrobenzoic acid).

nPCR Assay:

nPCR assay was carried out using Genei ™ Amplification Reagent Set MTB-25 for *M. tuberculosis*, manufactured by Bangalore Genei Bangalore, India. This test is based on the principle of single tube nested PCR method. The assay is a two-step sequential assay. In the first step in the IS region of *M. tuberculosis* complex DNA sequence, a 220 bp is amplified by specific external primers. In the second step, the nested primers are added to further amplify a 123 bp amplification product. A band of 123 bp was indicative of infection with *M. tuberculosis* complex. In this test false positive reactions that may be caused by previous amplicon contamination are prevented by the use of uracil DNA glycolase (UDG) and dUTP instead of

dTTP. DNA extraction was carried using Proteinase K according to the manufacturer's instructions. DNA extraction was done in a separate room. DNA amplification was carried out on the same day of receiving the sample. PCR inhibition was identified using an amplification internal control. Amplification product of 340 bp was indicative of successful amplification and DNA extraction. Its absence indicated inhibition of amplification or DNA amplification failed. (Figure-1)

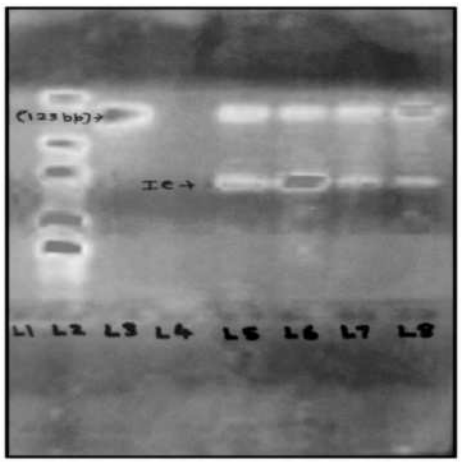

Figure - 1: Agarose gel electrophoresis analysis for DNA amplification products.
Lanes : L1 empty, L2 Molecular weight marker, L3 positive control (123 bp),
L4 negative control.
L5, L6, L7, L8 specimen positive for *M.tuberculosis* complex (123 bp).
IC: Internal control band (340 bp).

DNA Amplification:

A) First amplification:

Master Mix I was prepared, so that for each specimen the master mix I contained Amplification Premix I8.2 µl, Gene Hotstart Taq DNA Polymerase 0.33 µl and uracil DNA glycolase (UDG) 0.5 µl. To each 0.2ml PCR tube 9 µl of Master Mix-I was taken and 3 µl of extracted DNA was added. Positive and Negative controls were also included. DNA amplification was carried using the thermal cycler MJ Research using the calculated mode as suggested by the manufacturer. The first amplification profile was as follows: Step-1 was 22^0C for 10 minutes and initial denaturation at 94^0C for 5 minutes (No. of cycles -1). Step-2 was denaturation at 94^0C for 30 second, annealing at 68^0C for 1 minute and extension at 72^0C for 1 minute (No. of cycles - 20). Step-3 was final extension at 72^0C for 1 minute and storage at 4^0C (No. of cycles – 1).

B) Nested or Second Amplification:

For each specimen Master Mix-II contained Amplification premix-II 14.7 µl and Genei Hot Start Taq DNA polymerase 0.33 µl. Fifteen µl of the Master Mix-II thus prepared was added to the same PCR tubes used in the first amplification. Nested Amplification was performed using the following profile: Step-1 was initial denaturation at 94^0C for 5 minutes (No. of cycles-1). Step-2 was denaturation at 94^0C for 30 seconds, annealing at 68^0C for 30 seconds and extension at 72^0C for 30 seconds (No. of cycles 30). Step-3 was final extension at 72^0C for 7 minutes and storage at 4^0C (No. of cycles 1).

Analysis of amplified product:

Analysis of the amplified product was done using submarine electrophoresis using 2.5% agarose gel containing 10 µl of 10 mg per ml ethidium bromide dye solution for 100 ml gel. The gel was visualized under UV Transilluminator.

Nested PCR was also carried out on MOTT (Mycobacteria Other Than Tubercle) strains namely *M.chelonei, M.szulgai, M.phlei, M.avium and M.intracellulare* and also *M. tuberculosis* H37Rv obtained from Department of Microbiology, MGIM's Sewagram, district Wardha Maharashtra, India, to check the specificity of the primers.

