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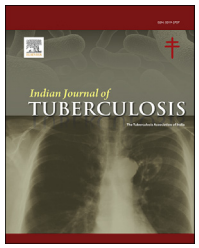

Editorial

Can sputum microscopy be replaced?

TB is diagnosed by finding *Mycobacterium tuberculosis* bacteria in a clinical specimen taken from the patient. In case of paucibacillary and extra-pulmonary forms, early confirmation of the diagnosis of tuberculosis is a challenging problem. Conventional methods available for diagnosis namely, tuberculin test, radiological examination and other imaging methods and sputum smear microscopy have their own advantages and their own limitations.

Since its inception, microscopy remains a cornerstone of tuberculosis control because it identifies sputum-smear-positive (most infectious) cases and is rapid and cheap, although has a limited specificity. The standard smear microscopy test has limitations. It usually finds it difficult to diagnose TB when the bacterial load is less than 10,000 per millilitre of the sputum sample, giving erroneous negative results for some patients.[1] Smear microscopy sensitivity is increased by using various fluorochrome dyes such as auramine and rhodamine, and Fluorescent-staining methods.

Some of the important methods which are adopted by RNTCP and are endorsed by WHO are: (i) **MGIT (Mycobacteria growth indicator tube)**: In this method growth is detected by a non-radioactive detection system using fluorochromes for detection and drug screening. (ii) Line Probe assays (LPA). LPA is a multiplex PCR based genotypic assay used for the screening of Rifampicin & Isoniazid drug resistance and *M. tuberculosis* complex simultaneously. (iii)The Xpert MTB/RIF – a rapid, fully-automated nucleic acid amplification test (NAAT). In addition, some test which was introduced recently shows some promises are described below:

i. Xpert MTB/RIF is costlier than microscopy but use of this test will increase the number of cases with microbiologically confirmed TB because of its higher sensitivity. WHO policy statement strongly advises that Xpert MTB/RIF be used as the initial diagnostic test in adults and in children who are at risk of MDR TB or HIV associated TB and these two groups should be prioritized for testing with Xpert MTB/RIF when resources are limited.[2] Following this RNTCP has also endorsed this test and issued guidelines to use this test as initial diagnostic test for the patient group mentioned above and has scaled up the procurement of Xpert MTB/RIF modules since 2016[3] and has placed them in almost all the districts across the country so that all can have access to universal DST. Although Xpert MTB/RIF is suitable for use at all levels of the health system, implementation in a diagnostic facility requires stable and uninterrupted electrical supply and also the challenges associated with instrument maintenance, training, quality assurance. To overcome the challenge, Cepheid, manufacturer of the instrument, continues to develop a new platform called the GeneXpert Omni. The Omni device is smaller, lighter and less expensive and suitable for use for point-of-care nucleic acid detection as it comes with a built-in 4-hour battery.[4]
ii. The World Health Organization (WHO) issued a recommendation in 2017 that Xpert MTB/RIF Ultra can be used as an alternative to the existing Xpert MTB/RIF test for the diagnosis of TB and detection of rifampicin resistance in all settings.[5] The Xpert MTB/RIF Ultra assay was redesigned to boost analytical sensitivity more than tenfold and to improve reliability of detecting mutations associated with rifampin resistance. In a multi centre study conducted by FIND it was found that the newly introduced Xpert MTB/RIF ultra assay was found to be non inferior to the original Xpert MTB/RIF for the diagnosis of TB as well as detection of rifampicin resistance, especially in smear negative culture positive cases this assay was found to be superior.[6]
iii. Loop mediated thermal amplification test (LAMP) is another molecular test endorsed by WHO which issued guidance in 2016 for the use of TB LAMP as a potential replacement for smear microscopy.[7] This test was found to be cheaper than Xpert MTB.RIF and the results are available in 40 min.[8]
iv. The TrueNat TB test is a new molecular test that can diagnosis TB in 1 h as well as testing for resistance to the drug rifampicin. The TrueNat machine is more of a point of care machine, which is not fully automated and which is designed for situations where there may not be electricity and where the need is for one test to be done at a time. With this test, samples can be tested as soon as a patient with symptoms of TB is seen. As the entire set-up is both battery operated and portable, it can be used at the most basic parts of the health care system.[9] It takes about 25 min to do the DNA extraction. It takes

another 35 min to diagnose TB. It takes an additional one hour for testing for rifampicin resistance.[10]

v. Gene amplification methods have been found to be highly sensitive and specific for diagnosis of tuberculosis directly from clinical specimens. Depending upon the bacteriological status and copy number of target sequence, sensitivity has ranged from 70 to 100 per cent whereas specificity between 80 and 100 per cent has been reported by different investigators. Whole-genome sequencing (WGS) of bacterial genomes allows simultaneous identification of all known resistance mutations as well as markers with which transmission can be monitored.[11] Whole genome sequencing is being used extensively in developed countries. New generation sequencing is cheap, easy to use and is manufactured by many companies worldwide. Gene Sequencing and analysis could allow individualized approach to treatment to ensure maximum safety efficacy without side effects.

The End TB Strategy by WHO calls for early diagnosis and prompt treatment of all persons of all ages with any form of drug-susceptible TB or DR-TB. This requires ensuring access to WHO-recommended rapid diagnostics and universal access to DST for all patients with signs and symptoms of TB. WHO defines universal access to DST as rapid DST for at least rifampicin, and further DST for at least fluoroquinolones and second-line injectable agents in all TB patients with rifampicin resistance.[12] WHO endorsed molecular methods are available now for the detection of TB as well as its drug resistance for first and second line drugs. New molecular detection tools including the faster and simpler NAAT and WGS, have resulted in a shorter time for diagnosis and, therefore, faster TB treatments.[13]

Although Xpert is currently being rolled out in multiple countries, this technology is challenging to deploy at the microscopy center level. Many companies are developing tools that can be used in lower-tier laboratories, countries which include the TrueNAT assay (Molbio Inc, Goa, India), Genedrive platform (Epistem, Manchester, UK), and EasyNAT (Ustar Biotechnologies, Hangzhou, China). While these products are already on the market, large scale evaluations are still on the way to collect evidence for policy development. Though currently there is no test which can replace microscopy, there are number of tests available in the market which have potential to replace microscopy and therefore the future of TB diagnosis looks brighter.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2018.08.005.

REFERENCES

1. Mudur,G.S. One-hour Test for TB. https://www.telegraphindia.com/india/one-hour-test-for-tb-183556.
2. *Policy Update- Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extra Pulmonary TB in Adults and in Children.* WHO; 2013. ISBN:978.92-41506335.
3. *WHO Monitoring of Xpert MTB/RIF Roll out.* Internet. Geneva: WHO; 2017. http;/www.int/areas-of-work/laboratory/mtb-rif-rollout/e/.
4. World Health Organization (WHO). *Global Tuberculosis Report.* Geneva: WHO; 2016.
5. WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. Available at: http://apps.who.int/iris/handle/10665/254792.
6. Dorman SE, et al. Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect Dis.* 2018;18:76–84.
7. The Use of Loop Mediated Thermal Amplification Test (TB LAMP) for the Diagnosis of Pulmonary TB; Policy guidelines (Internet). Geneva:WHO;c2017. http:/www.who.int/tb/publication/lamp in diagnosis-molecular/e/
8. Eiken.Genome SITE. *The Principle of LAMP Method*. Internet. Loopamp.eiken.co.jp.c; 2017. http://loopamp.eiken.co.jp/e/lamp/.
9. Chaitali N, et al. *Evaluation of the Indian TrueNAT Micro RT-PCR Device with GeneXpert for Case Detection of Pulmonary Tuberculosis*; September 2014. www.researchgate.net/publication/8233_.
10. *Cheaper, Indigenous TB Test Enters Final Validation Phase*. The Hindu; July 01, 2017. www.thehindu.com/sci-tech/health/cheaper-indigenous-tb-test-enters-final-validation-phase/article19193085.ece.
11. Brown CA, et al. Rapid whole-genome sequencing of *Mycobacterium tuberculosis* isolates directly from clinical samples. *J Clin Microbiol*. July 2015;53(7):2230–2237. https://doi.org/10.1128/JCM.00486-15.
12. World Health Organization (WHO). *Strategic and Technical Advisory Group for Tuberculosis (STAG-TB): Report of the 16th Meeting*. Geneva: WHO; 2016. Google Scholar.
13. Nurwidia Fariz, et al. Molecular diagnosis of tuberculosis. *Chonnam Med J*. 2018 Jan;54(1):1–9.

K.K. Chopra*
New Delhi Tuberculosis Centre, New Delhi, India
Indian Journal of Tuberculosis, India

M. Hanif.K.M
New Delhi Tuberculosis Centre, New Delhi, India

V.K. Arora
TB Association of India, India
Indian Journal of Tuberculosis, India

Himanshu Vashistha
New Delhi Tuberculosis Centre, New Delhi, India

**Corresponding author*. New Delhi Tuberculosis Centre, New Delhi, India. Tel.: +91 9811547066.
E-mail address: chopra_drkk@yahoo.co.in

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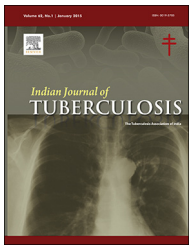

Case report

Tuberculosis and HIV co-infection; the deadly duos in vulva

Vikash Paudel [a,*], *Deepa Chudal* [b], *Dinesh Binod Pokhrel* [c]

[a] *Lecturer, Department of Dermatology and Venereology, Tribhuvan University, National Medical College, Birgunj, Nepal*
[b] *Lecturer, Department of Obstetrics and Gynecology, Tribhuvan University, National Medical College, Birgunj, Nepal*
[c] *Professor, Department of Dermatology and Venereology, Maharajgunj Medical Campus, Tribhuvan University Teaching Hospital, Institute of Medicine, Kathmandu, Nepal*

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ABSTRACT

Human Immunodeficiency Virus induced immune suppression leads the way for various infections with tuberculosis being the most common. Tuberculosis of the vulva is an extremely rare entity and is seen in only 1–2% of genital TB with increased risk in HIV co-infection. The co-infection places an immense burden on health care systems and poses particular diagnostic & therapeutic challenges with high mortality and morbidity. We present, here, a rare case of a 47 years postmenopausal female, who presented with itchy ulcerating lesions in the vulva with diagnostic dilemma turned to be vulval tuberculosis and during investigations, was found to be co-infected with HIV. The early diagnosis of TB and HIV in atypical looking lesions of vulva with high index of suspicion could lead to improved outcome.

1. Introduction

Tuberculosis (TB) is one of the most common and the oldest diseases known. It is increasing due to development of drug resistance; immune-suppression and immigration.[1] Human immune deficiency virus (HIV) induced immune suppression presents with a number of atypical infections being extra-pulmonary tuberculosis, the most common. Genital tuberculosis in HIV is rare entity with varied presentations.[2] With the recent advent of the AIDS pandemic, vulval lesions need to be scrutinized to rule out tuberculosis and properly treat the TB/HIV co-infection to reduce the morbidity and mortality associated with it. This article is probably first of its kind from Nepal to be published as the genital tuberculosis is rare entity, even vulval involvement is the rarest with clinical and diagnostic difficulty.

2. Case report

We report a case of 46-year-old post-menopausal, primipara widow who presented with multiple painful and itchy ulcerative discharging on the vulva for 4 months, misdiagnosed and wrongly treated as genital cancer, fungal infection and sexually transmitted infection. She also had history of an evening rise in temperature, with multiple nodes in axilla one and half years back, the histopathology of which was

* *Corresponding author*. National Medical College, Teaching Hospital, Department of Dermatology and Venereology, Bhediyahi-13, Parsa, Birgunj, Nepal. Tel.: +9779849948600.
E-mail address: vikashpoudel@iom.edu.np (V. Paudel).

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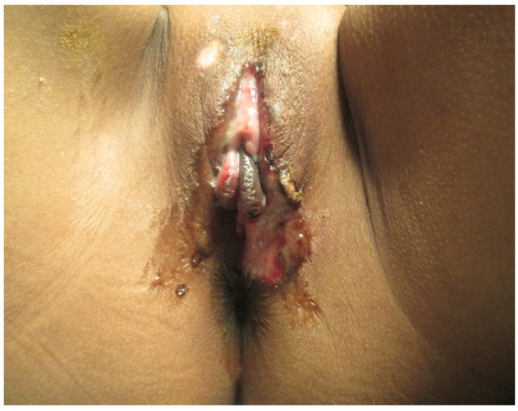

Fig. 1 – Ulcerative vulval lesion.

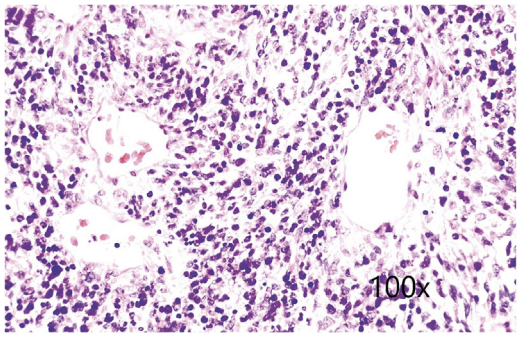

Fig. 2 – Histology of the ulcer 100×.

consistent with tubercular lymphadenitis for which anti-tubercular treatment was given for 10 months. With this background of uncommon presentation, resistance to treatment with conventional drugs and history of TB, patient was suspected for immune-suppression and found to be positive in ELISA and Western Blot.

On cutaneous examination, multiple ulcers were noted in the vulva involving both labia majora and single larger ulcers (3 × 4 cm) on the left side at the lower aspect of left labia minor extending up to perineum. Ulcers had irregular borders, indurated base, central necrosis, and were filled with mucous, jelly like discharge. (Fig. 1) Routine laboratory investigations showed hemoglobin 8.4 gm/dl, normocytic normochromic peripheral blood picture, WBC count 2100/cc (N86L13E1) and an erythrocyte sedimentation rate (ESR) of 60 in the first hour. Venereal disease research laboratory (VDRL) was negative. Western Blot was positive for HIV1 [(GAG p17, p24, p55 +ve), POL(p31, p51, p66 +ve), ENV (bp 41, bp 120, bp 160 +ve)]. Her CD 4+ count was initially 206 and within 2 weeks it was 6. Her chest X-ray was normal. The level of CEA and CA125 were within normal limit. (CEA: 4.5, CA125: 14.5). Tissue biopsy of the ulcer was performed. The histological report confirmed a ''caseating epithelioid cell granulomas'' which are the features suggestive of tuberculosis. An examination of the specimen with a Ziehl Neelsen stain revealed scattered acid-fast bacilli. (Fig. 2) PAS was negative. She was prescribed Cat II ATT, Cotrimoxazole and Anti-retroviral therapy as per the protocol of national guidelines i.e. Zidovudine, Lamivudine and Efavirenz. She died 2 weeks later starting ART and ATT.

3. Discussion

Tuberculosis is a common infectious disease caused by the *Mycobacterium tuberculosis*. A small proportion (5–15%) of the estimated 1.7 billion people infected with *M. tuberculosis* will develop TB. HIV is strong known risk factor for tubercular infection and progression to active disease.[1] TB is also the most common cause of AIDS-related death. Genital tuberculosis represents up to one fifth of extra-pulmonary tuberculosis. However, tuberculosis of vulva and vagina, an extremely rare entity, is seen in only 1–2% of genital tuberculosis cases.[2,3] It is also not very common in post-menopausal women.[4] Primary infection of female genital organs is very rare. It occurs secondary to primary disease in the lung, lymph nodes, urinary tract, bones, joints, and bowel as a result of bacillemia. Primary infection may also occur when the male partner has active genitor-urinary TB and transmission takes place by sexual intercourse.[5] Genital tuberculosis can present as pelvic inflammatory disease leading to infertility.[6] Whereas, vulval tuberculosis might present as hypertrophy, ulceration, nodules, abscess.[7] Diagnosis of genital tuberculosis is difficult because of its atypical presentations in HIV co-infection.[8–10]

In our case, we could not be established whether HIV or tuberculosis was the initial infection or it was a co-transmission by sexual route. TB/HIV co-infection is associated with higher mortality which is similar as in our case, the deadly duo residing in the vulva.[1,5] Even at the slightest suspicion, testing for tuberculosis and HIV should be undertaken because these are the infections which can present in polymorphous forms mimicking lesions of sexually transmitted diseases or carcinoma.[8,9]

Conflicts of interest

The authors have none to declare.

REFERENCES

1. WHO. TB/HIV Fact Sheet. Global Tuberculosis Report 2017. Accessed from http://www.who.int/entity/tb/publications/global_report/en/index.html Cited 15.12.17.
2. Ekaterina K. Extrapulmonary tuberculosis: are statistical reports accurate? *Ther Adv Infect Dis*. 2014;2(2):61–70.
3. Nogales-Ortiz F, Tarancon I, Nogales Jr FF. The pathology of female genital tuberculosis. A 31-year study of 1436 cases. *Obstet Gynecol*. 1979;53(4):422–428.
4. Akhlaghi F, Hamedi AB. Postmenopausal tuberculosis of the cervix, vagina and vulva. *Int J Gynaecol Obstet*. 2004;3:1–3.
5. Crofton J, Horne N, Miller F. *Clinical Tuberculosis*. 1st ed. London: Macmillan Education Ltd.; 1992:502–510.
6. Duggal S, Duggal N, Hans C, Mahajan RK. Female genital TB and HIV co-infection. *Indian J Med Microbiol*. 2009;27:361–363.
7. Nhlonzi G, Ramdial P, Nargan K, Lumamba K, Pillay B. Vulval tuberculosis: the histomorphological spectrum in patients with HIV co-infection and AIDS. *J AIDS Clin Res*. 2017;8(719):2.

8. Sardana K, Koranne RV, Sharma RC, Mahajan S. Tuberculosis of the vulva masquerading as a sexually transmitted disease. *J Dermatol*. 2001;28(9):505–507.
9. Mallya V, Yadav YK, Gupta K. Vulval tuberculosis masquerading vulval carcinoma. *J Postgrad Med*. 2012;58: 307–312.
10. Giannacopoulos K, Hatzidaki EG, Papanicolaou NC, Relakis KJ, Kokori HG, Giannacopoulou CC. Genital tuberculosis in a HIV infected woman: a case report. *Eur J Obstet Gynecol Reprod Biol*. 1998;80(2):227–229.

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Review article

Tuberculosis infection control measures at health care facilities offering HIV and tuberculosis services in India: A baseline assessment

K.S. Sachdeva [c], R.D. Deshmukh [a,b,*], N.S. Seguy [b], S.A. Nair [b], B.B. Rewari [a,b], R. Ramchandran [b], M. Parmar [b], V. Vohra [c], S. Singh [d], M. Ghedia [b,e], R. Agarwal [f], A.N. Shah [b,e], D. Balasubramanian [b], M. Bamrotiya [a], R. Sikhamani [a], R.S. Gupta [a], S.D. Khaparde [a]

[a] National AIDS Control Organization, Ministry of Health and Family Welfare, Basic Services Division, 6th Floor, Chandralok Building, 36-Janpath, New Delhi 110001, India
[b] World Health Organization, Country Office for India, 532, A Wing, Nirman Bhavan, Maulana Azad Road, New Delhi 110001, India
[c] National Institute of TB and Respiratory Diseases, Sri Aurobindo Marg, Near Qutub Minar, New Delhi 110030, India
[d] National TB Institute, 8, Avalon, Bellary Road, Bengaluru, Karnataka 560003, India
[e] Central TB Division, Ministry of Health and Family Welfare, Nirman Bhavan, Maulana Azad Road, New Delhi 110001, India
[f] Public Health Foundation of India, Plot no 47, Sec 44, Institutional Area Gurgaon, 122002 Haryana, India

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SUMMARY

Background: Tuberculosis (TB) is one of world's oldest infectious disease and ranks alongside HIV as leading infectious killer. Tuberculosis infection control especially in HIV and TB care facilities has warranted attention after the recent health care-associated outbreaks in South Africa. The aim of this study was to describe the tuberculosis infection control measures implemented by HIV and TB care facilities in five high HIV burden provinces in India.
Methods: Baseline assessment of 30 high burden Antiretroviral centers and TB facilities was conducted during Oct 2015–Dec 2015 by AIC trained staff using a structured format.
Results: Thirty HIV and TB care facilities in five high HIV burden provinces were enrolled. Facility infrastructure and airborne infection control practices were highly varied between facilities. TB screening and fast tracking at ART centers is happening at majority of centers however inadequate TB infection control training, poor compliance to administrative and personal protective measures and lack of mechanism for health care workers surveillance need attention.
Conclusions: Local specific TB infection control interventions to be designed and implemented at HIV and TB care facilities including implementation of administrative, environmental and use of personal protective equipment's with the training of staff members. Health care workers surveillance needs to be prioritized considering the rising instances of tuberculosis among Health care workers.

* *Corresponding author at*: National AIDS Control Organization, Ministry of Health and Family Welfare, Basic Services Division, 6th Floor, Chandralok Building, 36-Janpath, New Delhi 110001, India.
E-mail addresses: drdeshmukh.rajesh@gmail.com, rajyogi02@gmail.com (R.D. Deshmukh).
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1. Background

India accounts for one fourth of global Tuberculosis (TB) cases, with estimated 2.8 million new cases.[1] World Health organization policy on TB infection control,[2] National Framework for TB HIV collaborative activities in India[3] recommend TB infection control among People Living with HIV (PLHIV) as one of the fundamental strategy of prevention of tuberculosis among PLHIV. There is increasing risk of TB transmission in health care facilities and nosocomial outbreaks of multidrug-resistant TB and extensively drug-resistant TB have been reported earlier.[4–7] Infection control (IC) measures comprising of the administrative, environmental and personal protection measures are recommended at the health care facilities as per the National airborne infection control guidelines in India.[8]

Periodic assessment of TB infection control measures within the health care facilities is essential to reduce the risk of transmission in health care settings especially in HIV and TB health care facilities. There is limited experience in country on airborne infection control measures at HIV care settings. This paper aims at describing the public health practice of TB infection control assessment and TB infection control measures at high burden Antiretroviral Therapy centers (ART) in India.

2. Methods

This is a descriptive study of assessment of TB infection control measures at thirty high burden antiretroviral centers in India. The study setting included thirty antiretroviral in five high HIV burden states namely Andhra Pradesh (4), Karnataka (7), Maharashtra (8), Telangana (3) and Tamil Nadu (8). These sites were included as per the highest patient load attending the facility for HIV care. TB infection control assessments were conducted by thirty teams comprising of experts trained in Airborne Infection control (AIC) assessment at national and state level. Members from State and local facility members including a microbiologist, staff nurse ART nodal officer, District TB officer, facility engineer were members of the team. TB infection control assessments were conducted during Oct 2015 to Dec 2015 for three day at each of the 30 ART centers.

A standardized format based on the WHO recommended checklist for periodic assessment of TB infection control at health care facilities[9] was used by the team to assess each facility. Broadly the format included checklist and descriptive section for incorporating observations and recommendations.

2.1. *Ethical approval*

Permission and consent was obtained from the local facilities administrator as well as the central health departments of TB and HIV facilities prior to the assessment. Services were assessed, individual patient information was not collected and patients were not part of the assessment. Observations and recommendations of assessment were briefed to the local administrators prior to compilation at central level. No ethical approval was required for the study as it did not involve collection of private, sensitive data.

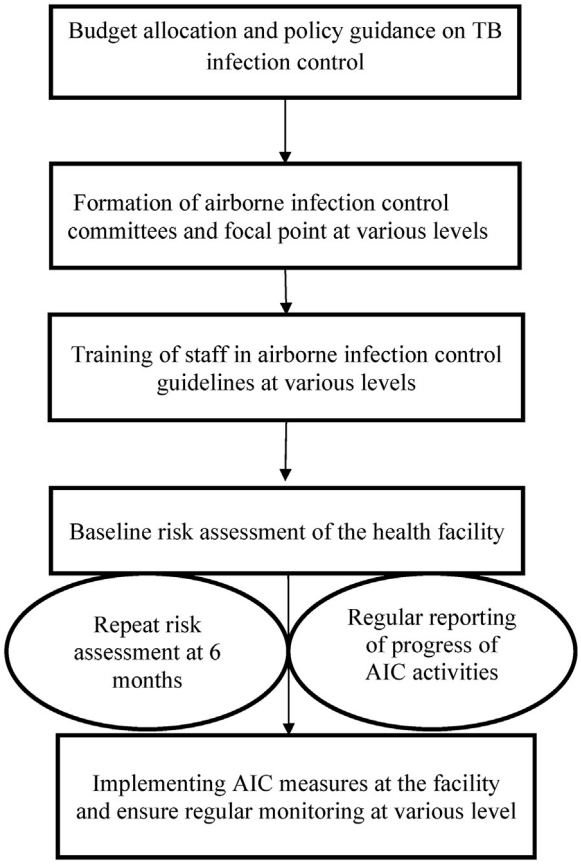

Fig. 1 – Task flow for the Airborne Infection Control Activities at the facility.

3. Results

All 30 facilities (100%) enrolled in the study and AIC practices were evaluated to determine the key IC parameters available across the services linked to ART centers including TB microscopic centers and treatment centers. The types of facilities evaluated included public sector medical college hospitals, district hospitals, sub-district hospital with ART center and also Revised National TB control Program (RNTCP) designated microscopic center and TB treatment facility.

At the facility level, Infection control committee was in place at 22(73%) of facilities, but as per the composition of committee, ART center nodal officer was part of committee members at only 16(53%) facilities. Similarly, Infection control committee met regularly at 16 (53%) facilities where minutes of meeting were available for review. Facility infection control plan was available in written form at only 11(37%) facilities.

Annual IC training plan for all levels of staff was available in 11(37%) facilities where training was conducted for infection control for all staff in last 2 years. ART center staff at 5(16%) facilities had undergone airborne infection control training and standardized training material for infection control was available at only 4(13%) facilities.

TB disease surveillance in health care workers was part of facility infection control plan at only 2 facilities, active screening for Tuberculosis for ART center staff was done at

only one facility. Three cases have been documented among ART center staff in past two years as per information available. Passive reporting of TB diagnosed or treated among staff was done at 5 (17%) facilities.

Fifteen i.e. 50% facilities had available signage to educate patients about cough hygiene, only 14 (47%) provided surgical face masks, tissue and waste bins for patients.

Patients with cough or other symptoms of TB promptly separated and fast tracked at 22 (73%) facilities, however overcrowding was reported at microscopic centers within the facility. Seventeen i.e. 57% facilities had well-ventilated, clearly designated sputum collection area away from others.

Although most of the ART centers had limited natural ventilation, none of the ART centers used ultraviolet (UV) germicidal irradiation and therefore cleaning, monitoring and maintenance plan for these environmental interventions (both UV and mechanical ventilation) was not evident at any of the facilities.

Personal protective equipment was not uniformly available in all facilities. Only 3 (10%) facilities had N-95 respirators readily available to all staff that have contact with patients with TB or suspected of having TB in the center. No facility offered fit-testing for use of these respirators.

Table 1 summarizes the findings of the airborne infection control practices at the sites.

Fig. 1 illustrates task flow for implementing AIC measures in the facility.

4. Discussion

In our study we reported baseline assessment situation of the facilities assessed by team of experts trained in Airborne Infection Control guidelines.

Within the administrative control ambit, during our assessment, we found very few facilities had infection control committees in place and wherever it was available, infection control committees were not meeting regularly. Facility infection control plan was not incorporating the airborne infection control activities plan and a designated focal point for the AIC in facility. Several studies have highlighted weak administrative and managerial support.[10,11] This signifies need for prioritization for AIC activities in the facilities at administrative level and designate a trained person who can monitor airborne infection control practices to ensure policies are in place in the health facility. Designated focal point for AIC has shown improvement in administrative, managerial and safe sputum collection practices.[12] It is important to have an AIC committee in place, with representation from various departments like microbiology, nursing, engineering. Regular meeting and actions on strengthening airborne infection control measures is equally important.

Fast-tracking is an important activity to identify chest symptomatic, screen patients and refer to the diagnostic facility, thereby reducing the waiting period within the facility and also helping to reduce the risk of transmission by segregating the symptomatic and not symptomatic patients. In our assessments this was evident in majority of facilities. The ART guidelines in India[13] strongly recommend fast-tracking by staff nurse and hence the processes are followed by several HIV treatment facilities. Our observations regarding non availability of airborne infection control specific Information, Education, Communication (IEC) correlated with other studies, which highlighted that the content of IEC material was general infection control.[14–16] Availability of TB infection control specific IEC material is very much required considering the risk of transmission of Tuberculosis in HIV care settings. An IEC plan to design and develop local specific content needs to be incorporated in the infection control plan.

Training of staff about airborne infection control guidelines has been seen as an important barrier in effectively implementing AIC measures in health care settings.[17,18] Regular training plan for health care workers regarding AIC guidelines will ensure policies translation in practice as seen in South Africa.[19] Regular availability of N95 respirators and use of personal protective equipment (PPE) by staff has been a challenge as highlighted in study from India.[20] Inclusion of

Table 1 – Summary of infection control practices at the 30 ART Centers health facilities in India.

S. No	Infection control policies and measures	Number yes (%) (n = 30)
1	Facility level infection control (IC) Committee or Bio-medical waste management committee in place?	22 (73)
2	Is the ART center nodal officer a member of Hospital Infection Control Committee?	16 (53)
3	Facility infection control plan available in written form?	11 (37)
4	Is there an institutional policy to provide N-95 or FF2 (or higher) respirators to staff who have contact with patients with DR TB and other infectious airborne diseases?	5 (16)
5	Is infection control education/training for staff being performed in last 2 years?	11 (37)
6	Is standardized training material on infection control available for staff?	4 (13)
7	Are signages for cough etiquette displayed?	15 (50)
8	Are patients routinely asked/monitored by staff nurse and care coordinator for cough or other symptoms (4S+/–) of TB upon entering/waiting in the facility?	22 (73)
9	Are patients with cough or other symptoms of TB promptly separated and fast tracked?	22 (73)
10	Does the ART center maintain the line-list for all suspected TB case referral?	21 (70)
11	Is TB infection control practices monitored daily?	6 (20)
12	Are sputum samples collected in well-ventilated, clearly designated area away from others?	17 (57)
13	Are surgical masks available for patients for cough or other TB symptoms?	14 (47)
14	Are N-95 or FF2 (or higher) respirators readily available for staff that have contact with presumptive TB cases or TB patients?	3 (10)
15	Is active TB screening done for ART staff?	1 (3)

procurement of N95 respirators and other PPEs in the annual budget plan will ensure the regular availability of N95 respirators in resource limited settings. Role of Upper-room ultraviolet germicidal irradiation (UVGI) is highlighted in studies earlier as important measure to prevent tuberculosis transmission specifically in areas where natural ventilation is lacking, waiting areas are crowded and respiratory isolation facilities are frequently unavailable.[21,22] Our assessment observed there was need for use of UVGI at several such facilities, but it was not available and not used at any of the assessed facilities. Proper procurement plan and maintenance of UVGI are important to reduce the risk of transmission in such facilities.

Several studies highlight the need for health care workers surveillance considering the higher risk and mortality reported in several parts of world.[23–26] Our observations about lack of systematic mechanism for health care workers surveillance has been highlighted in studies earlier.[27,28] Systematic implementation of infection control measures has been effective in reducing nosocomial transmission of multidrug-resistant strains to patients and health care workers.[29] There is need to build up administrative commitment to implement the health care workers surveillance in several high TB burden countries to prevent the mortality and morbidity due to TB among health care workers.

In conclusion, although airborne infection control guidelines are in place in India, there is gap in operationalization of these guidelines. Prioritization of airborne infection control activities, allocation of resources and availability of trained manpower to implement the guidelines is essential. There is urgent need to reduce the risk of occupationally acquired TB to health care workers in the facility.

Further research is needed to determine challenges in implementing the AIC guidelines and prioritization of airborne infection control measures, despite the knowledge of effectivity of such measures on reducing the risk of TB transmission.

Limitations: We were unable to evaluate all ART centers in the country, the sites included 30 high burden sites only, therefore there are facilities which have different load, and located in different provinces with varying AIC practices. Another possible limitation of this study was the fact that we are only able to report on what was seen during the visit to the facility on the day of the site visit. There may have been variations in follow up visits.

Authors' contribution

wSKS, DRD, SAN, RBB, PM, SA contributed to the concept, design. DRD, NS, RR, VV, GM, SS, AR, SR site visits, literature search, synthesis of information identified in the search, writing and editing of the manuscript, and data collection and analysis. All others contributed in the literature search, review of the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors have none to declare.

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REFERENCES

1. World Health Organization. *Global TB report. WHO/HTM/TB/2016.13.87*. Geneva, Switzerland: WHO; 2016.
2. World Health Organization. *WHO Policy on TB Infection Control in Health Care Facilities, Congregate Settings and Households. WHO/HTM/TB/2009.419*. Geneva, Switzerland: WHO; 2009.
3. National AIDS Control Organization and Central TB Division. *National Framework for HIV TB Collaborative Activities in India*. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2013.
4. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet*. 2006;368:1575–1580.
5. Alland D, Kalkut GE, Moss AR, et al. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods. *N Engl J Med*. 1994;330:1710–1716.
6. Pai M, Kalantri S, Aggarwal AN, Menzies D, Blumberg HM. Nosocomial tuberculosis in India. *Emerg Infect Dis*. 2006;12:1311–1318.
7. Bhanu NV, Banavalikar JN, Kapoor SK, Seth P. Suspected small scale interpersonal transmission of *Mycobacterium tuberculosis* in wards of an urban hospital in Delhi, India. *Am J Trop Med Hyg*. 2004;70:527–531.
8. Directorate General of Health Services. *Guidelines on Airborne Infection Control in Healthcare and Other Settings – In the Context of Tuberculosis and Other Airborne Infections*. New Delhi: Ministry of Health & Family Welfare, Government of India; 2010, April.
9. World Health Organization. *Guidelines for the Prevention of Tuberculosis in Healthcare Facilities in Resource-Limited Settings*. Geneva, Switzerland: WHO; 1999.
10. Kuyinu1 YA, Mohammed AS, Adeyeye OO, et al. Tuberculosis infection control measures in health care facilities offering TB services in Ikeja local government area, Lagos, South West, Nigeria. *BMC Infect Dis*. 2016;16:126.

11. Lin Y, Harries AD. Tuberculosis infection control measures in diabetes clinics in China: a rapid assessment of 10 hospitals. *Trop Med Int Health*. 2015;20(9):1196–1200.
12. Godfrey C, Tauscher G, Hunsberger S, et al. A survey of tuberculosis infection control practices at the NIH/NIAID/DAIDS supported clinical trial sites in low and middle income countries. *BMC Infect Dis*. 2016;16:269.
13. National AIDS Control Organization. *Antiretroviral Therapy Guidelines for ART HIV Infected Adults and Adolescents*. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2013.
14. Lai KK, Fontecchio SA, Kelley AL, Melvin ZS. Knowledge of the transmission of tuberculosis and infection control measures for tuberculosis among healthcare workers. *Infect Control Hosp Epidemiol*. 1996;17:168–170.
15. Woith WM, Volchenkov G, Larson JL. Russian health care workers' knowledge of tuberculosis and infection control. *Int J Tuberc Lung Dis*. 2010;14:1489–1492.
16. Messmer PR, Jones S, Moore J, et al. Knowledge, perceptions, and practice of nurses toward HIV positive AIDS patients diagnosed with tuberculosis. *J Contin Educ Nurs*. 1998;29:117–125.
17. Malangu N, Mngomezulu M. Evaluation of tuberculosis infection control measures implemented at primary health care facilities in Kwazulu-Natal province of South Africa. *BMC Infect Dis*. 2015;15:117.
18. Sissolak D, Marais F, Mehtar S. TB infection prevention and control experiences of South African nurses – a phenomenological study. *BMC Public Health*. 2011;11:262.
19. Farley JE, Tudor C, Mphahlel M, et al. A national infection control evaluation of drug-resistant tuberculosis hospitals in South Africa. *Int J Tuberc Lung Dis*. 2012;16(January (1)).
20. Malik M, Parmar KS, Kiran Rade S, et al. Airborne infection control in India: baseline assessment of health facilities. *Indian J Tuberc*. 2015;62(October (4)):211–217.
21. Escombe AR, Moore DAJ, Gilman RH, et al. Upper-room ultraviolet light and negative air ionization to prevent tuberculosis transmission. *PLoS Med*. 2009;6(3):e1000043.
22. Xu P, Peccia J, Fabian P, et al. Efficacy of ultraviolet germicidal irradiation of upper-room air in inactivating bacterial spores and Mycobacteria in full-scale studies. *Atmos Environ*. 2003;37:405–419.
23. Harries AD, Maher D, Nunn P. Practical and affordable measures for the protection of health care workers from tuberculosis in low-income countries. *Bull World Health Organ*. 1997;75(5):477–489.
24. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. *PLoS Med*. 2006;3(12):e494;ĆMenzies D, Fanning A, Yuan L, Fitzgerald M. Tuberculosis among health care workers. *N Engl J Med*. 1995;332(2):92–98.
25. Pearson ML, Jereb JA, Frieden TR, et al. Nosocomial transmission of multidrug-resistant *Mycobacterium tuberculosis*. A risk to patients and health care workers. *Ann Intern Med*. 1992;117(3):191–196.
26. Pai M, Joshi R, Dogra S, et al. Serial testing of health care workers for tuberculosis using interferon gamma assay. *Am J Respir Crit Care Med*. 2006;174:349–355.
27. Gopinath KG, Siddique S, Kirubakaran H, Shanmugam A, Mathai E, Chandy GM. Tuberculosis among healthcare workers in a tertiary care hospital in South India. *J Hosp Infect*. 2004;57:339–342.
28. Pai M, Gokhale K, Joshi R, et al. *Mycobacterium tuberculosis* infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. *J Am Med Assoc*. 2005;293:2746–2755.
29. Maloney SA, Pearson ML, Gordon MT, Del Castillo R, Boyle JF, Jarvis WR. Efficacy of control measures in preventing nosocomial transmission of multidrug-resistant tuberculosis to patients and health care workers. *Ann Intern Med*. 1995;122:90–95.

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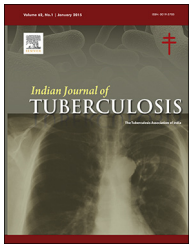

Original article

Prevalence of allergic bronchopulmonary aspergillosis in asthmatic patients: A prospective institutional study

Kalaiyarasan [a], *Anil Kumar Jain* [b,*], *Manmohan Puri* [c], *Devika Tayal* [d], *Ritu Singhal* [e], *Rohit Sarin* [f]

[a] *Junior Resident, Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

[b] *Chief Medical Officer, Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

[c] *Senior Consultant, Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

[d] *Head, Department of Biochemistry, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

[e] *Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

[f] *Director, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

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ABSTRACT

Background: Allergic bronchopulmonary aspergillosis (ABPA) is characterized by an allergic inflammatory response to colonization by *Aspergillus* species, most commonly *Aspergillus fumigatus*.

Aim: To study the prevalence of ABPA in asthmatic patients presenting to our institute.

Materials and methods: All consecutive asthma patients attending our allergy clinic Out Patient Department (OPD) over a period of 20 months were tested with skin prick test (SPT) for *Aspergillus* antigens and those who were found positive were further evaluated for ABPA using Greenberger's criteria.

Results: Seventy consecutive asthmatic patients were screened by SPT using *Aspergillus* antigens. Thirteen patients (18.57%) were found to be SPT positive, out of which nine patients (12.9%) were diagnosed as having ABPA using Greenberger's criteria. ABPA was common among 25–35 age group with no gender predilection. ABPA patients had longer duration of illness, predominantly mixed pattern in PFT, higher mean absolute eosinophil count (AEC) and serum total IgE compared to non-ABPA asthmatic patients. Specific IgE for *A. fumigatus* was positive in all ABPA patients and serum precipitins were positive in seven patients (77.58%). Chest X-ray abnormalities were seen in five patients (55.6%) and HRCT showed central bronchiectasis in eight patients (88.9%) with varying other radiological features. None were sputum fungal culture positive and five patients (55.6%) have been misdiagnosed as pulmonary tuberculosis in the past.

* *Corresponding author.*
E-mail address: akjain62@gmail.com (A.K. Jain).

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Conclusion: The prevalence of ABPA is significantly higher in bronchial asthma patients presenting to tertiary care centers and hence awareness is required among physicians for early diagnosis and management of ABPA to achieve better asthma control and to avoid permanent lung damage.

1. Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity mediated pulmonary disease characterized by an allergic inflammatory response to colonization of the airways by *Aspergillus* species, most commonly *Aspergillus fumigatus*.[1] ABPA usually occurs in patients of bronchial asthma and cystic fibrosis with a variety of clinical and radiological manifestations.

The first three cases of ABPA were reported by Hinson et al. in 1952 in England.[2] The first case of ABPA in the United States was reported in 1968, and the first childhood case of ABPA in the United States was reported by Slavin et al. in 1970.[3,4] In India, ABPA was first reported by Shah in 1971.[5] Data on childhood ABPA from India are very few.[6,7] The youngest case in India was first reported in 2006 by Gaur et al. where the patient was only four years old.[8] In 2017, Mathur et al. reported a case of ABPA in a child aged four years and nine days.[9]

The prevalence of ABPA is approximately 1–2% in asthmatics, 7–14% in corticosteroid-dependent asthmatics, and 1–15% in those with cystic fibrosis.[10–13] There is no gender predilection noted. Denning et al. estimated the prevalence of ABPA in adults with asthma to be 2.5% (range 0.72–3.5%) and concluded that the global burden of ABPA potentially exceeds 4.8 million people.[14] In India, several studies show a prevalence of 7–27.2% among asthmatic patients.[15–19]

2. Materials and methods

All consecutive patients of bronchial asthma between 15 and 55 years of age irrespective of sex, race and religion, attending the out-patient department of Allergy clinic, National Institute of Tuberculosis and Respiratory Diseases (NITRD), New Delhi over a period of 21 months (September, 2014 to May, 2016) were enrolled in this study. Approval for the study was obtained from our institute's research and ethical committees. Informed consent was obtained from patients enrolled in the study. Pregnant and lactating women, patients with history of smoking, patients with major medical illness were excluded.

132 bronchial asthma suspects were referred to our Allery clinic. On the basis of inclusion and exclusion criteria and the consent to participate in the study a total of 70 patients were included in to the study as initial diagnosis of bronchial asthma diagnosed as per Global Initiative for Asthma (GINA) guidelines.

All enrolled subjects were screened by skin prick test (SPT) using purified extracts of *A. fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor*, *Aspergillus tamarii* obtained from All Cure Pharma Pvt. Ltd. Grading of SPT was done as per Shivpuri's classification. Those who showed positive reaction to any *Aspergillus* antigen in SPT were further evaluated for ABPA using Greenberger's criteria[20] (Table 1).

Patients with all five major criteria were considered to have ABPA-CB (ABPA with central bronchiectasis). The presence of all major criteria in the absence of central bronchiectasis were considered ABPA-S (seropositive ABPA). Minor criteria were not used to diagnose ABPA rather was only used as a supportive for the diagnosis of ABPA.

Serum total IgE was quantitatively measured using ELISA technique manufactured by CALBIOTECH^{TM} and a value of $<$200 IU/mL was considered normal. Serum precipitins for *Aspergillus* was done using PLATELIA^{TM} *Aspergillus* IgG kit. ImmunoCAP® FEIA method was used to determine the specific IgE for *A. fumigatus* and levels $>$0.35 kUA/L was considered positive. Blood eosinophil counts greater than 500 cells/mm^3 was considered eosinophilia.

Wherever possible old chest radiographs of the patients were reviewed to look for any fleeting opacities, evidence suggestive of bronchiectasis, fibrosis, mucus plugging, consolidation. High resolution computerized tomography (HRCT) was done to look for central bronchiectasis and other radiological features.

3. Statistical analysis

SPSS version 21 was used for statistical analysis. Percentages were compared by Fisher's exact test and Means by Student's *t*-tests. p-value $<$0.05 was considered significant.