Sensitivity, Specificity, Positive predictive value and Negative Predictive value were calculated as per Parks text book of Preventive and social medicine. [16]

RESULTS

We have analyzed 50 pulmonary and 41 extrapulmonary specimens obtained from patients with suspected tuberculosis. Ziehl-Neelsen staining for AFB was positive in 19 (38%) pulmonary and 8(19.51%) extrapulmonary specimens. Culture was positive in 24 (48%) pulmonary and 9 (22%) extrapulmonary specimens. This leaves 26 specimens negative on culture in pulmonary group and 32 in extrapulmonary group. All culture isolates were identified as *M. tuberculosis*. nPCR gave a positive amplification result in 35 (70%) of pulmonary and 20 (48.78%) extrapulmonary specimens. nPCR was positive in all smear and culture positive specimens. There were 16 pulmonary and 12 extrapulmonary specimens which were nPCR positive but smear negative. nPCR gave a positive amplification in 11 out of 26 culture negative pulmonary specimens and 11 out of 32 extrapulmonary specimens (Table-1). As culture is the gold standard, with respect to culture these specimens would be false positive. Therefore with respect to culture for nPCR in pulmonary specimens the sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive values (NPV) are 100%, 57%, 68% and 100% respectively. Similarly for extrapulmonary specimens the values are SN- 100%, SP - 65%, PPV - 45%, and NPV -100%.

Table 1: Correlation between smear, culture and nPCR

nPCR	Smear +ve (n=19)	Smear -ve (n=31)	Culture +ve (n = 24)	Culture -ve (n = 26)
Pulmonary [N = 50]				
nPCR +ve (n=35)	19	16	24	11
nPCR -ve (n=15)	00	15	00	15
Extrapulmonary [N = 41]				
nPCR +ve (n=20)	8	12	9	11
nPCR –ve (n=21)	00	21	00	21

n = Number of specimens

As the culture results in the present study were low we did a final evaluation of results by studying each case on the parameters of clinical, microbiological, radiological evidence and response to anti-tubercular treatment to prove the presence of disease. We thought this to be a better way of evaluating the results. On this basis we found of the total 50 pulmonary cases, 33 to be diseased and 17 not diseased (Table-2). All 33 diseased were positive by nPCR (True positive). Of the 17 non diseased patients 15 were negative for amplification (True negative) and 2 were amplification positive (False positive). Thus the final values for nPCR were SN-100%, SP-88%, PPV-94% and NPV-100%. For microscopy and culture there were no false positives, but there were 14 and 9 false negatives respectively. Accordingly for microscopy and culture the final SN, SP, PPV & NPV were 57%, 100%, 100%, 54% and 72%, 100%, 100% and 65% respectively.

For the extrapulmonary specimens, of the total 41 specimens 21 proved to have disease. Out of these nPCR was positive in 20 specimens (True positive) and negative in one specimen (False negative). For all the 20 not diseased nPCR gave a negative amplification so there were no false positives (Table-2). For microscopy and culture there were no false positives. The final results for the three tests were, for microscopy SN, SP, PPV, & NPV were 40%, 100%, 100%, and 63% respectively. For culture the values were 45%, 100%, !00% & 65% respectively. For nPCR they were 95%, 100%, 100% & 95%

respectively. nPCR gave a negative amplification result for all the MOTT strains tested and a positive result for *M. tuberculosis* H37Rv.

Table 2: Final results for various tests after correlation with evidence of disease and response to treatment

	Pulmonary [N=50]		Extrapulmonary [N=41]	
	Disease present	Disease absent	Disease present	Disease absent
Smear +ve Smear –ve	19 (TP) 14(FN)	0 (FP) 17 (TN)	8 (TP) 12 (FN)	0 (FP) 21(TN)
Culture+ve Culture-ve	24 (TP) 09(FN)	0 (FP) 17 (TN)	9 (TP) 11 (FN)	0 (FP) 21(TN)
nPCR+ve nPCR -ve	33 (TP) 0 (FN)	02 (FP) 15 (TN)	20 (TP) 1 (FN)	0 (FP) 20(TN)

n = Number of specimens, TP = True Positive, FN= False Negative, FP = False Positive, TN= True Negative

DISCUSSION

In this study we explored the usefulness of nPCR for diagnosis of Tuberculosis. Smear microscopy for acid fast bacilli is an important test for diagnosis of tuberculosis. In this study smear positivity was 38% for pulmonary tuberculosis and 19.51% for extrapulmonary pulmonary tuberculosis. For pulmonary and extrapulmonary tuberculosis positivity of culture was 48% and 22% respectively. One reason for culture results to be low was slightly higher contamination rate of 8% and these specimens were considered as culture negative. It has been suggested that digestion and decontamination procedures should be as gentle as possible, with no more than an overall contamination rate of 5%. [17] nPCR was positive in all smear and culture positive specimens. In both pulmonary and extrapulmonary there were 11 specimens which were culture negative but nPCR positive. After the final evaluation of the 11 culture negative but nPCR positive pulmonary specimens, 9 proved to have disease as they responded to anti-tubercular treatment and 2 specimens were false positive as these patients responded to routine antibiotic treatment (Table-3). These were also negative in microscopy. The 2 false positive nPCR may be because of contamination somewhere during sample processing. This must not be because of previous amplicon transfer as we had used uracil DNA glycolase (UDG) and dUTP instead of dTTP, which eliminates previous amplicon transfer. [18,19] Of the nine specimens from patients proved to have disease, microscopy was positive in 3 specimens. Out of which 2 had culture contamination (thus considered culture negative) and one was negative in culture. This culture negative specimen was from a patient who was already on anti-TB drugs. This is consistent with the fact that patients can still harbor mycobacteria long after culture for mycobacteria have become negative. This may suggest that DNA amplification method could detect mycobacteria that are unable to grow in vitro. [20]

In the extrapulmonary specimens after the final evaluation of the 11 nPCR positive but culture negative specimens all proved to have disease. Of these 2 were positive in smear. Of these 2 one was culture contaminated (thus considered culture negative) and other was negative in culture and was from a patient on anti-TB therapy, thus harboring dead bacilli picked up by microscopy and nPCR. There was 1 false negative result for nPCR (tissue specimen) also negative in smear and culture. This patient responded to anti-TB treatment. This may be because of very low no of bacteria in the specimen. This is not because of inhibitors of nPCR as we had used amplification controls (IC) which can detect

inhibition and the DNA extraction did not fail. Obtaining a positive signal from the second target (IC) demonstrates successful amplification, there by validating the result for the primary target. Increased sensitivity is achieved because false negative result is avoided and because additional positive results are detected by retesting inhibitory specimens. When introduced into the unprocessed specimen, the IC can also monitor nucleic acid recovery during specimen preparation. [21]

Table 3: Comparison of culture and nPCR with TB

Pulmonary N=[50]	Culture +ve (n=24)		Culture –ve (n=26)	
	nPCR		nPCR	
	+ve	-ve	+ve	-ve
Disease present (n=33)	24	0	9	0
Disease absent (n=17)	0	0	2[a]	15
Extrapulmonary [N=41]	Culture +ve (n=9)		Culture –ve (n=32)	
	nPCR		nPCR	
	+ve	-ve	+ve	-ve
Disease present (n=21)	9	0	11	1[b]
Disease absent (n=20)	0	0	0	20

n = Number of specimens, a = False positive specimens,
b = False negative specimen

The comparison of nPCR and disease with specimens was interesting (Table-4). Of the 32 sputum specimens nPCR was positive in 24. Twenty four of these proved to have disease resulting in a SN & SP of 100% & 80% respectively. Pleural fluid, BAL & ICD also were good specimens for amplification with 100% SN & 100% SP for nPCR. In extrapulmonary specimens the best results were for pus, 11 out of 12 being positive for nPCR. All these were diseased resulting in SN & SP of 100%. For tissue there was one false negative resulting in a SN of 0% and SP of 100%.

Table 4: Specimen wise results for the three tests.