4. Results

Out of 70 asthmatic patients enrolled in the study, thirteen patients (18.57%) showed positive SPT for one or more *Aspergillus* antigen(s), out of which nine patients (12.9%) turned out to be ABPA and the remaining four patients did not fulfill the major criteria set for diagnosis of ABPA. Thus, the prevalence of ABPA among bronchial asthma patients is estimated to be 12.9% (CI 6.9–22.7%).

Table 2 summarizes the characteristics of the non-ABPA asthmatic patients and ABPA patients. Out of 9 ABPA patients in the study, seven patients (77.8%) were males, two patients (22.2%) were females and their age ranged from 25 to 52 years with a mean age of 35.33 $\pm$ 11 years. This is not statistically significant compared to non-ABPA asthmatic patients (p 0.202). The mean duration of illness was higher among ABPA patients compared to non-ABPA asthmatic patients and it was statistically significant (18.44 years vs 8.8 years, p 0.001).

Table 1 – Greenberger's criteria.[20]

Major criteria
(1) Asthma
(2) Central bronchiectasis on a computed tomography (CT)
(3) Immediate cutaneous reactivity to *Aspergillus* species
(4) Elevated total serum IgE (>417 IU/mL or >1000 ng/mL)
(5) Elevated specific serum IgE and/or IgG to *Aspergillus fumigatus*
Minor criteria
(1) Chest roentgenographic infiltrates
(2) Peripheral blood eosinophilia
(3) Serum precipitating antibodies to *Aspergillus fumigatus*
(4) Mucous plugs
(5) Sputum culture positive for *Aspergillus species*

Among all *Aspergillus* antigen SPT positive patients in this study ($n = 13$), *A. fumigatus* and *A. tamarii* antigens were positive in 10 patients (76.92%) each. *A. flavus* and *A. versicolor* were positive in four patients (30.76%) each, and *A. niger* was positive in three patients (23%).

Among diagnosed ABPA patients ($n = 9$), along with *A. fumigatus* SPT positivity, *A. tamarii* antigen was also positive in seven patients (77.8%), *A. versicolor* and *A. flavus* were positive in four patients (44.4%) each, *A. niger* was positive in three patients (33.3%) (Tables 3 and 4).

The mean absolute eosinophil count (AEC) was significantly elevated in ABPA patients compared to non-ABPA asthmatic patients (1078.89 vs 552.79 cells/mm^3, $p < 0.001$). Among ABPA patients, the mean serum total IgE was 871.37 IU/mL compared to 623.88 IU/mL among non-ABPA asthma group. But the difference was not statistically significant (p 0.118). Serum specific IgE to *A. fumigatus* was positive in all nine ABPA patients and serum precipitins were positive in seven ABPA patients (77.8%).

Chest X-ray findings were present in five out of nine ABPA patients (55.6%). Two patients had areas of consolidation of varying sizes, three patients had irregular infiltrations in various lung zones. In HRCT, central bronchiectasis was the most common finding seen in eight out of nine diagnosed ABPA cases (88.9%). HRCT chest showed no abnormality in one patient (11.1%, ABPA-S), only bronchiectasis in two patients (22.2%, ABPA-CB), and bronchiectasis along with other radiological findings in six patients (66.7%, Allergic Bronchopulmonary Aspergillosis with Central Bronchiectasis and Other Radiological Features, ABPA-CB-ORF). Other radiological findings seen in CT chest apart from bronchiectasis were centrilobular opacities, mucus plugs, calcification, consolidation, mediastinal lymphadenopathy and fibrosis.

The mean Forced Expiratory Volume in 1 second (FEV1) was 2.19 ± 0.62 among ABPA patients. Seven (77.8%) out of nine ABPA patients in our study had mixed pattern. Six patients (66.7%) had moderate to very severe impairment. Out of nine diagnosed ABPA patients in this study, none grew any *Aspergillus* sp. whereas in non-ABPA asthma group two patients had growth in fungal culture. One patient's sputum grew *A. fumigatus* and the other grew *A. flavus*. Five ABPA patients (55.6%) had received anti-tubercular treatment (ATT) in the past compared to only six patients (9.84%) in the non-ABPA asthma group. This difference is statistically significant (p 0.003).

Table 2 – Characteristics of non-ABPA asthma patients and ABPA patients.

Parameters	Non-ABPA asthma group ($n = 61$)	ABPA group ($n = 9$)	p value
Age (years)			
Mean ± SD	30.31 ± 10.9	35.33 ± 11	0.202
Range	15–53	25–52	
Sex			
Males	42 (68.86%)	7 (77.78%)	0.714
Females	19 (31.14%)	2 (22.22%)	
Duration of illness (years)			
Mean ± SD	8.8 ± 7	18.44 ± 10.32	**0.001**
Range	1–25	5–35	
Anti-tubercular treatment			
H/O ATT	6 (9.84%)	5 (55.56%)	**0.003**
FEV1			
Mean ± SD	1.99 ± 0.8	2.19 ± 0.62	0.505
Range	0.38–3.93	0.98–2.94	
% Pred FEV1			
Mean ± SD	63.57 ± 19.71	62.67 ± 16.16	0.816
Range	15–92	33–78	
AEC (cells/mm^3)			
Mean ± SD	552.79 ± 368.32	1078.89 ± 457.7	**<0.001**
Range	20–1760	520–1830	
Total IgE (IU/mL)			
Mean ± SD	623.88 ± 448.22	871.37 ± 343.86	0.118
Range	65.67–2651	434–1441	

p-Value <0.05 was considered significant.

5. Discussion

Majority of ABPA patients in our study belonged to 25–45 years (mean, 35.33 years) with no sex predilection. The mean age ranged from 31.2 to 36.25 years in several studies.[15,18,21–23] Although ABPA can occur at any age, majority of the cases are seen in 20–40 years of age, ABPA has also been reported in children[7,24] and even in infants.[25]

In our study, ABPA patients had significantly longer mean duration of illness compared to non-ABPA asthmatic patients, which was statistically significant (18.44 years vs 8.8 years, p 0.001). Similar observation was made in various studies.[18,21,23,26,27] Whether long standing asthma leads to the colonization of *A. fumigatus* and subsequent development of ABPA or the development ABPA leads to chronicity of asthma is debatable.

More than half of the ABPA patients ($n = 5$, 55.56%) in our study had received ATT in the past, sometimes more than once compared to only 6 patients (9.84%) among non-ABPA asthmatic patients. In India, several studies have reported that ABPA was misdiagnosed as pulmonary tuberculosis in as many as 17–58% of cases.[15,26,28,29] This may be due factors such as radiological similarities and lack of awareness among healthcare professionals.

Spirometry usually show an obstructive pattern of varying severity in ABPA patients with or without a restrictive pattern and reduction in DL_{CO}.[30] In long standing ABPA cases with extensive bronchiectasis and parenchymal fibrosis, an irreversible mixed pattern and a reduced diffusion capacity is usually seen.[31] Seven (77.8%) ABPA patients in our study

Table 3 – Frequency of different *Aspergillus* antigen positivity by SPT.

Antigen	Total patients ($n = 13$) Frequency (%)	ABPA patients ($n = 9$) Frequency (%)	Patients not fulfilling the criteria ($n = 4$) Frequency (%)
A. fumigatus	10 (76.92)	9 (100)	1 (25)
A. tamarii	10 (76.92)	7 (77.8)	3 (75)
A. versicolor	4 (30.76)	4 (44.4)	
A. niger	3 (23)	3 (33.3)	
A. flavus	4 (30.76)	4 (44.4)	

Table 4 – *Aspergillus* antigen sensitization pattern by SPT among ABPA patients.

No. of patients ($n = 9$)	Aspergillus SPT positivity
4	*A. fumigatus*, *A. tamarii*
1	*A. fumigatus*, *A. flavus*
1	*A. fumigatus*, *A. tamarii*, *A. versicolor*, *A. niger*
1	*A. fumigatus*, *A. tamarii*, *A. versicolor*, *A. flavus*
1	*A. fumigatus*, *A. flavus*, *A. versicolor*, *A. niger*
1	*A. fumigatus*, *A. tamarii*, *A. flavus*, *A. versicolor*, *A. niger*

showed mixed obstruction and restrictive pattern with moderate to severe impairment in lung function. *A. fumigatus* is the most common fungus causing severe asthma associated with fungal sensitivity (SAFS).[32] All four patients with moderate to severe obstruction have long standing symptom for 20–25 years, while only 2 patients out of five patients with mild obstruction have symptoms for 23–35 years. However, the development of ABPA is not dependent on severity of asthma but the development of ABPA leads to chronic asthma and increased severity of pre-existing asthma.[33] Hence all patients of asthma must be screened for risk of development of ABPA by SPT for *Aspergillus*.

In our study, the AEC was significantly elevated in ABPA patients ($p < 0.001$). Many studies have reported significantly elevated AEC among ABPA patients compared to non-ABPA patients.[16,18,21,23,34,35] But absolute eosinophil count has neither proved to be sensitive nor specific for the diagnosis of ABPA. Further AEC can vary based on the stage of ABPA, intake of oral corticosteroids or spontaneously.[30] AEC can be used as a supportive parameter in the diagnosis of ABPA provided medications like corticosteroids and other conditions common in tropical countries like tropical pulmonary eosinophilia (TPE) has been ruled out.

Among ABPA patients, the mean serum total IgE was 871.37 IU/mL compared to 623.88 IU/mL among non-ABPA asthma group (p 0.118). Total serum IgE may be mildly elevated in allergic asthma but it is markedly elevated in ABPA with levels typically exceeding greater than >417 IU/mL (1000 ng/mL). Similar to AEC, serum total IgE is neither sensitive nor specific for the diagnosis of ABPA as levels vary based on the stage of ABPA, intake of oral corticosteroids.

In this study, serum precipitins for *A. fumigatus* were positive in seven ABPA patients (77.8%) compared to only two patients (3.3%) in non-ABPA asthma group. This difference is statistically significant ($p < 0.001$). Results similar to our study were obtained in the several studies.[15,21,29]

In ABPA, the rates of culture positivity range from 39% to 60% depending on the number of specimens examined.[34,36] In our study none of the ABPA patients had any growth on fungal culture. This may be due to a small sample size of the study. Culture of *A. fumigatus* in sputum is supportive but not diagnostic of ABPA because the fungus can also be grown in other pulmonary diseases due to ubiquitous nature of the fungi.

Four patients who were SPT positive for *Aspergillus* antigens were not considered as having ABPA, due to not meeting the adopted criteria in this study. However, two of these patients, who were SPT positive for *A. tamarii* only, met all the criteria including central bronchiectasis but were negative for specific IgE against *A. fumigatus*, which is obvious. Further, specific IgE for *A. tamarii* is not available commercially. Such cases can be considered as having ABPA due to ''*Aspergillus* species other than *A. fumigatus*'' or ''atypical ABPA'' based on clinical and radiological features. For instance, there are two case reports which reported ABPA due to *A. niger* without bronchial asthma.[37,38]

6. Conclusion

ABPA is commonly seen in young age group, with no gender predilection. Any patient of long standing, poorly controlled bronchial asthma, with markedly elevated peripheral eosinophil counts, fleeting shadows/pulmonary infiltrates on serial chest radiographs should always be screened with *Aspergillus* skin testing and/or serum total IgE to rule out the possibility of ABPA.

A diagnosis of ABPA should not be excluded based only on the cut off values of total serum IgE as it is highly variable depending on stage of ABPA, intake of oral corticosteroids. In these cases, ABPA should be confirmed by serum specific IgE for *A. fumigatus* and HRCT. ABPA is commonly misdiagnosed as pulmonary tuberculosis in India due to the radiological similarity which delays the diagnosis of ABPA.

Currently, no uniform diagnostic criteria and diagnostic algorithm are followed for diagnosis of ABPA in India. Further studies are required to simplify the diagnosis and treatment algorithms in resource limited countries like India.

7. Limitations

Sample size of this study was small. This is an institutional study so results may be different from population based study.

As all of the asthmatic patients attending general OPD were not referred to our allergy clinic OPD, this study may not reflect true prevalence of ABPA and it may even be higher in the asthmatic patients attending our institute.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. *Eur Respir Rev*. 2011;20(121):156–174.
2. Hinson KFW, Moon AJ, Plummer NS. Broncho-pulmonary aspergillosis: a review and a report of eight new cases. *Thorax*. 1952;7(4):317–333.
3. Patterson R, Golbert TM. Hypersensitivity disease of the lung. *Univ Mich Med Cent J*. 1968;34(1):8–11.
4. Slavin RG, Laird TS, Cherry JD. Allergic bronchopulmonary aspergillosis in a child. *J Pediatrics*. 1970;76(3):416–421.
5. Shah JR. Allergic pulmonary aspergillosis. *J Assoc Phys India*. 1971;19(12):835–841.
6. Chetty A, Bhargava S, Jain RK. Allergic bronchopulmonary aspergillosis in Indian children with bronchial asthma. *Ann Allergy*. 1985;54(1):46–49.
7. Shah A, Bhagat R, Panchal N. Allergic bronchopulmonary aspergillosis with clubbing and cavitation. *Indian Pediatrics*. 1993;30(2):248–251.
8. Gaur S, Zu K, Kumar R. *Youngest Patient of ABPA in Indian Subcontinent – A Case Report*. 2006;37–40.
9. Mathur N, Mathur M, Singh V. Youngest case of allergic broncho pulmonary aspergillosis in India: a case report from arid zone of India. *J Clin Diagn Res*. 2017;11(4):OD03–OD4.
10. Schwartz HJ, Greenberger PA. The prevalence of allergic bronchopulmonary aspergillosis in patients with asthma, determined by serologic and radiologic criteria in patients at risk. *J Lab Clin Med*. 1991;117(2):138–142.
11. Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis and the evaluation of the patient with asthma. *J Allergy Clin Immunol*. 1988;81(4):646–650.
12. Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis – state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis*. 2003;37(suppl 3):S225–S264.
13. Basich JE, Graves TS, Baz MN, et al. Allergic bronchopulmonary aspergillosis in corticosteroid-dependent asthmatics. *J Allergy Clin Immunol*. 1981;68(2):98–102.
14. Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol*. 2013;51(4):361–370.
15. Agarwal R, Gupta D, Aggarwal AN, Behera D, Jindal SK. Allergic bronchopulmonary aspergillosis: lessons from 126 patients attending a chest clinic in north India. *Chest*. 2006;130(2):442–448.
16. Kumar R, Gaur SN. Prevalence of allergic bronchopulmonary aspergillosis in patients with bronchial asthma. *Asian Pac J Allergy Immunol*. 2000;18(4):181–185.
17. Maurya V, Gugnani HC, Sarma PU, Madan T, Shah A. Sensitization to *Aspergillus* antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. *Chest*. 2005;127(4):1252–1259.
18. Prasad R, Garg R, Sanjay. Dixit R. Study on prevalence of allergic bronchopulmonary aspergillosis in patients of bronchial asthma. *Internet J Pulm Med*. 2007;9(2).
19. Ghosh T, Dey A, Biswas D, Chatterjee S, Haldar N, Maiti PK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis among asthma patients in eastern India. *J Indian Med Assoc*. 2010;108(12):863–865.
20. Greenberger PA. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol*. 2002;110(5):685–692.
21. Kumar R. Allergic bronchopulmonary aspergillosis – review of 29 cases. *Indian J Tuberc*. 2000;47(4):237–240.
22. Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A, Jindal SK. Clinical significance of hyperattenuating mucoid impaction in allergic bronchopulmonary aspergillosis: an analysis of 155 patients. *Chest*. 2007; 132(4):1183–1190.
23. Prasad R, Garg R, Sanjay. Shukla AD. Allergic bronchopulmonary aspergillosis: a review of 42 patients from a tertiary care center in India. *Lung India*. 2009;26(2):38–40.
24. Shah A, Kala J, Sahay S. Allergic bronchopulmonary aspergillosis with hilar adenopathy in a 42-month-old boy. *Pediatric Pulmonol*. 2007;42(8):747–748.
25. Imbeau SA, Cohen M, Reed CE. Allergic bronchopulmonary aspergillosis in infants. *Am J Dis Children*. 1977;131(10): 1127–1130.
26. Natarajan S, Subramanian P. Allergic bronchopulmonary aspergillosis: a clinical review of 24 patients: are we right in frequent serologic monitoring? *Ann Thorac Med*. 2014; 9(4):216–220.
27. Shah A, Panjabi C. Allergic bronchopulmonary aspergillosis: a perplexing clinical entity. *Allergy Asthma Immunol Res*. 2016;8(4):282–297.
28. Radha TG, Viswanathan R. Allergic bronchopulmonary aspergillosis. *Respiration*. 1978;36(2):104–111.
29. Behera D, Guleria R, Jindal S, Chakrabarti A, Panigrahi D. Allergic bronchopulmonary aspergillosis: a retrospective study of 35 cases. *Indian J Chest Dis Allied Sci*. 1993;36(4):173–179.
30. Shah A, Panjabi C. Allergic aspergillosis of the respiratory tract. *Eur Respir Rev*. 2014;23(131):8–29.
31. Nichols D, Dopico GA, Braun S, Imbeau S, Peters ME, Rankin J. Acute and chronic pulmonary function changes in allergic bronchopulmonary aspergillosis. *Am J Med*. 1979;67(4): 631–637.
32. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J*. 2006;27(3):615–626.
33. Knutsen AP, Slavin RG. Allergic bronchopulmonary aspergillosis in asthma and cystic fibrosis. *Clin Dev Immunol*. 2011.
34. Chakrabarti A, Sethi S, Raman DS, Behera D. Eight-year study of allergic bronchopulmonary aspergillosis in an Indian teaching hospital. *Mycoses*. 2002;45(8):295–299.
35. Bedi RS. Allergic bronchopulmonary aspergillosis: review of 20 cases. *Indian J Chest Dis Allied Sci*. 1994;36(4):181–186.
36. McCarthy D, Pepys J. Allergic broncho-pulmonary aspergillosis. Clinical immunology. 2. Skin, nasal and bronchial tests. *Clin Exp Allergy*. 1971;1(4):415–432.
37. Hoshino H, Tagaki S, Kon H, et al. Allergic bronchopulmonary aspergillosis due to *Aspergillus niger* without bronchial asthma. *Respiration*. 1999;66(4):369–372.
38. Shah A, Maurya V, Panjabi C, Khanna P. Allergic bronchopulmonary aspergillosis without clinical asthma caused by *Aspergillus niger*. *Allergy*. 2004;59(2):236–237.

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Original article

A cross sectional study to assess the Tuberculosis Treatment Providers in the mid hills of India

S.K. Ajay [a,*], C. Sumit [b], B.K. Dimple [c], C. Bharti [b]

[a] District Programme Officer, Health & Family Welfare, Solan, Himachal Pradesh, India
[b] Assistant Professor, Department of Community Medicine, Dr YS Parmar Government, Medical College Nahan, Himachal Pradesh, India
[c] Medical Officer, Regional Hospital, Solan, Himachal Pradesh, India

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ABSTRACT

Background: A high level of knowledge about Tuberculosis amongst the Multi-Purpose Workers (MPWs) is the cornerstone for the successful implementation of Directly Observed Treatment Short-course (DOTS) strategy under the Revised National Tuberculosis control Programme of India. In this regard, the evaluation of MPWs, the major workforce of the Health Department of Solan district, has never been done in the past. Hence the present study was undertaken.

Methods: Objective: To evaluate the knowledge about Tuberculosis among the MPW DOT and non DOT Providers.

Design: A cross sectional study amongst 174 MPWs of the five Tubercular Units was conducted.

Informed written consent was obtained. A pretested self-administered questionnaire was used.

Data was analyzed in Microsoft Excel 2010 and IBM SPSS statistics version 21 software.

Results: 85.6 per cent of the study participants were or had been DOT Providers. Only 9.2 per cent of the workers had received RNTCP Modular training whereas, 87.4 per cent had received just the 'On the Spot training' about DOTS. The difference in knowledge by Gender distribution across the five TUs was found significant.

Conclusion: There is inadequate knowledge of tuberculosis amongst the MPW DOT and Non DOT Providers. Hence regular Modular and refresher trainings are recommended.

1. Background

According to the World Health Organization, Tuberculosis (TB) is a major public health problem and the leading cause of deaths worldwide. Out of the total incidence of 9.6 million cases of TB in 2015, worldwide, India accounts for about 2.2 million cases. The prevalence of TB in India is 2.5 million.[1] It is also estimated that a vast majority (40%) of Indian population has latent infection rather than the primary tubercular infection. The Revised National Tuberculosis Control Programme (RNTCP) started in 1997 in India envisages the prompt

* Corresponding author.
E-mail address: ajaysingh7279@gmail.com (S.K. Ajay).
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detection and treatment of TB and to cut further the chain of disease transmission. With its extensive network throughout the country the Programme was able to cover 1285 million people by the year 2015. About 902,732 people were diagnosed sputum smear positive out of the 9,132,306 people tested by sputum smear microscopy.[2]

In Himachal Pradesh, a northern state of India, the RNTCP was launched in 1995. Out of 7,100,100 people covered under RNTCP in the state, 7957 smear positive patients were diagnosed in 2015.[3] The annualized risk of infection in the state is 1.9% as compared to the national rate of 1%.[4]

Solan is one of the fastest growing districts of the State. It has witnessed a vast expansion of small and large scale industries, including the cement plants across the length and breadth of its landscape.[5] This has not only added to the environment and sanitation related health problems but also has put up the challenges associated with the migrant population dynamics. Majority of the TB patients in the district are being managed in the public sector under the stewardship of the RNTCP, launched in the district in the year 2000.[6] The vast organizational set up of the public health sector in the district, starting from the grass root level of the Health Sub Centres being manned by MPWs, to the state of the art Tertiary level Hospital and a TB Sanatorium, provide management for TB under the guidelines of the RNTCP. The case detection and the cure rate for the district was 84 per cent and 88 per cent respectively in 2013. To further improve the detection and the cure rate, we need to improvise the RNTCP functionaries. For this, first we need to assess the knowledge of the Multi-Purpose Workers (MPWs) who have been working as the DOT and Non DOT Providers, rendering various RNTCP services. The RNTCP defines various responsibilities of the MPWs, the first health functionary to be in direct contact with the masses. Their interaction with the people and solution of their health problems is the keystone to the success of the RNTCP. This warrants for an up to date knowledge of TB amongst the workers. Base line assessment of the knowledge of TB has never been done in the district. Hence this study was proposed with the objectives of the assessment of the knowledge of TB in the MPWs and comparison of the knowledge of TB amongst the DOT and the Non DOT Provider MPWs. Also an assessment of knowledge was done for the DOT Providers of ≤2 patients and DOT Providers of >2 patients.

2. Methods

2.1. Sampling technique

Purposive sampling technique was utilized.

2.2. Study area

5 Tubercular Units (Arki, Chandi, Dharampur, Nalagarh and Syri) of district Solan were enrolled for the study.

2.3. Study period

The study was conducted between 1st March and 30th August, 2015.

2.4. Study population

174 out of 181 MPWs from the five Tubercular Units of the district participated in the study. Seven workers were on leave during the time of the study.

2.5. Study Design

It was a cross sectional study with descriptive epidemiology.

2.6. Study Tools

A self-administered pre tested questionnaire was used to assess the knowledge on Various attributes related to the diagnosis and treatment of Tuberculosis.

Prior to the study an informed written consent from the MPWs was taken. The DOTS strategy Evaluation is a routine inbuilt important component of the functioning of the RNTCP of an area.

Hence the approval of the Chief Medical Officer of the district was obtained for the study.

2.7. Data statistics and analysis

The data was collected regarding the general characteristics of the DOT Providers, their training status and knowledge about the RNTCP and its diagnostic and treatment guidelines. Also a comparison was drawn on the basis of number of patients treated with DOTS. The data was analyzed in IBM SPSS Statistics version 21 and Microsoft Excel 2010 software. Pearson's Chi-Square test (χ^2) test was done to ascertain the statistical significance. The p values of lesser than 0.05 were considered significant.

3. Results

The Table 1 shows that the mean average age of the study participants was 45.84 years with 84.5% (147) being above 35 years of age. Majority of the MPWs were females (62.1%). A large proportion of the workers (87.4%) had undergone only the spot training. The duration of on spot training was for about 2 hours and such on spot trainings were held for three times in the very first month of the initiation of the treatment. The formal modular RNTCP training (3 days training) for MPWs had been received by only 16 (9.2%) of the workers. 149 (85.6%) of the study participants had been or were DOT Providers and 25 (14.4%) were Non DOT providers. A large number of MPWs (86.8%) had the experience of more than 5 years of service and the average mean years of service of 17.5 years. Table 2 depicts the difference detected across the blocks for attributes such as the knowledge about the mode of the spread of the disease and the main symptoms of the disease. It was observed that not all the DOT providers knew the cause of TB. It varied from 45.5% of male workers of Syri to 81% of Female workers of the block. The knowledge about the Communicability of the disease also varied from about 100% male workers of Arki and Nalagarh, all the Female workers of Arki, Nalagarh and Dharampur to 87% of male workers of the Blocks Chandi and Nalagarh. The knowledge about the DOTS strategy and the year of its

Table 1 – General attributes of the study participants.

Attribute		Frequency	Percentage
Gender	Male	66	37.9
	Female	108	62.1
Age in years	35 or less	27	15.5
	More than 35	147	84.5
Training in RNTCP	Modular	16	9.2
	Spot	152	87.4
	Not trained	6	3.4
Years of service	5 years or less	23	13.2
	More than 5 years	151	86.8
DOTS provision	DOT providers	149	85.6
	Non DOT providers	25	14.4

implementation throughout the Country, was also observed to vary across the blocks.

A difference between the Non DOT Providers and the DOT Providers about the knowledge of the fact that all primary infected persons of tuberculosis do not have symptoms was also observed in the study. Moreover, the difference also existed in Knowledge about other aspects of Tuberculosis such as the cause of TB, its communicability, its mode of spread and main symptoms of TB. A large number of the DOT providers (83.6% of the total 55) did not know the cause of TB. Also, about 76.3 per cent (of the total who did not know the symptoms) of the DOT providers were lacking the knowledge of the symptoms of Tuberculosis.

MPWs who were DOT Providers had a varied knowledge on the different aspects of diagnosis of tuberculosis. All the Male workers of the blocks Chandi and Arki and 100% Female workers of block Dharampur knew about the sputum microscopy as the most effective diagnostic test. Also all the Female DOT Providers (13) of block Syri had the knowledge of sputum microscopy being a better test than X-ray. None of the male workers of the blocks Chandi and Arki knew about the other diagnostic tests of TB apart from the sputum microscopy and X-ray. The workers across the blocks had poor knowledge about the number of sputum samples required for testing. It varied across the block, ranging from 12.5 per cent amongst Female MPW of block Syri to about 62 per cent of Male MPWs of block Nalagarh.

All the male DOT Providers of the blocks had the knowledge of TB being a completely curable disease. A difference in knowledge (Gender wise) about the duration of treatment for a new diagnosed TB case, about 4 and 2 anti-tubercular drugs being used for treatment and about the names of 4 anti-tubercular drugs, was found across the blocks. The workers had a varied knowledge about the total number of the patient categories under the RNTCP. The knowledge about the

Table 2 – Knowledge about salient features of Tb amongst the DOT providers.

Attributes	Chandi		Syri		Arki		Nalagarh		Dharampur	
	M** (n*** = 8)	F* (n = 16)	M (n = 11)	F (n = 16)	M (n = 8)	F (n = 15)	M (n = 19)	F (n = 27)	M (n = 20)	F (n = 34)
Cause of Tb	6	11	5	13	6	11	13	19	12	23
Communicability	7	15	10	14	8	15	19	27	18	34
Mode of spread	7	12	11	15	8	13	16	25	18	32
Do all primary infected have symptoms	4	3	5	12	0	3	7	10	16	28
Main symptoms of lung Tb	6	9	7	15	8	12	11	19	19	30
Name of strategy of medicine provision under RNTCP (DOTS)	7	13	9	15	7	10	17	24	19	30
Year in which DOTS was implemented throughout the country	0	0	0	2	2	2	1	4	4	2

Attributes	Chandi		Syri		Arki		Nalagarh		Dharampur	
	M (n = 8)	F (n = 16)	M (n = 11)	F (n = 16)	M (n = 8)	F (n = 15)	M (n = 19)	F (n = 27)	M (n = 20)	F (n = 34)
Cause of Tb	6	11	5	13	6	11	13	19	12	23
Communicability	7	15	10	14	8	15	19	27	18	34
Mode of spread	7	12	11	15	8	13	16	25	18	32
Do all primary infected have symptoms	4	3	5	12	0	3	7	10	16	28
Main symptoms of lung Tb	6	9	7	15	8	12	11	19	19	30
Name of strategy of medicine provision under RNTCP (DOTS)	7	13	9	15	7	10	17	24	19	30
Year in which DOTS was implemented throughout the country	0	0	0	2	2	2	1	4	4	2

* F - Females.
** M - Males.
*** n - study population.

Table 3 – Knowledge about the treatment and diagnostic features of tuberculosis among the DOT and Non DOT Providers.

Attribute	Knowledge	Non DOT providers (n = 25)	DOT providers (n = 149)	Total (N = 149)	Chi square value	p-Value
Most effective test (sputum microscopy)	Y[a]	23 (14.4)	137 (85.6)	160	0.00	0.67
	N[b]	2 (14.3)	12 (85.7)	14		
Sputum microscopy is better than X-ray	Y	22 (15.1)	124 (84.9)	146	0.36	0.39
	N	3 (10.7)	25 (89.3)	28		
Number of sputum samples needed	Y	16 (16.5)	81 (83.5)	97	0.80	0.25
	N	9 (11.7)	68 (88.3)	7		
Duration of treatment of new case	Y	20 (12.5)	140 (87.5)	160	5.63	0.03
	N	5 (35.7)	9 (64.3)	14		
Duration of retreatment case	Y	5 (9.3)	49 (90.7)	54	1.66	0.14
	N	20 (16.7)	100 (83.3)	120		
4 drugs for new case	Y	5 (17.9)	23 (82.1)	28	0.33	0.37
	N	20 (13.7)	126 (86.3)	146		
Name of 4 drugs	Y	2 (12.5)	14 (87.5)	16	0.05	0.58
	N	23 (14.6)	135 (85.4)	158		
DOTS strategy	Y	20 (13.2)	131 (86.8)	151	1.39	0.13
	N	6 (26.0)	17 (73.9)	23		

[a] Yes.
[b] No, figures in parenthesis indicate percentage.

duration of retreatment was found lowest in the Female workers of block Dharampur (14.81%) and highest i.e. 60% amongst the male workers of the same block.

All the Male DOT Providers of the blocks Chandi, Syri and Arki had received spot training at the beginning of their DOT provision to the patients whereas about 97% Female workers of Dharampur and 73.3% Female workers of Arki had received this training. The relation between HIV and TB was explained variably by the workers ranging from about 18.75% of Female workers of block Syri to 75% of Male workers of block Arki and Dharampur.

The workers had less knowledge about the relation of diabetes and TB i.e. it varied from about 8.8 per cent in the Female workers of Dharampur to about 75 per cent of male workers of block Arki.

The workers had varied knowledge about the number of categories of patients of tuberculosis under RNTCP. A large number of DOT providers (67) responded that there are 3 categories of TB patients. On the other hand 141 DOT providers did not know that there are 4 categories of TB patients.

Though a large number of DOT providers (137) knew about sputum microscopy as the most effective laboratory investigation, still about 12 of the DOT providers were unaware of this fact.

Also, a large proportion (89.3%) of the ones not knowing about the better examination out of the sputum microscopy and the X-ray, were the DOT providers. There was a statistically significant difference (p value = 0.03) in the knowledge about the treatment duration of a new case of TB between the Non DOT providers and the DOT providers (Table 3). A very large number (135) of the DOT providers were not knowing the name of main four anti tubercular drugs and another 126 of the total 149 DOT providers were even not knowing that there were 4 main anti tubercular drugs.

Further analysis shows that the difference in the knowledge about the number of anti-tubercular drugs available for a new case i.e. 4 drugs, was found statistically significant amongst the DOT providers of 2 or less than 2 patients as compared to the ones who had provided DOTs to more than 2 patients (Table 4). Similarly the difference in the knowledge about the name of the DOTS strategy was also found to be statistically significant with p-value of 0.01. However, there was not much difference in other attributes such as the cause of the disease, its mode of spread and main symptoms.

4. Discussion

The RNTCP defines a DOT Provider to be a person, other than the family member of the patient, one who is accessible, acceptable to the patient and accountable to the health system. The MPWs are the appropriate DOT Providers in this context. The programme has laid down several duties of MPWs for the management of tuberculosis.[7] Kaur A et al. in a cross sectional study found the knowledge about RNTCP, Tuberculosis and DOTs amongst the DOT providers as unsatisfactory and emphasized the need for adequate modular training and reorientation courses for the DOT providers.[8] The present study also highlights that only 9.2% of the workers had Undergone the Modular training and this study also has recommended for sufficient trainings to the DOT providers from time to time. In a similar study in Ujjain, India, Jain et al. showed that the knowledge of DOT Providers was not satisfactory and varied by the Gender of the workers.[9] Our study also highlights that the knowledge about the cause of TB was poor in the workers and there was significant difference by Gender across the five tubercular units, in the knowledge of attributes such as the mode of spread of TB and the main symptoms of the disease.

Hashim et al. in a study conducted in Iraq showed that though the knowledge of the health care workers was good (95%) but their practice of TB guidelines was poor (61.8% of the total workers). The study also concluded that the National TB programme of Iraq had a good impact on the knowledge of TB

Table 4 – Knowledge about salient features, diagnostic and treatment attributes of Tuberculosis amongst the DOT providers.

Attribute	Knowledge	DOTs provided to ≤2 patients (n = 88)	DOT provided to >2 patients (n = 61)	Total (N = 149)	Chi square value	*p*-Value
Cause of Tb	Y[a]	62 (60.2)	41 (39.8)	103	0.17	0.40
	N[b]	26 (56.5)	20 (43.5)	46		
Communicable	Y	83 (58.5)	59 (41.5)	142	0.46	0.39
	N	5 (71.4)	2 (28.6)	7		
Mode of spread	Y	82 (60.3)	54 (39.7)	136	0.98	0.24
	N	6 (46.2)	7 (53.8)	13		
Do all primary infected have symptoms	Y	46 (55.4)	37 (44.6)	83	0.32	0.19
	N	42 (63.6)	24 (36.4)	66		
Main symptoms	Y	72 (60.0)	48 (40.0)	120	0.67	0.39
	N	16 (55.2)	13 (44.8)	29		
Most effective test (sputum microscopy)	Y	79 (57.7)	58 (42.3)	137	1.37	0.19
	N	9 (75.0)	3 (25.0)	14		
Number of sputum samples needed	Y	49 (60.5)	32 (39.5)	81	0.15	0.41
	N	39 (57.4)	29 (42.6)	68		
Duration of treatment of new case	Y	82 (58.6)	58 (41.4)	140	0.73	0.45
	N	6 (66.7)	3 (33.3)	9		
4 drugs for new case	Y	9 (39.1)	14 (60.9)	23	4.46	0.03
	N	79 (62.7)	47 (37.3)	126		
Name of single anti tubercular drug	Y	23 (76.7)	7 (23.3)	30	4.81	0.02
	N	65 (54.6)	54 (45.4)	119		
DOTS strategy	Y	72 (55.0)	59 (45.0)	30	9.57	0.01
	N	16 (94.1)	1 (5.9)	17		

[a] Yes.
[b] No, figures in parenthesis indicate percentage.

amongst the workers.[10] Gupta (1997) had evaluated RNTCP and found that it had achieved satisfactory results in all the five Tubercular Units of district Kangra in Himachal Pradesh, India.[11] In another study by Gupta et al., the performance of RNTCP was evaluated for District Kangra and found a poor health management system.[12] The present study showed that the workers had poor knowledge about the duration of treatment (p value = 0.00). Similarly, Ibrahim et al. in a study in Nigeria in 2011 concluded that 71.1% of the health care workers had poor knowledge about the treatment duration.[13] The knowledge about the communicability varied from 100% to 87% across the Tubercular Units. Our study showed that the knowledge about the cause of TB, its communicability and mode of spread was high (86.6%, 85% and 86.6% respectively) in DOT Providers as compared to Non DOT Providers (13.4%, 15% and 13.4% respectively).

The present study shows that the age and years of service had no effect on the knowledge aspects. Similar findings were shown in a study conducted at Delhi by Singla et al., in 1998.[14] Our study also showed that there in not much difference in the knowledge about the aspects of tuberculosis in accordance with the number of patients being given DOTS for TB.

5. Conclusion

There is inadequate knowledge of tuberculosis, its diagnostics, treatment and DOTS strategy amongst the MPW DOT and Non DOT Providers of all the Tubercular units of the district. The lack of modular trainings has significant repercussions on the knowledge of the workers.

Recommendations

The district health authorities were recommended for holding regular Modular trainings and Refresher courses about Tuberculosis management for the MPWs.

Conflicts of interest

The authors have none to declare.

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REFERENCES

1. World Health Organization. *Global Tuberculosis Control 2015*. Geneva: WHO; 2015. Available from http://www.who.int/TB/global_report/ [cited 15.05.17].
2. TB FACTS.ORG. Information about Tuberculosis. Available from http://www.TBfacts.org/TB-statistics-India/ [cited 19.05.17].
3. World Health Organization. *Global Tuberculosis Report 2015*. 20th ed. Geneva: WHO; 2007.

4. Himachal Pradesh: Health at Glance. *Department of Health and Family Welfare*. 2006. Available from http://www.who.int/TB/global_report/ [cited 15.05.17].
5. Micro, Small & Medium Enterprises Development Institute. Brief Industrial Profile of Solan District. Government of India, Ministry of MSME. 2014–15. Available at www.msmedihimachal.nic.in [cited 09.09.17].
6. Himachal Pradesh Stat; revealing Himachal Pradesh statistically. Available from http://www.himachalpradeshstat.com/health/16/diseases/77/tuberculosis/17800/stats.aspx [cited 11.05.17].
7. Revised National Tuberculosis Control Programme. *Module for Multi Purpose Workers and other DOT Providers*. New Delhi: Central TB Division; 2005.
8. Kaur A, Kaur P, Singh R, et al. Knowledge and attitude of DOT providers and treatment outcome of TB patients of tuberculosis unit, Patiala. *Int J Health Sci Res*. 2014;4(4):1–8.
9. Jain M, Chakole SV, Pawaiya AS, et al. Knowledge, attitude and practice of DOTS providers under RNTCP in Ujjain, Madhya Pradesh. *Natl J Community Med*. 2012;3(4):670–674.
10. Hashim DS, Kubaisy WA, Dulayme AA. Knowledge, attitude and practices survey among health care workers and tuberculosis patients in Iraq. *East Mediterr Health J*. 2003;9(4):718–731.
11. Gupta SN, Gupta N. Evaluation of Revised National Tuberculosis Control Programme, District Kangra, Himachal Pradesh, India, 2007. *Lung India*. 2011;28(3):163–168.
12. Gupta SN, Gupta N, Gupta S. Surveillance data analysis of Revised National Tuberculosis Control Programme of Kangra, Himachal Pradesh. *J Fam Med Prim Care*. 2013;2(3):250–255.
13. Ibrahim ML, Hadjia IS, Nguku P, et al. Health care worker's knowledge and attitude towards TB patients under Direct Observation of Treatment in Plateau state, Nigeria. *Pan Afr Med J*. 2011;18(suppl 1):8.
14. Singla N, Sharma PP, Jain RC. Awareness about tuberculosis among nurses working in a tuberculosis hospital and in a general hospital in Delhi India. *Int J Tuberc Lung Dis*. 1998;2(12):1005–1010.

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Original article

The clinical utility of cycle of threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care center in India

Ashish K. Prakash [a,*], *B. Datta* [a], *J.P. Tripathy* [b], *N. Kumar* [c], *P. Chatterjee* [a], *A. Jaiswal* [a]

[a] *Department of Respiratory & Sleep Medicine, Medanta – The Medicity Gurgaon, India*
[b] *International Union Against Tuberculosis and Lung Disease, The Union South East Asia Office, New Delhi, India*
[c] *Department of Microbiology, Medanta – The Medicity Gurgaon, India*

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ABSTRACT

Background: There are knowledge gaps in the in-depth analysis of the most promising and robust diagnostic tool, GeneXpert MTB/RIF (CBNAAT). The cycle of threshold (CT) value of the CBNAAT test and its clinical implications has not been explored much.
Aims and objectives: The study aimed at (a) estimating the diagnostic accuracy and incremental yield of Xpert MTB/RIF in various specimens (b) establishing the association between CT value category (high, medium, low, very low) and culture time-to-positivity (TTP).
Methods: A total of 1000 samples, both pulmonary and extra-pulmonary were collected from presumptive TB cases in a large tertiary care hospital. Sensitivity and specificity of CBNAAT was calculated with culture as the gold standard. The association of CT value with culture TTP was also studied.
Results: The overall sensitivity of CBNAAT was 88.5%, with bronchial washing specimen being the most sensitive (92.3%) and pleural fluid being the least (66.7%). In smear negative individuals, the sensitivity of CBNAAT was 80.9%. The additional yield of CBNAAT over smear microscopy was 10.9%. It was observed that as we move from high to very low CT category, culture positivity decreases significantly ($p < 0.001$), whereas time taken for culture growth increases ($p < 0.001$).
Conclusion: CBNAAT is a robust test for accurate diagnosis of tuberculosis both pulmonary and extra-pulmonary, smear negative as well, especially in resource-limited settings. The correlation between CT value and culture TTP has potential in predicting bacillary load, though further studies are required.

1. Introduction

Despite significant progress, tuberculosis (TB) continues to be a global public health problem. In 2015 around 10.4 million people fell ill because of TB and 1.4 million died from TB. Over 95% of TB deaths happen in low- and middle-income countries. India shares nearly one-fourth of the global TB burden.[1] Early diagnosis and prompt treatment remains the hallmark of TB control. Smear microscopy and culture

* *Corresponding author at*: Department of Respiratory & Sleep Medicine, Medanta – The Medicity Gurgaon, India. Tel.: +91 9968014661.
E-mail addresses: ashishsinhadoctor@gmail.com, akp_vpci@yahoo.com (A.K. Prakash).
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techniques have been the mainstay of pulmonary TB (PTB) diagnosis for several decades now. While smear microscopy has poor sensitivity and quality control issues, conventional solid culture has a long turnover time up to 10–12 weeks.[2] Liquid culture techniques were developed for early detection of mycobacterial growth, but the turnaround time of 21 days is still quite substantial for a diagnostic test to curb transmission.[3]

The Cepheid Xpert MTB/RIF assay (Cepheid, Sunnyvale CA), was developed for rapid diagnosis of TB. It can detect both TB and rifampicin resistance.[4] The test is basically based on a heminested PCR test that detects the presence of *Mycobacterium tuberculosis* complex bacilli (MTB).[5] The target is an 81-base-pair region of the *rpoB*gene which is the rifampicin resistance determining region (RRDR). This is a single-use cartridge based system making it easy to operate, also called CBNAAT (Cartridge Based Nucleic Acid Amplification Test). There is no cross contamination and result can be obtained in only 100 min which can dramatically reduce the time for diagnosis of TB.[6] In a recent meta-analysis, Xpert as an initial replacement for smear microscopy showed a pooled sensitivity of 89% and specificity of 99%, and as an add-on test following a negative smear microscopy, pooled sensitivity and specificity were 67% and 99% respectively. The interpretation of the CBNAAT result is done on the basis of Cycle of Threshold (CT) value in PCR as high, medium, low and very low. CT value is a continuous variable and is inversely correlated with the concentration of the starting material.