Pulmonary					
Specimen	No	Smear +ve	Culture +ve	nPCR +ve	Disease present
Sputum	32	15	17	24	22[a]
Pleural fluid	14	2	5	8	8
BAL	2	1	1	1	1
ICD	2	1	1	2	2
Extrapulmonary					
Ascitic fluid	11	2	3	5	5
Pus	12	5	5	11	11
Aspirates	3	1	1	1	1
Urine	3	0	0	1	1
CSF	3	0	0	0	0
Tissue	4	0	0	0	1[b]
Blood	5	0	0	2	2

n = Number of specimen, a = Two false positive
b = One false negative

nPCR in the present study with respect to culture showed a 100% SN and NPV confirming the usefulness of the technique. Specificity and PPV was less because a large number of specimens were culture negative but nPCR positive. After the final evaluation microscopy and culture had no false positive and showed 100% specificity. nPCR had two false positives because of contamination this pointing towards the importance of good laboratory practices. nPCR gave a sensitivity of 100% for

pulmonary and 95% for extrapulmonary specimens which was much higher that the values for microscopy and culture, ascertaining the usefulness of nPCR in the diagnosis of tuberculosis.

In this study nPCR gave a negative amplification result with the MOTT bacteria and a positive amplification with *M. tuberculosis* H37Rv indicating the specificity of the IS6110 target for *M.tuberculosis* complex as was found by other workers. [4,18,22] We found no difficulty in detecting *M.tuberculosis* using this target, and the nested format suggesting it to be suitable for the diagnosis of tuberculosis confirming the findings of other workers. [23-25]

CONCLUSION

In conclusion, nPCR was found to be efficient in the diagnosis of tuberculosis. It correlates well with conventional techniques and is useful in the diagnosis of tuberculosis in specimens, where conventional techniques fail. The method is fast with results available in 10-12 hours. At the same time the importance of microscopy and culture can not be overlooked from the findings of the present study. Disadvantage of nPCR is that if MOTT is present in the specimen they will be missed by nPCR using primers targeting the *M.tuberculosis* complex. To conclude we find nested nPCR targeting IS6110 to be useful for the rapid diagnosis of tuberculosis.

REFERENCES

1. World Health Organization : Global Tuberculosis Control 2009:Available from:[htpp://www.who.int/tb/publical/global/2009/en] Geneva, Switzerland;2010.Who/HTM/Tb/2009.411 [Last accessed on 2011 Aug 07].
2. Sharma SK, Mohan A. Extrapulmonary Tuberculosis – Review Article. Ind J Med Res 2004;120: 316 -53
3. Bhargava A, Jain A, Agarwal SK. A comparison of liquid and solid culture media with radiometric system for detection of Mycobacterium tuberculosis in clinical specimens. Ind J Tub 2001;48: 9-12.
4. Borun M, Sajduda A, Pawlowska I, McFadden JJ, Dziadek J. Detection of Mycobacterium Tuberculosis in clinical sample using insertion sequence IS 6110 and IS 990. *Tuberculosis* 2001;81 : 271-278.
5. ULF Sjobring, Michel Meckleburg, Ase Bengard Anderson, Hakan Miorner. Polymerase chain reaction for detection of Mycobacterium tuberculosis. J Clin Microbiol 1990; 28 : 2200-2204.
6. Rodrigues C, Nukala R, Menon S, Hakimiyan A, Mehta AP. DNA amplification of IS 6110 in rapid detection of Mycobacterium tuberculosis. Ind J Med Microbiol 1997; 15 : 167- 171.
7. Koneman EW, Allen SD Janda WM, Schreckenberger PC, Winn, Jr WC. Color atlas and textbook of diagnostic microbiology: 5th ed. (Lippincott, Philadelphia, New York) 1997; 893-952.
8. Chaudhary M, Gupta S, Khare shashi,Lal S. Diagnosis of tuberculosis in an era of HIV pandemic: A review of current status and future prospects. Ind J Microbiol 2010;28(4): 281-90.
9. Rodrigues C. Diagnosis of tuberculosis: Time to usher in a new era . Ind J Med Microbiol 2011; 29(1) :2-3.
10. Honore-Bouakline S, Vincensini JP, Giacuzzo V , Lagrange PH, Hermann JL. Rapid diagnosis of extrapulmonary tuberculosis by nPCR . Impact of sample preparation and DNA extraction. J Clin Microbiol 2003; 41 : 22323-2329.
11. Negi SS , Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S . Diagnosis of Mycobacterium Tuberculosis in clinical samples. Ind J Med Microbiol 2007; 25 : 43-49.
12. BanavalikarJL, Bhalotra Bobby, Sharma DC, Goel Manoj K, Khandekar PS,