As countries continue to improve strategies for TB case-finding, it would be helpful to know the utility of Xpert MTB/RIF in varied specimens vis-à-vis sputum culture and the incremental value of each Xpert MTB/RIF test performed. Perhaps more importantly, programs require a better understanding of how patient and specimen characteristics influence the yield of these tests, so that their utility is maximized while financial and human resource are not hardened. The present study was done at a tertiary state-of-the-art private hospital with the objective of evaluating the accuracy and incremental yield of CBNAAT assay over smear microscopy in diagnosing PTB, taking culture as the gold standard for confirmed diagnosis. The cycle of threshold (CT) value of the CBNAAT test and its role in clinical decision making in terms of its ability to predict bacillary load via culture TTP has been less researched upon. The study also attempted to correlate the CT category of CBNAAT with culture positivity and time to positivity (TTP).

2. Materials and methods

2.1. Study setting

Medanta – The Medicity Hospital is a 1250-bed multi-specialty private hospital situated in Delhi National Capital Region, India. Medanta has nine multi-specialty institutes and over 20 specialty divisions and departments, which assists with clinical care, education and research. The hospital receives patients from whole of India as well as international patients from Nepal, Burma, Bhutan, Pakistan and Middle East. The TB laboratory is equipped with sputum smear microscopy, GeneXpert, mycobacterial culture, polymerase chain reaction (PCR) diagnostics, bacterial gene sequencing for identification and drug susceptibility testing (DST).[7]

2.2. Study design

A cross-sectional study was conducted from July 2014 to March 2016.

2.3. Study participants

All presumptive cases of tuberculosis visiting the outpatient/inpatient during the study period who provided respiratory samples (bronchial washing, endotracheal tube secretions and sputum), pleural samples (pleural fluid and biopsies) and others (samples from extra-pulmonary sites, lymph node biopsies, tissue samples, etc.) constitute the study participants. A total of 1000 samples were collected.

2.4. Data collection

Samples from various sites were sent for direct smear microscopy, culture as well as CBNAAT. During period of study as CBNAAT was not available in house it was outsourced to a CAP and NABL accredited laboratory, Quest diagnostic center. Since all the samples did not undergo all the three tests due to operational issues, the numbers in each category may vary. The direct smear was done by Ziehl–Neelsen (ZN). Culture was done on liquid media (MGIT – mycobacterium growth indicator tube) and solid culture using Lowenstein–Jensen (LJ) Medium. The culture was recorded weekly for mycobacterial growth. Since culture was used as a reference for this study, samples with contaminated cultures or whose culture samples not sent were excluded.

2.5. Data analysis

Smear microscopy and CBNAAT results were compared using culture as the gold standard. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with 95% confidence intervals (CIs) for both the diagnostic tests by the type of specimen. The yield of CBNAAT, smear microscopy and culture was calculated by the type of specimen. Incremental yield of GeneXpert over AFB smear microscopy was expressed as the number and proportion of TB cases diagnosed using CBNAAT that were not diagnosed by smear microscopy, divided by the total number of presumptive TB cases that received both the tests. Chi-square test was used to study the association of CT value with culture positivity and TTP.

2.6. Ethics approval

This study was approved by the Institutional Review Board of Medanta – The Medicity Hospital and Research Institute. Prior to offering the tests, patients were informed about the study and consent was taken.

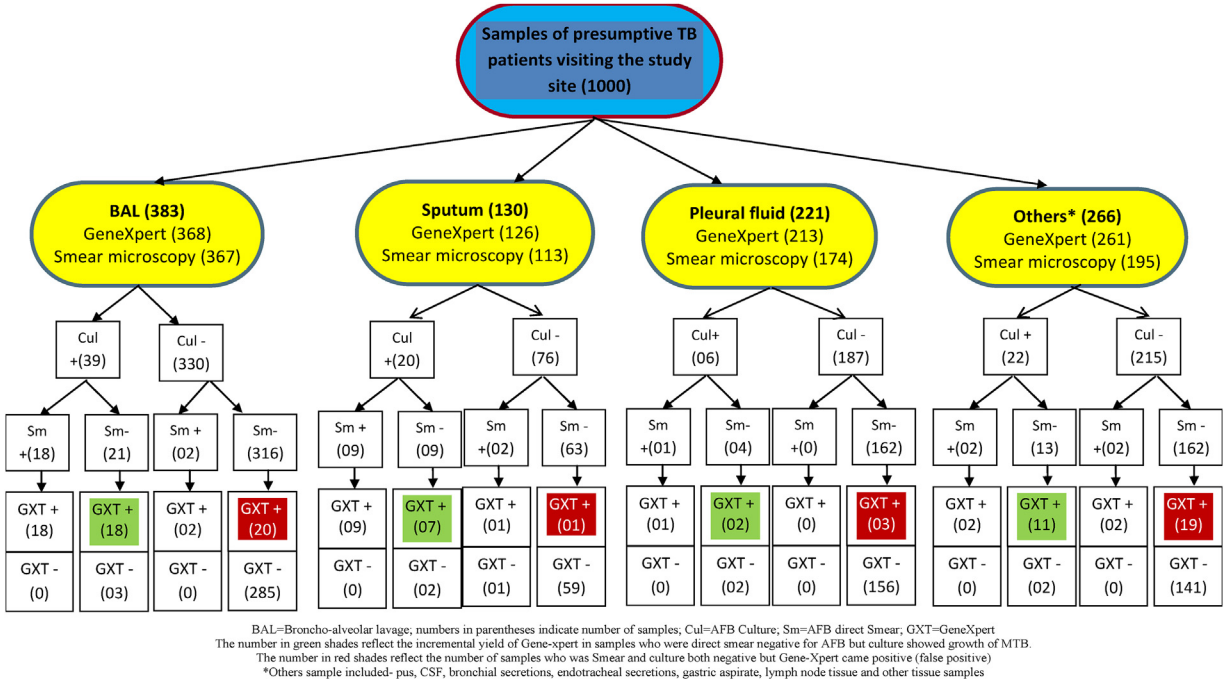

Fig. 1 – Laboratory tests performed on various specimens for tuberculosis.

3. Results

A total of 1000 samples were collected (bronchial washing – 383, sputum – 130, pleural fluid/biopsy – 221, others – 266) as showed in Fig. 1. CBNAAT detected MTB in 162 samples out of 968 (16.7%). Rifampicin resistance was detected in 25 (2.6%, 25/968) samples and 2 samples had indeterminate results. The highest yield was found in sputum specimens (29.4%, 37/126) and lowest in pleural fluid (4.2%, 9/213). The additional yield of GeneXpert over smear microscopy was 10.9%. However among pooled extra-pulmonary samples which included specimens such as pus, CSF, gastric aspirate, lymph node tissue and other tissue samples, the incremental value was 17.1% (Table 1).

The overall sensitivity of CBNAAT was 88.5% (79.9–94.3%), with bronchial washing specimen being the most sensitive (92.3%) and pleural fluid being the least (66.7%). The sensitivity of GeneXpert was much higher than that of smear microscopy (39.0%, 28.0–50.7%). The specificity of GeneXpert was 98.6% (97.6–99.2%), similar to that of smear microscopy 99.1% (98.2–99.7%) and with minimal variation between different types of specimens. In smear negative individuals, the sensitivity of CBNAAT was 80.9% (67.5–89.6%) whereas specificity was 93.7% (91.6–95.3%) (Table 2).

Among 804 samples which could not detect MTB in CBNAAT only one was AFB positive and only 10 were culture positive for MTB (all smear negative). All of them were smear-negative and the culture TTP was more than 5 weeks for most (07) of the samples in this group.

Table 1 – Diagnostic yield of CBNAAT, smear microscopy and culture and additional yield of CBNAAT over smear microscopy by type of specimen.[b]

Type of specimen	N	Gene Xpert %(+/total tested)	Smear microscopy %(+/total tested)	Additional yield[a] %(+/total tested)	AFB culture %(+/total tested)
Bronchial washing	383	16.6 (61/368)	5.7 (21/367)	11.3 (40/354)	10.6 (39/369)
Sputum	130	29.4 (37/126)	19.5 (22/113)	10.9 (12/110)	20.8 (20/96)
Pleural fluid	221	4.2 (9/213)	0.6 (1/174)	2.9 (5/171)	3.1 (6/193)
Others	266	21.1 (55/261)	2.6 (5/195)	17.1 (33/193)	9.3 (22/237)
Overall	1000	16.7 (162/968)	5.8 (49/849)	10.9 (90/828)	9.7 (87/895)

Bronchial washing; AFB – acid fast bacilli.
[a] Additional yield of CBNAAT over smear microscopy.
[b] All the samples did not undergo the entire three tests due to operational issues; hence the numbers in each category are varying.

Table 2 – Sensitivity and Specificity of CBNAAT and smear microscopy compared to culture by type of specimen and smear status.

Type of specimen	CBNAAT				Smear microscopy			
	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Bronchial washing	92.3 (79.1–98.4)	93.1 (89.7–95.6)	62.1 (52.0–71.2)	99.0 (97.1–99.7)	46.1 (30.1–62.8)	99.4 (97.8–99.9)	90.0 (68.4–97.4)	93.8 (91.8–95.3)
Sputum	90.0 (68.3–98.8)	95.9 (88.5–99.1)	85.7 (66.2–94.8)	97.2 (90.4–99.2)	50.0 (26.0–74.0)	96.9 (89.3–99.6)	81.8 (51.6–95.0)	87.5 (81.5–91.8)
Pleural fluid	66.7 (22.3–95.7)	97.8 (94.5–99.4)	50.0 (24.6–75.4)	98.9 (96.6–99.6)	20.0 (0.5–71.6)	100.0 (97.8–100.0)	100.0 (-)	97.6 (96.3–98.4)
Others	86.4 (65.1–97.1)	85.7 (80.2–90.1)	38.8 (30.4–47.8)	98.4 (95.4–99.4)	13.3 (1.7–40.5)	98.8 (95.7–99.8)	50.0 (13.1–86.5)	92.6 (91.1–93.8)
Smear negative	80.9 (67.5–89.6)	93.7 (91.6–95.3)	46.9 (36.4–57.7)	98.6 (97.4–99.3)	–	–	–	–
Overall	88.5 (79.9–94.3)	92.5 (90.4–94.2)	56.6 (50.2–62.8)	98.6 (97.6–99.2)	39.0 (28.0–50.7)	99.1 (98.2–99.7)	83.3 (68.2–92.1)	93.7 (92.6–94.7)

4. Cycle of threshold (CT) value of CBNAAT vs sputum culture positivity

Among 162 patients with CBNAAT MTB detected, 13 were in the high CT category, 52 were medium, 63 low while 34 were in very low CT category. The mean (±SD) of TTP for CT values as we move from high, medium, low and very low was 3.8(±1.4), 4.4(±1.4), 4.9(±1.1) & 5.3(±1.1) weeks respectively. We found that from high to very low CT category, direct smear and culture positivity decreases significantly ($p < 0.001$), whereas time taken for culture growth (in terms of weeks) increases ($p < 0.001$) (Table 3 and Figs. 2 and 3).

Among 13 high detected samples, culture growth was seen in 7 samples and for remaining 6 samples culture results were not available, smear was positive for AFB in 11 samples; only one sample (pleural fluid) was negative for AFB; other sample was bronchial washing for which smear result was not available. Both the samples which came high on CBNAAT and were not smear positive showed growth in culture early at 3 weeks. In the very low category, out of 34 samples, only two samples were smear positive one of which was culture negative, whereas growth was seen in 10 samples only. The two samples which was smear positive in the very low category did not showed growth on culture. On the contarary ten samples showed growth on culture had delayed TTP and were AFB smear negative. Hence there is disconcordance in very low Ct category samples (Table 4).

5. Discussion

This is the first Indian study with a large sample size to have studied the performance of Xpert MTB/RIF assay with respect to culture as the gold standard in pulmonary and extra-pulmonary specimens. The key findings of the study are: (a) high overall sensitivity which has emphasized much on CT value of CBNAAT with 88.5%, with bronchial washing specimen being the most sensitive (92.3%) followed by sputum (90.3%) and pleural fluid being the least (66.7%). (b) High sensitivity (80.9%) of CBNAAT among smear negatives. (c) Additional yield of GeneXpert over smear microscopy being 10.9%. (d) Significant correlation of CT value category with AFB direct smear, culture positivity and culture TTP ($p < 0.001$).

This assay identified more than 90% of all patients with culture-positive tuberculosis, and more than 80% of patients with smear-negative disease. This is an important finding in the context of a high TB burden country like India where rapid diagnosis of smear-negative TB cases is still a major challenge for the TB control program. The results of our study are comparable to a meta-analysis by Steingart et al. which reported pooled sensitivity and specificity of Xpert as 89% and 99% respectively and a sensitivity of 67% and specificity of 99% for smear negative TB.[8] Another recent meta-analysis also concluded that Xpert MTB/RIF assay is a sensitive and specific robust assay as compared to other conventional tests for accurate initial TB diagnosis and may be used as an add-on test to microscopy for smear negative patients.[9] Boehme et al. have also reported similar sensitivity of 77% in cases of smear negative and culture positive patients in a large multi-centric study in routine settings.[10]

Table 3 – Smear microscopy, culture results and culture time-to-positivity (TTP) by the type of CT category in CBNAAT.

CT category	N	Smear microscopy			Culture results			Culture growth at week						
		+	–	NA	+	–	NA	2	3	4	5	6	7	8
High	13	11	1	1	7	0	6	0	4	2	0	0	1	0
Medium	52	24	46	6	30	11	11	1	4	15	5	2	1	2
Low	63	11	46	6	30	28	5	0	1	9	13	6	0	1
Very low	34	2	24	8	10	20	04	0	0	3	3	2	2	0

CT – cycle of threshold; NA – not applicable.

The diagnostic performance of CBNAAT in sputum sample and bronchial washing was high with a sensitivity of 90.0% and 92.3% respectively. A large study conducted in India with genotyping, phenotyping and clinical validation of GeneXpert

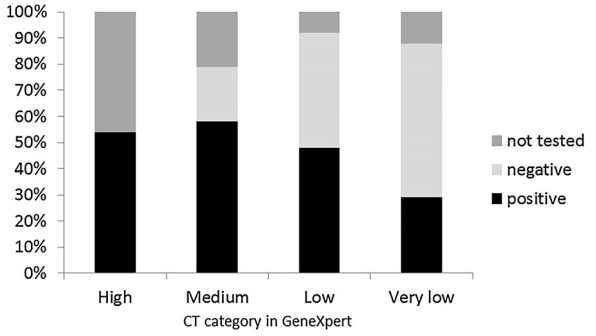

Fig. 2 – Proportion of culture positivity in different categories of CT values in CBNAAT.

Fig. 3 – Culture time-to-positivity in different categories of CT values in CBNAAT.

Table 4 – Analysis of samples of very low detected CBNAAT.

Sample	Total no	AFB smear+	AFB culture+	TTP in weeks (no)
Bronchial washings	12	0	4	4,5,6,7
Sputum	7	2[a]	3	4(2), 5
Lymph node	8	0	0	0
Pleural fluid	2	0	1	6
Others[b]	5	0	2	5,7
Total	34	2	10	

[a] The two AFB smear positive samples did not show growth on culture.
[b] Included – pus (2) and both showed growth in culture, chest wall sinus (1), thyroid tissue (1), lung tissue (1).

with large sample size of 761(EP) and 384 pulmonary specimens had shown sensitivity of 100% and 90.68%, specificity of 100% and 99.62% for pulmonary and extra-pulmonary samples.[11] Another large study in India also reported similar results for sputum and bronchial washing specimens.[12] The present study reported higher sensitivity for pleural fluid (66.7%) compared to earlier reports (can be because of better processing and centrifugation, further validation still needed) that have reported sensitivities ranging from 15% to 44% but high specificity up to 100%.[13–19] The sensitivity of other extra-pulmonary specimens (pus, CSF, gastric aspirate, lymph node tissue and other tissue samples) was similar to that of sputum specimen (86.4%). This is similar to the findings in a systematic review by Denkinger et al. which showed sensitivities in the range of 80–83% for various specimen types such as CSF, lymph node tissue or aspirates.[20] Another systematic review also reported high sensitivities ranging from 78% in gastric aspirates to 96% in lymph node tissue with a median sensitivity of 83%.[21]

Further analysis of the study data showed that CBNAAT could identify 90 (out of 828) additional cases who were smear negative among whom 38 were culture positive. This is a significant finding, particularly in the setting of a national TB control program in India where these numbers translate into millions of TB cases. In the absence of CBNAAT such patients are likely to be missed or undergo repeat testing for sputum smear microscopy and culture resulting in an unnecessary delay in initiation of treatment.

Post hoc analysis shows that a total of 59 culture negative samples were Xpert positive. Theron et al. clarified the significance of these results using short-term follow-up cultures, sequencing, and a suggestive radiologic picture saying that there was a 35% relative increase in the number of detected cases. However, larger studies are required in different settings to evaluate such discordant cases with long-term follow-up.[22]

The cycle of threshold (CT) value of the CBNAAT test and its role in predicting bacterial load mediated via culture positivity and culture TTP has been less researched upon. CT value is an arbitrarily designed cutoff point which just tells us about the concentration of target in the PCR reaction. The lower the CT value, the higher the starting concentration of DNA template and vice versa. The result is interpreted on the basis of C_T value as high (<16 cycles), medium (16–22 cycles), low (23–28 cycles) and very low (>28 cycles). The present study demonstrated a significant association between the CT category and culture positivity as well as TTP, which is a measure of bacterial load. Few other studies have supported this evidence demonstrating a good correlation between TTP and average CT values and

Box 1. CT values in clinical decision making and need for culture.

CT values	Treatment start	Clinical correlation	Culture report needed
High	+	Needed	–
Medium	+/–	Strongly needed	+/–
Low	+/–	Strongly needed	+/–
Very low	–	Needed	+

also providing a method for equating these variables, although the results of these studies were limited by small sample sizes.[22–24] These findings are important because sputum bacillary load at diagnosis is one of the strongest baseline predictors of long-term outcome among pulmonary TB patients, and CT value could thus be used for the prognostication of patients, however it needs further exploration.[25]

Though cycle of threshold is an arbitrary cutoff value in a continuous phenomenon of bacterial load, there should also be a clinical threshold to start the treatment. Based on the study results, samples with high CT value have high culture positivity rates and thus need to be put on treatment immediately without waiting for culture reports. On the contrary, if CT value is very low, we should wait for the culture reports before intervening due to poor AFB and culture positivity in those samples. In cases of medium and low CT value, clinical correlation is strongly needed to aid decision making. Though there is sizeable evidence to support clinical decision making in high and very low value CT values, there is still a gray zone regarding medium and low CT values. Further studies are needed to bridge the evidence gap in this area. There is lot of scope for further studies regarding correlation of clinical symptomatology and radiological findings vs CT values in CBNAAT. We summarize our discussion with the following suggestions (Box 1).

The study had few limitations. There is an inevitable referral bias due to the study being conducted at the tertiary care center. The study findings have limited relevance to high HIV prevalence settings. A key advantage of CBNAAT over smear microscopy is the simultaneous assessment for rifampicin resistance. However, in this study we are unable to comment on sensitivity for rifampicin resistance given that the drug susceptibility test was not done. All the samples did not undergo all three tests due to operational issues. However, a large sample size lends strength to this study and increases the generalizability.

6. Conclusions

To conclude, our study adds to the growing body of literature demonstrating the utility of CBNAAT for PTB and EPTB (extrapulmonary TB) diagnosis when applied to diverse types of clinical samples as well as among smear negatives cases. This study provides some evidence in using CT values, especially high and very low categories in making early clinical judgment regarding initiation of treatment based on its correlation with bacillary load which is mediated via culture positivity and TTP. This correlation can be further used for treatment response as surrogate to smear or culture conversion, as well as studying early bactericidal activity of new TB drugs.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. World Health Organization (WHO). *Globronchial Washing Tuberculosis Report 2016*. Geneva, Switzerland; 2016.
2. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6 (10):664–674.
3. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. *Diagn Microbiol Infect Dis*. 2005;52(3):247–254.
4. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48(1):229–237.
5. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363(11):1005–1015.
6. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol*. 2011;6(9):1067–1082.
7. Medanta – The Medicity [Internet]. Available from: http://www.medanta.org/index.aspx [cited 25.05.17].
8. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Systematic Review*. (1):2014;(1):CD009593.
9. Kaur R, Kachroo K, Sharma JK, Vatturi SM, Dang A. Diagnostic accuracy of Xpert test in tuberculosis detection: a systematic review and meta-analysis. *J Glob Infect Dis*. 2016;8 (1):32–40.
10. Boehme CC, Nicol MP, Nabeta P, et al. 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*. 2011;377(9776):1495–1505.
11. Singh UB, Pandey P, Mehta G, Bhatnagar AK, Mohan A, Goyal V, et al. Genotypic, phenotypic and clinical validation of GeneXpert in extra-pulmonary and pulmonary tuberculosis in India. *PLoS ONE*. 2016;11(2):e0149258. http://dx.doi.org/10.1371/journal.pone.0149258.
12. Sharma SK, Kohli M, Yadav RN, et al. Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. *PLOS ONE*. 2015;10(10):e0141011.
13. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the CBNAAT MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol*. 2011;49(12):4138–4141.
14. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert((R)) MTB/RIF assay. *Expert Rev Anti Infect Ther*. 2012;10(6):631–635.

15. Lusiba JK, Nakiyingi L, Kirenga BJ, et al. Evaluation of Cepheid's Xpert MTB/Rif test on pleural fluid in the diagnosis of pleural tuberculosis in a high prevalence HIV/TB setting. *PLOS ONE*. 2014;9(7):e102702.
16. Meldau R, Peter J, Theron G, et al. Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN-gamma for the evaluation of pleural tuberculosis: a prospective cohort study. *BMC Pulm Med*. 2014;14:58.
17. Friedrich SO, von Groote-Bidlingmaier F, Diacon AH. Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. *J Clin Microbiol*. 2011;49(12):4341–4342.
18. Tortoli E, Russo C, Piersimoni C, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J*. 2012;40(2):442–447.
19. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated CBNAAT MTB/RIF system. *J Clin Microbiol*. 2011;49(4):1202–1205.
20. 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):435–446.
21. Maynard-Smith L, Larke N, Peters JA, Lawn SD. Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC Infect Dis*. 2014;4(1):709.
22. Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *Am J Respir Crit Care Med*. 2011;184(1):132–140.
23. Theron G, Pinto L, Peter J, et al. The use of an automated quantitative polymerase chain reaction (Xpert MTB/RIF) to predict the sputum smear status of tuberculosis patients. *Clin Infect Dis*. 2012;54(3):384–388.
24. Theron G, Peter J, Calligaro G, et al. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for TB diagnosis using specimens from different body compartments. *Sci Rep*. 2014;4(1):5658.
25. Perrin FMR, Woodward N, Phillips PPJ, et al. Radiological cavitation, sputum mycobacterial load and treatment response in pulmonary tuberculosis. *Int J Tuberc Lung Dis*. 2010;14(12):1596–1602.

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Original article

Delineating the factors associated with recurrence of tuberculosis in programmatic settings of rural health block, Himachal Pradesh, India

Dinesh Kumar [a,*], *Chirag Goel* [b], *Avi Kumar Bansal* [c], *Ashok Kumar Bhardwaj* [d]

[a] *Department of Community Medicine, Dr. Rajendra Prasad Government Medical College, Kangra, Himachal Pradesh, India*

[b] *Model Rural Health Research Unit (MRHRU), Haroli, Una, Himachal Pradesh, India*

[c] *National JALMA Institute of Leprosy and Other Mycobacterial Diseases (NJIL&OMD), Agra, Uttar Pradesh, India*

[d] *Community Medicine, Dr. Radhakrishanan Government Medical College, Hamirpur, Himachal Pradesh, India*

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ABSTRACT

Background: Tuberculosis (TB) recurrence observed to be an important event in its treatment and has future implications under national TB control efforts. The present study was carried out to assess the recurrence rate along with its risk factors among patients undergoing treatment for TB under Revised National TB Control Program (RNTCP).

Material and methods: Total 204 patients in health block of district Una, Himachal Pradesh were studied using pretested structured interviewer-administered questionnaire. Along with univariate a non-hierarchal multi-way frequency analysis (MFA) was done to study the one and multi-way effects between the discrete variables included in a hypothesized model. The variables were under-nutrition, pulmonary TB, injecting drug use (IDU), multi-drug resistant (MDR) TB, and past TB (recurrent cases).

Results: Total 29 cases (14.2%) had recurrence (17.7/100,000 population) with significantly high fraction for alternate residence (Recurrent: 50.0%, Non-recurrent: 47.4%; $p = 0.001$), Multi-drug resistance (MDR) TB (Recurrent: 13.8%, Non-recurrent: 2.3%; $p = 0.003$), and sputum negative patients (Recurrent: 51.7%, Non-recurrent: 14.5%; $p = 0.000$). Non-recurrent cases had significantly high fraction for sputum positive cases (Recurrent: 48.3%, Non-recurrent: 72.1%; $p = 0.011$), and extra-pulmonary TB (Recurrent: 00.0%, Non-recurrent: 13.4%; $p = 0.036$). MFA observed all significant one-way effects. Significant two-way effects were IDU and pulmonary TB ($p = 0.001$), MDR and past TB ($p = 0.004$), IDU and past TB ($p = 0.019$), and IDU and MDR-TB ($p = 0.039$).

Conclusion: Proportion of TB recurrence was expected with a significant difference between the history of change of residence, MDR-TB, pulmonary and extra-pulmonary nature of the disease. Hypothesized model observed with a significant association of IDU, pulmonary TB, MDR-TB and past TB.

* *Corresponding author.*
E-mail address: dinesh9809@gmail.com (D. Kumar).

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1. Background

India harbors about thirty percent of the global tuberculosis (TB) burden and about 2 million people develop and 5,00,000 die due to the disease.[1] Evidence had reported the recurrence of TB in 10–15% of sputum positive patients across India.[2,3] Recurrence leads to additional cases for treatment, which causes almost consistent transmission of disease in their close contacts, and contributes to disease endemicity. There were a difference in the reported figures, as it was about ten percent in India, whereas, a review of multinational studies observed about four percent recurrence.[4] In an another set of studies, a considerable variation in recurrence rates of 0–14%, was reported.[5] High recurrence rate has posed a threat of the multi-drug-resistant (MDR) TB despite an effective treatment strategy. Relapse because of the same infection or due to a new strain of bacteria has considered being an important parameter to ensure the efficacy of the national tuberculosis control program.[6,7] Disease epidemiology and characteristics of the national disease control program mainly the coverage and quality of drugs has influence over the disease recurrence. Specific factors like drug irregularity, initial drug resistance, poor treatment adherence, smoking, and alcoholism observed to be associated with the recurrence.[2,8] Co-morbid conditions like under-nutrition, HIV, and diabetes mellitus (DM) are also considered to be the potential contributors for disease recurrence.[2,9]

Revised National TB Control Program (RNTCP) is the largest program in India which has subjected about 13 million patients on treatment and averted about two million deaths.[10] The program also observed with a successful decline in the caseload in state of Himachal Pradesh.[11] Since the program has shown its effectiveness but, reasons for insidious accumulation of old cases (recurrent one) need to be explored under programmatic conditions. Such a exercise was planned to assess the recurrence rate along with its risk factors among patients undergoing treatment for TB under RNTCP in a health block of Himachal Pradesh. Differential recurrence rate over health blocks along with the delineation of associated reasons for the disease recurrence were perceived to be beneficial for local managers. Due attention to the reasons will help to ensure deliberations over the new strategies to reduce disease recurrence rate in future.

2. Material and methods

A sample size of 196 was calculated with an estimated prevalence of 15.0% among TB patients with 80.0% study power and at a 5.0% level of significance. A cross-sectional study was carried out in Haroli health block of district Una, Himachal Pradesh for a period of one year. In the present study, the interview was conducted in 204 patients with TB who were living in sixty villages with a total population of 163,865. Interviewed patients were already registered under the RNTCP and were on treatment. The residential details of the patients were obtained from the RNTCP register and all patients were visited at their place of residence. A case with a past history of tuberculosis with treatment, irrespective of the source, in last one year before initiation of treatment for a the current episode of TB, was considered to be a recurrent case. A trained interviewer collected the data using a pretested structured questionnaire after obtaining an informed consent. The collected information was for basic variables like socio-demographic, economic, education, etc., and specifically for the door to window area, the presence of DM, MDR-TB, substance use like smoking, injecting drug use (IDU) and alcohol, and anthropometries like height and weight.

Data were entered and analyzed in Epi-Info (version 7) for windows. Student *t*-test and χ^2-test were used to test the statistically significant difference in the distribution of continuous and discrete variables respectively. Thereafter, a hypothetical model was developed with an association between the past-history of TB (recurrent cases), under-nutrition, IDU, Pulmonary TB, and MDR-TB. All four variables included in the model were discrete in nature. A log-linear technique, non-hierarchical, multi-way frequency analysis (MFA) was carried out to study the significance of one-way or higher order effect in the model. The analysis was chosen due to its non-parametrical nature without any distributional assumptions. The analysis assessed relationship among four discrete variables where none was considered as a dependent variable. The model was assessed for its fitness with the data and further analysis was carried out to delineate the different combinations of variables that had a significant effect on the fitted model, once removed. Prior approval of Institution Ethics Committee (IEC) was obtained before initiation of the study.

3. Results

Of surveyed patients, about thirty-eight percent of cases were living in villages where the proportion of the socially disadvantaged population was relatively high (>19.0%), though its distribution was statistically similar in both the groups of recurrent and non-recurrent cases. In the study area, a total 29 patients had recurrent TB (14.2%), with a burden of 141/100,000 for all cases, 94/100,000 for incident cases, and 17.7/100,000 population for recurrent cases. Patients had a mean age of 43.9 years (range: 18–90 year) and was statistically similar in both groups. Most (66.2%) of the patients were males, which were similar in both the groups. Almost half of the patients had the history of stay at other than the present address and its proportion was significantly high ($p = 0.001$) in recurrent cases. The frequency of change of residence was more in recurrent (4.1 ± 3.4) than the non-recurrent (1.0 ± 4.4) cases. Overall, patients were staying in the houses with average of four rooms (including kitchen) (Recurrent: 2.8 ± 1.6; Non-recurrent: 3.8 ± 1.8; $p = 0.960$). Separate kitchen facility was present in the majority (about 75%) of the houses with the statistical similarity in both the groups. The average area of windows and doors were about one-fourth of the entire house (Recurrent: 27.8 ± 11.1; Non-recurrent: 26.5 ± 19.9; $p = 0.814$). There was no statistical difference in the presence of type-2 diabetes mellitus (DM). Current smokers and alcohol users were statistically indifferent but cases using IDU for pleasure was observed to high in non-recurrent cases as compared to recurrent cases (Table 1).

Table 1 – Demographic and house profile of patients with Tuberculosis (TB) in the health block of district Una, Himachal Pradesh, 2016–17.

Variables	Recurrent (29)	Non-Recurrent (175)	p value	Total (204)
Village with socially deprived proportion ≥19.0%	33.3	38.6	0.600	37.9
Male (%)	53.3	77.3	0.550	66.2
Age in years (Mean ± SD)	43.9 (16.8)	43.1 (17.7)	0.868	43.9 (18.9)
Age group (%)				
<20	3.7	8.2	0.627	7.6
21-40	33.3	38.6	0.653	37.9
41-60	37.0	39.2	0.878	38.9
61-80	22.2	11.7	0.278	13.1
>80	3.7	2.3	NC	2.5
Resided other than the current address (%)	50.0%	47.4	0.001*	49.7
Average number of times change of place (Mean ± SD)	4.1 (3.4)	1.0 (4.4)	0.076	1.2 (4.2)
Average rooms with kitchen in the house (Mean ± SD)	2.8 (1.6)	3.8 (1.8)	0.960	3.8 (1.8)
Separate Kitchen (%)	73.3	76.7	0.627	75.6
Commonly used cooking fuel (%)				
Firewood/Cow-dung	40.0	42.0	0.927	40.5
LPG	53.3	42.3	0.342	45.6
Average area of windows/doors (Mean ± SD)	27.8 (11.1)	26.5 (19.9)	0.814	26.6 (18.8)
Door and window area ≤25.0% (%)	53.8	62.6	0.397	61.3
Known case of type-2 DM (%)	14.8	17.1	0.772	16.8
Current Smoker (%)	10.3	14.0	0.598	13.4
Currently consuming alcohol (%)	11.1	4.7	0.172	5.5
IDU for pleasure (%)	3.7	18.6	0.053	16.6

NC, not calculated.

Overall, the fraction of MDR-TB was 4.0% and was significantly high (p = 0.003) in recurrent cases (13.8%) as compare to non-recurrent cases (2.3%). All cases were pulmonary in recurrent cases and about seventy-two percent in non-recurrent cases; sputum positive were high in non-recurrent (p = 0.011) and negative in recurrent cases (p = 0.000). EPTB cases were observed only in the non-recurrent group. In pulmonary cases, cases with high bacterial load (≥1 acid-fast bacilli per oil immersion field) were statistically high (p = 0.021) in recurrent (58.8%) as compared to the non-recurrent group. Almost all cases were receiving treatment form government facilities (Table 2).

Assessment of self-reported social participation found that almost all had perceived enough social support. It was more from the caregivers (Recurrent: 100.0%; Non-recurrent: 93.0%; p = 0.367) than from the village members (Recurrent: 96,3%; Non-recurrent: 87.8%; p = 0.400). Assessment for participation in the recreational activities found that 68.3% had participation before the disease and it was high (p = 0.000) in non-recurrent cases (75.6%). Very few (3.5%) were devoid of participation after the disease but it turned out to be significantly high (p = 0.006) in recurrent cases (14.8%) (Table 2). Assessed for anthropometry, it was found that the majority (39.9%) had normal body mass index (BMI) and 36.8% were undernourished (Recurrent: 51.9%; Non-recurrent: 34.3%; p = 0.071). Overweight and obesity were observed in 12.4% and 10.9% cases without any significant difference in recurrent and non-recurrent cases (Table 3).

The MFA was done with a well-fitted model with the inclusion of under-nutrition, current IDU, pulmonary TB, MDR-TB and recurrent TB. One-way with higher order (df: 31, p = 0.000) and two-way with higher order effects (df: 26, p = 0.000) were observed to be statistically significant, so removal of one-way and two-way effects had a significant effect on the model fit. The partial association observed that all the four variables had a significant (p = 0.000) one-way effect. The variables with a significant two-way effects were, IDU and pulmonary TB (p = 0.001), MDR and recurrent TB

Table 2 – Disease and behavior for treatment in patients with Tuberculosis (TB) in health block of district Una, Himachal Pradesh, 2016–17.

Variables	Recurrent (29)	New (175)	p value	Total (204)
Multi-Drug Resistance Tuberculosis (%)	13.8	2.3	0.003*	4.0
Pulmonary (%)	100.0	86.6	0.079	88.6
Sputum Positive	48.3	72.1	0.011*	68.7
Sputum negative pulmonary cases (%)	51.7	14.5	0.000*	14.4
High load of bacteria (%)	58.8	30.6	0.021*	34.0
Extra-Pulmonary TB	0.0	13.4	0.036*	11.4
Treatment from government facilities (%)	93.3	96.5	0.707	95.5
Received enough support from care givers (%)	100.0	93.0	0.367	94.0
Received enough support from village members (%)	96.3	87.8	0.400	86.9
Participation in recreational activities before TB (%)	22.2	75.6	0.000*	68.3
Devoid of participation in recreational activities after TB (%)	14.8	1.7	0.006*	3.5

Table 3 – Body mass index (BMI) of patients with Tuberculosis (TB) in health block of district Una, Himachal Pradesh, 2016–17.

Variables	Recurrent (29)	New (175)	p value	Total (204)
Undernourished: < 18.5 Kg/m^2	51.9	34.3	0.071	36.8
Normal: 18.5–23.9 Kg/m^2	25.9	42.2	0.134	39.9
Overweight: 24.0–27.5 Kg/m^2	18.5	11.4	0.562	12.4
Obesity: >27.5 Kg/m^2	3.7	12.0	0.292	10.9

($p = 0.004$), IDU and recurrent TB ($p = 0.019$), and IDU and MDR-TB ($p = 0.039$). Significant parameter estimates were observed for all one-way effects except for recurrent TB ($p = 0.052$), whereas only two-way effects, IDU and MDR TB was found to be significant ($p = 0.008$). However, the parameter estimate was marginally significant for the two-way effects, MDR and recurrent TB ($p = 0.052$). Backward elimination statistics observed a significant effect on the model upon removal of two-way effect, IDU and pulmonary TB at the ninth step with a non-significant likelihood ratio (1.047) and Pearson chi-square (0.666) test statistics.

4. Discussion

Present study observed about fourteen percent patients had recurrent TB, which had statistical similarity with non-recurrent cases for their average age, gender distribution, housing conditions, average area for ventilation, type of fuel used for cooking, and co-morbid conditions. The difference was found for the history of residing other than the current address where the frequency of change of residence was more in recurrent than the non-recurrent cases. MDR-TB, sputum positivity and high bacterial load were significantly high in recurrent cases. Lack of social participation in recreational activities was common in recurrent that the non-recurrent cases.

In the current study, the fraction of recurrent cases observed to be similar as observed by the earlier studies.[12–14] Evidence also stated an association of high rate of recurrence of TB with the presence of type-2 DM and smoking,[15–17] whereas, current study observed statistically indifferent proportion for DM and smoking in both recurrence and non-recurrence cases. Stigma related to TB has received quite well deserved attention from various national and international agencies. In the year 2016, the meeting in The Netherlands discussed stigma measurement.[18] The current study revealed that almost all patients reported about positive social support but there was a lack of participation in recreational activities, which was more in patients with TB recurrence. However, the patients reported positive social support but lack of participation in recreational activities, more in recurrent cases observed a gap in social attitude and practice.

Current study reported a relatively better performance of the program as recurrence rate was quite similar to earlier studies. There had been a better uptake of the government health care facilities by the patients with TB. However, the history of current smoking during the disease and even the IDU observed to be a concern for local program managers. Additionally, under-nutrition was quite high in all patients; more in recurrent (51.9%) as compare to non-recurrent (34.3%) cases. Evidence has observed that the under-nutrition, sputum-positivity, and smoking are well-associated host-related factors with TB recurrence.[8,12,15,19] Drug resistance and high bacterial load were also observed to be associated with recurrence.[12,13] Current study observed a similar prevalence for current smoking, alcohol, and diagnosed case of type-2 DM between recurrent and non-recurrent cases but MDR-TB, IDU, and high bacterial load observed to be significantly more in recurrent cases. The MFA observed that the IDU and MDR-TB each had significantly associated with recurrent TB, whereas, IDU had also observed to be associated with pulmonary and MDR-TB independently.

Most of the recurrence cases were mostly pulmonary and manifested again within a median period of five to six years of the last completed treatment.[20,21] The duration of recurrence depends on the disease epidemiology as studies in low-incidence countries reported a TB recurrence ranging from 70 to 410/10,000 population, which was more in early years of the follow-up period.[21–23] It is well studied that the recurrence rate observed to be different in the settings of varied disease epidemiology like high versus low incidence of TB and adequate treatment to the patients.[23] Current study area observed a recurrence rate of 17.7/100,000 population with TB incidence of 94/100,000 population. In the studied population, the recurrence of TB certainly appears to be relatively low but a cohort study would be more reasonable.

The generated findings in the current study certainly have limitation due to the low case to variables ratio, which in turn affected the expected cell frequency. Follow-up a current cohort will disentangle the actual risk factors for recurrence like consistent use of smoking, IDU use, loss to follow-up, primary or secondary infection. A cross-sectional study, due to its inherent limitations used to generate the research questions in the contextual settings, e.g. Does IDU is an independent risk factor for recurrence of TB in the study area? Such consistent efforts will help to give a specific recommendation to national TB control program to design a new approach to tackle recurrence in the local settings. The program generates a vast database at the patient level but usually limits itself to this sort of analysis due to its own limitations, whereas, such an in-depth patient or programmatic level of analysis will certainly help the program managers.

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Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2018.07.001.

REFERENCES

1. Jha P, Gajalakshmi V, Gupta PC, et al. Prospective study of one million deaths in India: rationale, design, and validation results. *PLoS Med*. 2006;3(2):e18.
2. Mehra RK, Dhingra VK, Nish A, Vashist RP. Study of relapse and failure cases of CAT I retreated with CAT II under RNTCP–an eleven year follow up. *Indian J Tubercul*. 2008;55(4):188–191.
3. Mukherjee A, Sarkar A, Saha I, Biswas B, Bhattacharyya PS. Outcomes of different subgroups of smear-positive retreatment patients under RNTCP in rural West Bengal, India. *Rural Rem Health*. 2009;9(1):926.
4. Hill AR, Manikal VM, Riska PF. Effectiveness of directly observed therapy (DOT) for tuberculosis: a review of multinational experience reported in 1990-2000. *Medicine (Baltim)*. 2002;81(3):179–193.
5. Cox HS, Morrow M, Deutschmann PW. Long term efficacy of DOTS regimens for tuberculosis: systematic review. *BMJ*. 2008;336(7642):484–487.
6. Chiang CY, Riley LW. Exogenous reinfection in tuberculosis. *Lancet Infect Dis*. 2005;5(10):629–636.
7. Verver S, Warren RM, Beyers N, et al. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. *Am J Respir Crit Care Med*. 2005;171(12):1430–1435.
8. Thomas A, Gopi PG, Santha T, et al. Predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India. *Int J Tubercul Lung Dis*. 2005;9(5):556–561.
9. Narayanan S, Swaminathan S, Supply P, et al. Impact of HIV infection on the recurrence of tuberculosis in South India. *J Infect Dis*. 2010;201(5):691–703.
10. Verma R, Khanna P, Mehta B. Revised national tuberculosis control program in India: the need to strengthen. *Int J Prev Med*. 2013;4(1):1–5.
11. Gupta SN, Gupta N, Gupta S. Surveillance data analysis of revised national tuberculosis control program of Kangra, Himachal Pradesh. *J Fam Med Prim Care*. 2013;2(3):250–255.
12. Moosazadeh M, Bahrampour A, Nasehi M, Khanjani N. The incidence of recurrence of tuberculosis and its related factors in smear-positive pulmonary tuberculosis patients in Iran: a retrospective cohort study. *Lung India*. 2015;32(6):557–560.
13. Cox H, Kebede Y, Allamuratova S, et al. Tuberculosis recurrence and mortality after successful treatment: impact of drug resistance. *PLoS Med*. 2006;3(10):e384.
14. Huyen MN, Buu TN, Tiemersma E, et al. Tuberculosis relapse in Vietnam is significantly associated with Mycobacterium tuberculosis Beijing genotype infections. *J Infect Dis*. 2013;207(10):1516–1524.
15. Anaam MS, Ibrahim MI, Al Serouri AW, Bassili A, Aldobhani A. A nested case-control study on relapse predictors among tuberculosis patients treated in Yemen's NTCP. *Public Health Action*. 2012;2(4):168–173.
16. Khanna A, Lohya S, Sharath BN, Harries AD. Characteristics and treatment response in patients with tuberculosis and diabetes mellitus in New Delhi, India. *Public Health Action*. 2013;3(suppl 1):S48–S50.
17. Przybylski G, Dabrowska A, Golda R, Gadzinska A, Trzcinska H. The analysis of smoking tobacco of patients with tuberculosis–data from ten years observation in Regional Center of Pulmonology in Bydgoszcz. *Przegl Lek*. 2012;69(10):953–957.
18. Macintyre K, Bakker MI, Bergson S, et al. Defining the research agenda to measure and reduce tuberculosis stigmas. *Int J Tubercul Lung Dis*. 2017;21(11):87–96.
19. Sadikot RT. Identifying patients at high risk of tuberculosis recurrence. *Int J Mycobacteriol*. 2016;5(suppl 1):S66.
20. Hung CL, Chien JY, Ou CY. Associated factors for tuberculosis recurrence in Taiwan: a nationwide nested case-control study from 1998 to 2010. *PLoS One*. 2015;10(5). e0124822.
21. Korhonen V, Soini H, Vasankari T, Ollgren J, Smit PW, Ruutu P. Recurrent tuberculosis in Finland 1995-2013: a clinical and epidemiological cohort study. *BMC Infect Dis*. 2017;17(1):721.
22. Dobler CC, Crawford AB, Jelfs PJ, Gilbert GL, Marks GB. Recurrence of tuberculosis in a low-incidence setting. *Eur Respir J*. 2009;33(1):160–167.
23. Panjabi R, Comstock GW, Golub JE. Recurrent tuberculosis and its risk factors: adequately treated patients are still at high risk. *Int J Tubercul Lung Dis*. 2007;11(8):828–837.