Bose M. Identification of *M. tuberculosis* by polymerase chain reaction in clinical specimens. Ind J Tub 1998; 45 : 15-18.
13. Maurya AK , Kant S, Nag VL, Kushwaha RAS, Dhole TN. Detection of 123bp fragment of IS element IS6110 of M. tuberculosis for diagnosis of extrapulmonary tuberculosis. Ind J Med Microbiol 2012;30:182-6.
14. Parandam Vijayalaxmi, Narayanan Shujatha, Narayanan PR. Utility of polymerase chain reactions using two probes for diagnosis of tubercular pleuritis in comparison to conventional methods . Ind J Med Res *2000; 112 : 47-51.*
15. Mauricio Morishi Ogusku and Julia Ignez Salem. Analysis of different primers used in PCR method : Diagnosis of tuberculosis in the state of Amezonas, Brazil. J Bras Pneumol 2004.
16. JE Park. Screening for disease. In : K Park, editor. Text book of preventive and social medicine. 20th ed. Banarsidas Banot, Jabalpur, India : 2009. P. 1123 - 130.
17. Mycobacteria. Chapter 48, In Bailey and Scott's Diagnostic Microbiology 9th ed. Ellen Jo Baron. Lancer R Peterson, Sydney M Finegold, Eds.(Mosby, USA) 1994 : 538
18. Kox LFF, Rhienthony D, Medo Miranda A, Udomansantisuk N, Ellis K, J Van Leeuwen, S Van Heusden. A more reliable PCR for the detection of Mycobactrium tuberculosis on clinical samples. J Clin Microbiol 1994;32 : 672-678.
19. Ashok Ratan. PCR for the diagnosis of tuberculosis : where are we now? Ind J Tub 2000; 47 :79-82.
20. Chia C Pao, Yen Benedict TS, Jinn-Bang You, Juehn-Shin Maa, Ellen H Fiss, Chau-Hsiung Chang. Detection and identification of Mycobacterium tuberculosis by DNA amplification. J Clin Microbial 1990;28 : 1877-1880.
21. Maurice Rosentraus, Zuan Wang, Sheng-Yung Chang, David De bonville, Joanne.P. Spodro.*An* internal control for routine diagnostic PCR; Design properties and Effects on clinical performance. J Clin Microbiol 1998; 36 (1) : 191-197
22. Pasricha Gunisha, HN Madhavan, U-Jayanthi, KLily Therese. Polymerase chain reaction using IS 6110 primer to detect *Mycobactrium tuberculosis* in clinical samples. Ind J Pathol Microbiol 2001; 44: 97-102.
23. P Sharma, P Gill, P Aggarwal. Nested PCR for the diagnosis of extrapulmonary tuberculosis: Need of the hour. Ind J Med microbial 2013 31(2): 199-210
24. Nandagopal B, Shankar S, Lingesan K, Appu KC, Sridharen G, Gopinathan AK.Evaluation of n =PCR targeting IS6110 of *M. tuberculosis* for detection of the organism in the leukocyte fraction of blood samples. Ind J Med microbiol 2010;28 (3) : 227-32
25. S Sankar, B Balakrashnan, B Nandgopala,K Thangarayu, SNatraja, comparative evaluation of two NPCR's targeting different genomic regions of *Mycobactrium tuberculosis* Ind J Med microbiol 2010 : 28 (4),303-307.

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EFFECT OF ANTITUBERCULOSIS TREATMENT ON SERUM ZINC LEVEL IN TUBERCULOSIS

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ABSTRACT

INTRODUCTION: Tuberculosis is a major public health problem worldwide. One third population of world is infected with M. tuberculosis. Zinc is micro-mineral essential for human growth, development and immune function. Deficiency of this micronutrient impairs overall immune function and resistance to infection. Present study was conducted to evaluate the level of serum zinc in patients with tuberculosis at various period of treatment.

METHODS: Total 92 subjects included were evaluated from OPD, IPD and DOTS centre of MGM group of Hospitals, Navi Mumbai, India. Among them there were 50 patients with Tuberculosis and 42 normal healthy controls. In all the subject estimation of Zinc was carried out. Out of 50 tuberculosis patients, 31 patients were assessed for zinc after two months and six months (i.e. completion) of DOTS therapy. The assessment was done according to the cut off values of serum zinc i.e. 60-120 µgm/dl.

RESULTS: Serum zinc levels were significantly low ($p<0.001$) in study group as compared to the healthy control. Serum zinc concentration of the patients increased gradually after anti-tuberculous therapy and reached normal levels after completion of therapy.

CONCLUSION: In the present study serum zinc levels of patients with pulmonary tuberculosis was found low as compared to healthy control subjects. Supplement of zinc during the treatment can be important for the betterment of the treatment of this disease.

KEY WORDS: Tuberculosis, Zinc Therapy

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INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by organism *Mycobacterium tuberculosis*, aerobic non motile bacillus. The *tuberculosis* bacteria can attack any part of the body, most commonly the lungs. It spreads through air, when infectious people cough, sneeze, talk or spit; they propel TB germs known as bacilli into air. Inhalation of very small numbers of these bacilli will lead to M. tuberculosis infection.[1] One third population of world is infected with M. tuberculosis. The vast majority of these have latent infections. Annually, more than 8 million people develop tuberculosis (TB), and approximately 1.8 million cases result in death.[2] although tuberculosis is a curable disease, it continues to be prevalent and causes death. Without aggressive public health measures and continued research, effective treatments or vaccine development, this treatable disease will continue to be rampant.[3] Zinc is micro-mineral essential for human growth, development and immune function. Deficiency of this micronutrient impairs overall immune function and resistance to infection.[4]

MATERIAL AND METHODS

Study was carried out in 92 subjects. There were 42 normal healthy individuals and 50 patients with Tuberculosis. All the subjects were selected from OPD, IPD and DOTS centre of MGM group of Hospitals, Navi Mumbai and estimation of serum zinc was carried out. Out of 50 tuberculosis patients, 31 patients were assessed after two months and six months (i.e. completion) of DOTS therapy. The patients were given the combination of drugs consisting of rifampicin, isoniazid, pyrizinamide and ethambutol as per RNTCP (DOTS) regimen. The control group comprises of healthy subjects of both sexes. Venous Blood samples were obtained from tuberculosis patients (at the time of diagnosis, after two months and on completion of DOTS therapy) and healthy controls. The diagnosis of tuberculosis was based on clinical, radiological, sputum Acid Fast Bacillus (AFB) smear positivity and tuberculin skin test positivity.

Serum Zinc was estimated by Coral Kit method. Zinc in an alkaline medium reacts with Nitro- PAPS to form a purple colored complex. Intensity of the colored complex is directly proportional to the amount of zinc present in the sample.

$$\text{Zinc} + \text{Nitro-PAPS} \xrightarrow{\text{alkaline medium}} \text{purple colored complex}$$

RESULTS

Control group: Forty two normal healthy individuals aged between 20 to 60 years of age [27 males (64.28%) and 15 females (35.72%)] were included as controls. In this group the mean±SD of the serum Zinc was found to be 93.30 ± 10.03 µgm/dl.

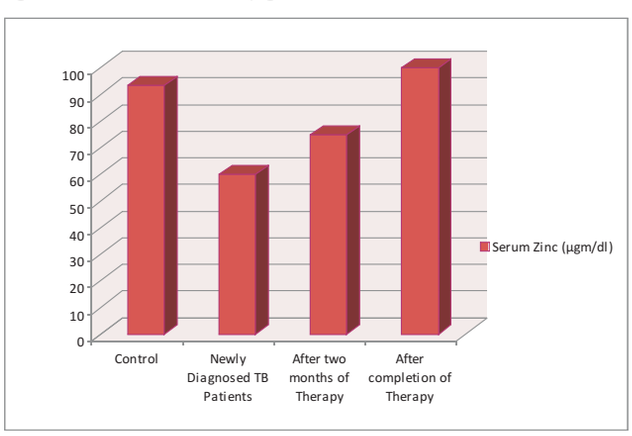
Study group: Fifty newly diagnosed tuberculosis patients were included in the study. Out 50 patients, in 31 patients the serum zinc levels was also estimated during treatment and on completion of therapy. In present study, mean serum zinc levels in newly diagnosed tuberculosis patients was (59.88 ± 7.82 µgm/dl). The mean serum zinc level in control subjects was found to be (93.30 ± 10.03 µgm/dl). The mean serum zinc level in patients after two months and on completion (six months) of anti tubercular therapy was found to be (74.70 ± 6.50 µgm/dl) and (99.82 ± 14.23 µgm/dl) respectively. No significant difference was observed in mean serum zinc level in different sex and age groups among these patients.