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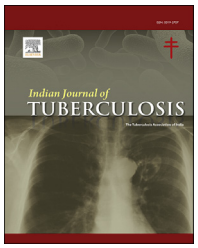

Original article

Treatment seeking pathways in pediatric tuberculosis patients attending DOTS centers in urban areas of Delhi☆

S. Dhaked [a,*], N. Sharma [b], K.K. Chopra [c], A. Khanna [d], R. Kumar [b]

[a] Department of Community Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi, India
[b] Department of Community Medicine, Maulana Azad Medical College, New Delhi, India
[c] New Delhi Tuberculosis Centre, India
[d] Lok Nayak Hospital Chest Clinic, Delhi, India

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ABSTRACT

Background: The treatment seeking pathways prior to initiation of Direct Observed Treatment Short-course Therapy (DOTS), provides the extent of patient and health system delays among pediatric tuberculosis (TB) patients.

Objectives: The study attempted to understand the treatment seeking pathways of pediatric TB patients under revised national tuberculosis control program (RNTCP).

Study design and setting: It was a prospective observational study carried out from January 2015 to December 2015. A predesigned, pretested and semi-structured questionnaire was used to interview 141 caregivers of pediatric patients (0–14 years) at two chest clinics selected purposively.

Results: Thirteen different treatment seeking pathways were identified and fever was the commonest symptom (41.8%) for seeking care from 1st health facility. Median time taken from onset of symptoms to first consultation varied from 1 to 144 weeks. More than half of the study subjects were first taken to a private practitioner (64.5%) followed by a pharmacist (19.1%) and trust in provider was the commonest reason for choosing the first care-provider in 52 (41.1%), followed by easy access or convenience in 49 (34.8%).

Conclusion: A significant delay was found in treatment initiation of patients with extra pulmonary tuberculosis (EPTB), those belonging to lower socio-economic class families, low literacy level of parents, who went to private facility first and availed more than three health facilities before diagnosis.

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* *Corresponding author*. Tel.: +91 9910120989.
E-mail address: sunitadhaked@gmail.com (S. Dhaked).

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1. Introduction

India accounts for one fourth of the global tuberculosis (TB) burden. In 2015, an estimated 28 lakh cases occurred and 4.8 lakh people died due to TB. The proportion of children among new TB patients reported was 6% in 2016. In a state like Delhi, more than 10% TB patients were children.[1] Tuberculosis in children is mainly due to failure of TB control in adults.[2] This orphan disease exists in the shadow of adult TB and is a significant child health problem, but is neglected because as cases are usually smear negative it is considered to make a relatively minor contribution to spread.[3] Diagnostic difficulties associated with pediatric TB could be contributing to overall diagnostic delays and thus an increased period of infectiousness in pediatric patients.[4] Health system delays included misdiagnosis and parents being sent to multiple institutions before having their child attended by a facility that was properly equipped to test the child for TB.[5] In India, health care is predominantly sought from the private sector.[6] A large majority of the private sector are individual providers, who are both formal (qualified) and informal (nonqualified) providers.[7,8] Symptoms of TB are such that patients often mistake it for routine illness and tend to seek first clinical advice either from a retail chemist or an informal provider - as they would do for other illnesses. Temporary relief followed by recurrence of same symptoms, drives the patient to seek clinical opinion once again from the same provider or other similar providers. Confounded by persistent symptoms without any relief from a number of providers, the patients may finally turn up at the appropriate health facility (like DOTS center) at a later stage of their disease.[9] The present study documented the treatment seeking pathway prior to initiation of Direct Observed Treatment Short-course Therapy (DOTS), providing the extent of patient and health system delay among pediatric TB patients in Delhi and the factors associated with such delays.

2. Methods

It was a cross sectional study conducted at two chest clinics viz. Karawal Nagar Chest clinic (13 DOTS-Direct Observed Treatment Short-course Therapy centers and 7 DMCs – Designated Microscopy Centers) and at Lok Nayak chest clinic (7 DOTS centers and 3 DMCs) which are located in eastern and central part of Delhi, respectively. It was carried out from January 2015 to December 2015. All Pediatric (up to 14 years.) tuberculosis patients who were referred for registration from the selected chest clinics to respective DOTS centers, during February–April 2015 were included in the study and seriously ill cases or those referred for admission were excluded from the study. A predesigned, pretested, semi-structured questionnaire was used.

Statistical analysis: The data was entered in MS-Excel and analyzed by using SPSS software version 17. Qualitative data was expressed in percentages with 95% confidence interval and quantitative data was expressed in mean ± Standard Deviation (SD). Chi square test/Fisher's Exact test was used for qualitative variables. P-value < 0.05 was considered significant.

Ethical consideration: The study was approved by the ethical committee of Maulana Azad Medical College. A written and informed consent was taken from guardian of all subjects and assent was taken from 7 to 14 years old subjects. No pressure was exerted on subjects for participation. Confidentiality and privacy was ensured at all stages of the study period. Caregivers were counseled regarding the importance of completion of treatment and its benefits.

3. Results

3.1. *Socio-demographic features*

Out of the total study subjects, 107 (75.9%) were registered at Karawal Nagar chest clinic and 34 (24.1%) at Lok Nayak chest clinic. Extra-pulmonary TB [99 (70.2%)] were almost three times more than pulmonary TB [42 (29.8%)]. Majority 69 (48.9%) of the families of the patients belonged to lower middle class, according to modified BG Prasad's (2015) classification. Majority of the primary care givers were mother [84 (59.7%)] of the children, however 48 (34.0%) cases were primarily taken care of by fathers in relation to giving medication and taking child to clinic, as mothers were not considered competent for such care.

3.2. *Treatment seeking pathways of pediatric TB patients*

3.2.1. *First symptom for which health care facility visited*

The commonest symptom, which made the caregivers of pediatric patients seek health care facility/personnel was fever in 59 (41.8%), followed by nodular skin swelling in 38 (27.0%) and cough in 24 (17.0%). Cough was the commonest symptom 24 (57.1%) for pulmonary tuberculosis patients and fever was the commonest symptom, in 41 (41.4%) for extra-pulmonary patients for which they were taken to first health facility by the caregivers.

3.2.2. *Major reasons for choosing first health care facility*

Trust in provider was the commonest reason cited by the caregivers for choosing the first care-provider in 52 (41.1%), followed by easy access or convenience in 49 (34.8%). Commonest reason for going to a private facility first, was trust in provider in 52 (44.1%) followed by easy access in 45 (38.1%). The reasons cited for seeking government health facility first were, low cost in 11 (47.8%) followed by trust in services in 6 (26.1%).

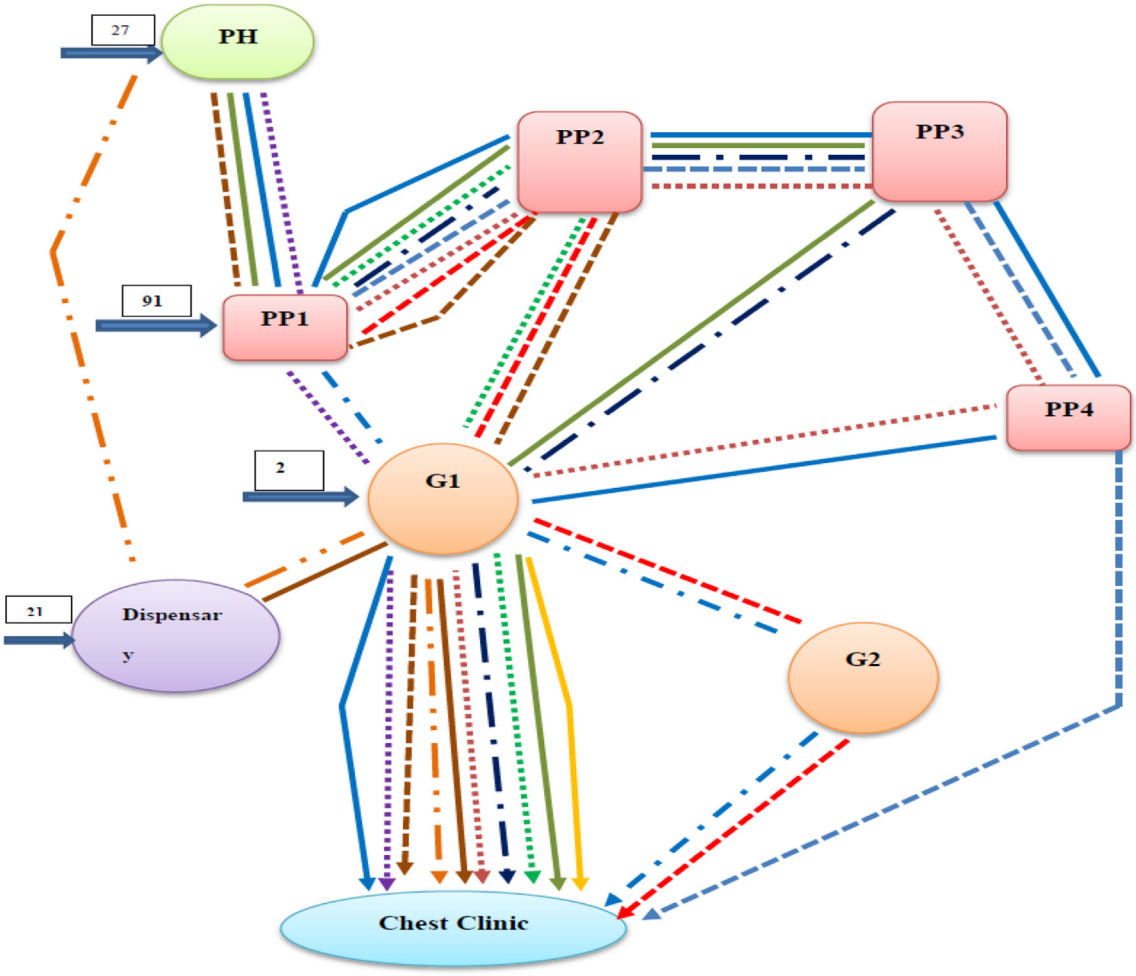
3.2.3. *First source of treatment*

More than half of the study subjects were first taken to a private practitioner for health care i.e. by 91 (64.5%) followed by a pharmacist by 27 (19.1%) and public clinic/health center/dispensary by 21 (14.9%). Only two (1.4%) patients were directly taken to a higher level government facility (they had altered sensorium and were diagnosed as TB meningitis), because of the severity of patient's condition as perceived by the caregivers.

3.2.4. *Treatment seeking pathways for TB diagnosis and initiation of treatment*

Treatment seeking pathways were traced from the onset of symptoms to final diagnosis and initiation of treatment for pediatric TB patients at the chest clinic. Thirteen different pathways were identified as given in Fig. 1. and Table 1.

As shown in Table 2, median time taken from onset of symptoms to diagnosis ranged from 4 to 162 weeks. Majority i.e. 97 (68.8%) were referred by another government facility while 31 (22.0%) were referred by self/relative/friend/neighbor followed by private practitioners i.e. 13 (9.2%). Mean number of sources visited by the caregivers prior to diagnosis were



Note:

PH1, Pharmacist visited by the caregivers of patients

PP1, PP2, PP3, PP4 First, second, third and fourth private facility visited by the caregivers of patients

G1 and G2, first & second government health facility visited by the caregivers of patients

CC - chest clinic

→ No. in the box present above the arrow depicts no. of patients visiting a facility directly

Fig. 1 – Treatment seeking pathways for pediatric TB patients.

Table 1 – Treatment seeking pathway followed by the caregivers of the patients.

Pathway diagram	Pathway	Number N (%)
	G1→CC	2 (1.4)
	PP1→G1→G2→CC	2 (1.4)
	PP1→PP2→G1→G2→CC	7 (5.0)
	PH1→PP1→PP2→PP3→G1→CC	6 (4.3)
	PP1→PP2→G1→CC	22 (15.6)
	PP1→PP2→PP3→G1→CC	28 (19.9)
	PP1→PP2→PP3→PP4→CC	3 (2.1)
	PP1→PP2→PP3→PP4→G1→CC	27 (19.1)
	Dispensary→G1→CC	20 (14.2)
	PH1→Dispensary→G1→CC	3 (2.1)
	PH1→PP1→PP2→G1→CC	4 (2.8)
	PH1→PP1→G1→CC	9 (6.4)
	PH1→PP1→PP2→PP3→PP4→G1→CC	8 (5.7)

3.78 ± 1.196 and was significantly higher for extra pulmonary tuberculosis (EPTB) patients (4.23 ± 1.1) as compared to pulmonary tuberculosis (PTB) patients (2.71 ± 0.55) (P < 0.005).

Patient delay in seeking treatment was found to be significantly higher for the female children, having EPTB and presented with non-specific complains like nodular swelling, skin lesion, eye redness etc., who resided in JJ clusters and resettlement colonies and belonged to families of low socioeconomic status. (P-value < 0.05) (Table 3).

Health facility delay was significantly lower in patients who first taken to Govt. facility and visited less than 3 health facility before reaching to TB facility hospital/chest clinic. (P-value < 0.05) (Table 4).

On binary logistic regression, factors which were significantly associated with patient delay in treatment seeking were, female child, having EPTB and who were taken to private facility first and factors which were significantly associated with the health facility delay were children aged more than 6 years of age (Table 5).

Table 2 – Different time intervals taken by the caregivers between recognition of the first symptoms and start of treatment (in weeks).

Measure	T1[a]	T2[b]	T3[c]	T2+T3[d]	T (T1+T2+T3)[e]
Range	1–144	2–92	1–8	3–96	4–162
Median	10	12	2	15	28
Q1–Q3	8–20	8–16	2–3	10–20	18.5–37.5

[a] Onset of symptoms to the first consultation at any health facility (T1) (in weeks).
[b] First health facility to referral to a TB facility (T2) (in weeks).
[c] Referral to TB facility till the diagnosis and initiation of treatment (T3) (in weeks).
[d] Time taken from first consultation to diagnosis (T2 + T3) (in weeks).
[e] Total time included from onset of symptoms to diagnosis (T1 + T2 + T3) (in weeks).

4. Discussion

Due to paucity of data on pediatric TB patients, the studies conducted on the adult TB population have been compared with the present study.

Conventionally, data on delay is divided by mean or median as there is no consensus on cut-off for different types of delay among different populations. In present study private practitioner was the first source visited for treatment by more than

Table 3 – Distribution of study subjects according to factors associated with patient delay in seeking treatment.

Factors		Patient delay ≤10 weeks N (%)	Patient delay >10 weeks N (%)
Gender**	Male	37 (46.8)	14 (22.6)
	Female	42 (53.2)	48 (77.4)
Locality* (urban)	JJ cluster, Resettlement colony	50 (63.3)	50 (80.6)
	Regular colony, Walled city, Unauthorized colony	29 (36.7)	12 (19.4)
Socio-economic status*	Lower and lower middle	62 (78.5)	57 (91.9)
	Middle and upper	13 (21.5)	5 (8.1)
Type of TB**	EPTB	45 (57.0)	54 (87.1)
	PTB	34 (43.0)	8 (12.9)
Symptoms for which first health facility visited**	Nodular swelling, skin lesion, eye redness etc.	10 (12.7)	31 (50.0)
	Cough, fever, abdominal pain, bone/joint pain, chest pain, altered sensorium, breathlessness	69 (87.3)	31 (50.0)
1st health facility availed**	Govt. facility	22 (27.8)	1 (1.6)
	Private, Pharmacist	57 (72.2)	61 (98.4)
No. of health facilities availed**	≤3	46 (58.2)	12 (19.4)
	>3	33 (41.8)	50 (80.6)
Distance from Govt. facility#,**	>3 km.	21 (95.7)	1 (4.3)
	≤3 km.	57 (48.3)	61 (51.2)

Chi square test used to compare the groups. *P < 0.05, **P value < 0.005, #Fischer's exact test was used.

half of caregivers of study subjects (64.5%) followed by a pharmacist. Studies in adult TB patients in India showed that more than half of TB patients in India first approach the private health care sector, where TB is often not diagnosed and treated successfully.[10,11] Symptoms of TB are not specific and patients are unable to determine the seriousness of the illness. They tend to seek first clinical advice either from a retail chemist or an informal provider - as they would do for other illnesses. Reports suggest that even formal private providers indulge in irrational treatment protocols, without proper diagnosis.[12] Trust in provider was the commonest reason cited by the caregivers for choosing the first care-provider in about half of the caregivers (41.1%), followed by easy access or convenience in 34.8%. However the study on adult TB patients by Grover et al reported the commonest reason for going to first health facility was, easy access and a known provider.[10] In the present study approximately two third of the tuberculosis patients (68.8%) were referred to a TB facility by another Govt. facility and in the study by Grover et al, adult EPTB patients showed similar results as 68% patients were referred by another Govt. facility, thereby indicating the better take-up or implementation of the programme in the government sector.[10] Referral by the private practitioners was only 13 (9.2%) in the present study and is a matter of grave concern as they are the first health-care providers for the majority. In the present study median patient delay in seeking care was found to be 10 weeks, (onset of symptoms to the first consultation at any health facility). Various studies in adult EPTB patients have also reported median patient delay of 12.8 weeks (90 days) in Ethiopia and 8.6 weeks in New York.[13,14] However most of the studies in

Table 4 – Distribution of study subjects according to factors associated with health facility delay in diagnosis and initiating treatment.

Factors		Health facility delay ≤ 15 weeks N (%)	Health facility delay > 15 weeks N (%)
Age*	Less than 6 years	5 (6.7)	12 (18.2)
	More than 6 years	70 (93.3)	54 (81.8)
Locality* (urban)	JJ cluster, Resettlement colony	46 (60.5)	54 (83.1)
	Regular colony, Walled city, Unauthorized colony	29 (39.5)	12 (16.9)
Family size*	≤3	30 (40.0)	45 (24.2)
	>3	16 (60.0)	50 (75.8)
Socio-economic status*	Lower and lower middle	59 (78.7)	60 (90.9)
	Middle and upper	16 (21.3)	6 (9.1)
Type of TB**	EPTB	41 (54.7)	58 (87.9)
	PTB	34 (45.3)	8 (12.1)
1st health facility availed**	Govt. facility	20 (26.7)	3 (4.5)
	Private, Pharmacist	55 (73.3)	63 (95.5)
Distance from higher Govt. facility#,**	>3 km.	20 (87.0)	3 (13.0)
	≤3 km.	55 (46.6)	63 (53.4)

Chi square test used to compare the groups. *P < 0.05, **P value < 0.005, #Fischer's exact test was used.

Table 5 – Predictors of patient delay and health facility delay of more than the median value.

Factors		P-value	Odds ratio	95% CI
Patient delay	Gender (female)	0.003	3.992	1.599–9.965
More than 10 weeks	Extra pulmonary TB	0.016	3.765	1.277–11.10
	Symptoms like nodular swelling, skin lesions, eye redness etc.	0.007	3.958	1.468–10.668
	First health facility is private practitioner or pharmacist	0.040	9.711	1.109–85.019
Health facility delay more than 15 weeks	Extra pulmonary TB	0.0001	6.775	2.426–18.914
	Age more than 6 years	0.015	5.113	1.374–19.026

adult TB patients reported lesser median patient delay as compared to children such as 6 weeks in Norway and 1.6 weeks in Vietnam, while a study done in India on adult EPTB patients reported 3.5 weeks of median patient delay.[15,16,10] This longer delay could be because symptoms of TB in children are non-specific in nature as compared to adults. The median health facility delay observed was 15 weeks which was significantly higher in EPTB patients (16 weeks) as compared to PTB patients (10 weeks). A much lower health facility delay as compared to our study was reported from studies in Ethiopia and Norway was 1.5 weeks and 6 weeks respectively.[13,15] Adult EPTB patients in India reported median health facility delay as 4.5 weeks which is lesser than the present findings in children.[10] The reason for these findings could be the lack of knowledge and non-specific symptoms of childhood TB (mostly in extra pulmonary type) in health care providers. Highest patient and health facility delay was seen in patients in whom site of EPTB was skin, which could be because of non-specificity of symptoms. However the study by Grover et al reported the highest delay in adult patients with ocular TB but the delay was lesser as compared to pediatric patients.[10] This could be because the nature of symptoms of EPTB in children which is often confused with other diseases leads to misdiagnosis. The study showed that the median health system delay was longer than the median patient delay but treatment initiation was prompt once the patient reached the revised national tuberculosis control program (RNTCP) system. Longer duration of delay was seen for those consulting multiple providers until diagnosis, which has also been reported in another study by Paz et al and choosing a less qualified provider or a private sector provider as first provider.[4]

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Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2018.06.007.

REFERENCES

1. *TB India 2017, Annual Status Report*. New Delhi: Indian Medical Association for Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of India; 2017 Mar, 173 p.
2. Hanumantappa R, Shoba A, Manjula VD. Treatment outcomes of childhood tuberculosis with DOTS strategy in Kottayam, Kerala. *Indian J Community Health*. 2012 Dec;24(4):280–284.
3. Donald P. *A Research Agenda for Childhood TB*. Geneva: World Health Organization; 2007, 124 p.
4. Paz-Soldan VA, Alban RE, Dimos Jones C, Powell AR, Oberhelman RA. Patient reported delays in seeking treatment for tuberculosis among adult and pediatric TB patients and TB patients co-infected with HIV in Lima, Peru: a qualitative study. *Front Publ Health*. 2014 Dec 22;2:281.
5. Uplekar MW. Public-private mix for DOTS: demanding, but delay will only hamper TB control. *Int J Tubercul Lung Dis*. 2003 Dec;7(12):1113–1114.
6. Health Care in India. *Emerging Market Report 2007*. India: PriceWaterhouseCoopers; 2007, 26 p.
7. Bajpai V, Saraya A. Healthcare financing: approaches and trends in India. *Natl Med J India*. 2010 Jul-Aug;23(4):231–235.
8. Charles N, Thomas B, Watson B, Raja Sakthivel M, Chandrasekeran V, Wares F. Care seeking behavior of chest symptomatics: a community based study done in South India after the implementation of the RNTCP. *PLoS One*. 2010 Sep 20;5(9).
9. Grover M, Bhagat N, Sharma N, Dhuria M. Treatment pathways of extrapulmonary patients diagnosed at a tertiary care hospital in Delhi, India. *Lung India*. 2014 Jan-Mar;31(1):16–22.
10. Kapoor SK, Raman AV, Sachdeva KS, Satyanarayana S. How did the TB patients reach DOTS services in Delhi? A study of patient treatment seeking behavior. *PLoS One*. 2012 Aug 6;7(8):1–6.
11. Udwadia ZF, Pinto LM, Uplekar MW. Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? *PLoS One*. 2010 Aug 9;5(8).
12. Mesfin MM, Tasew TW, Tareke IG, Kifle YT, Karen WH, Richard MJ. Delays and care seeking behavior among tuberculosis patients in Tigray of Northern Ethiopia. *Ethiop J Health Dev*. 2005;19:7–12.
13. Sarmiento K, Hirsch-Moverman Y, Colson PW, El-Sadr W. Help-seeking behavior of marginalized groups: a study of TB patients in Harlem, New York. *Int J Tubercul Lung Dis*. 2006 Oct;10(10):1140–1145.

14. Farah MG, Rygh JH, Steen TW, Selmer R, Heldal E, Bjune G. Patient and health care system delays in the start of tuberculosis treatment in Norway. *BMC Infect Dis*. 2006 Feb 24;6:33.
15. Lonnroth K, Thuong LM, Linh PD, Diwan VK. Delay and discontinuity-A survey of TB patients' search of a diagnosis in a diversified health care system. *Int J Tubercul Lung Dis*. 1999 Nov;3(11):992–1000.
16. Beyers N, Gie RP, Schaaf HS, et al. Delay in the diagnosis, notification and initiation of treatment and compliance in children with tuberculosis. *Tuber Lung Dis*. 1994 Aug;75(4):260–265.

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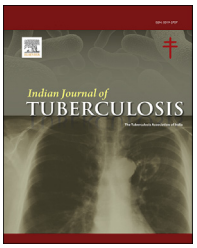

Original article

Tuberculosis diagnostic and treatment practices in private sector: Implementation study in an Indian city

V.K. Chadha [a,*], Bhoomika Bajaj Bhalla [a], Sowmya B. Ramesh [b], J. Gupta [a], N. Nagendra [a], R. Padmesh [a], J. Ahmed [a], R.K. Srivastava [a], R.K. Jaiswal [a], P. Praseeja [a]

[a] Epidemiology and Research Division, National Tuberculosis Institute, Bangalore, Karnataka, India
[b] Department of Family Medicine, P D Hinduja Hospital, Bangalore, India

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ABSTRACT

Setting: Implementation study in private health facilities in an Indian metropolis.
Objectives: Improve Tuberculosis (TB) care by private practitioners (PPs).
Methods: PPs from a defined city area were imparted short training in TB care and linkages made with public facilities; subsequent practices were recorded.
Results: Of 364 presumptive TB patient records, 70 (19.3%) did not conform to its definition. Of the conforming, 174 (59.2%) had presumptive pulmonary TB (PTB), 53 (18%) presumptive extra-pulmonary (EPTB) and 67 (24%) had both. Of conforming presumptive PTB, most underwent Chest X-ray and sputum examination in private laboratories. Tissue based diagnostics were not advised for most presumptive EPTB patients. Of 101 cases diagnosed with TB, 82% were new, 23% known diabetic and 4.7% human immune deficiency virus (HIV) reactive out of 64 tested. Most were notified and initiated treatment within 15 days of diagnosis. One-fourth was prescribed standard treatment regimen and treatment was not directly observed for most. One third was initial defaulters or lost during treatment; 62% of PTB and 46% EPTB cases initiated on treatment in private were successfully treated. Of successfully treated PTB cases, 61% had undergone follow-up sputum examination.
Conclusion: Much intensified support mechanisms are needed to improve TB care in private sector.

1. Introduction

Tuberculosis (TB) continues to be a major scourge in India. Organized TB services are primarily delivered through public health care sector in the form of Revised National TB Control Program (RNTCP) based on directly observed treatment short course (DOTS) strategy introduced from 1997 and expanded in phased manner to achieve full geographical coverage by 2006.

* *Corresponding author*. Epidemiology and Research Division, National Tuberculosis Institute, No.8, Avalon, Bellary Road, Bangalore, India.
E-mail address: vineet2.chadha@gmail.com (V.K. Chadha).
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However, India has a large private health care sector. Pathway-to-care studies have shown that majority of TB patients first approach private health care providers and that atleast half of TB patients are treated in private sector.[1–4] Studies have also revealed disparate diagnostic and treatment practices in the private sector resulting in delay or missed diagnosis and poor treatment success rates as reported from a few areas.[1,4–8] Realizing the role of private providers (PPs) in delivering standard TB care services, some of the recent projects aimed at providing free TB diagnostic and treatment services led to significant increase in case notifications and demonstrated treatment success of about 75%.[9] However, these projects contracted services of Public Private Interface Agencies (PPIAs) and have been too resource intensive precluding their scale up. Therefore, we at the National Tuberculosis Institute, Bangalore (NTI) in collaboration with state health department and local medical professional associations designed a relatively lesser resource intensive Public Private Mix (PPM) project for implementation for a period of one year on a pilot basis in Bangalore, a metropolitan city in the southern state of Karnataka with the broad aim to improve and document TB diagnostic and treatment practices of PPs. Authors of this manuscript have the conviction that the project implementation plan, achievements, pitfalls and challenges shared hereunder will be of use to different health agencies aiming to improve TB care in private sector.

2. Material and methods

The project aimed to involve all mapped private health care facilities (PHFs) – standalone clinics, hospitals (all disciplines available), nursing homes (all discipline may not be available) and private laboratories, providing health care for profit through PPs having allopathic qualification, during 2015–16 in the geographical jurisdiction of Dasappa Tuberculosis Unit (TU) of Bangalore city, inhabited by 0.86 million population. TU is responsible for managing RNTCP services including necessary support to PPs. Standard operating procedures for the project were developed with inputs from state RNTCP officials and professional associations like Indian Medical Association (IMA), Indian Association of Pediatricians (IAP) after their sensitization about scope of the proposed project.

Eight Continuing Medical Education (CME) workshops – five in NTI and three in selected hospitals were conducted for PPs and lectures delivered on standards of TB care in India, newer diagnostic tools, recommended diagnostic algorithms, treatment of drug susceptible and drug resistance TB and the participants were apprised of objectives and procedures of the present implementation study. Demonstration was also given on various modes of TB case notification to the public health authority. CME participants were given credit points by state branch of Indian Medical Council. Each PHF was subsequently visited by NTI medical officer and field staff to re-appraise PPs in project modalities. Three-day training in sputum microscopy was provided to laboratory technicians of 3 private laboratories at NTI and on the spot training was imparted in 8 other laboratories. Besides, individual PPs were provided a table calendar depicting diagnostic algorithms, treatment regimen, list of Designated Microscopy Centers (DMCs) and integrated counseling and human immune deficiency virus (HIV) testing centers in public sector, list of cartridge based nucleic acid amplification test (CBNAAT) laboratories in private sector, treatment centers under RNTCP and contact details of key RNTCP officials. PPs were informed to avail free CBNAAT facility for presumptive pediatric and extra-pulmonary TB patients and patients living with HIV (PLHs) available in public sector at Dasappa TU and Rajiv Gandhi Institute of Health Sciences, a tertiary care center located in another part of the city. For this purpose, RNTCP field staff consented to collect sputum specimen from PHFs on demand. Each PP was provided with state of the art 'Individual Presumptive TB patient cards' to be filled for each such patient to record identification particulars, contact details, presenting signs & symptoms with duration, co-morbidities if any, personal habits (current smoking, alcoholic use) investigations advised, place where carried out with results, medications advised, diagnosis made and details of health facility where anti-TB treatment (ATT) initiated. For patients diagnosed to be suffering from TB, they were advised to record in 'Individual TB Treatment Card' the contact details, disease classification, results of investigations at diagnosis and follow-up, results of counseling and HIV testing, TB medications prescribed with date and duration, treatment outcome, adverse drug reactions if any; drug administration was to be directly observed and recorded in the duplicate treatment card with a treatment supporter-family member/PHF staff. PPs were provided referral forms for investigations and/or treatment at public health centers if so desired. Separate workshops were conducted with RNTCP staff to provide necessary support to patients and PHFs as the case may be.

PHFs were facilitated for registration in TB notification system if not already done and provided with notification registers.

Personal interviews were conducted with diagnosed TB patients/family members (in case of deceased patients), after an interval of one year from diagnosis to find out their symptom, disease and survival status and corroborate the duration for which ATT taken.

Protocol was approved by the Ethics Committee of NTI. Invitations for participation in the project were issued to individual PHFs and those responded were included in the project.

2.1. *Definitions*

Presumptive TB[10]

- Presumptive pulmonary TB: cough or unexplained fever ≥2 weeks/chest pain >1 month/hemoptysis anytime in last 6 months
- Presumptive extra-pulmonary TB: unexplained fever ≥2 weeks/significant weight loss/organ specific symptoms or signs[10]
- Presumptive pediatric TB: cough or unexplained fever ≥2 weeks/failure to thrive[10]

Microbiologically confirmed TB case: positive on smear/culture/Xpert MTB/RIF (Xpert)

Clinically diagnosed TB case: microbiologically unconfirmed, decision to treat for TB

Standard ATT Regimen

- New cases: atleast four 1st line oral anti-TB drugs in intensive phase and two oral drugs in continuation phase, daily/thrice weekly with doses as recommended by RNTCP.
- Previously treated cases: atleast four 1st line oral anti-TB drugs and streptomycin in intensive phase and two oral drugs in continuation phase, daily/thrice weekly with doses as recommended by RNTCP

TB treatment outcome

- Successfully treated: Taken full course of treatment and smear negative at the end of treatment if sputum examination performed in case of PTB patients
- Unsuccessfully treated: Lost to follow-up (interrupted treatment ≥1 month) or died during treatment or failure (smear positive at the end of treatment)

3. Results

There were a total of 86 PHFs in the area – 62 standalone clinics with only out-patient facilities, 10 with inpatient facilities – 6 hospitals, 4 nursing homes and 14 laboratories providing microscopy services – one also had Xpert MTB/RIF. Of 133 PPs in these PHFs, 129 attended CMEs.

Twenty PHFs were issued study formats and referral forms. During the one-year study period, eleven PHFs – 4 hospitals, 2 nursing homes, and 5 clinics filled in 364 presumptive TB patient cards and 101 TB patients' cards.

Of 364 patients with presumptive TB cards, 206 (56.6%) were male; 250 (68.6%) ≥ 45 years old, 16 (4.4%) had history of close contact with a known TB case, 37 (10.2%) had history of previous ATT, 139 (38.2%) were known to have diabetes, 8 (2.2%) were known HIV positive, 38 (10.4%) current smokers and 15 (4.1%) regular alcohol users (Table 1). Cough was the predominant presenting symptom followed by fever, pain or swelling in joints, abdominal pain and significant weight loss (Table 2). Two hundred ninety-four (80.7%) confirmed to definition of presumptive TB and 70 (19.3%) did not. Of the confirming, 174 (59.2%) had presumptive PTB, 53 (18%) presumptive EPTB and 67 (24%) had both. Of the non-confirming, 39 (55.7%) had pulmonary symptoms, 1 (1.4%) had symptoms pertaining to extra-pulmonary site, 3 (4.3%) had both pulmonary and extra-pulmonary symptoms and information was not available for 27 (38.5%) (Fig. 1).

Of conforming 174 presumptive PTB patients, 153 (87.9%) had cough ≥2 weeks and remaining had one or more other symptoms (table not given). Among them, sputum smear microscopy was performed in 159 (91.4%), sputum culture in 15 (8.6%) and Xpert in 12 (6.9%) (Fig. 1). Altogether, 165 (94.8%) patients underwent sputum examination – 153 (92.7%) in private laboratories, 1 in government laboratory and information was not recorded for 11 (6.6%). One specimen was examined in 90 (54.5%), two in 17 (10.3%), three in 3 (1.8%) and information was not recorded for 55 (33.3%).

Chest X-ray (CXR) was done in 150 (86.2%) presumptive PTB patients; of 110 with recorded results, 47 (42.7%) were normal, 40 (36.4%) had some pulmonary abnormality. Frequency distribution of type of pulmonary abnormality as recorded by the PPs included cavity-6, consolidation-10, infiltration-6, lung abscess-1, opacity-8, bronchiectasis-3, pleural effusion-4, ill defined shadow-2; as many as 23 (20.9%) X-ray results were not in consonance with description of any X-ray lesion.

Other investigations carried out are given in Fig. 1. Eighty-six (49.4%) were given a course of general antibiotics.

Investigations carried out in patients with presumptive EPTB, both presumptive PTB and EPTB and not conforming to the definition are presented in Fig. 1; 66% of presumptive EPTB and 91% with both presumptive PTB and EPTB were subjected to sputum smear examination.

Table 1 – Presumptive TB patients-selected host characteristics (N = 364).

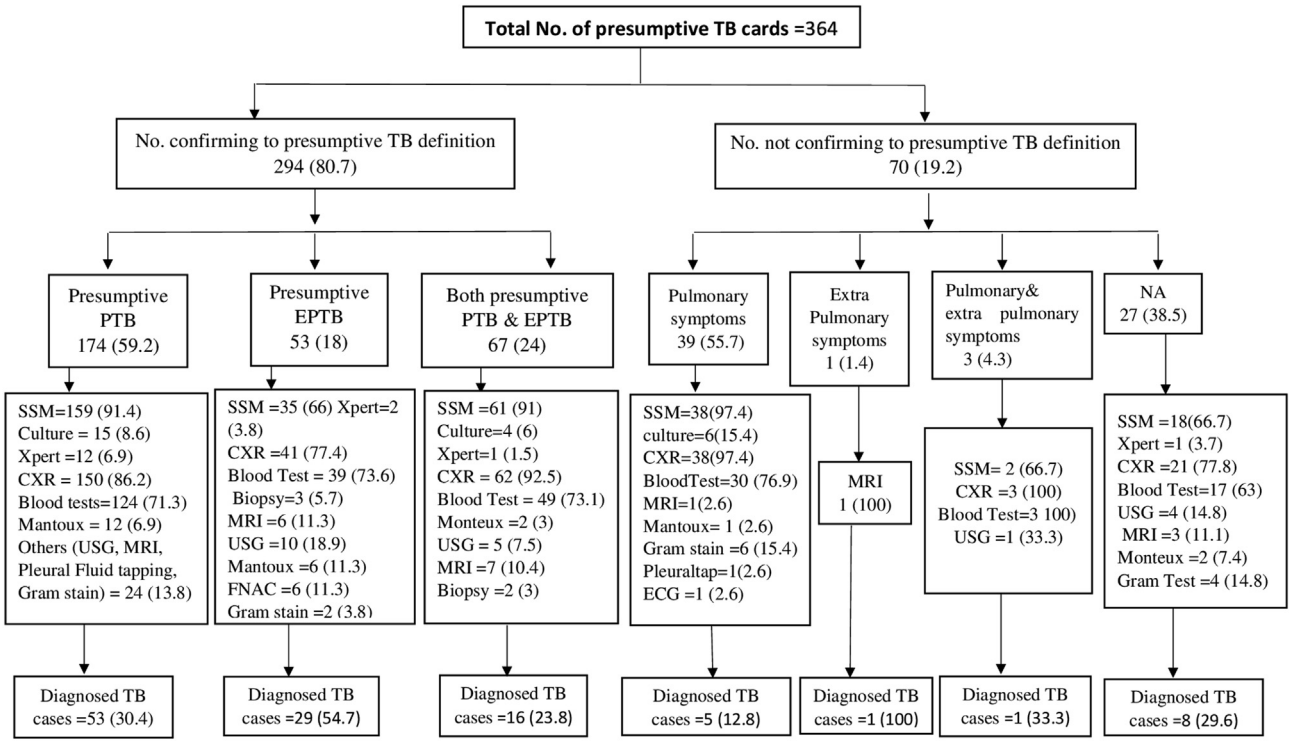
	No. (%)
Sex	
Male	206 (56.6)
Female	158 (43.4)
Age-group	
0–14	11 (3.0)
15–24	35 (9.6)
25–34	32 (8.8)
35–44	36 (9.9)
45–54	69 (19.0)
55–64	79 (21.7)
>=65	102 (28.0)
History of close contact with a known TB case	
Yes	16 (4.4)
No	348 (95.6)
History of previous Anti-TB treatment	
Yes	37 (10.2)
No	327 (89.8)
Diabetes Mellitus	
Known to have diabetes	139 (38.2)
Non-diabetic/status unknown	225 (61.8)
HIV status	
known to be HIV reactive	8 (2.2)
Status unknown/HIV negative	356 (97.8)
Current smoking status	
Smokers	38 (10.4)
Non-smokers	326 (89.6)
Current alcohol use	
Regular user	15 (4.1)
Non-user	349 (95.9)

( ): percentages.

Table 2 – Distribution of symptoms and signs as recorded in presumptive TB cards (N = 364).

Symptoms/signs present*	Number (%)
Cough ≥2 weeks	200 (55.0)
<2 weeks	123 (34.0)
Fever ≥1 month	79 (21.7)
<1 month	238 (58.3)
Pain or swelling in joints	92 (25.3)
abdominal pain	52 (14.3)
Significant weight loss	47 (12.9)
Chest pain	24 (6.6)
Swelling of lymph node	13 (3.6)
Hemoptysis	11 (3.0)

*With or without other symptoms/signs; ( ): percentages.



() : Percentage ,PTB :Pulmonary TB, EPTB :Extra Pulmonary TB, SSM: sputum smear microscopy, Xpert : Xpert MTB/RIF, CXR: Chest X-ray, MRI: Magnetic radio-imaging, USG: Ultra-sonography, FNAC: Fine needle aspiration cytology, ECG:Elctro-Cardio-Graphy, NA: Information not available

Fig. 1 – Presumptive TB patients and investigations undertaken.

Among patients conforming to presumptive TB definition, 98 (33.3%) were diagnosed to have TB; there were 15 additional cases among those not conforming to presumptive TB definition (Fig. 1).

Of the total 113 TB cases diagnosed, treatment cards were available for 101, of whom 61 (60.4%) were male and 44 (43.5%) ≥45 years of age (Table 3). Sixty-four (63.4%) had PTB, 34 (33.6%) EPTB and information was not recorded for 3. Eighty-three (82.2%) were new cases and 17 (16.8%) previously treated; information was not recorded for one. Twenty-three (22.8%) were known diabetic while the diabetic status of others was unknown. Sixty-four (63.4%) were subjected to counseling and HIV testing – 16 (24.6%) at Government, 49 (75.4%) at private facilities; 3 (4.7%) were HIV reactive. Twelve (11.9%) were smokers and 9 (8.9%) regular alcohol users (Table 3).There were 12 (11.9%) initial defaulters and disease classification was not recorded for three. Of the remaining 86, 67 (77.9%) initiated ATT within 15 days of diagnosis, 42 (48.8%) visited ≥2 health facilities post-diagnosis before initiating ATT.

Of 74 cases initiated on ATT in private, 45 were PTB and 29 EPTB; basis of diagnosis is presented in Fig. 2. Fifteen (20.3%) received standard ATT regimen, 57 (77.0%) other than standard, 1 received ATT trial and the type of regimen was not known for one case. Anti-TB drugs were self-administered by 61 (82.4%) patients and under observation in 13 (17.5%). Overall, twenty-eight (62.2%) PTB and 13 (44.8%) EPTB cases were successfully treated (Fig. 2).

Treatment outcome of those given anti-TB drugs in the private, by type of case (new/previously treated) and treatment regimen (standard/non-standard) is given in Table 4. Overall 41 (55%) were successfully treated, 21 (28%) were lost to follow up, 6 (8%) died and one was not evaluated. Of 13 new cases treated with standard regimen, 12 (92%) were successfully treated while only 23 (50%) of 46 new cases on non-standard treatment regimen and 6 (50%) of 12 previously treated were successfully treated. Of the total treated in private, 6 cases died during treatment and 21 were lost to follow-up. Of the latter, diagnosis had been changed for six, 3 stopped drugs due to feeling better, 2 had migrated, one stopped on PP's advice, one due to other illness and the reason for lost to treatment follow-up (LTFU) for remaining 8 could not be ascertained. Of successfully treated PTB cases, 17 (60.7%) had undergone follow-up sputum examination and 18 (64.3%) CXR during treatment (Table 5). At one-year post-treatment follow-up, 8 (LTFU-6, Treatment completed-2) were still symptomatic, an additional patient died post-treatment and 46 (62.1%) were non-symptomatic.

Of 12 cases who initiated ATT at public facilities, 8 had PTB and 4 EPTB (Fig. 2); all received standard regimen under observation. Six (75.0%) PTB and all EPTB cases were successfully treated; 2 PTB patients died during treatment. All successfully treated PTB cases had undergone follow-up sputum examination. All successfully treated including EPTB cases were asymptomatic at one-year follow-up.

Table 3 – Diagnosed TB patients-selected host characteristics (N = 101).

	No. (%)
Sex	
Male	61 (60.4)
Female	40 (39.6)
Age-group	
0–14	5 (5.0)
15–24	20 (19.8)
25–34	17 (16.8)
35–44	15 (14.9)
45–54	18 (17.8)
55–64	9 (8.9)
>=65	17 (16.8)
Disease classification	
Pulmonary TB	64 (63.4)
Extra-pulmonary TB	34 (33.6)
Not recorded	3 (3.0)
Type of case	
New	83 (82.2)
Previously treated	17 (16.8)
Not recorded	1 (1.0)
Diabetes Mellitus	
Known to have diabetes	23 (22.8)
Non-diabetic/status unknown	78 (77.2)
HIV status	
HIV reactive	3 (3.0)
HIV non-reactive	61 (60.4)
HIV status unknown	37 (36.6)
Current smoking status	
Smokers	12 (11.9)
Non-smokers	89 (88.1)
Current alcohol use	
Regular user	9 (8.9)
Non-user	92 (91.1)

( ): percentages.

Of 113 cases diagnosed, 95 (94.1%) including six for whom diagnosis was changed subsequently were notified.

4. Discussion

This study gave us fresh perspectives on the challenges of TB care in private sector; there were some definite positive outcomes as well.

Though participation in CMEs was satisfying, only a small proportion of PHFs actively participated in this implementation study despite several visits by NTI field staff. Of 86 PHFs, 20 consented to participate. Others were mostly standalone clinics and either said they were referring all TB symptomatic to RNTCP or other specialists and thus not interested to participate or simply did not consent to participate due to constraints in maintaining records. Of the twenty PHFs that participated, nine did not record any presumptive or confirmed TB case record as envisaged under the project and no data could be obtained from them. Thus the project could not achieve the desired result on the coverage issue, which remains a challenge in private sector involvement.

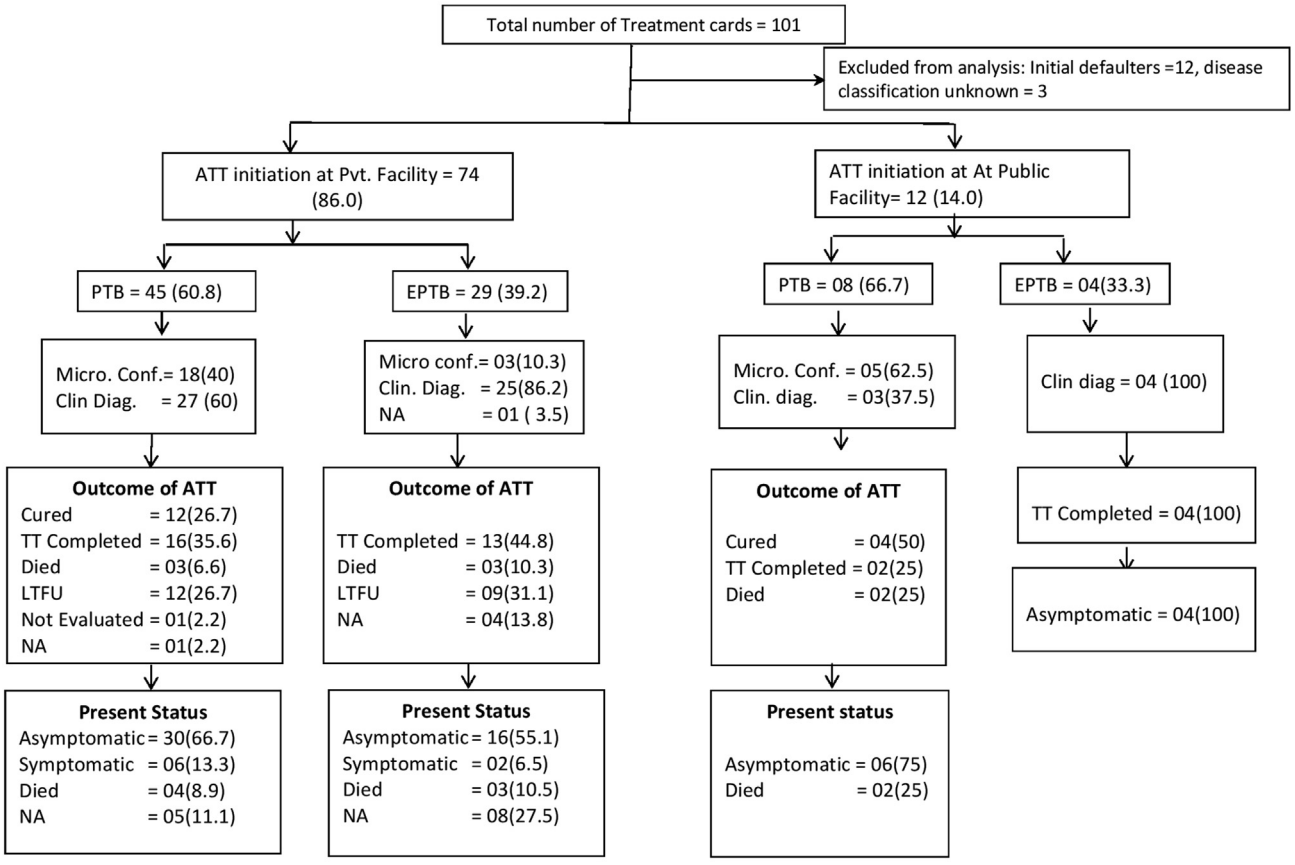
Proportion of presumptive patients out of total patient-footfalls could not be ascertained. About 20% of presumptive patients so labeled did not conform to definitions but there were additional TB cases among them. That 15 of the 70 (21%) cases not confirming to presumptive TB definition were detected to be having TB would suggest that definitions need to be revised. However, these definitions of presumptive TB have been based on extensive research. We reckon that there may be errors in finding out the exact duration of symptoms which needs to be done diligently.

A higher proportion of presumptive as well as diagnosed TB patients having diabetes than hitherto reported in India is another grim reminder of its increasing trend[11], despite the fact that we had not insisted on diabetes screening for all, since RNTCP guidelines were not uniformly implemented in the country at the time of conceptualizing the project.

Sputum examination performed in 95% of conforming presumptive PTB patients was a significant change from earlier experiences in PPM projects including initial phases of PPIA projects.[8,9,12] Sensitization of PPs leading to satisfactory proportion adopting sputum examination was also demonstrated in Chennai.[7,13] However, most of it in our study was performed on one specimen in private laboratories lacking quality assurance mechanisms, even though list of DMCs available within reasonable distance and commitment of DMC staff to provide services to referred patients had been informed.

As per the current RNTCP recommended diagnostic algorithm,[14] most were also subjected to CXR. However, correct reporting of CXR result was lacking in significant proportion as also seen in public sector in one of our earlier studies.[15] Of those with legibly recorded results, 36% had some pulmonary abnormality which was similar to findings in an earlier study.[16] This data may be important for program managers; RNTCP mandates that all those with any pulmonary CXR abnormality should be subjected to sputum examination.[14] About 50% were also prescribed a course of antibiotics. PPs also advised sputum examination in about 2/3rd of presumptive EPTB patients presumably as supportive investigation in diagnostic workup; however, tissue based diagnostics were advised only in a minority.

Other positive outcomes of the project were typing of diagnosed TB cases as new/previously treated, proportion of microbiological confirmation at 41% and provider initiated counseling and HIV testing in 64%, which were higher than hitherto reported from private sector.[5] Delay of >15 days in initiating treatment was observed in 20% cases. Only 1/4th of cases were prescribed standard treatment regimen despite the short training and provision of reference material by us; treatment with non-standard regimen has also been observed in previous studies in India.[4–8] Most patients in the present study were not linked to direct observation of treatment, which is a cause of concern and demands closer private public collaboration. Proportion of TB cases treated successfully in the private was 55%, which was similar to some earlier reports from private sector but lower than some PPM projects[1,9,17]; importantly about a third were either initial defaulters or lost during treatment. Notably, treatment success rates was satisfactory in new cases treated with standard ATT regimen in the private sector and expectedly much poorer among those treated with non-standard regimen and those with previous history of treatment albeit the numbers were small,



(): Percentage; ATT: anti-TB treatment; PTB: pulmonary tuberculosis, EPTB: Extra-pulmonary tuberculosis, micro. Conf.: microbiologically confirmed; Clin. Diag.: clinically diagnosed. LTFU: Loss to treatment follow-up, NA: Information Not Available

Fig. 2 – Diagnosed TB cases-ATT initiation and basis of diagnosis and treatment outcome.

Table 4 – Treatment outcome by type of case and regimen, among those initiated on treatment in private sector (N = 74).

Type of case	Type of regimen	Treatment outcome					
		No. successfully treated	LTFU	Died	Not Known	Transferred out	Total N (%)
New	Standard	12 (92.3)	–	1 (7.7%)	–	–	13
	Non-standard	23 (50%)	15 (32.6)	3 (6.5)	4 (8.7%)	1 (2.2%)	46
	ATT trial	–	1 (100)	–	–	–	1
	Not Known	–	1 (100)	–	–	–	1
	Total N (%)	35 (57.4)	17 (27.9)	4 (6.6)	4 (6.6)	1 (1.7)	61
Previously treated	Standard	2 (100)	–	–	–	–	2
	Non-standard	4 (40)	3 (30)	2 (20)	1 (10)	–	10
	Total N (%)	6 (50)	3 (25)	2 (16.7)	1 (8.3)	–	12
Not Known	Non-Standard	–	1 (100)	–	–	–	1
Total N (%)		41 (55.4)	21 (28.4)	6 (8.1)	5 (6.8)	1 (1.3)	74

( ): percentages; -: zero.

underscoring the role of professional associations and RNTCP managers in correcting the knowledge gap of PPs. Though most diagnosed TB cases were notified, change of diagnosis in a proportion underlies need to establish a system for prior verification. However, poor TB notifications from the area in the post project period were disheartening.

We had initially planned to provide free testing by Xpert for all presumptive patients by transporting specimen to NTI. Though Xpert machine was available at NTI, cartridges could not be procured due to reasons beyond authors' control which became a major limitation in achieving the aims as envisaged. Another limitation was non-diligence of PPs in recording all required information despite our efforts.

It is recommended that CME programs for PPs should include refresher training on CXR reading, INDEX TB guidelines for EPTB,[18] more intensified training on standard treatment regimen and emphasis to reduce delay in treatment initiation. Study findings re-emphasize the need for support

Table 5 – Follow-up investigations carried out for patients completed treatment in private health facilities.

	Pulmonary TB	Extra-pulmonary TB
Follow-up investigations	(N = 28)	(N = 13)
Sputum smear examination	17 (60.7)	1 (7.6)
Chest X-ray	18 (64.3)	3 (23.1)
Blood test	7 (25)	7 (53.8)
USG/CT/MRI	3 (10.7)	4 (30.7)
Others (Urine test/Xpert)	1 (3.5)	1 (7.6)
Not recorded	4 (14.3)	5 (38.5)

USG: Ultra-sonography; CT: computerized tomography, MRI: Magnetic radio-imaging.

by public health managers by necessary linkage with laboratories for histo-pathology/microbiology, provide support to private laboratories in quality assurance, preventing initial default, supporting patient in treatment adherence including incentivizing patients and providers as envisaged in National Strategic Plan (NSP) for TB elimination, 2017–2025.[19] Other components of NSP including free diagnostics and ATT if implemented effectively will enhance the quality of TB care in private sector. We conclude that mitigating challenges in order to improve TB care in private demands much intensified efforts.

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Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2018.06.010.

REFERENCES

1. Uplekar M, Juvekar S, Morankar S, Rangan S, Nunn P. Tuberculosis patients and practitioners in private clinics in India. *Int J Tubercul Lung Dis*. 1998;2(4):324–329.
2. Satyanarayana S, Nair SA, Chadha SS, et al. From where are tuberculosis patients accessing treatment in India? Results from a cross-sectional community based survey of 30 districts. *PLoS One*. 2011;6(9):e24160. https://doi.org/10.1371/journal.pone.0024160. Epub 2011 Sep 2.
3. Arinaminpathy N, Batra D, Khaparde S, et al. The number of privately treated tuberculosis cases in India: an estimation from drug sales data. *Lancet Infect Dis*. 2016;16(11):1255–1260. https://doi.org/10.1016/S1473-3099(16)30259-6.
4. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. *Int J Tubercul Lung Dis*. 2014;18(3):255–266.
5. Achanta S, Jaju J, Kumar AM, et al. Tuberculosis management practices by private practitioners in Andhra Pradesh, India. *PLoS One*. 2013 Aug 13;8(8):e71119. https://doi.org/10.1371/journal.pone.0071119.
6. Udwadia ZF, Pinto LM, Uplekar MW. Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? *PLoS One*. 2010;5(8):e12023. https://doi.org/10.1371/journal.pone.0012023.
7. BronnerMurrison L, Ananthakrishnan R, Sukumar S, et al. How do urban Indian private practitioners diagnose and treat tuberculosis? A cross-sectional study in Chennai. *PLoS One*. 2016;11(2):e0149862. https://doi.org/10.1371/journal.pone.0149862.
8. Satyanarayana S, Subbaraman R, Shete P, et al. Quality of tuberculosis care in India: a systematic review. *Int J Tubercul Lung Dis*. 2015;19(7):751–763.
9. WHO Country Office for India, Central TB Division-Directorate General of Health Services, Government of India. *Universal Access to TB Care: Concurrent Assessment Report*; May 2016. www.tbcindia.nic.in. Accessed October 11, 2017.
10. Chaudhuri Arunabha D. Recent changes in technical and operational guidelines for tuberculosis control programme in India – 2016: a paradigm shift in tuberculosis control. *J Assoc Chest Phys*. 2017;5(1):1–9.
11. India Tuberculosis-Diabetes Study Group. *Screening of Patients with Tuberculosis for Diabetes Mellitus in India*. Tropical Medicine and International Health; 2013. https://doi.org/10.1111/tmi.1208.
12. Murthy KJR, Frieden TR, Yazdani A, Hreshikesh P. Public-private partnership in tuberculosis control: experience in Hyderabad, India. *Int J Tubercul Lung Dis*. 2001;5(4):354–359.
13. Krishnan N, Ananthakrishnan R, Augustine S, et al. Impact of advocacy on the tuberculosis management practices of private practitioners in Chennai City, India. *Int J Tubercul Lung Dis*. 2009;13(1):112–118.
14. Central TB, Division-Directorate General of Health Services, Government of India. *Technical and Operational Guidelines for Tuberculosis Control in India*; 2016. https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4573&lid=3177. Accessed December 24, 2017.
15. Chadha VK, Praseeja P, Hemanthkumar NK, et al. Are registered sputum smear-negative tuberculosis patients in Karnataka, India, diagnosed by national algorithm? *Int J Tubercul Lung Dis*. 2014;18(12):1491–1495.
16. Somashekar N, Chadha VK, Praseeja P, et al. Role of pre-Xpert screening using chest X-ray in early diagnosis of smear-negative pulmonary tuberculosis. *Int J Tubercul Lung Dis*. 2014;18(10):1243–1244.
17. Subramaniyam S, Chadha VK, Manuvel C, et al. Treatment outcome of tuberculosis patients in a clinic of Bangalore. *Int J Tuberc Lung Dis*. 2014;61(3):189–194.
18. WHO Country Office for India, Central TB Division-Directorate General of Health Services, Government of India. *INDEX TB Guidelines-guidelines for Extra-pulmonary Tuberculosis in India*. World Health Organization; 2016.
19. Central TB Division-Directorate General of Health Services, Government of India. *Revised National Tuberculosis Control Program. National Strategic Plan for Tuberculosis Elimination 2017–2025*. March 2017.

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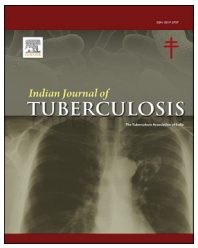

Original article

Time to sputum culture conversion and treatment outcome among the first cohort of multidrug resistant tuberculosis patients in a high burden country

O.M. Ige [a,*], Regina E. Oladokun [b]

[a] Department of Medicine, College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria
[b] Department of Paediatrics, College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria

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ABSTRACT

Background: Sputum conversion considered the most important interim indicator of the efficacy of anti-tuberculosis treatment was assessed at varying time points among the first cohort of multidrug resistant tuberculosis (MDR-TB) patients in a National TB Control Programme.

Methods: A retrospective study was conducted for the period between 2010 and 2013, at the premiere MDR-TB treatment center in Nigeria. Genexpert, culture and drug susceptibility tests were carried out. Total duration of treatment was 20 months.

Results: A total of 115 patients were studied consisting of 76 (66.1%) males and 39 (33.9%) females with ages ranging between 15 and 65 years. Median time to sputum conversion was 2.06 months (95% confident interval [CI] = 1.82, 2.30). At the end of the first month, 43 (37.4%) patients sputum converted, increasing to 104 (90.4%) at the end of three months. There was no significant interaction with Human Immunodeficiency Virus (HIV) status. Overall treatment success was 69.4%. The default rate was 8.7% (10/115) and 25 (21.7%) deaths were recorded.

Conclusion: The treatment success rate in the study was high with most of cases with or without HIV infection, achieving sputum culture conversion within 2 months of commencing treatment. Expansion of MDR-TB treatment services is necessary to reduce the death rate.

1. Introduction

Multidrug-tuberculosis (MDR-TB) constitutes a huge public health burden in Nigeria as the country ranks 9th among the 27 high burden MDR-TB countries of the world.[1] It is estimated that MDR-TB among new (primary MDR-TB) and retreatment cases is 2.9 and 14 respectively.[2] The situation is compounded by the concurrent Human Immunodeficiency Virus (HIV) epidemic.[3] A higher prevalence of MDR-TB has been reported

* *Corresponding author.*
E-mail address: olusojiige@yahoo.com (O.M. Ige).
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in HIV seropositive compared to seronegative individuals.[4] Though the current prevalence of HIV/MDR-TB co-infection in Nigeria is not known, the prevalence of HIV among tuberculosis (TB) patients increased from 2.2% in 1991 to about 27% in 2008.[5]

Through a collaborative mechanism between the Federal Ministry of Health and partners, the first specialized treatment centre with 25 bed capacity was established at the University College Hospital, Ibadan. Under the DOTS-Plus strategy, treatment of MDR-TB cases commenced in 2010. Thereafter, resources have been mobilized for many more MDR admission facilities across the country. The World Health Organization (WHO) had recommended a total treatment duration of 20 months in most cases of treatment of MDR-TB which was adopted by the National programme.[6,7]

Sputum culture remains the gold standard for monitoring pulmonary MDR-TB. Sputum conversion is considered the most important interim indicator of the efficacy of anti-TB treatment.[8] The accepted method for monitoring treatment of MDR-TB is periodic sputum culture. The importance of sputum culture conversion in the management of patients with MDR-TB cannot be overemphasized. Predictors of sputum culture conversion and treatment outcomes are important in order to improve on the strategies for reducing the development and spread of MDR-TB.

From some DOTS Plus programs in different parts of the world, sputum culture conversion rates ranging from 77 to 88% with a median of 60% after two to three months of treatment initiation had been reported.[9–11]

Reports on MDR-TB in Nigeria are scarce. Among the first cohort of MDR cases hospitalized in the University College Hospital, Ibadan, Nigeria, the sputum conversion rate, time to initial sputum culture conversion as prognostic markers for end-of-treatment outcome were assessed. The optimum time points for when sputum culture conversion can be considered as a marker for final treatment outcome were compared among MDR patients with and without HIV infection.

2. Methodology

Study location: The study was carried out at the University College Hospital, Ibadan where the pioneer MDR-TB treatment centre in Nigeria was established.

2.1. Patients

MDR-TB cases admitted between July 2010 and December 2013 were enrolled into the study. Xpert MTB/Rif (GeneXpert) was carried out to ascertain the presence of rifampicin resistance while MDR-TB was confirmed by sputum culture on solid media and Drug Sensitivity Testing (DST). Each patient had a chest X-ray to document radiologic evidence of pulmonary TB. Screening for HIV status using enzyme linked immunosorbent assay (ELISA) was carried out after pre-test counselling and positive tests were confirmed by Western Blot. Sociodemographic and clinical information were obtained including occupation and Body Mass Index (BMI).

2.2. Treatment of MDR-TB

All the patients had been treated previously and had failed the first-line antituberculosis therapy. Treatment for the MDR-TB was the conventional standardized regimen of the Nigerian National tuberculosis programme (NTP) consisting of Kanamycin (Km), Levofloxacin (Lfx), Cycloserine (CS), Prothionamide (Pto), and Pyrazinamide (Z) for intensive phase of 8 months followed by a continuation phase of 12 months with all the drugs of the intensive phase without Kanamycin or amikacin.[7]

HIV co-infected patients were receiving first line antiretroviral therapy (ART) therapy consisting of Tenofovir/Lamivudine/Efavirenz as fixed drug combination and cotrimoxazole prophylaxis. All the patients had both nutritional and psychosocial support. Directly Observe Treatment (DOT) was applied throughout the entire treatment period of 20 months.

Monitoring of response to treatment was assessed by monthly weight, monthly sputum culture during the intensive phase and every 2 months in the continuation phase till the end of treatment. The chest X-rays were carried out at baseline, 6 months, 12 months and 18 months as well as at the end of treatment.

2.3. Definitions

We defined a positive culture as one or more colonies of *Mycobacterium tuberculosis*.

Culture conversion was deemed to have occurred if there were two or more negative consecutive TB cultures taken at least 30 days apart. Time to conversion was the interval between the date of MDR-TB treatment initiation and the date of the first of two negative consecutive cultures.

Conversion date was the date of specimen collection of the first of the two consecutive negative cultures taken 30 days apart. A patient was said to be cured if treatment had been completed and there were at least five consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. A case was categorised as treatment completed if treatment has been completed but the clinical definition of cure is not met as a result of lack of bacteriological results or where fewer than five cultures were performed in the final 12 months of treatment. Treatment failure; Treatment will be considered to have failed if two or more of the five cultures recorded in the final 12 months of the therapy are positive. A default when treatment was interrupted for two or more consecutive months for any reason. Died; A patient who dies for any reason during the course of drug resistant tuberculosis (DR-TB) treatment.

Transfer out refers to when a patient has been transferred to another reporting and recording unit with unknown treatment as well as final outcome. Treatment success is defined as cured and completion of treatment. Poor Outcome is death, failure or default.

2.4. Statistical analysis

Data were coded and analysed using the statistical software SPSS Version 20.0 (SPSS Inc. Chicago IL). Chi-square and

Fisher's exact tests where appropriate were used to measure the associations between discrete variables. The time to initial sputum culture conversion was analysed with the Kaplan–Meier method and assessed differences between groups with the log-rank test. To estimate the association of 3 month and 6 month sputum culture conversion with successful treatment outcome, odds ratios (ORs) were calculated and 95% CIs with random-effects multivariable logistic regression. Using bivariate analysis, factors associated with the treatment outcomes was calculated and the proportion of patients in each outcome category was compared among HIV negative versus HIV positive cases. P values of less than 0.05 were considered significant. The cohort analysis was done at 24 months to provide the overall assessment of the treatment outcome.

2.5. *Ethical consideration*

Approval for the study was obtained from the University of Ibadan/University College Hospital Ethical Review Committee.

3. Results

3.1. *Baseline characteristics*

A total of 115 cases of MDR-TB were enrolled within the study period. There were 76 (66.1%) males and 39 (33.9%) females with ages ranging between 15 and 65 years. The mean ages in males and females were 37.34 and 30.54 respectively. There were 7 (6.1%) cases below 20 years while 83.4% were within the 20–49 years age category. Only 12 (10.4%) were aged 50 years and above. With regards to BMI, 72 (62.6%) were underweight, 39 (33.9%) had normal weight while only 4 (3.5%) were overweight. Out of the 115 patients studied 22 were confirmed HIV positive given a sero-prevalence of 19.1%. The DST for the first line anti-tuberculosis drugs showed 100% resistance to Rifampicin, Isoniazid and Pyrazinamide but resistance to Ethambutol was recorded in 54 (47.0%) and Streptomycin 78 (67.8%).

Notably, there were no significant associations between treatment outcomes (failure or success) and each of the patients' characteristics (Table 1). Although, the odds of treatment failure were higher in patients aged <20–29 years and 40–49 years than 50–>60 years, those from urban than rural areas, (1+) and (2+) Acid fast bacilli (AFB) test than (3+) as well as those who received Ethambutol than regimen without Ethambutol. However, the odds of treatment failure for the various characteristics were not statistically significant.

3.2. *Treatment outcome*

Fig. 1 shows that overall, 68.7% (79/115) of patients had treatment success comprising a cure rate of 42/115 (36.5%) and treatment completion rate of 37/115 (32.2%). Treatment failure with continued sputum culture positivity at the end of therapy occurred in only 1 patient. The default rate was 8.7% (10/115), and no patient was transferred out to other MDR-TB treatment centers. A total of 25 (21.7%) deaths were recorded.

Table 1 – Baseline demographic characteristics and association with treatment success.

Characteristic	Total	Failure (%)	Success (%)	p	OR (95% CI)
Ages					
<20–29	32	12 (37.5)	20 (62.5)	0.673	1.80 (0.41, 7.99)
40–49	71	22 (31.0)	50 (69.0)	0.961	1.32 (0.33, 5.35)
50–>60	12	3 (25.0)	9 (75.0)	–	1
Sex					
Female	39	12 (30.8)	27 (69.2)	0.901	0.96 (0.42, 2.22)
Male	76	24 (31.6)	52 (68.4)	–	1
Domicile					
Urban	73	26 (35.6)	47 (64.4)	0.269	1.77 (0.75, 4.17)
Rural	42	10 (23.8)	32 (76.2)	–	1
HIV status					
Positive	22	6 (27.3)	16 (72.7)	0.832	0.79 (0.28, 2.21)
Negative	93	30 (32.3)	63 (67.7)	–	1
BMI (kg/m^2)					
<18.5	72	29 (40.3)	43 (59.7)	0.934	2.02 (0.20, 20.42)
18.5–24.9	39	6 (15.4)	33 (84.6)	0.201	0.54 (0.24, 1.24)
>25	4	1 (25.0)	3 (75.0)	–	1
AFB					
1+	33	11 (33.3)	22 (66.7)	0.781	1.26 (0.51, 3.10)
2+	15	6 (40.0)	9 (60.0)	0.565	1.68 (0.53, 5.38)
3+	67	19 (28.4)	48 (71.6)	–	1
DST (E)					
Yes	54	18 (33.3)	36 (66.7)	0.810	1.19 (0.54, 2.63)
No	61	18 (29.5)	43 (70.5)	–	1
DST (S)					
Yes	78	23 (29.5)	55 (70.5)	0.692	0.77 (0.34, 1.77)
No	37	13 (35.1)	24 (64.9)	–	1

BMI: Underweight: <18.5; Normal: 18.5–24.9; Overweight: 25–29.9; AFB: Acid fast bacilli; DST: Drug sensitivity testing.

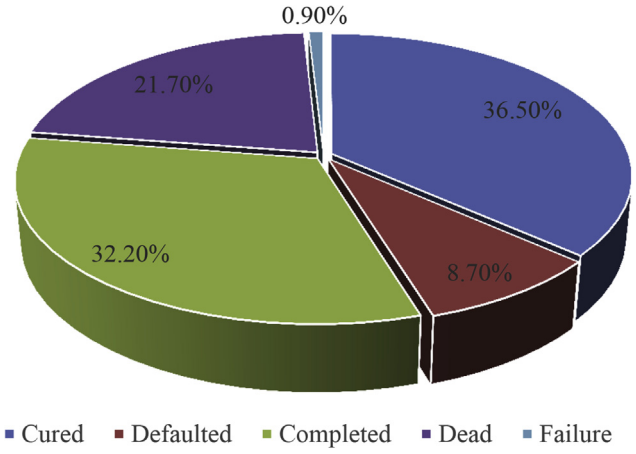

Fig. 1 – Treatment outcome of 115 MDR-TB patients.

3.3. *Sputum culture conversion time*

Of the 115 cases of MDR-TB, 105 converted while 10 cases were lost to follow up (censored). Fig. 2a displays the probability that a case of MDR-TB sputum converted at a particular time during treatment. Notably, the probability of conversion by: 1 month was 0.3, 2 months 0.8 and it rose to almost 1.0 at 7–8 months. The overall mean time to conversion among cases was 2.06 months (95% CI = 1.82, 2.30).

Similarly, the probability that a case converted at a particular time during the follow up among HIV positive compared with HIV negative individuals is displayed in Fig. 2b. The pattern of curve for both HIV positive and HIV negative cases appear similar. The mean time to conversion among HIV positive cases (2.48 months; 95% CI = 1.80, 3.16) was not significantly different from those of HIV negative cases (1.96 months; 95% CI = 1.72, 2.20); Log Rank (Mantel–Cox) $\chi^2 = 2.703$, df = 1, p = 0.100.

Table 2 shows the number of patients that converted at the end of each month of therapy. At the end of the first month, 43 (37.4%) patients sputum converted. The number of patients achieving sputum conversion increased to 91 (79.1%) and 104 (90.4%) at the end of the second and third months respectively. At the fourth month 110 (95.7%) patients had achieved sputum conversion, increasing to 113 (98.3%) at the end of the 5th month. From the 7th month, the sputum conversion rate remained static till the end eight months of the intensive phase and through the continuation phase of treatment. Between the 7th and 8th month there was one case each of default and death. One (0.9%) patient showed continued sputum positivity and was considered as a case of treatment failure.

From the end of the 5th month, 3 (2.6%) patients remained culture positive; one of whom died, and one defaulted leaving one patient with persistent culture positivity till the end of treatment.

4. Discussion

The treatment outcome in the study showed that the standardized MDR-TB treatment regimen recommended by the national TB programme appeared to be effective as a high success rate was recorded. About one in five patients were HIV co-infected. Neither the sociodemographic nor the baseline laboratory statistics was associated with treatment success. The mean sputum conversion time was about 2 months and there was a high probability of converting by two months.

In this study 68.7% of the patients had treatment success as shown by the cure rate of 36.5% plus treatment completed rate of 32.2%. The success rate in this study is comparable with reports from previous studies.[12–19] The high success rate in the study could partly be explained by the fact that our patients had not been previously exposed to second line medications. All our patients also were managed using the DOTS plus strategy for the initial 8 months before continuation with ambulatory care. The patients also received maximum support including nutritional, and psychosocial. Some other studies on treatment outcome on MDR-TB patients have documented lower success rates varying from 39 to 51.2%.[19–22] Differences in treatment outcomes vary with the treatment delivery strategy and type of regimen. Optimal strategies for treatment of MDR-TB are important to reduce the public health threat posed by the disease.

The default rate recorded in the study was low (6%). In a study carried out by Lockman et al in Estonia, a high default rate among MDR-TB patient of 35% was also reported.[23] Other authors from India, Taiwan and Korea have also reported high default rates varying between 15 and 39%.[20,21,24,25] We believe that the reason for the low default rate at our centre was due to intense motivation of patients at enrolment and discharge from hospitalisation. Regular contact was maintained after discharge and as much as possible prompt tracking and intervention were put in place for defaulting patients. Supervision of treatment was continued during the ambulatory continuation phase and was facilitated by the national TB DOTS officers and partner support.

The death rate of 24% observed in this study was high which expectedly affected the overall success rate of the treatments. The explanation lies in the delay in accessing treatment in patients who had failed first-line anti-tuberculosis treatment before the second line treatment became available in the national TB programme. After the MDR-TB treatment was rolled out, patients had to wait on a queue for long periods of time as the second line treatment was commenced in phases, starting initially in the University College Hospital (UCH) pilot site with only 23 bed spaces. The referrals to the treatment centre included a cluster of patients in very poor clinical condition. Majority of the deaths occurred at the early weeks of commencement of treatment.

Treatment outcomes with high death rates in MDR-TB patients in some other settings have also been reported. Singla et al in India reported a death rate of 19%.[26] An average delay of 5 months in the diagnosis of MDR-TB and a subsequent delay of approximately 3.3 months in initiating treatment was revealed in their study. Schaaf et al in their study in Western Cape reported a death rate of 33% among 240 MDR-TB patients.[27] Similarly, in a study undertaken by Vella et al at Tugela Ferry in KwaZulu–Natal from 2005 to 2007, the mortality rate among MDR-TB patients after one year's treatment was 75%.[28] The South African situation is thought to be unique in that the health services face the problem of the high burden of HIV and TB, high TB–HIV co-infection rates and high incidence of

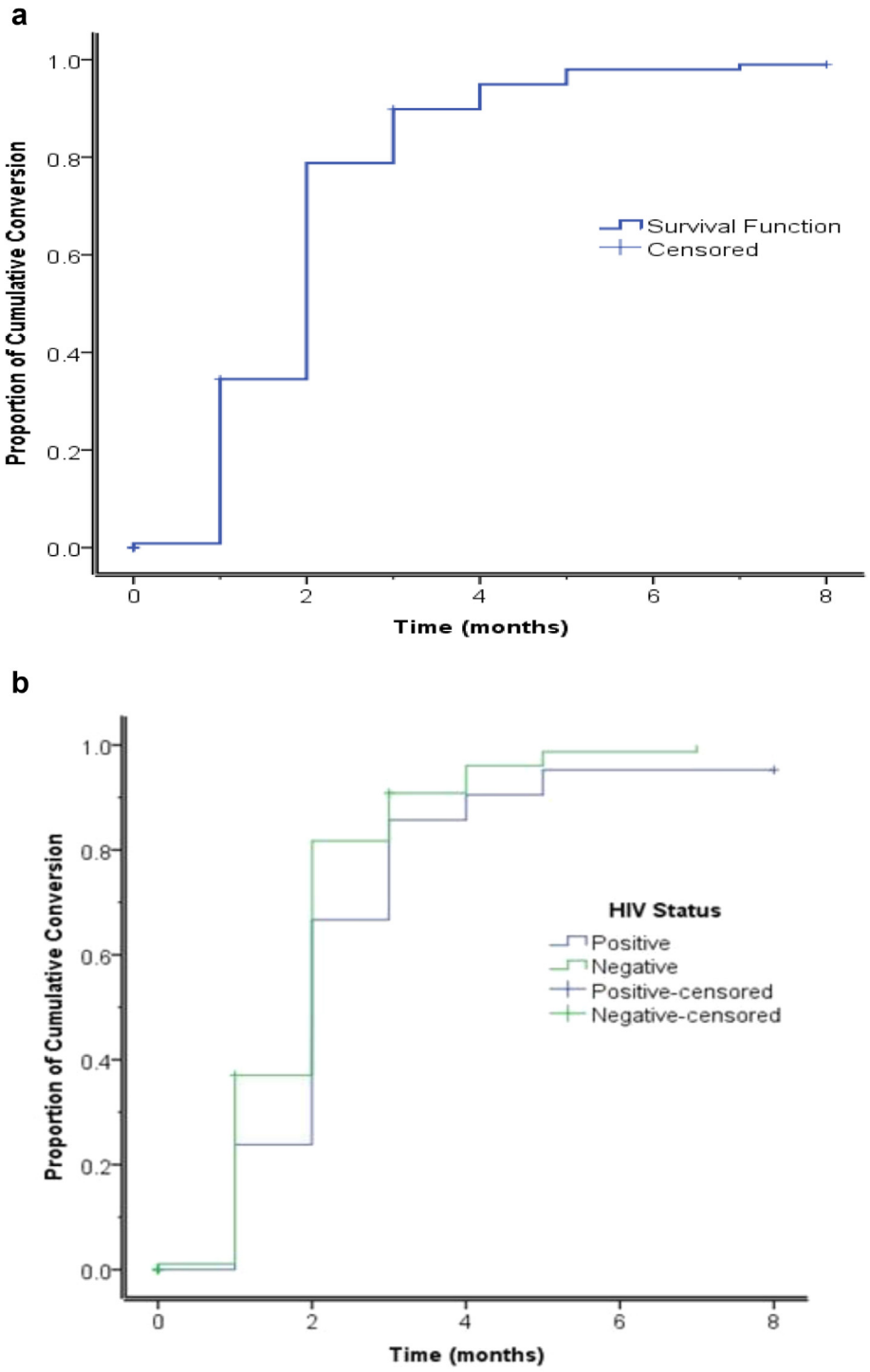

Fig. 2 – a: Survival graph showing cumulative conversion by time among the cohort of 115 MDR-TB cases. b: Sputum conversion time in HIV positive compared with HIV negative individuals.

MDR-TB as well as pockets of high incidence of extensively drug resistant tuberculosis (XDR-TB).[17,29]

In the present study, one in five patients was HIV co-infected. No differences were observed in the sputum conversion and treatment outcome between HIV positive and HIV negative MDR-TB cases. As the ART was being administered under the DOTS strategy while the patients were hospitalized, meaning that the HIV was under better control. This could explain the similar outcome in both groups of patients. Similarly, Hafkin et al, found no difference in the proportion of or time to initial sputum culture conversion between an HIV-infected and a non-infected cohort of MDR-TB patients in Botswana.[10] Increased mortality in HIV-positive compared to HIV-negative patients with drug-resistant TB was reported in some other studies.[17,29,30] The importance of integrating TB and HIV care cannot be overemphasized.

Table 2 – Treatment outcome in relation to sputum conversion.

Time sputum conversion occurred	Number of patients	Cured	Defaulted	Completed	Dead	Failure
1 month	43	16 (37.2%)	4 (9.3%)	13 (30.2%)	10 (23.3%)	0 (0%)
2 months	91	31 (34.1%)	7 (7.7%)	33 (36.3%)	19 (20.9%)	1 (0%)
3 months	104	38 (36.5%)	8 (7.7%)	36 (34.6%)	21 (20.2%)	1 (1%)
4 months	110	40 (36.4%)	9 (8.2%)	36 (32.7%)	24 (21.8%)	1 (1.0%)
5 months	113	42 (37.2%)	9 (8.0%)	37 (32.7%)	24 (21.2%)	1 (0.9%)
6 months	113	42 (37.2%)	9 (8.0%)	37 (32.7%)	24 (21.2%)	1 (0.9%)
7 months	113	42 (37.2%)	9 (8.0%)	37 (32.7%)	24 (21.2%)	1 (0.9%)
≥8 months	115	42 (36.5%)	10 (8.7%)	37 (32.2%)	25 (21.7%)	1 (0.9%)

The mean time of sputum culture conversion in this study was about two months. Furthermore, there was a high probability of sputum converting by two months. The large percentage of patients' sputum converting early as reported in this study lends credence to the likelihood of shortening the duration of MDR-TB treatment. Achieving a rapid sputum culture conversion goes a long way in reducing the clinical and public health risks that MDR-TB poses. The landmark Bangladesh study which utilized a 9 months regimen showed a bacteriologically favourable outcome (84.4%), minimal failure rate of 1% and about 90% relapse-free cure.[31] The shorter MDR-TB regimen lasts between 9 and 12 months comprising an initial phase of 4–6 months of kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol, followed by 5 months of moxifloxacin, clofazimine, pyrazinamide, and ethambutol. The shorter regimen will not only be cheaper, but will also ensure improved treatment success, reduce infectious capacity and selection of resistances. An observational study of a nine-month MDR-TB treatment regimen in Africa was conducted with patients in nine francophone countries in Africa which showed high treatment success rates of 82% on a nine-month treatment regimen.[32,33] The shorter regimen is also being evaluated in a randomized controlled trial (standardized treatment regimen of anti-tuberculosis drugs for patients with multi-drug-resistant tuberculosis STREAM study).[34] With the available evidence, the WHO has recently recommended the shorter MDR-TB regimen in its guidelines.[35] As the country joins other African countries in rolling out a phased shorter regimen, it is hoped that carefully selecting eligible patients and optimised laboratory services would give the desired outcome in MDR-TB patients.[36]

The limitation of the study is that though, only one patient had persistent sputum positivity up to the end of the 20 months (treatment failure), the high default and death rates make this outcome unrepresentative of the final treatment failure rate. Secondly, DST to second line drugs could not be done to ascertain the level of resistance to second line drugs in the patient that failed treatment.

5. Conclusion

The study showed that the treatment success rate was high with most of cases with or without HIV infection, achieving sputum culture conversion within 2 months of commencing treatment. A high death rate was recorded which calls for expansion of MDR-TB treatment services for early initiation and access in patients that have failed first line therapy.

Conflicts of interest

The authors have none to declare.

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REFERENCES

1. World Health Organization. Multidrug and extensively drug resistant TB (M/XDR-TB): 2014 global report. http://www.who.int/tb/publications/global_report/en/. Accessed 12 November 2014.
2. Federal Ministry of Health, Nigeria. *National Drug Resistance TB Prevalence Survey Report*. August 2012.
3. Wells CD, Cegielski JP, Nelson LJ, et al. HIV Infection and multidrug-resistant tuberculosis – the perfect storm. *J Infect Dis*. 2007;196(suppl):S86–S107.
4. Sethi S, Mewara A, Kumar S, et al. Prevalence of multidrug resistance in Mycobacterium tuberculosis isolates from HIV seropositive and seronegative patients with pulmonary tuberculosis in north India. *BMC Infect Dis*. 2013;13:137.
5. Federal Ministry of Health, Nigeria. *National Tuberculosis and Leprosy Control Programme*. Workers' manual. 5th ed. Department of Public Health; 2010.
6. Falzon D, Jaramillo E, Schünemann HJ. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. *Eur Respir J*. 2011;38:516–528.
7. *Guidelines for the Clinical Management and Control of Drug Resistant Tuberculosis*. 5th ed. Federal ministry of health, department of public health; 2011:34–49.
8. World Health Organization. *Guidelines for Establishing DOTS-plus Pilot Projects for the Management of Multidrug-resistant Tuberculosis (MDR-TB)*. WHO/CDS/TB/2000.279. Geneva: World Health Organization; 2000.
9. Holtz TH, Sternberg M, Kammerer S, et al. Time to sputum culture conversion in multidrug-resistant tuberculosis and

relationship to treatment outcome. *Ann Intern Med*. 2006;144:650–659.
10. Hafkin J, Modongo C, Newcomb C, Lowenthal. Impact of the human immunodeficiency virus on early multidrug-resistant tuberculosis treatment outcomes in Botswana. *Int J Tuberc Lung Dis*. 2013;17(3):348–353.
11. Brust J, Lygizos M, Chaiyachati K, et al. Culture conversion among HIV co-infected multidrug-resistant tuberculosis patients in Tugela Ferry, South Africa. *PLoS One*. 2010;6(1), e15841.
12. Park SK, Kim CT, Song SD. Outcome of chemotherapy in 107 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *Int J Tuberc Lung Dis*. 1998;2:877–884.
13. Leimane V, Riekstina V, Holtz TH, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. *Lancet*. 2005;365:318–326.
14. Chan ED, Laurel V, Strand MJ, et al. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. *Am J Respir Crit Care Med*. 2004;169:1103–1109.
15. Tahaoğlu K, Törün T, Sevim T, et al. The treatment of multidrug-resistant tuberculosis in Turkey. *N Engl J Med*. 2001;345:170–174.
16. Holtz TH, Sternberg M, Kammerer S, et al. Time to sputum culture conversion in multidrug-resistant tuberculosis: predictors and relationship to treatment outcome. *Ann Intern Med*. 2006;144:650–659.
17. Orenstein EW, Basu S, Shah NS, et al. Treatment outcome among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *Lancet Infect Dis*. 2009;9:153–161.
18. Joseph P, Desai VB, Mohan NS, et al. Outcome of standardized treatment for patients with MDR-TB from Tamil Nadu, India. *Indian J Med Res*. 2011;133:529–534.
19. Rao NA, Mahfooz Z, Irfan M. Treatment outcome of multi-drug resistant tuberculosis in a tertiary care hospital in Karachi. *J Pak Med Assoc*. 2009;59(10):694–698.
20. Park SK, Lee WC, Mitrick CD, Hart L, Scung KJ. Self-administered standardized regimens for multi drug resistant tuberculosis in South Korea. *Int J Tuberc Lung Dis*. 2004;8:361–368.
21. Ching YC, Enarson DA, Yu MC, et al. Outcome of pulmonary multidrug- resistant tuberculosis: a 6-year follow-up study. *Eur Respir J*. 2006;28:980–985.
22. Sua'rez PG, Floyd K, Portocarrero J, et al. Feasibility and cost-effectiveness of standardized second- line drug treatment for chronic tuberculosis patients: a national cohort study in Peru. *Lancet*. 2002;359:1980–1989.
23. Lockman S, Kruianer A, Binkin NJ, et al. Clinical outcomes of Estonian patients with primary multidrug resistant versus drug-susceptible tuberculosis. *Clin Infect Dis*. 2001;32:373–380.
24. Kim HJ, Hong YP, Kim SJ, Lew WJ, Lee EG. Ambulatory treatment of multidrug resistant pulmonary tuberculosis patients at a chest clinic. *Int J Tuberc Lung Dis*. 2001;5:1129–1136.
25. Prasad R. Long term treatment outcome in multi drug resistant tuberculosis (MDR-TB). *Chest*. 2004;126 836S:35–39.
26. Singla R, Sarin R, Khalid UK, et al. Seven-year DOTS-Plus pilot experience in India: results, constraints and issues. *Int J Tuberc Lung Dis*. 2009;13:976–981.
27. Schaaf HS, Marais BJ. Management of multidrug-resistant tuberculosis: a survival guide for physicians and pediatricians. Faculty of Health Sciences, Stellenbosch University, and Tygerberg Children's Hospital, Cape Town, South Africa *Public Health Action*. 2011;3:156–165.
28. Vella V, Racalbuto R, Guerra C, et al. Household contact investigation of multidrug-resistant and extensively drug-resistant tuberculosis in a high HIV prevalence setting. *Int J Tuberc Lung Dis*. 2011;15(9):1170–1175.
29. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-Analysis. *PLoS One*. 2009;4(9), e6914.
30. Isaakidis P, Cox HS, Varghese B, Montaldo C, Da Silva E. Ambulatory multi-drug resistant tuberculosis treatment outcomes in a cohort of HIV-infected patients in a slum setting in Mumbai, India. *PLoS One*. 2016;12, e28066.
31. Aung KJM, Van Deun A, Declercq E, et al. Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tuberc Lung Dis*. 2014;18(10):1180–1187.
32. Piubello A, Harouna SH, Souleymane MB, et al. High cure rate with standardised short-course multidrug-resistant tuberculosis treatment in Niger: no relapses. *Int J Tuberc Lung Dis*. 2014;18(10):1188–1194. https://doi.org/10.5588/ijtld.13.0075.
33. Kuaban C, Noeske J, Rieder HL, Ait-Khaled N, Abena Foe JL, Treburg A. High effectiveness of a 12-month regimen for MDR-TB patients in Cameron. *Int J Tuberc Lung Dis*. 2015;19(5):517–524.
34. Nunn AJ, Rusen ID, van Deun A, et al. Evaluation of a standardized treatment regimen of anti-tuberculosis drugs for patients with multi-drug-resistant tuberculosis (STREAM): study protocol for a randomized controlled trial. *Trials*. 2014;15:353. https://doi.org/10.1186/1745-6215-15-353.
35. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. http://apps.who.int/iris/handle/10665/250125/.
36. Sotgiu G, Tiberi S, Centis R, et al. Applicability of the shorter 'Bangladesh regimen' in high multidrug-resistant tuberculosis settings. *Int J Infect Dis*. 2017;56:190–193.