Table 1: Comparisons of Serum Zinc in Control groups and different study groups.

Parameter	Control Group (mean ± SD)	Study Groups		
		Newly Diagnosed TB Patient (mean ± SD)	Patients after two months of therapy (mean ± SD)	Patients on completion of therapy (mean ± SD)
ZINC (µgm/dl)	93.30 ± 10.03	59.88 ± 7.82*	74.70 ± 6.50	99.82 ± 14.23

* $p<0.001$ (statistically highly significant)

Figure 1: Serum Zinc (µgm/dl)



DISCUSSION

In our study we observed that the mean serum zinc concentration was significantly decreased in tuberculosis patients as compared to the control ($P<0.001$). Also we found that the mean serum zinc concentration was significantly increased in the patients after the completion of DOTS therapy ($P<0.001$). We found serum zinc concentration of the patients increased gradually after anti-tuberculous therapy and reached normal levels after completion of therapy.

Similar study was carried out by Hassan Ghulam et al.[5] and reported that the mean serum zinc concentration in pulmonary tuberculosis group prior to therapy showed a significant fall ($P < 0.05$) in average serum zinc levels in contrast to the control group. In India, Ray M et al.[6] studied the plasma zinc status of 50 children with tuberculosis and compared the observations with 10 healthy and 10 malnourished children without tuberculosis at 0,1,2,3 and 6 months of anti-tubercular therapy. The children with tuberculosis had significantly lower plasma zinc levels than those without the disease, irrespective of the nutritional status. Similarly, Karyadi et al.[7] from Indonesia studied the nutritional status of patients with active pulmonary tuberculosis and compared the values with those of healthy controls and found poor nutritional status and significantly low serum zinc levels in tuberculosis patients compared to control.

Contrary to previous studies and the present one, Ciftci et al.[8] from Turkey studied 22 pulmonary TB patients and 18 healthy subjects and found an increase in the levels of zinc; however the mechanism of this increase was not explained. Similar study from Japan reported higher mean serum zinc level (12.39 ± 2.17 mmol/L) in patients with pulmonary TB.[9]

The reason for the low serum zinc levels in TB could be multi-factorial. Firstly, a change in distribution of zinc in the body tissues is known to occur in chronic infections, with a net flow of zinc to the liver for the synthesis of acute phase reactants including metalloenzymes. Secondly, zinc may be utilized by tuberculosis for growth and multiplication.[10]

CONCLUSION

In the present study serum zinc levels of patients with pulmonary tuberculosis was evaluated and it was found that these patients had low serum concentration of zinc as compared to control subjects. This was likely due to the redistribution of zinc from plasma to other tissues, reduction of hepatic production of zinc-carrier protein α_2-macroglobulin and a rise in the production of metallothionin, a protein that transports zinc to the liver. The concentration of serum zinc was found to be increased after the anti tuberculosis therapy. Zinc supplementation can be suggested as a treatment protocol for the betterment of treatment.