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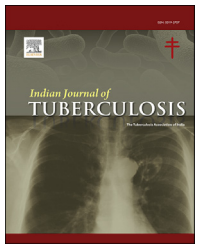

Original article

Identification of Non-Tuberculous Mycobacterium by LPA (CM/AS) assay, HPLC and biochemical test: which is feasible for RNTCP?

George Sebastian [a,b,*], *Sharath Burugina Nagaraja* [c], *Telagi Vishwanatha* [d], *Krishna Hemalatha* [b], *Narayana Vijayalakshmi* [b], *Prahlad Kumar* [a]

[a] *Bharathiar University, Coimbatore, India*
[b] *National Tuberculosis Institute, Bangalore, India*
[c] *ESIC Medical College and PGIMSR, Rajajinagar, Bangalore, India*
[d] *Maharani Science College for Women, Bangalore, India*

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ABSTRACT

Introduction: Non-tuberculous mycobacteria (NTM) causing clinical disease have become increasingly common and more diverse. The development of fast, inexpensive, and reliable tests to identify nontuberculous mycobacteria is need of the hour especially under the Revised National TB Control Programme (RNTCP). The Aim of the study was to check the Diagnostic efficacy of the GenoType Mycobacterium CM/AS assay compared with HPLC and Biochemical Test for Identification of Non-Tuberculous Mycobacteria under the Revised Tuberculosis Control Programme.

Methods and result: It is a cross-sectional study, the suspected NTM culture isolates from the RNTCP accredited laboratories were sent to NRL for speciation and Identification. The culture positive isolates were subjected to Biochemical Identification Test, HPLC and LPA CM/AS. The LPA had 98.23% sensitivity, 50% specificity, 99.56% positive predictive value (PPV) and 20% Negative predictive value (NPV) when compared to HPLC considering Biochemical test as Gold reference standard. The comparison of HPLC and LPA for identification of each species using Mc Nemers Chi square test shown no significant difference between these tests.

Conclusion: Considering Cost, Time and ease of performing the techniques, we recommend first do the basic biochemical test to rule out MTBC from NTM. Then do HPLC and further if results are unclear do LPA CM/AS kit for species confirmation.

Significance and impact of study: NTM are emerging as important causative agents of pulmonary and extra pulmonary disease, the ability to recognize disease caused by NTM and subsequently treat such disease has become increasingly important. The identification of NTM up to its species level using HPLC and LPA CM/AS should gain importance in all TB reference Laboratories.

* *Corresponding author*. Laboratory Division, National Tuberculosis Institute, Bellary Road, Bangalore-03, Karnataka, India. Tel.: +91 9483584564 (Mobile).
E-mail address: georgesebastian05@gmail.com (G. Sebastian).

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1. Introduction

The disease caused by species of the genus *Mycobacterium* are primary cause of morbidity and mortality in the world.[1] The identification and speciation of *Mycobacterium* is of paramount importance as some of them have clinical implications while some remain as saprophytes. It is relevant to have substantial knowledge of the affected species as it influences the patient management and the anti-mycobacterium drugs are quite species specific.

The conventional methods to identify *mycobacterium* are sputum microscopy, culture and biochemical assay techniques. Although sputum smear microscopy for acid fast bacilli provides rapid diagnosis for mycobacteria, it cannot differentiate *Mycobacterium tuberculosis* from Non-Tuberculous mycobacteria (NTM). Nevertheless, speciation of NTM by conventional methods requires a battery of biochemical test besides periodic observation on growth rate and colony morphology. However, it is time consuming and labor intensive and requires an experienced skilled personnel to interpret the results.[2]

There are other alternative techniques available for NTM speciation such as thin layer chromatography, gas chromatography, High Performance Liquid Chromatography (HPLC Agilent 1200 series), Line Probe Assay (LPA) CM/AS (common mycobacterium/additional species) Kit (Hain Life Science, Germany) and other molecular techniques which are based on the principles of hybridization and amplification. The introduction of such newer molecular technologies has led to a marked improvement in accuracy as well as the turnaround time; however, these technologies in developing countries are currently restricted to National Reference Laboratories (NRL). Currently, the Revised National TB Control Programme (RNTCP) in India does not have any provisions or guidelines to detect the NTM species. However, the programme has made a way to screen sputum samples for rifampicin resistance of those patients who do not respond to first line anti-tubercular treatment (ATT) usually known as presumptive drug resistant cases. The sample screening tests are done at intermediate reference laboratories (IRL) or culture drug susceptibility testing (CDST) laboratories. The patients who are not rifampicin resistance but not responding to first line drugs continue to receive the same first line ATT treatment.

We conducted a study at a NRL in India to determine the feasibility of introducing a rapid diagnostic tool to detect NTM in National TB control Programme. We compared the HPLC and LPA (CM/AS) against the conventional biochemical tests under programmatic settings, (a) to identify the mycobacterial species (b) to study the turnaround time, cost incurred for each test and the technical skills required to conduct these tests.

2. Materials and methods

A cross sectional study was conducted during August 2014 to July 2016 at National Tuberculosis Institute (NTI), Bangalore, India which is a National Reference Laboratory.

2.1. Sputum sample processing at IRL or CDST laboratory

Routinely, under the programme, the sputum samples of presumptive drug resistant TB patients are collected from the field to screen for drug resistance. Such samples are processed at the RNTCP IRL/CDST laboratories; initially, the sputum is subjected to smear examination using Ziehl-Neelsen (ZN) technique; those found positive for acid fast bacilli (AFB) are processed by NALC-NaOH method and the deoxyribonucleic acid (DNA) is extracted using LPA kit (HAIN Life Science, Gmbh. Nehren, Germany) according to the guidelines issued by the manufacturer. The samples which are sputum smear positive and "TUB" band negative on LPA are interpreted as presumptive case of NTM.

For the purpose of this study, an additional sample from the same patient (presumptive NTM) was collected and processed using NALC-NaOH method. The sediment from both these isolates was cultured on Mycobacterium growth indicator tube (MGIT) liquid culture (BD Biosciences, Sparks, Md.) and solid Lownstein Jensen (LJ) media. The positive culture growth was subjected to Immuno-Chromatographic Test (ICT) (SD BioLine, MPT 64, South Korea) to confirm the isolate as NTM.

Such samples were further sent to NRL for identification and speciation using HPLC (Agilent Technologies, Germany) and LPA (CM/AS) kit. Those samples which are sputum smear negative were subjected to cartridge nucleic acid amplification test (CBNAAT) [Xpert/MTB RIF Cepheid, USA] as per the standard programme protocol.

2.2. Work flow process followed at National Reference Laboratory

Upon receipt of isolates from IRL/CDST laboratories at NRL, the isolates were sub-cultured on to a MGIT liquid culture tubes and also onto a LJ culture media to get a fresh growth of culture isolate. The culture was then subjected to three different diagnostic tests like biochemical identification, HPLC and LPA (CM/AS).

2.3. Biochemical tests (BT)

Biochemical Test (BT) analysis began by determining growth rate and pigment production for each isolate in LJ media. To determine the optimal temperature for growth, each isolate was incubated at several temperatures (25 °C, 37 °C, and 42 °C). The BT such as niacin accumulation, nitrate reduction positive isolates were reported as *M. tuberculosis* and negative isolates were further characterized with battery of biochemical test like Iron uptake, 5% NaCl tolerance, 1mM and 3mM aryl sulfatase, growth in macconkey's agar, and urease.

2.4. HPLC

Another culture isolate from the solid LJ media was subjected to HPLC analysis and was processed according the standard protocol.[3] The total run time was twenty minutes. Peaks were identified on the basis of their retention times, relative to that of the internal standard and were labeled as suggested by the

Centre for Disease Control.[4] Fig. 2 shows the HPLC chromatogram of different NTM species.

2.5. LPA (CM/AS) kit

The LPA (CM/AS) is a commercially available kit which is developed to differentiate and identify different species of NTM from cultures. It is a very accurate and reliable test to identify NTM species, which require a specialized set up and trained laboratory personnel. The assay has two kits: the CM (Common Mycobacteria), which identifies 15 Mycobacterium species, including *M. tuberculosis* complex while the AS (Additional Species) kit aims to differentiate 16 additional less common NTM species available for differentiation. The LPA using CM/AS kit was performed as per manufactures instruction. Fig. 3 shows Species level identification using LPACM/AS kit.

Among the three methods the conventional biochemical test is considered as the gold standard. The species identified by each method was cross checked using the biochemical test. In general, the sensitivity and specificity of HPLC is 99.47% and 99%; while that of LPA (CM/AS) is 81% and 99% respectively.

3. Result

Of the 270 culture isolates received, only 233 isolates were considered for evaluation and final analysis. The remaining 35 culture isolates were excluded due to cultures being contaminated and no growth. All the 233 culture isolates were subcultured in solid LJ, and MGIT liquid culture. The samples which were found positive by MGIT (n = 233) was subjected to ICT of which 19 culture isolates were found positive for ICT, and were reported as *M. tuberculosis* Complex. The positive culture (n = 233) from LJ/MGIT was subjected to LPA (CM/AS) kit. The culture positive samples on LJ were taken for HPLC as well as for basic biochemical identification test like niacin accumulation, nitrate reduction (n = 235). Out of these 19 isolates were found to be BT positive for *M. tuberculosis complex*. The remaining species which were negative (n = 216) were further characterized with other BT like Iron uptake, 5% NaCl tolerance, 1mM and 3mM aryl sulfatase, growth in Macconkey's agar and urease test (Table 1).

The most common NTM species identified were 44 (26.8%) were *Mycobacterium chelonae*, 21 (12.8%) were *Mfortuitum fortuitum*, 15 (9%) were *Mycobacterium gordonae*, 15 (9%) were *M. tuberculosis complex*, 10 (6.1%) were *Mycobacterium kansasii*, 8 (4.9%) were *Mycobacterium simiae*, 4 (2.4%) were *Mycobacterium thermophile*, 2 (1.2%) were *Mycobacterium gastri*, 1 (0.6%) were *Mycobacterium scrofulaceum*, 1 (0.6%) were *Mycobacterium avium* and 8 (4.9%). There was high concordance of NTM species identification using HPLC and LPA (CM/AS) against BT (Table 2).

3.1. BT

The total turnaround time incurred for each BT for the detection of *M. tuberculosis* i.e., niacin accumulation and nitrate reduction was one day; while for NTM isolation it took around fourteen days for completion. The cost of BT ranged between 1 and 2 US$ per sample (see Fig. 1).

3.2. HPLC

The total turnaround time for processing the sample using HPLC was around five days and the entire assay was carried

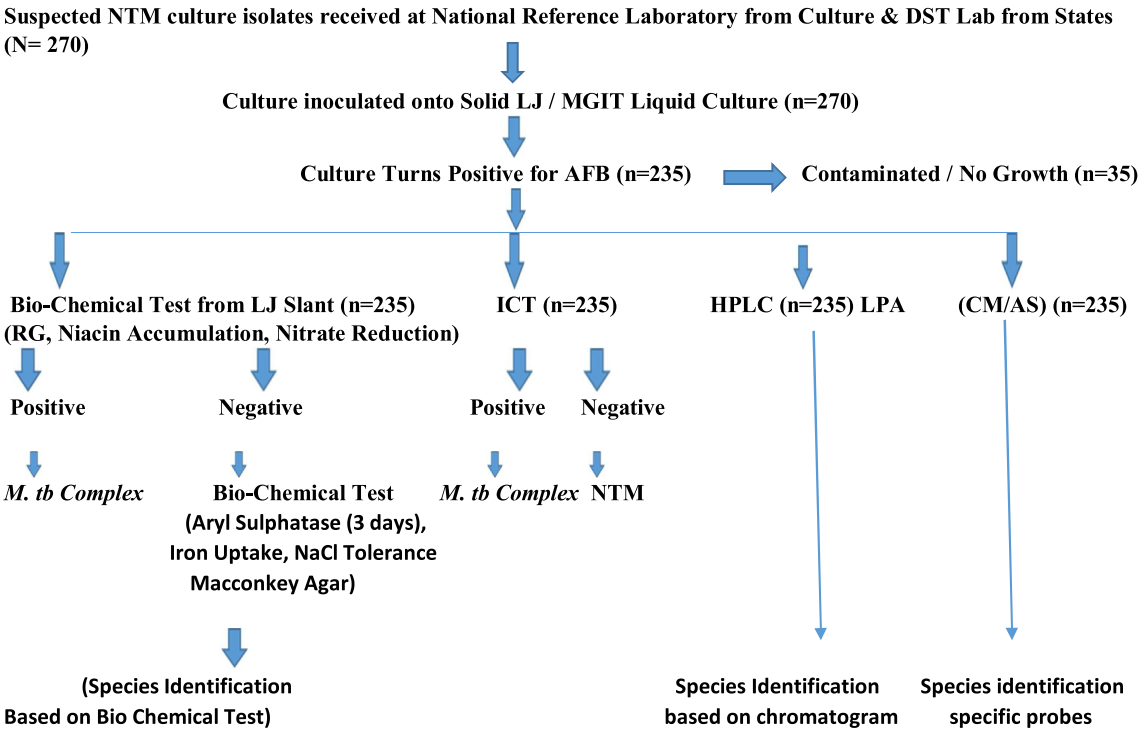

Fig. 1 – Flow chart for the NTM isolation using different diagnostic tools available.

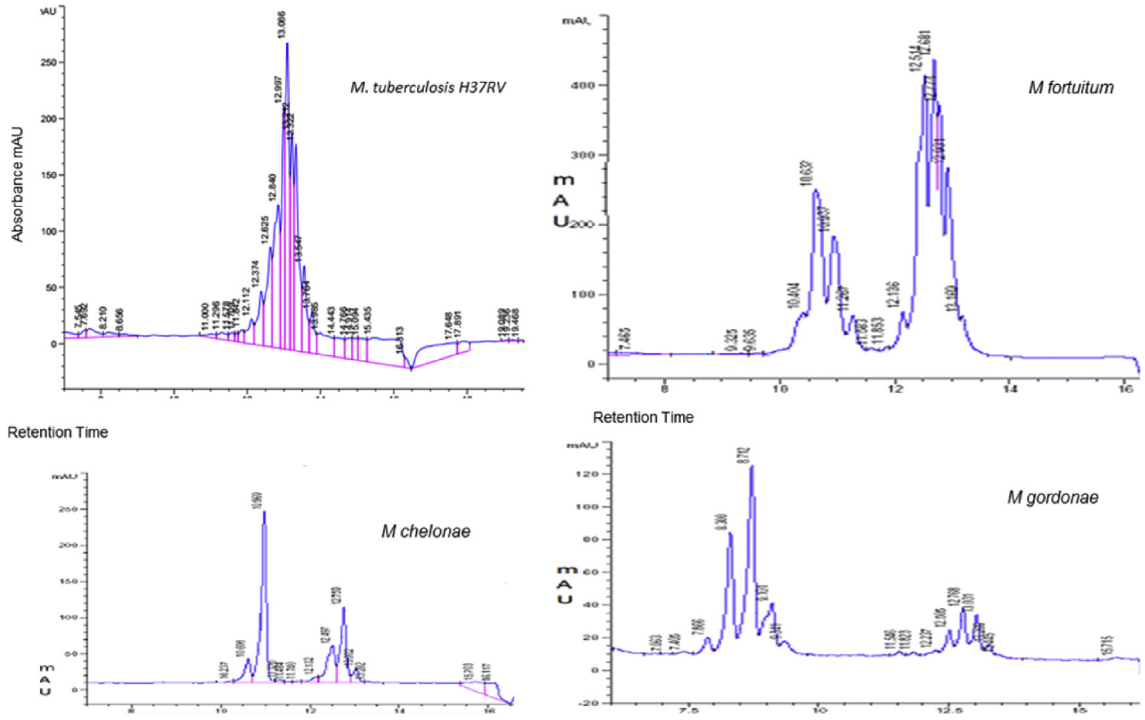

Fig. 2 – Shows the HPLC chromatogram of different NTM species.

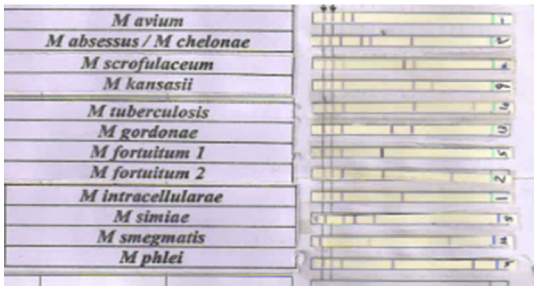

Fig. 3 – LPA Species level identification using CM/ AS kit.

out by a single technologist. An approximately five samples can be run using the HPLC in a single day and the direct cost involved would be around 4 US$ per sample.

3.3. LPA (CM/AS)

The total turnaround time for processing the sample using LPA (CM/AS) kit was around one to two days, and LPA (CM/AS) was done by a single trained technologist. An approximately twelve samples can be run using the LPA in a single day and the cost involved would be around 25 US$ per sample.

Table 1 – List of Biochemical Test and the Species identification.

Species	Nitrate Reductase	Niacin Accumulation	Aryl Sulphatase (3 days)	Iron Uptake	Tolerance to Nacl	McConkey Agar	Urease	Colony morphology
M. tuberculosis Complex	+	+	–	–	–	–	–	R
M avium	–	–	–	+	–	–	–	Sm
M. chelonae	–	–	+	–	–	+	+	Sm
M. flavescens	+	–	+	–	+	–	+	Sm
M. fortuitum	+	+	+	+	+	+	+	Sm
M. gastri	–	–	+	–	–	–	+	Sm
M. gordonae	–	–	+	–	–	–	+	Sm
M. interjectum	–	–	–	–	–	–	+	Sm
M. kansasii	+	–	+	–	–	–	+	R
M.phlei	+	–	+	–	–	–	+	Sm
M. scrofulaceum	–	–	+	–	–	–	+	Sm
M. simiae	–	+	–	+	–	–	+	Sm
M. terrae	+	–	+	–	–	–	–	Sm
M. thermophile	–	–	+	–	–	–	–	Sm

Table 2 – Represents the Species level identification and Frequency distribution of different NTM species.

Species	BT	HPLC	LPA
H37 RV	19	19 (100%)	19 (100%)
M avium	5	5 (100%)	5 (100%)
M. chelonae	80	79 (98.7%)	80 (100%)
M. flavescens	1	1 (100%)	0 (0%)
M. fortuitum	40	39 (97.5%)	39 (97.5%)
M. gastri	10	10 (100%)	10 (100%)
M. gordonae	29	28 (96.5%)	29 (100%)
M. interjectum	6	6 (100%)	6 (100%)
M. kansasii	16	16 (100%)	16 (100%)
M. phlei	1	1 (100%)	1(100%)
M. scorfulaceum	9	9 (100%)	9 (100%)
M. simiae	11	11 (100%)	11 (100%)
M. terrae	2	2 (100%)	0 (0%)
M. thermophile	6	6 (100%)	3 (50%)

BT: Biochemical Test.
NTM: Non Tuberculous Mycobacteria.
HPLC: High Performance Liquid Chromatography.
LPA: Line Probe Assay.

4. Discussion

It is one of the first studies conducted in the country to compare the feasibility of introducing either HPLC or LPA (CM/AS) or BT as a rapid diagnostic tool for detection of NTM under programmatic settings.

In the recent years, there has been an increased awareness of NTM as disease-causing agents. The NTMs share common clinical and radiological similarities with MTB especially among the older age groups, people with preexisting lung conditions, advanced HIV disease may take long to treat often with poor outcome as compared with MTB.[5–7] In India, the isolation rate of NTM ranges from 0.5% to 8.6%.[8] Central India reported the increase in prevalence of NTM from 1.0% (2005) to 3.5% (2008) and 88.6% of the NTM isolated had clinical relevance.[8] The diagnosis and treatment of NTM infection are mostly species specific and quite a few may be resistant to conventional ATT drugs; hence, their identification up to species level is of paramount importance. The commonest NTM species encountered in our study were *M. chelonae, M. fortuitum*, *M. gordonae* and *M. kansasii* which was similar to the findings of other studies in India.[9–11] The *M. gordonae* species which can be commonly isolated from the tap water has potential to cause clinically significant disease.[12] As NTM strains are resistance to first line ATT drugs, the infection due to NTM are considered as TB treatment failure, and subsequently patients are treated for drug-resistant TB (DR-TB) disease.[13] There remains a vacuum to measure the magnitude of NTM speciation in RNTCP due to lack of feasible diagnostic tool to detect NTM.

The HPLC serve as a rapid diagnostic tool which can identify and speciate the organism. For interpretation of HPLC results there is a need to have a good collection and library of reference standards.[3] To interpret the results of our study we utilised the standard reference strains of 15 NTM species available at NTI laboratory. Approximately 5–6 samples results can be obtained using HPLC in a day.

The LPA (CM/AS) kit is reliable, consumes lesser time, easy to perform and interpret. It requires a dedicated work area with strict aseptic precaution to run the assay. While such molecular methods are expensive and require precise technical skills to perform molecular assays they are prone for contamination.[14,15] The limiting factor for its use currently is the higher cost. At least ten samples have to run in a batch for the optimal utilization of the kit. In anticipation of more number of samples the laboratories usually store the cultures in a cold storage which cumulatively delays the reporting of NTM speciation. The reasons for contamination and no growth in our study could be due to the batching up of the culture isolates in the laboratory or delay occurred due to transportation of the isolate to NRL.

In our study, the sensitivity and specificity of HPLC was found to be 99.47% and 99% and that of LPA (CM/AS) was around 81% and 99% when compared to the conventional biochemical tests. The low sensitivity of LPA (CM/AS) was due to the fact that it failed to identify two species namely *M. flavescens* and *M. terrae* and few strains of *M. thermophile* which was identified by HPLC.

An important component of our study was the cost analysis and turnaround time (TAT) for each technique. In a direct comparison, the cost of BT used for *M. tuberculosis* identification were least expensive (1 US$) followed by biochemical identification for NTM (2 US$). The TAT for biochemical identification was around fourteen days to complete the identification process. The cost incurred for HPLC was around 4 US$ and TAT was around 5 days. The LPA (CM/AS) kit was the most expensive (25 US$ for each sample).

We perceive that the more efficient strategy in identification of mycobacteria under the RNTCP could be achieved by a combination of three methods. When the primary culture is obtained in IRL/CDST laboratory, the basic biochemical test to identify MTB should be done to rule out MTB from NTM, based on the result if the culture is found to be an NTM, the culture should be subjected to HPLC for species identification. If the ICT is "unclassified" then an effort should be done to speciate using LPA (CM/AS). To conclude, if cost is not a concern to the programme the best technically feasible and user friendly diagnostic tool for NTM is LPA (CM/AS) kit.

Contributorship statement

GS, BNS, PK conceived the study. VL performed the assay, GS, BNS, TV wrote the paper.

Ethics approval

The National Tuberculosis Institute board of ethics committee was obtained from the ethical clearance for the study.

Informed consent

Informed Consent was taken before the sample collection.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Stout JE, Koh WJ, Yew WW. Update on pulmonary disease due to non-tuberculous mycobacteria. *Int J Infect Dis*. 2016;45:123–134.
2. Hawker PM. The role of the polymerase chain reaction in the diagnosis of Mycobacterial infections. *Rev Med Microbiol*. 1994;4:21–32.
3. Butler WR, Guthertz LS. Mycolic Acid Analysis by High-Performance Liquid Chromatography for Identification of Mycobacterium Species Mycolic Acid Analysis by High-Performance Liquid Chromatography for Identification of Mycobacterium Species. *Clin Microbiol Rev*. 2001;14(4):704–726.
4. Glickman SE, Kilburn JO, Butler WR, et al. Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a Mycobacterium library. *J Clin Microbiol*. 1994;32(3):740–745.
5. Griffith DE, Timothy A, Barbara A, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175(4):367–416.
6. Sakatani M. Nontuberculous mycobacteriosis; the present status of epidemiology and clinical studies. *Kekkaku*. 1999;74:377–384.
7. Singh S, Gopinath K, Shahdad S, et al. Nontuberculous mycobacterial infections in Indian AIDS patients detected by a novel set of ESAT-6 polymerase chain reaction primers. *Jpn J Infect Dis*. 2007;60(1):14–18.
8. Jani M, Rodrigues C, Mehta A. The neglected and often ignored: Nontuberculous mycobacteria. *Journal of Global Infectious Diseases*. 2011;3(1):94.
9. Devi DRG, Sridaran D, Indumathi VA, et al. Isolation of Mycobacterium Chelonae from wound infection following Laparoscopy: a case report. *Indian J Tubercul*. 2004;51(9):149–152.
10. Jesudason MV, Gladstone P. Non tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital in South India. *Indian J Med Microbiol*. 2005;23(3):172–175.
11. Kalita JB, Rahman H, B K. Delayed post-operative wound infections due to non-tuberculous Mycobacterium. *Indian J Med Res*. 2005;122(6):535–539.
12. Shirin A, Mazumder MD, Anna Hicks MD, et al. *Mycobacterium Gordonae* Pulmonary Infection in an Immunocompetent Adult. *N Am J Med Sci*. 2010;2(4):205–207.
13. Gopinath K, Singh S. Non-Tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger? *PLoS Neglected Trop Dis*. 2010;4(4):1–4.
14. Burman WJ, Reves RR. Review of False-Positive Cultures for Mycobacterium tuberculosis and Recommendations for Avoiding Unnecessary Treatment. *Clin Infect Dis*. 2000;31(6):1390–1395.
15. Couto I, Machado D, Viveiros M, et al. Identification of nontuberculous mycobacteria in clinical samples using molecular methods: a 3-year study. *Clin Microbiol Infect*. 2010;16(8):1161–1164.

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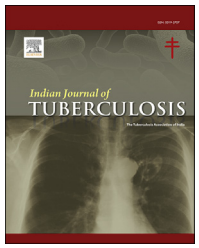

Original article

Potential of adjunctive *Mycobacterium w* (MIP) immunotherapy in reducing the duration of standard chemotherapy against tuberculosis

Mamta Chahar [a,2], *Krishan Dutta Rawat* [a,b,2], *P.V.J. Reddy* [a], *Umesh Dutt Gupta* [a], *Mohan Natrajan* [a], *Devendra Singh Chauhan* [a], *Kiran Katoch* [a], *Godavarthi B.K.S. Prasad* [c], *Vishwa Mohan Katoch* [d,*,1]

[a] *National JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR), Tajganj, Agra, 282004, UP, India*
[b] *Department of Bio & Nanotechnology, Guru Jambheshwar University of Science & Technology, Hisar, 125001, India*
[c] *Department of Biochemistry, Jiwaji University, Gwalior, 474011, India*
[d] *Former Secretary, Department of Health Research, Govt of India and Director-General, Indian Council of Medical Research, Ansari Nagar, New Delhi, 110029, India*

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ABSTRACT

Introduction: The need to shorten the treatment duration in tuberculosis has always been felt. Immunotherapy in combination with chemotherapy has been considered a promising approach for this purpose into tuberculosis. We studied the adjuvant immunotherapeutic activity of *Mycobacterium indicus pranii* (MIP *or Mw*) in combination with conventional chemotherapy using guinea pig of pulmonary tuberculosis infected with *Mycobacterium tuberculosis* H37Rv via aerosol.

Methods: Experimental animals treated with standard chemotherapy and immunotherapy (MIP) separately and in combination of both. Guinea pig lungs evaluated following infection and subsequent therapy at predefine time point. Various cytokine mRNA expressions levels were quantified by quantitative reverse transcriptase PCR at the 4th, 8th and 12th week post-infection of *M. tuberculosis*.

Results: We determined the time required for bacterial clearance from guinea pig lungs. Standard chemotherapy (RvCh) compared to the animals where chemotherapy plus Mw immunotherpay (RvChMwT) was given. It took 12 weeks to achieve bacterial clearance in the RvCh group while this was achieved in 8 weeks in RvChMwT group. Pro-inflammatory cytokines (IFN-γ, IL-2, IL-12p35 and TNF-α) level were higher in RvCh, RvChMwT and RvMwT group, while the IL-10 and TGF-β were suppressed.

Conclusion: Cytokine expression level showed that *Mw* in conjunction with chemotherapy enhances the effect of pro-inflammatory cytokines (such as, IFN-γ, IL-2, IL-12 and TNF-α) and reduces the production and effect of anti-inflammatory cytokines (like IL-10 and TGF-

* *Corresponding author.*
E-mail address: vishwamohan_katoch@yahoo.co.in (V.M. Katoch).
[1] Present address: NASI-ICMR Chair at Rajasthan University of Health Sciences (RUHS), Jaipur.
[2] Equal contribution.
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β) thereby restoring the pro-inflammatory / anti-inflammatory cytokines balance. Thus, the present study indicates that subject to rigorous testing by other parameters, *Mw* (MIP) as adjunct immunotherapy has potential for reducing treatment duration.

1. Introduction

Tuberculosis (TB) continues to be a formidable public health challenge in several parts of the world and contributes considerably to illness and death around the globe.[1] The current TB epidemic is being sustained and fuelled by two important factors: the human immunodeficiency virus (HIV) infection and increasing resistance of *Mycobacterium tuberculosis* to the anti-TB drugs.[2–4]

At present, tuberculosis chemotherapy requires a long treatment duration (usually 6–9 months) which has a negative impact on treatment compliance. Therefore, efforts have been focused on the development of new treatment regimens that are of shorter treatment duration but are effective in reducing the bacterial burden in tissues. The host immune system is a critical factor both for containment and cure of *M. tuberculosis* infection. Potential immunoadjuvants can complement the current chemotherapy to enhance the immune specially the mycobactericidal response of the host. If adjunct chemotherapy succeeds in enhancing bacterial clearance, this will also have potential role in reducing/curtailing transmission, which is critical in reaching the target of ending TB.

Infection of tuberculosis is mainly controlled by the cell mediated immunity (CMI).[5] Cytokines play key role in CMI response and are broadly functionalised into two groups, namely pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type). Induction of pro-inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-2, IL-12 and tumour necrosis factor (TNF)-α provides protection against invading pathogens but their production in excess can lead to uncontrolled tissue damage.[6–8] Anti-inflammatory type cytokines such as IL-10 and transforming growth factor (TGF)-β inhibit the immune response and their production also counteracts the pro-inflammatory mediated actions.[9] Presence of *M. tuberculosis* infecion may up-regulate the IL-10 and TGF-β cytokines by down-regulating the proinflammatory cytokines.[9] Therefore, during and after anti-tuberculosis treatment, a good balance between Th1 and Th2 responses is necessary in order to control the disease. This balance could be achieved by enhancing the Th1 response and reducing the Th2 response.[10] In tuberculosis, IFN-γ acts to strengthen the immune system against *M. tuberculosis* by activating the macrophages. Up-regulation of IL-2 and IL-12 cytokines help in production of IFN-γ and hence mounts an effective immune response.[11] TNF-α assists in granuloma formation and maintenance.[12] It has been reported that deficiency of TNF-α causes disorganized granuloma after infection in mice.[13,14]

Undoubtedly, chemotherapy is the most efficient treatment of tuberculosis but it has to be taken regularly for 6–9 months. The long duration of treatment is the main reason of the poor adherence to the therapy and often results into treatment failure among non-compliant cases and contributes towards the emergence of multi drug resistance (MDR) which is a major problem in developing countries.[15] Hence, an effective adjunct to the standard therapy is the need of time in order to treat tuberculosis more effectively. *Mycobacterium indicus pranii* (*Mw*) is an well known immunomodulator, known to protect against *M. tuberculosis* infection in mice[16] and has been evaluated as one of the possible alternatives to solve this problem for many years.[17] Moreover, several other TB vaccines and immunomodulatory candidates have been studied as adjunct to chemotherapy to enhance the efficacy of treatment in varying degree of success.[5,6,18,19] *Mycobacterium indicus pranii*, formerly known as *Mycobacterium w* (*Mw*/MIP), is a cultivable, non-pathogenic and rapidly growing saprophyte classified in Runyon's group IV along with other rapid growers like *M. fortuitum*, *M. smegmatis*, *M. chelonae* and *M. vaccae* on the basis of its growth and metabolic properties.[20,21] Gupta and co-workers found that *Mw* has higher immunogenicity and protective efficacy than BCG when given as a prophylactic vaccine by aerosol route in the mouse model of tuberculosis. Rawat et al reported levels of expression of certain chemokines in *M. tuberculosis* infected versus *Mw*/MIP immunized animals.[23] The use of heat killed *Mw* vaccine in immunotherapeutic mode has been found to be successful in patients with borderline-lepromatous or lepromatous leprosy.[24–26] Patel and Tripathi observed that TB patients who received the adjuvant Mw immunotherapy had rapid sputum conversion and clinical improvement.[27,28] *Mw* also showed some protection against tuberculosis in contacts of leprosy cases.[29] Mw immunotherapy has also been shown to be very effective for enhancing the immune recovery in HIV infected patients.[30] Over the years *Mw* has emerged as a broad spectrum immunomodulator and *Mw* immunotherapy also showed significant effect in lung cancer, neck cancer and head cancer treatment.[31,32]

In the present study, the efficacy of *Mw* as an adjunct to standard chemotherapy for tuberculosis was evaluated in experimental guinea pig model. It was also evaluated whether *Mw* has any efficacy as an immunotherapeutic agent when given in the absence of chemotherapy.

2. Methodology

2.1. *Mycobacterial strains*

M. tuberculosis reference strain, H37Rv (obtained from the Mycobacterial Repository Centre of our Institute) was used for aerosol infection of guinea pigs. For immunotherapy of infected animals, the saprophytic non-pathogenic species,

heat killed inactivated *Mycobacterium indicus pranii* (*Mw*/MIP), was obtained from Cadilla Pharmaceuticals Ltd, Ahmadabad. Before aerosol infection, H37Rv was cultured in Middlebrook 7H9 broth (Difco Laboratories) supplemented with oleic acid-albumin-dextrose-catalase (OADC). The log phase mycobacterium culture was harvested and CFU was estimated before storing the aliquots at −70 °C.

2.2. Animal model

Female outbred guinea pigs of 250–300 g weight (procured from Central Drug Research Institute, Lucknow, India) were used for this study. All the experiments were performed after taking the approval of the Institutional Animal Ethical Committee. All the animals were maintained under animal biosafety level 3 conditions in isolator cages within an air-filtered environment under a 12 h light–dark cycle for the entire period of the experiment and fed standard guinea pig food and water ad libitum. A pathogen free environment was provided to the animals of healthy control (NH) group. Guinea pigs were infected via aerosol route by aerosol Generator device (Glas-Col, USA). A bacterial suspension of 4.37×10^7/ml was used to deliver ~100 bacilli of *M. tuberculosis* (H37Rv) per guinea pig. After 4 weeks post infection of H37Rv strain, various treatments were started in three groups of animal while one group was kept un-treated (Rvo). First group (RvCh) was treated with chemotherapy (first line anti-tuberculosis drugs: rifampicin, isoniazid, ethambutol and pyrazinamide) five days/week by oral route from 4th week to 16th week post infection.[33] Second group (RvMwT) was treated with immunotherapy (*Mw*) by subcutaneous route while third group (RvChMwT) was treated with chemotherapy in conjunction with immunotherapy. Doses of chemotherapy administered to the animals were according to their body weight – i.e. rifampicin (RIF) 10 mg/kg, ethambutol (EMB) 15 mg/kg, isoniazid (INH) 5 mg/kg and pyrazinamide (PZA) 25 mg/kg.[33] All drugs were obtained from Sigma Chemical (USA) and administered by oral gavages via instillation in the back of the mouth. INH, PZA and EMB were dissolved in distilled water while RIF was initially dissolved in 100% DMSO (Merck, Germany) and diluted in H_2O to obtain a final 5% DMSO concentration. At first, double doses of immunotherapy (5×10^8 heat killed bacilli of *Mw* per dose) were given at 4th week post-infection. After that a single dose of immunotherapy (*Mw*) was given at two weeks intervals up to 14th week post-infection.

2.3. Experimental design and procedures

Bacterial load and fundamental immune responses in all the groups of animal were examined at five time points (day 1, 4th, 8th, 12th, and 16th week) post-infection. After one day of infection, three animals were randomly chosen for checking the success of aerosol infection. The infection was also checked before starting various interventions at 4th week post-infection by randomly dissecting three animals. For the treated groups, various interventions were started from the end of 4th week post infection and three animals per group were sacrificed at 8th, 12th and 16th week time point. The right caudal lobe of lung was fixed in 10% buffered formalin for histopathological study. The left caudal lobe of lungs was stored in liquid nitrogen for expression studies of cytokines genes. For determining the colony-forming units (CFUs) counts, the remaining lung and spleen were homogenized in Middlebrook 7H9 broth (Difco Laboratories) using polytrone homogeniger (Glas-Col, USA). Homogenates and their dilution were plated onto Middlebrook 7H11 plates (Difco Laboratories) supplemented with OADC and cycloheximide (50 μg/mL), carbenicillin (100 μg/mL), polymyxin B (200 U/mL), and trimethoprim (20 μg/mL).[34] After incubation at 37 °C for 21 days, the colonies were counted upto 28 days.

Histopathology: The right caudal lobe of the lung was fixed in 10% buffered formalin. Subsequently, they were removed from the fixative and placed into a mould (metallic). After that, they were transferred to a tissue processor which was programmed for desired length of time for each step of the processing schedule. Paraffin blocks of processed tissues were prepared and mounted onto wooden blocks. After trimming the paraffin blocks of tissue were cut into sections (size 5μ) with the help of a rotary microtome. Tissue sections were floated onto a water bath (at 50 °C) and picked onto coated slides placed on a hot plate (<50 °C for 30 min) and stained with Hematoxylin and Eosin (H&E) and Fite-Faraco as per standard protocol. Photomicrographs were taken by photo microscope (Olympus, Japan) and analysed using the Image-Pro software (Olympus, Japan).

RNA isolation and real-time reverse transcriptase-polymerase chain reaction (RT-PCR):

Lung tissues were ground in liquid nitrogen and transferred into a pre-chilled 2 mL micro-centrifuge tube. Total RNA isolation was done by using RNeasy mini kit (Qiagen. USA) as per the manufacturer's instructions followed by quantification on NanoDrop2000c (Implen, Germany) and RNA quality was checked by gel electrophoresis on 1.2% Agarose denaturing gel (Sigma, USA). These RNA samples were used to study the *in-vivo* cytokine genes expression profiling by real time reverse-transcripase-PCR (RT-PCR) in various groups of animals and time intervals of the study.

The RT-PCR was performed on the selected cytokine genes using primers designed with the Primer3 software (Table 1). Total 500 ng RNA was converted into cDNA using the cDNA Synthesis kit (Sigma, USA) according to the manufacturer's instructions. Reactions were performed in duplicate with cDNA using SYBR Green I kit (Roche, Germany). on Light-Cycler480 instrument (Roche, Germany) using following parameters; initial denaturation at 95 °C for 10 minutes followed by amplification steps at 95 °C for 10 s, 60 °C for 20 s and 72 °C for 15 s for 45 cycles followed by melting curve analysis at 95 °C for 10 s, 65 °C for 15 s, 95 °C for continuous acquisition mode signal time as per manufacturer's protocol. The transcript level of each gene was expressed as a fold change for all test samples relative to the control sample. Similarly, each gene expression was quantified at 4th, 8th and 12th week post infection. The real time PCR amplification results were expressed as threshold cycles (C_t) normalized against the housekeeping genes GAPDH.[35] Relative fold changes were calculated by $2^{-\Delta\Delta Ct}$ formula for each gene, where $\Delta\Delta C_t = (C_t$, Target gene — C_t, reference gene) of test sample/(C_t, Target gene — C_t, reference gene) of control sample.[36]

Table 1 – Sequences of primers used in real time RT-PCR with amplicon size.

S.No.	Targets Gene		Sequences	Length (bp)
1.	IFN-γ	Forward	TCAGAGCCAAATCGTCTCCT	139
		Reverse	CACCTTGTTGCTGCTGTTGT	
2.	TNF-α	Forward	ATCTACCTGGGAGGCGTCTT	121
		Reverse	ACAGGGCAATGACTCCAAAG	
3.	IL-2	Forward	CGTGGAGCAGGTGCTAAAT	111
		Reverse	GTTTCGGATCCCTTTAGGCT	
4.	IL-12p35	Forward	ACCGTGAAAGCCTGTGTACC	129
		Reverse	GCACAGGGCCATCATAAAAG	
5.	IL-10	Forward	TTGGCAGGGTGAAGACTTTC	120
		Reverse	GGATCATTTCCGATAGGGCT	
6.	TGF-β	Forward	TACCTCAAGCAACCAGCTCCT	119
		Reverse	CTGAAGCGAAAGCCCTCTAA	
7.	GAPDH	Forward	ATGGATTCTACCCACGGCAAC	99
		Reverse	GATCTCGCTCCTGGAAGATC	

Abbreviations: Interleukin (IL), tumour necrosis factor (TNF), interferon (IFN) and housekeeping gene-glyceraldehye-3-phosphate dehydrogenase (GAPGH).

2.4. *Statistical analyses*

CFU data analysis and cytokine genes expressions of all the groups were carried out for each time point by "Two-way ANOVA" using Bonferroni post test analysis when $p < 0.05$. For statistical analysis and generation of graphs, Prism 5 software (Version 5.01; GraphPad Software Inc., USA) was used.

3. Results

3.1. *Growth kinetics of* M. tuberculosis

We evaluated the immunotherapeutic potential of *Mw* (Heat killed) using guinea pig model. The animals were first challenged with *M. tuberculosis* H37Rv and various interventions (chemotherapy and immunotherapy, as detailed in Methods section) were started 4 weeks after post-infection in three groups while one group where no treatment was given (Rvo) was used as a control group. In order to determine the growth kinetics of *M. tuberculosis* in various groups of guinea pigs, CFU counts at day one, 4th, 8th, 12th and 16th week post-infection were enumerated (Fig. 2). In the beginning, the bacillary loads (mean $\log_{10}$ CFU) in the lungs of animals at day one and 4 week post-infection were 2.127 ± .055 and 5.952 ± .036, respectively. After 4 weeks of treatment or 8th weeks of post infection, the mean CFU of all the treated groups were compared to untreated group. The RvMwT group mean CFU was found to be 0.416 $\log_{10}$ which is significantly lower ($p < 0.01$) than Rvo group. Both the groups treated with RvCh (4.227 ± .045 $\log_{10}$ CFU) and RvChMwT (3.125 ± .065) had shown better effect than the group treated with *Mw* immunotherapy RvMwT. A clear influence of *Mw* immunotherapy in conjunction with chemotherapy was evident after 4 weeks of treatment as a significantly lower ($p < 0.001$) bacillary load was observed for the RvChMwT group (treated with chemotherapy in conjunction with *Mw* immunotherapy) as compared to the RvCh group (chemotherapy alone) and RvMwT (immunotherapy alone) groups.

After 8 weeks of treatment (i.e. 12 weeks of post infection), the CFU count was reduced to zero in the RvChMwT group. On the other hand, no significant reduction in the CFU counts was observed for the Rvo and the RvMwT groups, the values being 4.714 ± .085 and 4.936 ± .047 $\log_{10}$. The CFU counts of the RvCh group treated with chemotherapy alone was significantly lower ($p < 0.001$) than that in the Rvo and RvMwT groups. At the end of 12 weeks of treatment, the bacillary load in the lungs of animals of RvCh group treated with chemotherapy alone was found to be undetectable while all the animals in

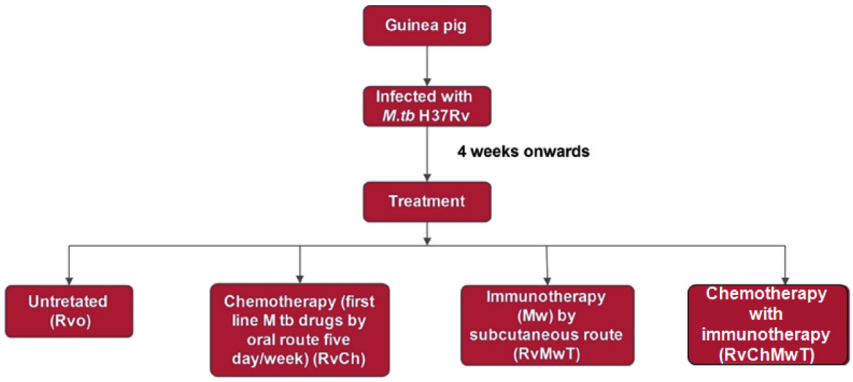

Fig. 1 – Schematic presentation of Animal experiments.

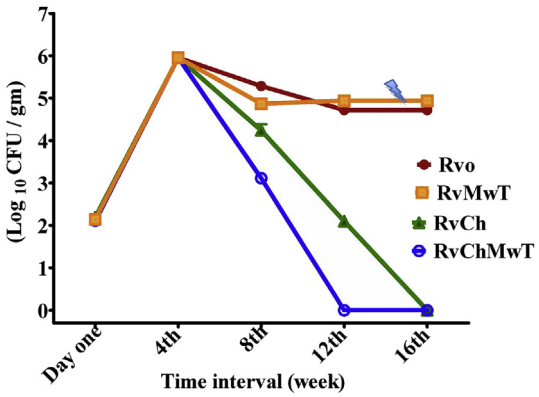

Fig. 2 – The growth kinetics of the *M. tuberculosis* (H37Rv strain) in the lungs of guinea pigs is shown at different time points in all the experimental groups. The growth curves are represented by colony forming units per gram tissue (CFU/gm) on the Y-axis. The RvChMwT group showed significantly lower CFUs from the 8th week onwards and no CFUs were detectable at 12th week time point. In contrast, the RvCh group showed slower clearance of infection (no detectable CFUs) at 16th week time point. All the animals belonging to the Rvo and RvMwT groups died between 12 and 14 week post infections (represented by the lightning symbol in the figure).

Rvo and RvMwT groups died between 14th the 16th week of infection due to heavy bacterial load.

3.2. *Histological examination*

Establishment/progression of TB infection can be determined locally at the tissue level (at the infection site) in the form of granuloma. Therefore, identification of granulomas was done on guinea pig tissues to assess the interaction between the *M. tuberculosis* and host immune system. Microscopic examinations of the lungs of guinea pigs at 4th week post-infection in untreated group revealed substantial interstitial inflammation and varying degrees of necrosis, nevertheless, there were also organized granulomas evident (Fig. 3a). Average diameter of granuloma in Rvo group at 4th week time point was 1.687 mm with 72.5% of infiltration. The lung of guinea pig showed numerous multifocal to confluent necrogranulomas throughout the parenchyma. The necrogranulomas consisted of central area of caseous necrosis that was often moderately calcified, surrounded by large numbers of macrophages and epithelioid cells, and low to moderate number of multinucleated giant cells (Fig. 3b). Fite - Faraco staining (Fig. 3c and d) of the lung sections revealed numerous medium-sized slender acid fast bacilli in cytoplasm of macrophage and epithelioid cells in necrogranulomas along the edge of the necrotic centre. Bacilli were also present in moderate number in macrophage and epithelioid cells of the smaller granulomas. Freely lying acid fast bacilli in bronchioles were admixed with cellular debris and there were also bacilli in the cytoplasm of intra bronchiolar macrophages.

3.3. *Cytokines expression*

To study the adaptive immune responses elicited by various interventions, we investigated the expression profiles of four proinflamatory type (IFN-γ, IL-2, IL-12p35 and TNF-α) and two anti-inflammatory (IL-10 and TGF-β) cytokines by using real-time quantitative-PCR from 4th week post-infection. The data of fold expressions relative to the healthy control group of all the selected cytokines at each time points (4th, 8th and 12th week) are shown graphically in Fig. 4a–f. At 4th week post-infection, all the pro- and anti-inflammatory cytokines except IL-12p35 showed up-regulation. After four weeks of different treatments (i.e. 8 weeks of post-infection), cytokine expression profiles changed in all groups. TGF-β was down regulated in all the groups at 8th week post infection (4th week post treatment) and a significant difference ($p<0.01$) was found in RvCh, RvMwT & RvChMwT (treated) and Rvo (untreated) groups in compared to 4th week of post-infection (Fig. 4e). However, IL-10 cytokine gene significantly over expressed in RvChMwT group whereas it was significantly ($p<0.001$) down regulated in Rvo, RvCh, and RvMwT group (Fig. 4g). The cytokine IL-2 was significantly ($p<0.05$) over expressed in RvCh group as compared to Rvo group (Fig. 4c) while TNF-α was significantly over expressed ($p<0.001$) in RvMwT and RvChMwT as compared to RvCh and Rvo (Fig. 4b). The expression of IFN-γ and IL-12p35 was significantly higher ($p<0.001$) in RvChMwT group as compared to all the other groups (Rvo, RvCh and RvMwT) at 8th week post-infection time point (Fig. 4a, b, and d). Cytokine IFN-γ, TNF-α, IL-2 and IL-12p35 expression levels were found to be suppressed at 12th week post infection as compared to 8th week post infection in all groups however significantly suppressed in IFN-γ and TNF-α cytokine were not only significant different in all treatments groups (RCh, RvMwT and RvChMwT) in comparison to untreated group but also showed significant difference among treatment (RvMwT/RvChMwT, RvCh/RvMwT and RvCh/RvChMwT) groups at 12th week post-infection. Cytokine IL-12p35 was not found significantly different in Rvo/RvCh and Rvo/RvChMwT but was found significantly suppressed in Rvo/RvMwT and RvCh/RvMwT, RvMwT/RvChMwT groups. Although IL-2 exhibited significant variance in RvMwT and RvChMwT groups but no significant difference was observed in RvCh group as compared to the untreated (Rvo) group. Cytokine IL-10 and TGF-β showed higher expression levels in RvMwT and Rvo groups while exhibited suppression in RvCh and RvChMwT groups at 12th week post-infection. RvCh, RvMwT and RvChMwT showed significant difference as compared to Rvo group whereas RvCh and RvChMwT groups showed significant difference in compared to RvMwT group at 12th week post-infection. Overall, expression levels of cytokine TGF-β and IL-10 (Fig. 4e and f) were significantly lower in RvChMwT and RvCh as compared to Rvo and RvMwT while TNF-α, IL-2 and IL-12p35 cytokines were significantly upregulated in RvChMwT and RvCh groups as compared to Rvo and RvMwT groups. Only cytokine IFN-γ (Fig. 4a) was found to show different pattern in comparison to other pro-inflammatory type cytokines at 12th week post infection (i.e. after 8 weeks of treatment).

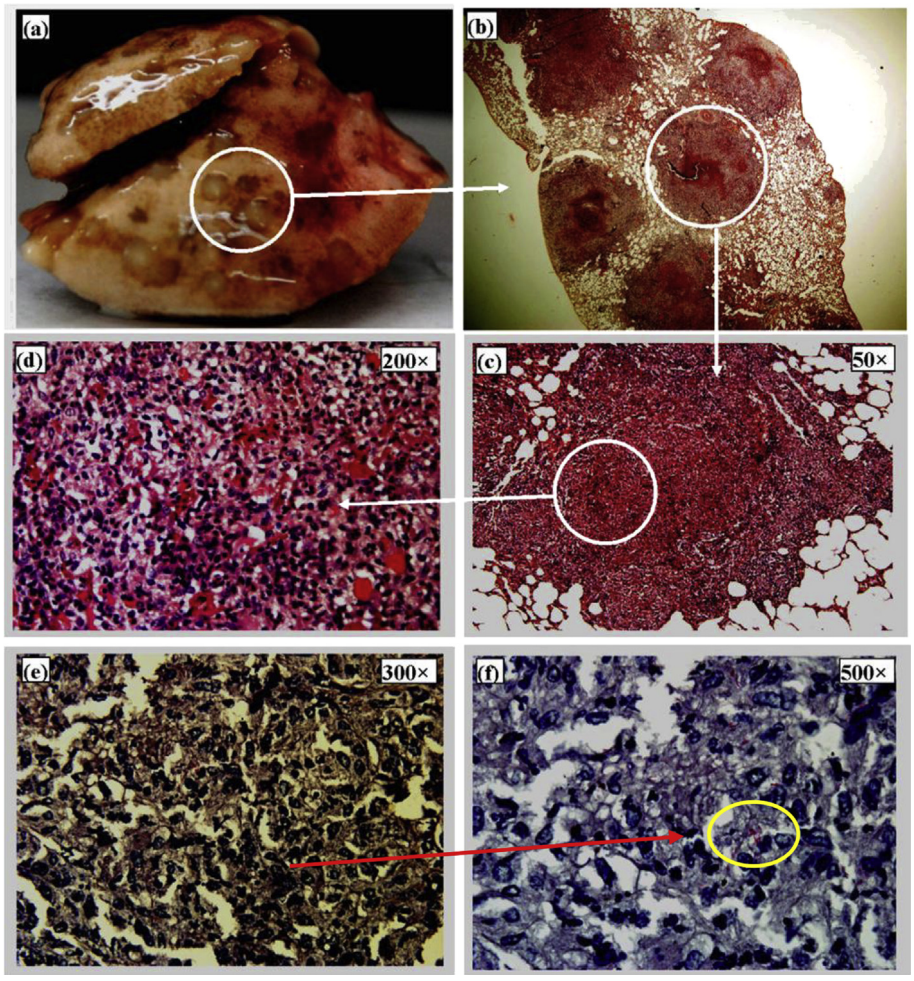

Fig. 3 – (a) Representative photographs of lungs of infected guinea pig at 4th week post infection of *M. tuberculosis* (b) caseous granulomas revealing by haematoxylin & eosin staining at, 10x magnification, (c) 50x magnification, (d) 200x magnification. AFB bacilli in guinea pig lung tissue at 4th week post infection revealing by Fite-Faraco staining (e) 300x magnification (f) 500x magnification.

4. Discussion

Earlier reports concluded that in animal models *Mw* is effective against tuberculosis infection as immunotherapy in combination with standard chemotherapy when administered by aerosol route rather than subcutaneously.[22] As immunization via aerosol route is not a practical and convenient approach in humans, the present study has investigated whether *Mw* as a immunotherapy in combination with chemotherapy administered for longer time period by parenteral route will have effect on immune response and bacterial killing/clearance. It was observed that *Mw* immunotherapy in combination with chemotherapy rapidly and significantly reduced the CFU counts from the guinea pig lungs. The animals belonging to the group RvChMwT did not show any CFU counts at the 12th week of post infection (8 weeks after starting treatments) while other groups including the chemotherapy alone group (RvCh) had significantly higher CFU counts at the same stage. This shows that combination of chemotherapy with *Mw* immunotherapy can enhance the clearance of infection. The animal group which had only *Mw* immunotherapy (RvMwT) does not show significant decrease in CFU counts as compared to chemotherapy (RvCh) as well as chemotherapy + immunotherapy (RvChMwT) groups. These results also indicate that *Mw* immunotherapy alone is insufficient to control active tuberculosis disease. Present study thus confirms earlier findings that the combination of chemotherapy and immunotherapy was found to be more effective to reduce bacterial burden from the lungs tissue.[17]

It is known that effective cell mediated immunity (CMI) is required for controlling *M. tuberculosis* infection. IFN-γ and IL-2 are two important components of CMI against intracellular bacterial pathogens in guinea pigs as well as in humans.[37,38] Presence of anti-inflammatory cytokines such as IL-10 and TGF-β inhibits the immune response by down-regulating the IFN- γ.[8,39]

In the present study, the pro-inflammatory and anti-inflammatory type cytokine responses in various experimental groups were analysed to understand host immune response against *M. tuberculosis*. It was observed (Fig. 4a–f) that the expression levels of the pro-inflammatory cytokines (IFN-γ, IL-2, IL-12p35 and TNF-α) were higher in the treated groups

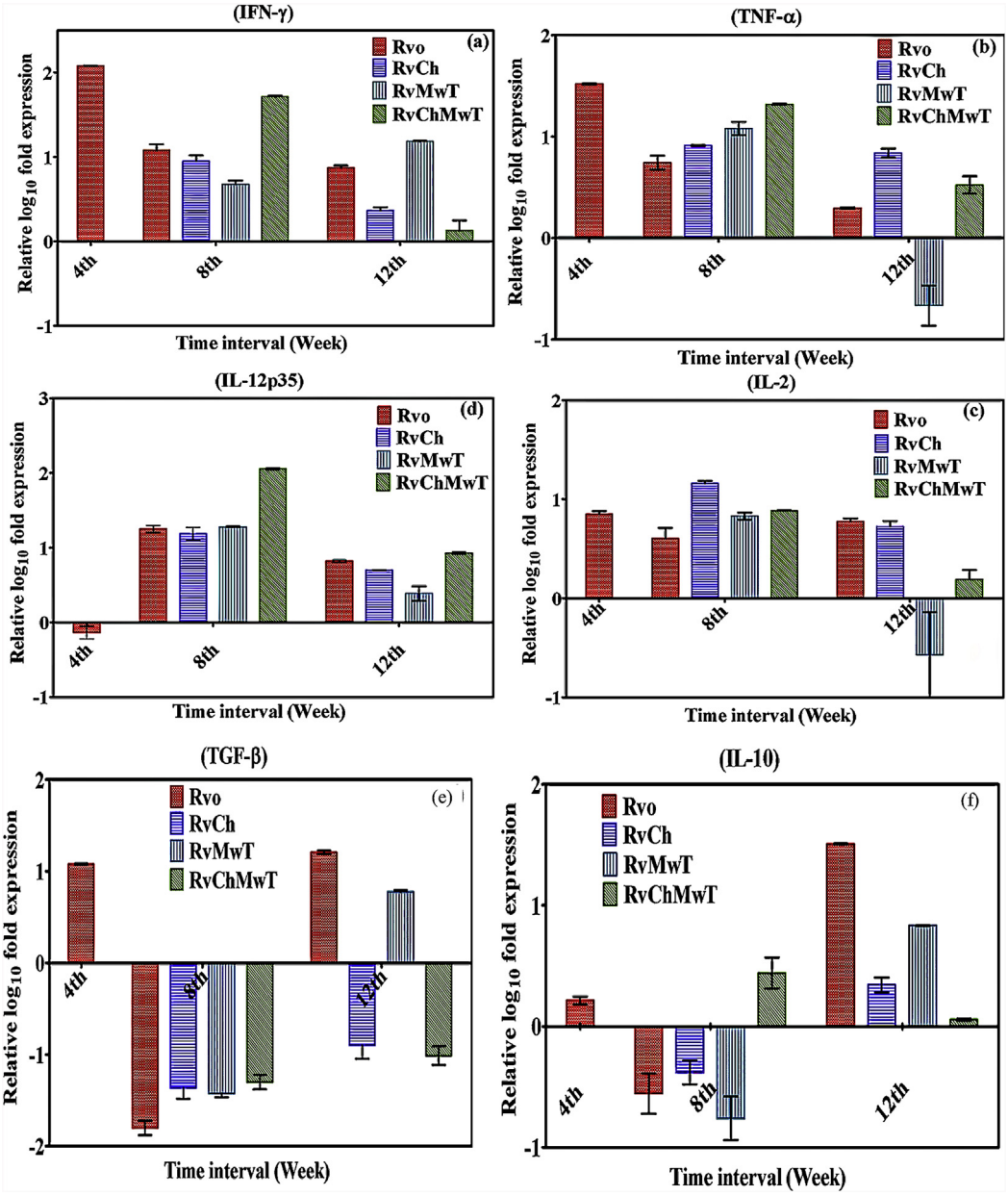

Fig. 4 – (a–g). Cytokine expression profiles of IFN-γ (a), TNF-α (b), IL-2 (c), IL-12p35 (d), TGF-β (e) and IL-10 (f) in the lungs of various therapeutic groups of guinea pigs at 4, 8 and 12th week of post infection. The RT-PCR was performed on the selected cytokine genes using the primers listed in Table 1. The data were first normalized to GAPDH (one of the housekeeping gene) levels and then normalized to the values of healthy control group to obtain ΔΔCt values. The relative fold expression values were measured by $2^{-\Delta\Delta Ct}$ method and are graphically represented as mean (±Standard error) in all experimental groups at various time points. The bars indicate the average ratios for three independent biological replicates. All the groups treated with chemotherapy (RvCh and RvChMwT) showed relatively higher levels of Th1 cytokines (IFN-γ, TNF-α, IL-2 and IL-12p35) and lower levels of anti-inflammatory cytokines (IL-10 and TGF-β), compared to the untreated (Rvo) and immunotherapy alone (RvMwT) groups.

(RvCh, RvChMwT and RvMwT) as compared to the untreated group (Rvo) at 8th week time point (i.e. 4 weeks after treatment). Moreover, IFN-γ, IL-12p35 and TNF-α cytokines levels were significantly higher in chemotherapy + immunotherapy (RvChMwT) group significantly as compared to other groups. The pattern of imunomodulation of these cytokines derived by *Mw* immunization appear to support pro-inflammatory response during the course of therapy. However, the anti-inflammatory cytokines (IL-10 and TGF-β) were suppressed only in RvCh and RvChMwT groups (and not in RvMwT) as compared to the Rvo group. These results indicate that *Mw* immunotherapy alone did not elicit a long term protective response. Up-regulations of both pro-inflammatory and anti-inflammatory cytokines were observed at the 4th week post-

infection in all the groups indicating an early phase of the disease establishment where both pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) cytokines were trying to dominate over each other, as reported previously.[8,38]

As the time scale move from 8th week to 12th week of post infection in Rvo and RvMwT groups, levels of pro-inflammatory cytokines (IFN-γ, IL-2, IL-12p35 and TNF-α) decreased whereas the IL-10 and TGF-β increased. As a result, IL-10 and TGF-β inhibited the anti-mycobacterial Th1 immune response and thus facilitated the survival of tubercle bacilli in these groups. Thus, in the absence of any efficient intervention such as chemotherapy; immune system fails to control the multiplication in *M. tuberculosis* in lung tissue. This could explain our observation that the animals in Rvo and RvMwT groups died between 12th to 14th week after infection due to heavy bacterial load and very low degree of protective response. Both the groups where chemotherapy was given (RvCh and RvChMwT) showed significant up-regulation of IFN-γ, TNF-α, IL-2 and IL-12p35, as compared to the Rvo and RvMwT groups at the 8th week time point after infection. Consequently, anti-inflammatory cytokines (IL-10 and TGF-β) were possibly suppressed by pro-inflammatory cytokines in these groups leading to gradual clearance of infection, though mechanisms postulated have been considered earlier,[40,41] but need more prospective studies.

From this study, it is evident that chemotherapy (RvCh) and *Mw* immunotherapy in combination with chemotherapy (RvChMwT) generated favourable conditions for *M. tuberculosis* clearance inside the guinea pigs lungs. Previously it has been reported that *Mw* can play an important role to generate a strong immune response when given as an adjunct to standard chemotherapy for tuberculosis in mice,[17,42] guinea pigs[23] and humans.[28,29] The multiple doses of heat killed *Mw* have been recommended for immunotherapy in order to stimulate pro-inflammatory immune response so that it could dominate over the anti-inflammatory response.[19] TGF beta and TNF alpha have been postulated to have roles in infections including in tuberculosis.[43,44] With immunotherapy, the immune response appears to become more competent due to the up-regulation of pro-inflammatory cytokines and synergistic bactericidal effect could potentiate the standard chemotherapy, thereby facilitating rapid clearance of bacteria from the infected guinea pigs by chemotherapy.[6,45] However, the reason(s) for rapid decline in anti-inflammatory response after the subsequent doses of *Mw* is not known though that could be due to the reduced bacterial load during that phase.[46] It is generally believed that during the first month of chemotherapy, a maximum bactericidal activity is exerted, after which the bacterial killing slows down owing to the cessation and mineralization of the granuloma, where the residual bacteria survive due to poor diffusion of the drug.[47] It has also been thought that, following an early effective granulomatous response, intra-granulomatous granulocytes found at the later stage might disrupt the formation of granuloma and consequently speckled bacteria would be reachable for the bactericidal activity of the anti TB drug combinations.[46,48] Enhanced killing/clearance of viable *M. tuberculosis* in lungs shows that adjunct immunotherapy with the combination of chemotherapy appears to be a potentially promising approach to reduce the treatment duration in tuberculosis. While the data presented in this study provides strong evidence to try this approach in humans, the results cannot be straightway extrapolated to treatment of disease in humans. Similarly, information about cytokine responses is also limited to few important cytokines. It is thus essential to carry out well designed clinical trials in humans which should also include comprehensive analysis of cytokine/chemokine profiles at different time points. Further, if adjunct chemotherapy succeeds in enhancing bacterial clearance, this will also have potential role in reducing/curtailing transmission, which is critical in reaching the target of ending TB.

5. Conclusions

Immunotherapy with chemotherapy can potentially reduce the treatment duration for TB. A four weeks shorter period was required for the bacterial clearance in guinea pigs where standard anti-TB chemotherapy was given in combination with *Mw* immunotherapy, as compared to the animals where only chemotherapy was given. The results also indicate that in the absence of chemotherapy *Mw* immunotherapy alone might not be able to contain the established infection. This study also provides direct evidence about the beneficial effect of *Mw* as immunotherapy as an adjunct to chemotherapy in experimental tuberculosis as observed by reduction of viable bacilli as well as evidence of pro-inflammatory/Th1 type response. In-depth studies are required to study the efficacy of immunotherapy with *Mw* (MIP) as adjunct to chemotherapy in terms of clinical outcomes, bacteriological clearance, relapses as well as to gain better understanding of host response in terms of cytokine and other chemo-attractant molecules level which may contribute to progress of tuberculosis infection in positive/negative manner.

Conflicts of interest

The authors have none to declare

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2018.08.004.

REFERENCES

1. WHO. *Global Tuberculosis Report*. Geneva: World Health Organization; 2012.

2. Dye C, Scheele S, Dolin P, et al. Global burden of tuberculosis. *JAMA*. 1999;282:686.
3. Corbett EL, Marston B, Churchyard GJ, et al. Tuberculosis in sub-saharan africa: opportunities, challenges, and change in the era of antiretroviral treatment. *Lancet*. 2006;367:926–937.
4. Murray CJ, Ortblad KF, Guinovart C, et al. Global, regional, and national incidence and mortality for hiv, tuberculosis, and malaria during 1990-2013: a systematic analysis for the global burden of disease study 2013. *Lancet*. 2014;384:1005–1070.
5. Rook GA, Lowrie DB, Hernandez-Pando R. Immunotherapeutics for tuberculosis in experimental animals: is there a common pathway activated by effective protocols? *J Infect Dis*. 2007;196:191–198.
6. Guo S, Zhao J. Immunotherapy for tuberculosis: What's the better choice? *Front Biosci*. 2012;17:2684–2690.
7. Ly LH, McMurray DN. The yin-yang of Tnf alpha in the Guinea pig model of tuberculosis. *Indian J Exp Biol*. 2009;47:432–439.
8. Sharma S, Bose M. Role of cytokines in immune response to pulmonary tuberculosis. *Asian Pac J Allergy Immunol*. 2001;19:213–219.
9. Hernandez-Pando R, Orozco-Esteves H, Maldonado HA, et al. A combination of a transforming growth factor-beta antagonist and an inhibitor of cyclooxygenase is an effective treatment for murine pulmonary tuberculosis. *Clin Exp Immunol*. 2006;144:264–272.
10. He XY, Xiao L, Chen HB, et al. T regulatory cells and th1/th2 cytokines in peripheral blood from tuberculosis patients. *Eur J Clin Microbiol Infect Dis*. 2010;29:643–650.
11. Gallegos AM, van Heijst JW, Samstein M, et al. A gamma interferon independent mechanism of CD4 T cell mediated control of m. Tuberculosis infection in vivo. *PLoS Pathog*. 2011;7, e1002052.
12. Flynn JL, Chan J. Immune evasion by mycobacterium tuberculosis: living with the enemy. *Curr Opin Immunol*. 2003;15:450–455.
13. Orme IM. The immunopathogenesis of tuberculosis: a new working hypothesis. *Trends Microbiol*. 1998;6:94–97.
14. Xie X, Li F, Chen JW, et al. Risk of tuberculosis infection in anti-tnf-alpha biological therapy: from bench to bedside. *J Microbiol Immunol Infect*. 2014;47:268–274.
15. Roy E, Lowrie DB, Jolles SR. Current strategies in tb immunotherapy. *Curr Mol Med*. 2007;7:373–386.
16. Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of Mycobacterium tuberculosis h37rv in mice of different inbred strains following immunization with a leprosy vaccine based on mycobacterium w. *Vaccine*. 1991;9:10–14.
17. Gupta A, Ahmad FA, Ahmad F, et al. Efficacy of Mycobacterium indicus pranii immunotherapy as an adjunct to chemotherapy for tuberculosis and underlying immune responses in the lung. *PLoS one*. 2012;7(7), e39215.
18. Doherty TM. Immunotherapy for TB. *Immunotherapy*. 2012;4:629–647.
19. Gupta UD, Katoch VM, McMurray DN. Current status of TB vaccines. *Vaccine*. 2007;25:3742–3751.
20. Katoch VM. A report on the biochemical analysis of Mycobacterium w. *Lepr India*. 1981;53:385–389.
21. Reddi PP, Amin AG, Khandekar PS, et al. Molecular definition of unique species status of Mycobacterium w; a candidate leprosy vaccine strain. *Int J Lepr Other Mycobact Dis*. 1994;62:229–236.
22. Gupta A, Geetha N, Mani J, et al. Immunogenicity and protective efficacy of "*Mycobacterium w*" against *Mycobacterium tuberculosis* in mice immunized with live versus heat-killed *M.w* by the aerosol or parenteral route. *Infect Immun*. 2009;77:223–231.
23. Rawat KD, Chahar M, Reddy PVJ, et al. Expression of CXCl10 (IP-10) and CXCl11 (I-tac) chemokines during Mycobacterium tuberculosis infection and immunoprophylaxis with mycobacterium indicus pranii (mw) in Guinea pig. *Infect Genet Evol*. 2013;13:11–17.
24. Talwar GP, Zaheer SA, Mukherjee R, et al. Immunotherapeutic effects of a vaccine based on a saprophytic cultivable mycobacterium, Mycobacterium w in multibacillary leprosy patients. *Vaccine*. 1990;8:121–129.
25. Katoch K, Katoch VM, Natrajan M, et al. Treatment of bacilliferous bl/ll cases with combined chemotherapy and immunotherapy. *Int J Lepr Other Mycobact Dis*. 1995;63:202–212.
26. Zaheer SA, Beena KR, Kar HK, et al. Addition of immunotherapy with mycobacterium w vaccine to multi-drug therapy benefits multibacillary leprosy patients. *Vaccine*. 1995;13:1102–1110.
27. Patel N, Deshpande MM, Shah M. Effect of an immunomodulator containing mycobacterium w on sputum conversion in pulmonary tuberculosis. *J Indian Med Assoc*. 2002;100:191–193.
28. Patel N, Trapathi SB. Improved cure rates in pulmonary tuberculosis category ii (retreatment) with Mycobacterium w. *J Indian Med Assoc*. 2003;101:680–682.
29. Katoch K, Singh P, Adhikari T, et al. Potential of *Mw* as a prophylactic vaccine against pulmonary tuberculosis. *Vaccine*. 2008;26:1228–1234.
30. Kharkar R. Immune recovery in hiv with Mycobacterium w. *J Indian Med Assoc*. 2002;100:578–579.
31. Sur PK, Dastidar AG. Role of Mycobacterium w as adjuvant treatment of lung cancer (non-small cell lung cancer). *J Indian Med Assoc*. 2003;101:118–120.
32. Sarkar SKDC. Role of Mycobacterium w as an adjuvant treatment of head and neck cancer. A randomised trial. *J Clin Oncol (Meeting Abstracts)*. 2005;23:5588.
33. Faujdar J, Gupta P, Natrajan M, et al. Mycobacterium indicus pranii as stand-alone or adjunct immunotherapeutic in treatment of experimental animal tuberculosis. *Indian J Med Res*. 2011;134:696–703.
34. Ahmad Z, Klinkenberg LG, Pinn ML, et al. Biphasic kill curve of isoniazid reveals the presence of drug-tolerant, not drug-resistant, *Mycobacterium tuberculosis* in the Guinea pig. *J Infect Dis*. 2009;200:1136–1143.
35. Dheda K, Huggett JF, Bustin SA, et al. Validation of housekeeping genes for normalizing rna expression in real-time PCR. *Biotechniques*. 2004;37:112–114, 116, 118-119.
36. Huggett J, Dheda K, Bustin S, et al. Real-time RT-PCR normalisation; strategies and considerations. *Genes Immun*. 2005;6:279–284.
37. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv Immunol*. 2007;96:41–101.
38. da Silva MV, Figueiredo AA, Machado JR, et al. T cell activation and proinflammatory cytokine production in clinically cured tuberculosis are time-dependent and accompanied by upregulation of il-10. *PLoS One*. 2013;8, e65492.
39. Yagi R, Zhu J, Paul WE. An updated view on transcription factor gata3-mediated regulation of th1 and th2 cell differentiation. *Int Immunol*. 2011;23:415–420.
40. Harris J, De Haro SA, Master SS, et al. T helper 2 cytokines inhibit autophagic control of intracellular mycobacterium tuberculosis. *Immunity*. 2007;27:505–517.
41. Peresi E, Oliveira LR, da Silva WL, et al. Cytokine polymorphisms, their influence and levels in brazilian patients with pulmonary tuberculosis during antituberculosis treatment. *Tuberc Res Treat*. 2013;285094:27.
42. Singh IG, Mukherjee R, Talwar GP, et al. In vitro characterization of T cells from mycobacterium w-vaccinated mice. *Infect Immun*. 1992;60:257–263.

43. Wahl SM. Transforming growth factor beta: the good, the bad, and the ugly. *J Exp Med*. 1994;180:1587–1590.
44. Koga M, Terawaki S, Tatsukawa R, et al. Development of miliary tuberculosis related to anti-tumor necrosis factor-alpha inhibitor therapy for pustular psoriasis. *J Dermatol*. 2013;40:476–477.
45. Bermudez LE. Production of transforming growth factor-beta by mycobacterium avium-infected human macrophages is associated with unresponsiveness to IFN-gamma. *J Immunol*. 1993;150:1838–1845.
46. Petrofsky M, Bermudez LE. Neutrophils from mycobacterium avium-infected mice produce tnf-alpha, il-12, and il-1 beta and have a putative role in early host response. *Clin Immunol*. 1999;91:354–358.
47. Ordway DJ, Shanley CA, Caraway ML, et al. Evaluation of standard chemotherapy in the Guinea pig model of tuberculosis. *Antimicrob Agents Chemother*. 2010;54:1820–1833.
48. Peter SN. The role of adjunctive Mycobacterium w immunotherapy for tuberculosis. *J Exp Clin Med*. 2010;2:124–129.

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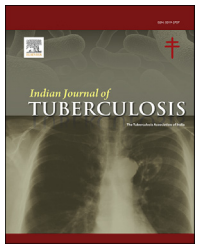

Original article

Metformin associated inflammation levels regulation in type 2 diabetes mellitus-tuberculosis coinfection patients – A case report

Bernadette Dian Novita [a,*], *Endang Isbandiati Soediono* [a], *Jusak Nugraha* [b]

[a] *Department of Pharmacology, Faculty of Medicine, Widya Mandala Catholic University, Surabaya, Indonesia*
[b] *Department of Clinical Pathology, Faculty of Medicine, University of Airlangga/ Dr. Soetomo Hospital, Surabaya, Indonesia*

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ABSTRACT

IFN-γ elevation is one of the indicators of successful treatment in active tuberculosis (TB) infection due to macrophage and Th-1 activation in inducing autophagy process. However, IL-10 also inhibits interferon-mediated mycobactericid activities by blocking IFN-γ; signaling pathways in autophagy. Therefore, ratio IFN-γ/IL-10 has to be greater than 1 (>1) then IFN-γ remains has anti-mycobacterium. Metformin (MET) is a potent combination drug to elevate anti-TB efficacy and able to regulate inflammation.

In this study, an observational clinical study was done in diabetes mellitus (DM)-TB co-infection outpatients at Surabaya Paru Hospital. This study evaluated how MET therapy; affected inflammation. MET was used at least 2 months, accompanying with insulin and; anti-TB and as comparison to non MET group.

The result in this study MET increased both pro-inflammatory and anti-inflammatory cytokines, thus MET may consider as adjunct therapy in DM-TB coinfection patients due; to its ability in Th-1 and Th-2 immuno-regulating response, especially to enhance IFN-γ; and to reduce insulin associated IL-10 upregulation.

1. Introduction

Mycobacterium tuberculosis (*M. tuberculosis*) infection or known by TB infection is a leading cause of global morbidity and mortality thus, requiring long-term therapy.[1,2] There are five phases of *M. tuberculosis* infection and divided into two main phase. Firstly is invasion phase (phase 1–2) and secondly is immunological phase, this phase is happened due to the immunology response during interaction of *M. tuberculosis* and host (phases 3–5).[3] Invasion phase occurs when *M. tuberculosis* reaches the pulmonary alveoli and becomes colony in the lung with its ability to avoid the phagocytosis. In phase 2, *M. tuberculosis* multiplies in immature nonactivated macrophages to form a lesion called tubercle. In invasion phase, anti TB works well to eliminate *M. tuberculosis*. However, the efficacy of anti TB reduce in immunology phase, which the host

* *Corresponding author.*
E-mail address: novita@ukwms.ac.id (B.D. Novita).
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body starts limit *M. tuberculosis* by developing caseous necrosis as immune response against tuberculin-like antigens released by *M. tuberculosis* in phase 3, then becomes liquefaction phase in phase 4–5 to limited tuberculosis extracellular multiplication.[3,4] The aimed of anti-TB therapy is curing patients, preventing death, preventing recurrence, cutting off transmission chains and preventing germ resistance by eradication of *M. tuberculosis*. The effectiveness of rifampicin, isoniazid, pyrazinamide and ethambutol is influenced by host immune response.[5,6]Therefore, new approach is needed to enhance anti TB efficacy during immunology phase. One of offered suggestion was compiled MET during intensive phase of anti-TB therapy.

Metformin hydrochloride (MET), biguanide, use in type 2 diabetes mellitus by 1) inhibiting the production of hepatic glucose; 2) reducing intestinal glucose absorption; and 3) improving glucose uptake and utilization.[5,7,8] MET is known affecting inflammation mediators, both pro-inflammation, such IFN-γ, IL-β and also anti-inflammation such IL-10.[8–10] Interferon (IFN)-γ is a potent cytokine that indicates antimicrobial effect and also modulates the production or activities of several cytokines and chemokines.[11–13] IFN-γ activates macrophages and dendritic cells to perform autophagy to *M. tuberculosis*, and diminished of IFN-γ relates to anti-tuberculosis therapy failure.[14–18]

2. Materials and methods

2.1. Study design

In this study, an observational clinical study was done in diabetes mellitus (DM)-TB co-infection outpatients at Surabaya Paru Hospital. It involved two groups, MET group as observation group, and non MET group. The MET group was receiving MET therapy with doses from 1000 mg to 1500 mg along with insulin and anti-TB during the intensive periods. The enrolled patients criterias: 1) patient DM with a new case of TB co-infection, who were given insulin and anti-TB regimens; 2) positive sputum smear; 3) Patient's age was 25–60 years old; 4) has normal liver function and renal function; 5) not in hypoxia condition, presenting by peripheral oxygen saturation level must be higher than 92%.

The levels of IFN-γ and IL-10 was measured before and after this observation period and as a clinical result, we also evaluated the smear reversion in DM-TB coinfection patients in both groups.

2.2. Diagnosis and management therapy

The diagnosis of TB was established by 1) clinical symptoms and signs of TB, such: chronic productive cough, unintentional weight loss; 2) positive sputum smear of acid-fast bacilli (AFB) by microscopic Ziehl-Neelsen-stained sputum slides; and 3) chest radiographs with suggestive features of TB. The diagnosis of DM was established by fasting blood sugar (FBS) >120 mg/dL; HbA1c > 7%.

Patients diagnosed with TB were registered and treated with anti-TB for 6 months in accordance to WHO guidelines.[19,20] Insulin use for achieving good glycemic control in the patients in this study. These following drugs were used: MET (Metformin(R)), insulin (Humulin(R)), rifampicin (RIF), isoniazid (INH), pyrazinamide (PYR), ethambutol (ETH). MET were given 1000–1500 mg in the divided daily dose for at least two months or during the intensive phase of anti-TB therapy.

2.3. Acid fast bacilli smears (AFB) smears

Sputum smears were examined two times: 1) before treatment in order to diagnose and 2) after the intensive phase of anti-TB treatment in order to do evaluation.

2.4. Cells culture and ELISA

Cells and ELISA. Cells. PBMC was obtained from patients' whole blood and 1×10^6 were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 μg mantoux and 0.7 μg penicilline. Supernatant were harvested after 72 hours and prepare for ELISA methods in order to measure the levels of IFN-γ and IL-10. **ELISA.** IFN-γ (RnD DIF50) and IL-10 (RnD P113058) were used as measurement kits.

3. Result

3.1. Characteristic of patients

This study's ethical clearance was approved by ethical committee of Surabaya Paru Hospital with no. 09.01/KERS/102.6/2016 and written informed consent obtained from all participants after information for consent was given by the investigators. During this study period, there were 476 cases of new TB infection and 156 cases (~30%) of that were type 2 DM-TB co-infection. 42 patients were eligible participated in this observational studies. All the basic conditions in both groups were homogeneous ($p > 0.05$) (as seen in Table 1).

In order to prevent MET associated lacto acidosis (MALA) during MET therapy in this study, all patients has been determined as mention at enrolled patients criterias (data was as seen in Table 1). Moreover, there was no incidence of lactic acidosis event during this observation period.[21]

During observation weeks (intensive phase of anti-TB therapy), we also obtained FBS levels periodically and the

Table 1 – Characteristic of type 2 DM-TB coinfection during observation period of study.

Parameters	MET group	Non MET group	p (difference)
Ages (years old)	44.59 ± 8.64	48.40 ± 8.17	0.863
HbA1c (%)	8.82 ± 1.91	9.52 ± 2.02	0.379
Oxygen saturation (SpO_2) (%)	98.06 ± 0.73	97.47 ± 0.83	0.308
BUN (mg/dL)	0.95 ± 0.16	0.93 ± 0.13	0.980
Creatinine serum (U/L)	23.92 ± 11.92	27.3 ± 12.01	0.103
SGOT (U/L)	17.63 ± 6.16	14.44 ± 6.48	0.354
SGPT (U/L)	19.22 ± 8.73	16.09 ± 7.56	0.509

Participants characteristic condition before observation periods. HbA1c was measured after 2 months observation period.

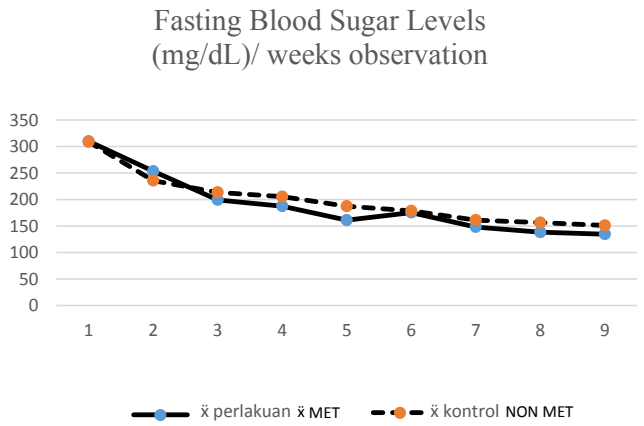

Fig. 1 – Fasting blood sugar levels of type 2 DM-TB coinfection during observation period of study.

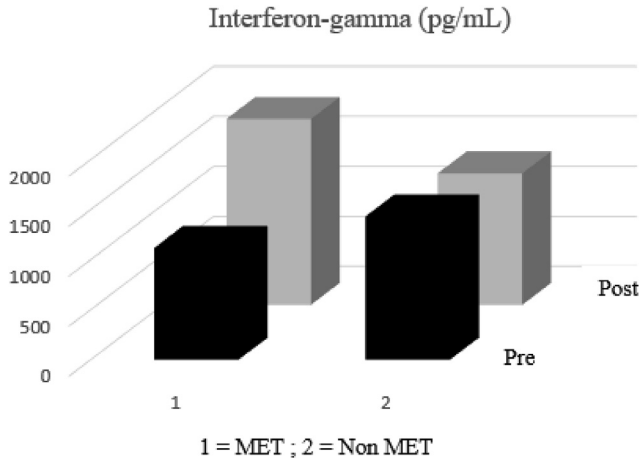

Fig. 2 – Interferon (IFN)-γ level of type 2 DM-TB patient before and after observation period. Difference of interferon gamma level pre and post observation in MET group was significantly different from non MET group.

FBS levels were also similar in both groups (as seen in Fig. 1). This data show that IFN-γ and IL-10 elevation in this study might be not directly influenced by hyperglycemia condition.

For sputum smears, as seen in Table 2 shows that prior to the intensive phase of anti-TB therapy none of the subjects were having negative AFB in both groups. The highest number of AFB count (+3) in MET group was 40.9% and in non MET group was 35%. After 2 months MET therapy accompanying with insulin and anti-TB regimens, all patients in MET group were AFB reversion (negative smears result), while only 75% of non MET group had AFB reversion. Using the Fisher's exact test, results of different test $p = 0.046$ ($p < 0.005$), which means there is a significantly difference of AFB smears reversion between the MET group and the non MET group.