REFERENCES

1. *http://www.who.int/mediacentre/factsheets/fs104/en*
2. *The Global Tuberculosis Control 2011.2011 Geneva: World Health Organization.*
3. *Perlin D, Cohen A, Perlin DS. The Complete Idiot's Guide to Dangerous Diseases & Epidemics 2002.*
4. *Walker CF, Black RE. Zinc and the risk for infectious disease, Annu Rev Nutr. 2004, 24: 255-275.*
5. *Ghulam H, Kadri SM, Manzoor A,Waseem Q, Aatif MS, Khan GQ, Manish K. Status of Zinc in pulmonary tuberculosis. J Infect Dev Ctries 2009; 3(5): 365-368.*
6. *Ray M, Kumar L, Prasad R. Plasma zinc status in Indian Childhood tuberculosis: impact on antituberculosis therapy. Int. J. Tuberc Ling Dis. 2: 719-25*
7. *Karyadi E, Schultink W, Ronald HH et al. Poor Micronutrient Status of Active Pulmonary Tuberculosis Patients in Indonesia. American Scoiety for Nutritional Sciences, 2000; 2953-8.*
8. *Ciftci TU, Cifti B, Yis O, Guney Y, Bilgihan A, Ogretensoy M. Changes on serum selenium, copper, zinc levels and cu/zn ratio in patients with pulmonary tuberculosis during therapy. Biol. Trace Elen Res. 2003; 95:65-71.*
9. *Kassu A, Yabutani T, Mahmud ZH , Mohammad A, Nguyen N, Huong BT, Hailemariam G, Diro E, Ayele B and Wondmikun Y. Alterations in serum levels of trace elements iin tuberculosis and HIV infections. Eur. J. Clin. Nutr., 2006; 60(5): 580-6.*
10. *Liu X et al. [Determination of trace elements in serum of tuberculosis patients]. Jiu WSY, 2000; 29(6): 395-6 [in Chinese]*

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Date -1 /10/2018

To,
The Dean
Mahatma Gandhi Mission Medical College
Kamothe, Panvel

Sub:- Sanction of grant-in-aid for the Operational Research proposal under RNTCP.

Ref:- The State Operational Research Committee meeting held on 25th June, 2018 at Disha Hall, Parivartan Building, Arogya Bhavan, Pune.

The following Operational Research proposal submitted by the Principal Investigator (PI) of your institute was discussed in State Operational Research Committee Meeting held on 25th June, 2018 under RNTCP and it has been approved.

Sr. no	Name of the PI	Name of the Department & Medical College	Topic
1	Dr. P. V. Potdar, Head and Prof.	Respiratory Department, MGM Medical College and Hospital Kamothe, Panvel	Design and evaluate the optimum algorithm to rule out TB in HIV patient via Intensify TB case finding in persons infected with HIV by screening with high quality Investigation.

The Principal Investigator (PI) will sign a Memorandum of Undertaking (MOU) with the TB programme manager on behalf of the society for the release of funds. The MOU will include the objectives for which he will utilize the funds and the timeline for the study. It will also include the commitment from him to return the funds if the study cannot be taken up due to any reason, and other relevant causes. Funds will be released on the name of the institution of the Principal Investigator, so that the College / Department can ensure the completion of the study project / will return the funds in the event that the Principal Investigator is moved from the college during the course of the study.

A Grant-in-aid of **Rs. 1,96,000 (Rs. One lac Ninety Six Thousand only)** for the above OR proposal will be released under the "New FMR Code - 10.2.9 Research for Medical Colleges from RNTCP funds by District TB Officer, Raigad. 50% of the grant-in-aid will be released

initially and remaining 30% after receiving the report of data analysis and 20% will be released after receipt of the four hardcopies of the final documents.

Joint Director of Health Services
(Leprosy & TB) Pune

Copy to –

1. The DTO Raigad – To follow up with the respective medical college & Principal Investigator and release the grant-in-aid amount of Rs. 1,96,000 (Rs. One lac Ninety Six Thousand only) under the "FMR Code 10.2.9 Research for medical colleges" from RNTCP funds as per the guidelines.

   The additional budget of Rs. 1,96,000/- is sanctioned from State level approved PIP of Rs. 12.20 Lac for the year 2018 – 19.

2. S Dr. P. V. Potdar, Head and Prof. of Respiratory Department, MGM Medical College and Hospital Kamothe, Navi Mumbai

3. The RNTCP Medical Consultants by email – mhconsultants@rntcp.org

4. The OR Committee Members ..... (All)

Copy with complements to –

Dr. Babaji Ghewade, Chairman State OR Committee, Maharashtra & Professor Respirator Medicine, Chief Medical Superintendent, Acharya Vinoba Bhave Rural Hospital and Jawaharlal Nehru Medical College, Wardha

Dr. Rajesh B. Goel
Registrar
MGM Institute of Health Sciences
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AWARD FROM MAHARASHTRA GOVERNMENT

Maharashtra, Government Award 2019

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