3.2. *IFN-γ, IL-10 and ratio IFN-γ, IL-10*

3.2.1. *IFN-γ*

IFN-γ is activated by Th1 and NK Cells to induce macrophage and dendritic cell activation thus provide protection against TB infection.[22,23] Increased of IFN-γ in chronic TB infection is a cellular immune response. Currently, IFN-γ release assay (IGRA) is used as one of the tools of diagnosis of latent TB infection and IFN-γ elevation is one of the indicators of successful treatment in active TB infection.[24]

Using *Wilcoxon-Mann Whitney*, nonparametric statistical test, Fig. 2 shows that IFN-γ level before treatment between MET group and non MET group were alike ($p > 0.005$), thus it shows that patients in both groups, before treatment, were in the similar stage of IFN-γ. The differences before and after observation period was significant in MET group ($p < 0.005$) while in non MET group was not. Referring to negative AFB in MET group after 2 months intensive therapy (as seen in Fig. 2), it supports that IFN-γ has effect as mycobactericid.

Table 2 – Acid Fast Bacilli Sputum Smears result of type 2 DM-TB patients before and after observation period.

AFB result	MET group (N %)		Non MET group (N %)	
	Before	After	Before	After
Negative	0 (0%)	22 (100%)	0 (0%)	15 (75%)
Scanty/ + 1	9 (40.9%)	0 (0%)	6 (30%)	5 (25%)
+2	4 (18.2%)	0 (0%)	7 (35%)	0 (0%)
+3	9 (40.9%)	0 (0%)	7 (35%)	0 (0%)
Total	22 (100%)	22 (100%)	20 (100%)	20 (100%)

3.2.2. *IL-10*

IL-10 has ability to inhibit the Th-1pro-inflammation cytokines, including IFN-γ.[23–25] Using *Wilcoxon-Mann Whitney*, nonparametric statistical test, Fig. 3 shows that IL-10 level before treatment between MET group and non MET group were alike ($p > 0.005$), thus it shows that patients in both groups, before treatment, were in the similar stage of IL-10. Although IL-10 level was increased, the differences before and after observation period was not significant between MET and nongroup ($p > 0.005$).

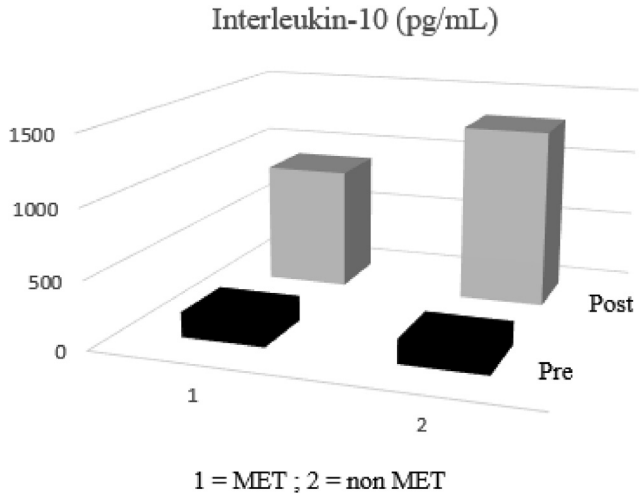

Fig. 3 – Interleukin (IL)-10 level of type 2 DM-TB patient before and after observation period. Difference of interleukin-10 level pre and post observation in MET group was not significantly different from non MET group.

3.2.3. *Ratio IFN-γ/IL-10*

Ratio IFN-γ/IL-10 shows that immune processes inside the host were more dominated by pro-inflammatory or anti-inflammatory cytokines after intensive phase of anti-TB with or without MET therapy. Whenever the IFN-γ/IL-10 ratio is greater than 1 (>1), thus the host's immunity defense system was dominated by pro-inflammation condition.[26]

Using *Wilcoxon-Mann Whitney*, Table 3 shows that ratio IFN-γ/IL-10 were not significant difference before and after the intensive phase of anti-TB therapy between MET and non MET group ($p > 0.005$). However, IFN-γ/IL-10 ratio difference variation after MET combined anti-TB and insulin was narrower than non MET group.

4. Discussion

IFN-γ is the chief cytokine involved in the protective immune response against mycobacterial infection.[11,27,28] The main function of IFN-γ is macrophage and dendritic cells activation, thus in this study autophagy marker was also high[21] and it referred to its mycobactericid functions. Predominantly IFN-γ is also contributed to less severe forms of pulmonary TB.[23] Moreover, IFN-γ also enhances the antigen presentation through the induction of the expression of molecules from the major histocompatibility complex (MHC) class I and class II and promoting the differentiation of CD4 T lymphocytes to the Th1 subpopulation.[11,22] Furthermore as conclusion in this study MET associated to inflammation regulatory in DM-TB coinfection patients. However, IFN-γ relates to CD8 T-lymphocytes or cytotoxic T-cells also contributes to lung tissue damaged, thus IFN-γ activity needs to be controlled.[22,23]

IL-10 a major anti-inflammatory cytokines plays important role in metabolic disorder such diabetes due its affect to insulin sensitivity.[29] IL-10 is produced by macrophages and Th-2 during *M. tuberculosis* infection It suppresses macrophage function and inhibits pro-inflammation cytokines such IFN-γ, TNF-σ and IL-1β. The increase in IL-10 levels appears to support the mycobacterial survival in the host[23] due to the inhibition of autophagy targeting signals through IL-10 activated SOCS3, and then, SOCS3 inhibits the Janus kinase-2 (Jak2)/signal transducer and activator of transcription (Stat) pathway in activating macrophage autophagy.[27,28] In this study, the increasing of IL-10 may happen not only due to macrophage related Th-2 activation but also due to insulin attenuated anti-inflammation regulatory.[29–32]

Table 3 – Ratio Interferon (IFN)-γ/Interleukin (IL)-10 level of type 2 DM-TB patient before and after observation period.

Ratio IFN-γ/IL-10	MET group	Non MET group	Between groups differences
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Before	8.99 ± 5.67	11.65 ± 6.17	0.247
After	2.18 ± 0.53	6.74 ± 4.35	0.212
Delta	0.904 ± 0.808	2.68 ± 6.17	0.433

Based on this result, MET therapy may consider as new strategy in enhancing anti TB efficacy due to its two main ability: 1) MET controlled IL-10 secretion thus alter host immune response against TB infection; and; 2) MET also affects to insulin sensitivity thus enhanced insulin therapy.

Regulating pro-inflammatory and anti-inflammatory cytokines is a critical role in the immunity and progression of inflammation. Knowing the use of "old" drug, MET, for new strategy in conquering TB was the purpose of this case-study. As conclusion in this study, MET increased both pro-inflammatory and anti-inflammatory cytokines, thus MET may consider as adjunct therapy in type 2 DM-TB coinfection patients due to its ability in Th-1 and Th-2 immuno-regulating response. However, the further study requires in knowing MET attenuated host sensitivity against *M. tuberculosis* infection in a larger number of DM-TB coinfection patients.

Conflicts of interest

All participants in this study were voluntary involved and funding was written in the acknowledgement.

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REFERENCES

1. WHO. Collaborative framework for care and control of tuberculosis and diabetes. *World Heal Organ*. 2011;314(5805):2, 978 92 4 150225 2.
2. Kementerian Kesehatan Republik Indonesia. *Pedoman Nasional Pengendalian Tuberkulosis*. 2014:24–25.
3. Caire-Brändli I, Papadopoulos A, Malaga W, et al. Reversible lipid accumulation and associated division arrest of *Mycobacterium avium* in lipoprotein-induced foamy macrophages may resemble key events during latency and reactivation of tuberculosis. *Infect Immun*. 2014;82(2):476–490. https://doi.org/10.1128/IAI.01196-13.
4. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D, Dieli F, Caccamo N. Functional signatures of human CD4 and CD8 T cell responses to *Mycobacterium tuberculosis*. *Front Immunol*. 2014;5(8):180. https://doi.org/10.3389/fimmu.2014.00180.
5. Brunton L, Chapner B, Knollmann B. In: Brunton L, Chapner B, eds. *The Pharmacological Basis of Therapeutics-Goodman & Gillman*. 12th ed. San Diego, California: Mc Graw Hill Medical; 2011.

6. V Almeida D. Revisiting anti-tuberculosis activity of pyrazinamide in mice. *Mycobact Dis*. 2014;04(02):2–7. https://doi.org/10.4172/2161-1068.1000145.
7. Katzung BG, Mastres SB, Trevor AJ. *Basic & Clinical Pharmacology*. 14th ed. Singapore: Mc Graw Hill Education (Asia); 2018.
8. Marupuru S, Senapati P, Pathadka S, Miraj SS, Unnikrishnan MK, Manu MK. Protective effect of metformin against tuberculosis infections in diabetic patients: an observational study of south Indian tertiary healthcare facility. *Braz J Infect Dis*. 2017;21(3):312–316. https://doi.org/10.1016/j.bjid.2017.01.001.
9. Dallaglio K, Bruno A, Cantelmo AR, et al. Paradoxic effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis*. 2014;35(5):1055–1066. https://doi.org/10.1093/carcin/bgu001.
10. Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. *Ann Oncol*. 2016;27(12):2184–2195. https://doi.org/10.1093/annonc/mdw410.
11. Chee CBE, KhinMar KW, Gan SH, et al. Tuberculosis treatment effect on T-cell interferon-gamma responses to *Mycobacterium tuberculosis*-specific antigens. *Eur Respir J*. 2010;36(2):355–361. https://doi.org/10.1183/09031936.00151309.
12. Cuccu B, Freer G, Genovesi A, Garzelli C, Rindi L. Identification of a human immunodominant T-cell epitope of mycobacterium tuberculosis antigen PPE44. *BMC Microbiol*. 2011;11(1):167. https://doi.org/10.1186/1471-2180-11-167.
13. Abbas AK, Licthman AH. *Cellular and Molecular Immunology*. 7th ed. Elsevier Inc.; 2012. https://doi.org/10.1016/B978-0-7020-3369-8.00001-X.
14. Lemos MP, Rhee KY, McKinney JD. Expression of the leptin receptor outside of bone marrow-derived cells regulates tuberculosis control and lung macrophage MHC expression. *J Immunol*. 2011;187(7):3776–3784. https://doi.org/10.4049/jimmunol.1003226.
15. Kresno SB. *Imunologi: Diagnosis Dan Prosedur Laboratorium*. V. Badan Penerbit Fakultas Kedokteran Universitas Indonesia; 2013.
16. Bertholet S, Horne DJ, Laughlin EM, et al. Effect of chemotherapy on whole-blood cytokine responses to *Mycobacterium tuberculosis* antigens in a small cohort of patients with pulmonary tuberculosis. *Clin Vacc Immunol*. 2011;18(8):1378–1386. https://doi.org/10.1128/CVI.05037-11.
17. Sakai S, Kauffman KD, Sallin MA, et al. CD4 T cell-derived IFN-γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. *PLOS Pathog*. 2016;12(5):e1005667. https://doi.org/10.1371/journal.ppat.1005667. Fortune SM, ed.
18. Herzmann C, Ernst M, Ehlers S, et al. Increased frequencies of pulmonary regulatory T-cells in latent *Mycobacterium tuberculosis* infection. *Eur Respir J*. 2012;40(6):1450–1457. https://doi.org/10.1183/09031936.00214611.
19. Menzies D, Sterling TR. Treatment of *Mycobacterium tuberculosis* infection: time to get a move on? *Ann Intern Med*. 2014;161(6):449. https://doi.org/10.7326/M14-1719.
20. van Deun A, Monedero I, Rieder HL, et al. In: Caminero J, ed. *Guidelines for Clinical and Operational Management of Drug-Resistant Tuberculosis*. 2013.
21. Novita BD, Pranoto A, Wuryani, Soediono EI, Mertaniasih NM. A case risk-study of lactic acidosis risk in metformin use in type 2 diabetes mellitus tuberculosis co-infection patients. *Indian J Tubercul*. 2017. https://doi.org/10.1016/j.ijtb.2017.05.008.
22. Abbas AK, Lichtman A. In: Abbas AK, Lichtman A, Pillai S, eds. *Cellular and Molecular Immunology*. 7th ed. Philadelphia, PA: Saunders; 2012.
23. Cavalcanti YVN, Brelaz MCA, Neves JKDAL, Ferraz JC, Pereira VRA. Role of TNF-alpha, IFN-gamma, and IL-10 in the development of pulmonary tuberculosis. *Pulm Med*. 2012. https://doi.org/10.1155/2012/745483.
24. Matsushita I, Le Hang NT, Hong LT, et al. Dynamics of immune parameters during the treatment of active tuberculosis showing negative interferon gamma response at the time of diagnosis. *Int J Infect Dis*. 2015;40:39–44. https://doi.org/10.1016/j.ijid.2015.09.021.
25. Singhal J, Agrawal N, Vashishta M, et al. Suppression of dendritic cell-mediated responses by genes in calcium and cysteine protease pathways during *Mycobacterium tuberculosis* infection. *J Biol Chem*. 2012;287(14):11108–11121. https://doi.org/10.1074/jbc.M111.300319.
26. Alam K, Ghousunnissa S, Nair S, Valluri VL, Mukhopadhyay S. Glutathione-redox balance regulates c-rel-driven IL-12 production in macrophages: possible implications in antituberculosis immunotherapy. *J Immunol*. 2010;184(6):2918–2929. https://doi.org/10.4049/jimmunol.0900439.
27. Lin C, Chien S, Chen C, et al. IFN- g induces mimic extracellular trap. *J Interf Cytokine Res*. 2015;36(2):1–13. https://doi.org/10.1089/jir.2015.0011.
28. Lin C, Lin C, Lee K, et al. Escape from IFN- γ -dependent immunosurveillance in tumorigenesis. *J Biomed Sci*. 2017;24(10):1–9. https://doi.org/10.1186/s12929-017-0317-0.
29. Barry JC, Shakibakho S, Durrer C, et al. Hyporesponsiveness to the anti-inflammatory action of interleukin-10 in type 2 diabetes. *Sci Rep*. 2016;6(1):21244. https://doi.org/10.1038/srep21244.
30. Dobrian AD, Ma Q, Lindsay JW, et al. Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. *Am J Physiol Endocrinol Metab*. 2011;300(2):E410–E421. https://doi.org/10.1152/ajpendo.00463.2010.
31. Clark I, Atwood C, Bowen R, Paz-filho G, Vissel B. Tumor necrosis factor-induced cerebral insulin resistance in Alzheimer's disease links numerous treatment rationales. *Pharmacol Rev*. 2012;64(4):1004–1026. https://doi.org/10.1124/pr.112.005850.
32. Hartman ME, O'Connor JC, Godbout JP, Minor KD, Mazzocco VR, Freund GG. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem*. 2004;279(27):28045–28050. https://doi.org/10.1074/jbc.M404368200.

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Case report

Concomitant methicillin-resistant *Staphylococcus aureus* infection in tubercular sacroiliitis masquerading as anti-tubercular drug resistance: Role for molecular diagnosis

Narendran Pushpasekaran [a,*], *Deepak Gupta* [a], *Abhishek Kashyap* [b], *Sumit Arora* [b], *Ajay Kumar Gupta* [c]

[a] *Senior Resident, Department of Orthopaedics, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi 110002, India*
[b] *Associate Professor, Department of Orthopaedics, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi 110002, India*
[c] *Professor and Head, Department of Orthopaedics, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi 110002, India*

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ABSTRACT

A 23-year-old female on anti-tubercular therapy for tuberculous sacroiliitis presented with right sided gluteal and thigh abscess. Suspecting treatment failure, surgical evacuation of purulent material was done. The bacteriological isolation showed positivity for methicillin-resistant *Staphylococcus aureus*. Although the microbiological and histopathology examination of the specimen were negative for tubercular isolates, the cartridge based –nucleic acid amplification tests revealed positive genes for *Mycobacterium tuberculosis* and additional primers showed sensitivity for rifampicin and isoniazid. She was adequately treated with vancomycin for six weeks and anti-tubercular drugs for eight months and followed till the bony ankyloses at 18 months. This is a rare case based scenario wherein concomitant staphylococcal infection in tubercular sacroiliitis masqueraded as anti-tubercular drug resistance. The cartridge-based nucleic acid amplification test for tuberculosis is a rapid and sensitive modality in identifying mycobacteria even mixed infections and also determine drug resistance. There are fewer consensuses in the literature regarding the drugs and duration of anti-tubercular regime for tuberculous sacroiliitis with most regimes using four drugs between six to eighteen months.

1. Introduction

Tuberculous sacroiliitis (TS) is not an uncommon entity in countries endemic to tuberculosis.[1] Though successfully treated with usual anti-tubercular drugs, the deterioration of patient's clinical and biological parameters are major concerns for diagnosis and drug susceptibility.[2–4] In a rare occurrence of tuberculous sacroilitis, a concomitant community acquired *staphylococcus* infection masqueraded as anti-tubercular drug

* Corresponding author at: Department of Orthopaedics, 6th Floor, Lok Nayak Jai Prakash Hospital, New Delhi 110002, India. Tel.: +91 9911670437.
E-mail address: drnaren247ortho@gmail.com (N. Pushpasekaran).

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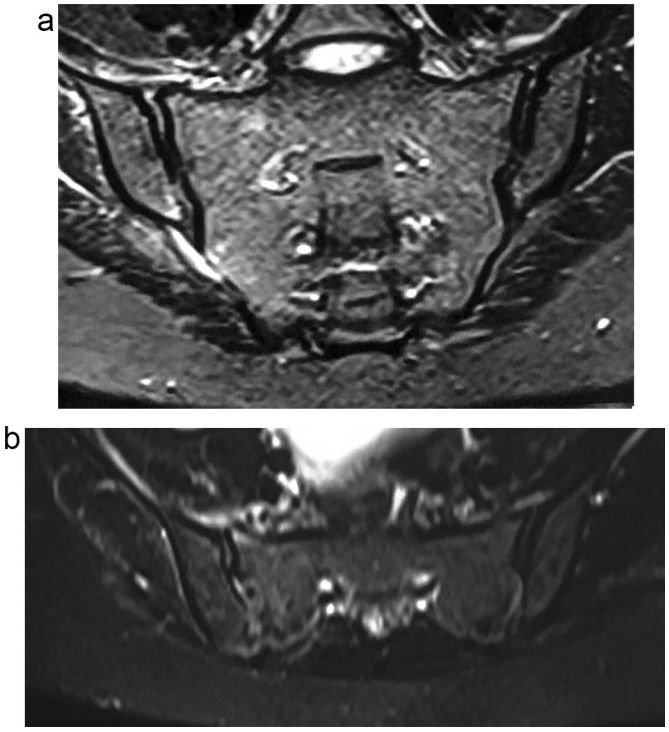

Fig. 1 – MRI of sacrum and both SI joints at initial presentation. (A) Coronal T2 image showing mild erosions, effusion, and minimal fluid collection at the inferior region of the right SI joint; (B) axial T1 fat suppression image.

resistance. This is a unique and complex entity in the natural history of TS as the clinical presentations are altered, isolation of organisms with drug sensitivity testing may be compromised.[5] Through this report, we highlight the importance of the current molecular methods in determining *Mycobacteria* and its drug resistance in case of suspected treatment failures.

2. Case report

A 23-year-old female, six months postpartum, presented with complaints of chronic lower backache and gluteal pain for last 5 months that was progressively debilitating, increasing with physical activities and disrupted sleep. Her pain was not associated with stiffness, periodicity or diurnal variation. She denied any family history with inflammatory arthritis. She had occasional complaints of low-grade fever with no loss in weight or appetite. Physical examination revealed tenderness over sacrum and sacroiliac joint (SI) sites. The SI joint stress tests (Gillet and Gaenslen) were positive. However, neurological examination was normal. Analysis of patient's blood sample showed that the hemoglobin (Hb) level was 9.8 g/dl, total leucocyte count (TLC) 11,000/mm^3, differential lymphocyte count 60%, albumin level 3.5 g/dl, erythrocyte sedimentation rate (ESR) 60 mm after the first hour, and C-reactive protein (CRP) 4 mg/dl. History and serology were negative for immunological disorders. Further, Mantoux test and tubercular gamma interferon assay were strongly positive. The plain radiographs of the chest and lumbosacral spine appeared normal; however, the magnetic resonance imaging (MRI) of the sacrum and both SI joints demonstrated effusions and mild erosions in the right side (Fig. 1). These initial reports led to the provisional diagnosis of TS. She was started on a four-drug anti-tubercular regime (isoniazid, rifampicin, pyrazinamide, and ethambutol).

After about five weeks of anti-tubercular regime, the patient presented to the emergency department with restlessness, a high-grade fever (101 °F), and excruciating lower back and gluteal pain. Diffuse swelling, warmth, and tenderness were noted in the right lower back, gluteal region, and anterolateral thigh region. Blood parameters read features of toxemia with Hb-8.2 g/dl, TLC 23,000/mm^3, neutrophil differential count of 90%, CRP – 16 mg/dl, and ESR – 120 mm/h. Ultrasonogram of the right lower back, upper, and lateral thigh showed a large hypoechoic collection posterior to the SI joint, which extended between the gluteus maximus and medius muscle up to the anterior aspect of the thigh. Repeat MRI of sacrum with SI joints and adnexa showed the features of infective arthritis of both sacroiliac joint with fluid collection extending anteriorly on the right side into the iliopsoas region, and laterally as well as posteriorly beneath the gluteus maximus muscle (Fig. 2).

Under general anesthesia, the right SI joint was approached posteriorly. The brownish purulent material was collected, and found to extend in the plane between gluteus maximus and medius/tensor fascia and into the anterior thigh. A separate curvilinear incision was made in the lateral thigh to evacuate the collection. The anterior approach to SI joint was

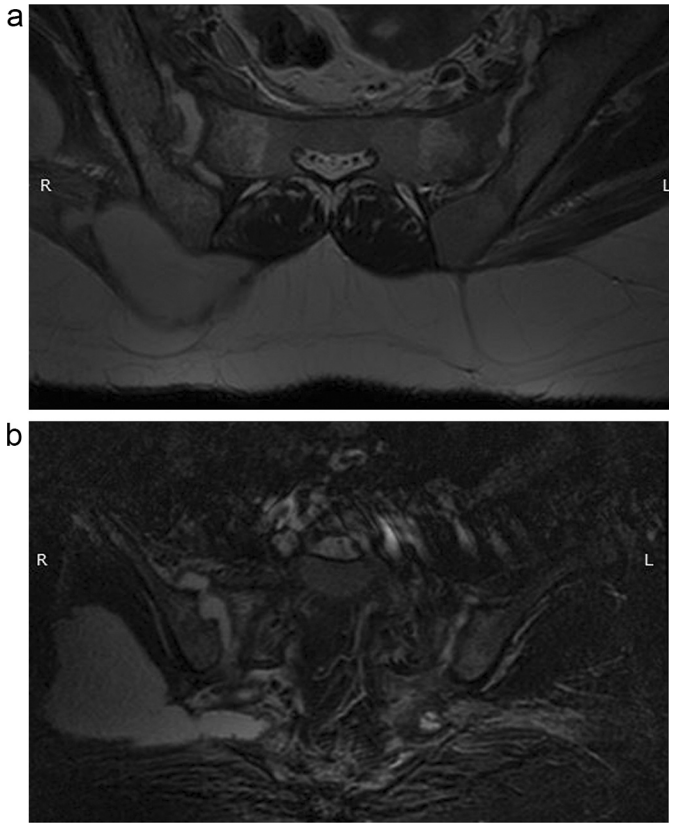

Fig. 2 – MRI of the sacrum, both SI joints, and adnexa at the time of presentation in emergency. (A) Axial T2 fat suppression image showing collection in both the sacroiliac joints extending interiorly into the iliopsoas region. Moderate collection posterior to the right SI joint and extending between gluteus maximus and medius also noted; (B) coronal stir image showing a large collection lateral to the right gluteus medius.

also performed to evacuate the collection in the iliopsoas and anterior SI joint (Supplementary Video 1). A total of 550 ml was evacuated and sent for microbial, sensitivity, and histopathology tests. The Ziehl Neelsen staining method to isolate mycobacteria was negative and the modified Lowenstein Jensen medium showed no growth for mycobacteria. However, the cartridge based-nucleic acid amplification tests (CB-NAAT) for *rpo*B gene sequence specific to Mycobacteria showed positivity and the primers to identify rifampicin and isoniazid resistance were negative. The fluid cultures showed growth for methicillin-resistant staphylococcal aureus (MRSA), sensitivity to vancomycin.

Along with the anti-tubercular regime, she was given intravenous vancomycin of 1 g at every 12th hour for 4 weeks. Her TLC was 12,000/mm^3, ESR of 40 mm/h, and CRP of 4 mg/dl after the 4th week of post-surgery. Her anti-tubercular drugs were provided under category 1 of direct observational short-course therapy (DOTS). Her constitutional symptoms and pain scores improved during the course of treatment and returned to normal activities by the end of 4 months post debridement. Her MRI at 5th month of post debridement showed signs of joint space obliteration and sclerosis changes in the sides of sacrum and ilium with no signs of residual collection (Fig. 3). Although the MRI scans showed mild inflammatory activity over the scar site, she was clinically asymptomatic, and her continuous phase was extended for another two months. At 18 months of follow-up, her plain radiographs and computed tomography (CT) scan of the pelvis showed complete bony ankylosis of right SI joint and patchy ankylosis of left SI joint (Fig. 4).

3. Discussion

The natural history of TS is indolent with vague and overlapping clinical symptoms, which are usually masked in pre-disposing factors for individuals like pregnancy, postpartum, and immune-compromised status.[1] Similar presentations of seropositive or seronegative spondyloarthropathies, absence of constitutional symptoms, and benign radiological appearance until gross destruction are common causes for delayed presentation and diagnosis.[4] However, rapid deterioration of clinical symptoms and raise in blood parameters over days and weeks should raise suspicion of non-tubercular organisms.

The gold standard for diagnosing extra pulmonary tubercular infections is demonstrated by the presence of mycobacteria in the specimen collected by aspiration or open

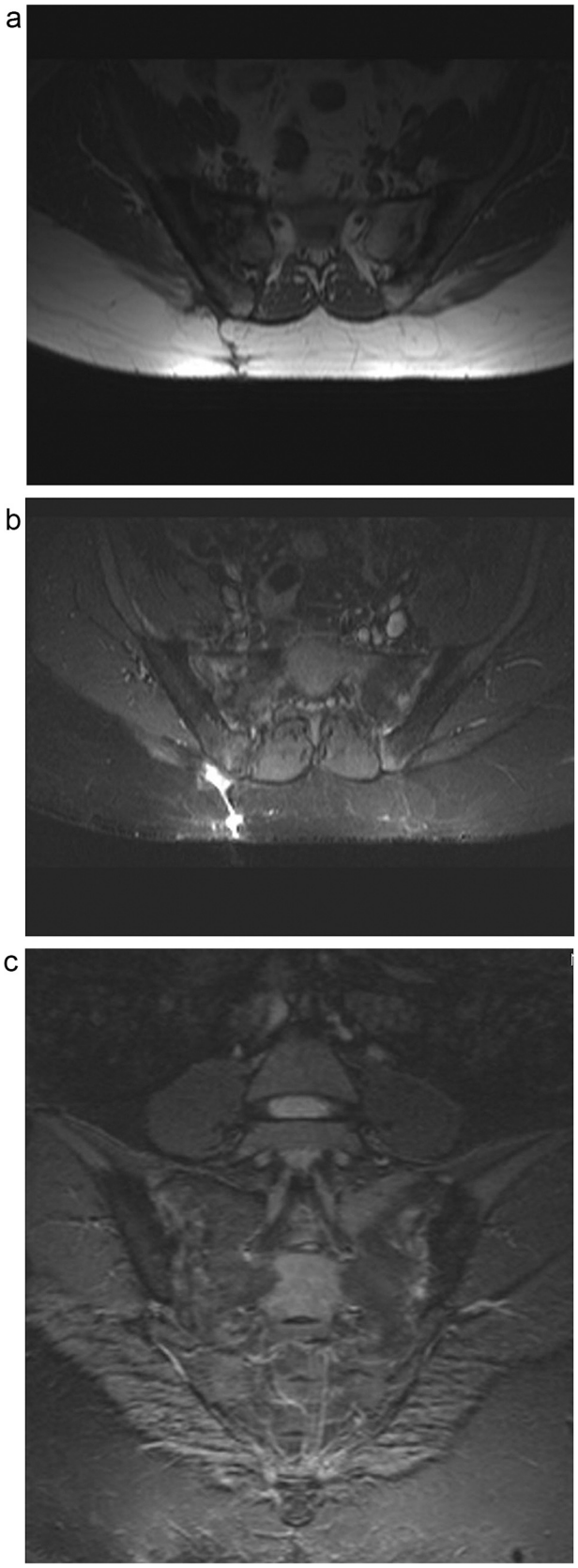

Fig. 3 – MRI of sacrum, SI joint, and adnexa at 6th month of follow-up (A, B, and C) axial T1, T2, and coronal T1 images, respectively. Showing a decreased joint space, marrow sclerosis, and no residual abscess. Mild hyper-intensity was noted in the scar site (B); however, the patient did not show any clinical signs of inflammatory activity.

biopsies; however the sensitivity for the same is between 15 and 27%, owing to the pauci-bacillary nature of osteo-atricular tuberculosis and varying characteristics of collection.[6,7] In the algorithm given by Tuli for endemic countries, diagnosis of spinal tuberculosis can be made with relative reliability using clinical, biological and radiological features for early presentations. Open biopsies or debridement are resorted to when treatment response is inadequate or diagnosis inconclusive.[7] Gao et al. in their retrospective analysis of 15 patients with TS diagnosed by clinical, biological, radiological, cytology and histopathology had performed biopsies or aspiration on all patients but bacilli was demonstrated only in 6 patients.[3] However, all 15 patients were treated successfully with anti-tubercular drugs.

Although not a common practice, we diagnosed TS in our 23 year post-partum female based on the equivocal clinical, biological and radiological findings. Due to the very minimal nature of collection (<5 cc), an aspiration was not done. Although she was administered with the anti-tubercular drugs, she subsequently presented with excruciating pain, large swellings in the lower back, gluteal region and toxemia. We anticipated treatment failure or inconclusive diagnosis.

The microbial array of tests showed MRSA growth, sensitivity to vancomycin, and negative tubercular isolates; however, CB-NAAT showed positive for mycobacterial genes (*rpo*B) and negative results for rifampicin and isoniazid resistance, respectively. The concomitant MRSA infection masqueraded as treatment failure which was not a common occurrence. In extra pulmonary infections, CB-NAAT showed an improved sensitivity in the isolation of mycobacteria even in very small samples, inactive bacilli, and mixed infections.[9] The use of additional primers for detecting rifampicin and isoniazid resistance provided an early detection of anti-tubercular drug resistance.[10]

We had successfully treated our patient with intravenous vancomycin for four weeks and anti-tubercular drugs under DOTS category one for a total duration of ten months (3 months extended intensive phase with 4 drugs and continuous phase of 7 months with two drugs). At present, there is no consensus in literature with regard to the anti-tubercular drugs and duration for treating TS. Many retrospective studies on TS supported the use of anti-tubercular drugs in a staged manner between 6 and 18 months.[3,5,7,8]

The natural history in treated TS is bony ankylosis, and the pyogenic infection can be normal, subchondral sclerosis, or ankylosis.[5,11] To our knowledge, this is a unique presentation of TS with concomitant MRSA infection and appropriately treated with end result of bony ankylosis.

4. Conclusion

The mixed infection of the sacroiliac joint, *Mycobacterium tuberculosis* and MRSA infections are extremely rare entities. The cartridge-based nucleic acid amplification test for

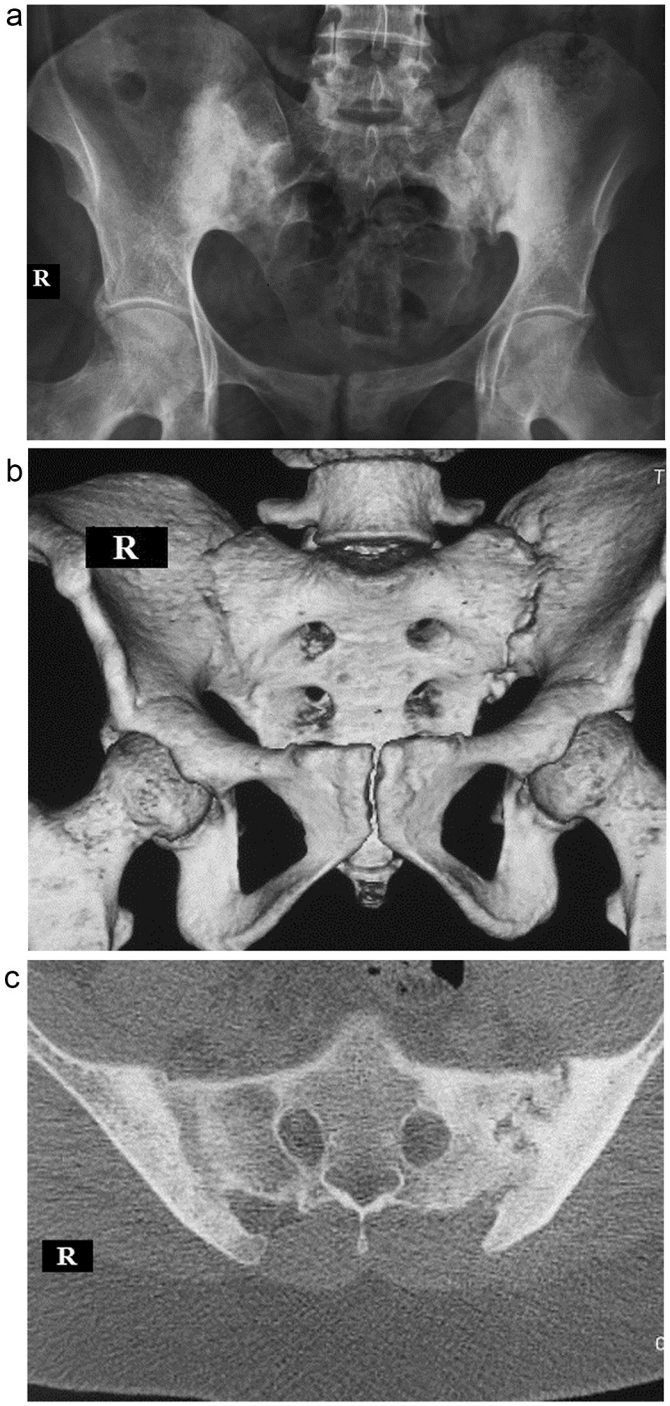

Fig. 4 – (A) Plain Radiograph AP view, (B) reconstructed CT pelvis, (C) axial CT scan at 18th month of follow-up. Showing sclerosis around both SI joint region and complete ankyloses of the joint on the right side and incomplete on the left side.

tuberculosis is a sensitive modality in mixed infections. At present, there is no consensus in the literature regarding the anti-tubercular regime for TS with various regimes recommending the usage for a period of about 6 to 18 months. The outcome in this scenario was bony ankyloses of the sacroiliac joint.

5. Case description

A 23 female with equivocal findings of tubercular sacroilitis was started on anti-tubercular drugs. During the course of treatment, she deteriorated and presented to emergency in toxemia and gluteal abscess. Evacuation of purulent material

showed MRSA positivity and all tests failed to identify or grow mycobacteria, though the biological tests showed positivity. However, the CB-NAAT tests showed positive genes for mycobacteria and sensitive to the first line drugs, which otherwise would have been labelled anti-tubercular drug resistance. She was adequately treated with vancomycin and category 1 DOTS regime and followed till bony ankyloses.

Salient features of discussion:

- A unique presentation of mixed infections of sacroiliac joint and its management.
- A rare scenario and perplexed situation wherein a concomitant community acquired MRSA infection in tubercular sacroilitis masqueraded as anti-tubercular treatment failure.
- The importance of using molecular tests to diagnose mycobacterial infections and its anti-tubercular drug resistance.
- The discussion also includes an algorism for diagnosis in endemic areas and case series highlighting the difficulty in isolating mycobacteria.
- End result of the sacroiliac joint in case of a mixed infection.
- Documentation of a successful anti-tubercular regime and parameters to be considered for decision making in anti-tubercular drugs and duration.

Lessons from case report:

- Mixed infections of sacroiliac joint can masquerade as anti-tubercular drug resistance and must be considered as a rare possibility in anti-tubercular treatment failures.
- The use of CB-NAAT or GENE-XPERT in identifying mycobacterial infections is over-whelming and need of hour in the management of osteo-articular tuberculosis.

Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijtb.2018.05.022.

REFERENCES

1. Kim , Lee , Yoo , Suh JS.. Sacroiliac joint tuberculosis. Classification and treatment. *Clin Orthop Relat Res.* 1999;358:215–222.
2. Ramlakan S, Govender S. Sacroiliac joint tuberculosis. *Int Orthop.* 2007;31:121–124.
3. Gao , Kong , Tong , et al. Tuberculous sacroiliitis: a study of the diagnosis, therapy and medium term results of 15 cases. *J Int Med Res.* 2011;39:321–335.
4. Prakash J. Sacroiliac tuberculosis – a neglected differential in refractory low back pain – our series of 35 patients. *J Clin Orthop Trauma.* 2014;5:146–153.
5. Papagelopoulos , Papadopoulos , Mavrogenis , Themistocleous , Korres , Soucacos PN.. Tuberculous sacroiliitis. A case report and review of the literature. *Eur Spine J.* 2005;14:683–688.
6. Cormican , Hammal , Messenger , Milburn HJ.. Current difficulties in the diagnosis and management of spinal tuberculosis. *Postgrad Med J.* 2006;82:46–51.
7. Tuli SM.. General principles of osteoarticular tuberculosis. *Clin Orthop Relat Res.* 2002;398:11–19.
8. Dunn R. The medical management of spinal tuberculosis. *SA Orthop J.* 2010;9:37–41.
9. Hristea , Otelea , Paraschiv , et al. Detection of *Mycobacterium tuberculosis* resistance mutations to rifampin and isoniazid by real-time PCR. *Indian J Med Microbiol.* 2010;28:211–216.
10. Hillemann , Rüsch-Gerdes , Boehme , Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J Clin Microbiol.* 2011;4:1202–1205.
11. Haanpää , Hannonen , Kaira , Laurikainen , Möttönen , Oka M. Clinical sequelae and sacroiliac joint changes by computed tomography after recovery from septic sacroiliitis. *Clin Rheumatol.* 1989;8:197–201.

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Case report

Two unusual reports of urogenital tuberculosis: One 'putty' kidney and another in association with benign prostatic hyperplasia

Krishnendu Mondal *, *Rupali Mandal*

Department of Pathology, Sonoscan Healthcare, Malda, India

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ABSTRACT

In India urogenital tuberculosis is the second commonest form of extra-pulmonary tuberculosis. Kidney is the highest and prostate is the least affected urogenital organ. But the extreme stage of renal tuberculosis named as 'putty' kidney is a rare manifestation. In general most cases of urogenital tuberculosis are quasi-symptomatic, and therefore an uttermost apprehension is needed from physicians to intercept such cases at the earliest. In this presentation we describe a case of 'putty' kidney, and another incidental association of prostate tuberculosis with benign prostatic hyperplasia.

1. Introduction

10–42% tuberculosis (TB) patients experience extrapulmonary dissemination. In India urogenital TB (UGTB) stands second to lymphatic TB constituting 10–14% of all extrapulmonary cases.[1–3] The epididymis, kidney, ureter, bladder and seminal vesicle share its dominant distribution. The least affected, prostate shows involvement in ~1% post-mortem specimens.[4] Kidney TB (KTB) is noted in 3% of all TB patients.[2] Its range of manifestation extends from minimal granuloma formation to widespread polycavernous caseation, called 'cement' or 'putty' kidney.[3,5] One such rare example of 'putty' kidney is now presented in this report; alongside another case of prostate TB (PTB) incidentally identified on transurethral resection of prostate (TURP) for benign prostatic hyperplasia (BPH).

2. Case reports

2.1. Case 1

A 46-year-old non-diabetic immunocompetent man complained about frequency, dysuria, and right-sided loin ache. His full blood count, uric acid, urea, creatinine and creatinine clearance remained normal. Urinalysis revealed pyuria, trace

* Corresponding author at: c/o-Barendra Nath Mondal, Vill-Fularhat, P.O. & P.S. Sonarpur, South 24 Parganas, 700150 West Bengal, India.
E-mail address: krishnendu.kriss@gmail.com (K. Mondal).

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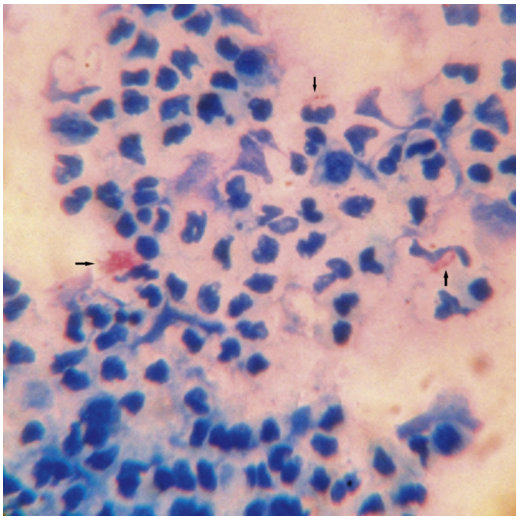

Fig. 1 – (Case 1) Urinalysis shows pyuria and multiple AFBs (arrows) in singles as well as in clusters [Ziehl–Neelsen stain, 1000×].

proteinuria, and a negative culture result. With a suspicion of UGTB his urine was screened for acid fast bacilli (AFB) for three consecutive days, two of which came positive (Fig. 1). Afterwards *Mycobacterium tuberculosis* (MTB) was isolated on BACTEC culture and polymerase chain reaction (PCR). Simultaneous abdominal radiography demonstrated a diffusely calcified right kidney, which was invisible on intravenous urography. His thoracic skiagram came normal. The patient was instituted upon intensive therapy with isoniazid, rifampicin, pyrazinamide and cycloserine for 4 months, with ofloxacin added for first 2 months. Continuation therapy was provided with initial three drugs for 5 more months.

During this phase the patient underwent right nephroureterectomy. Grossly the resected surface of kidney exposed multiple caverns delineated by thin fibrous septa, replacing the entire renal parenchyma including the pelvis. These cavities were filled with extensive greyish-white caseous necrotic debris, i.e. morphologically reminiscent of 'putty' kidney (Fig. 2). Histopathologically, there were only confluent zones of necrosis studded with calcific granules. AFB was not found on Ziehl–Neelsen stained sections, though the PCR readily detected the MTB there.

2.2. *Case 2*

After months-long suffering from lower urinary tract symptoms, a 65-year-old patient was diagnosed with BPH. The serum electrolytes and creatinine were normal. Prostate specific antigen (PSA) was measured 6.07 ng/ml (normal: 0.22–4.1 ng/ml), with the free-to-total PSA ratio being 0.3 (<0.1 favor prostatic cancer). Urinalysis detected microscopic haematuria and pyuria, but the culture remained sterile. Preoperative chest radiograph visualized a fibro-calcific healed TB focus in left lung. Anyway the patient never received any therapy for that. Simultaneously TURP was performed. Histologically, there was multiple variable-sized dilated, cystic and micropapillary hyperplastic glands lined by bimodal basal and secretory cells. In cohabitation, within the stroma there were epithelioid histiocytes in syncytial aggregates, characteristic of non-caseating granuloma. These were mantled by lymphoplasmacytic cells, and interspersed with Langhans-type giant cells. Some granulomas also developed central zone of amorphous caseous necrosis (Fig. 3). Ziehl–Neelsen stain failed to isolate any Mycobacterium thereof. But the PCR produced a confirmatory diagnosis of TB. Therapeutically isoniazid, rifampicin and pyrazinamide, with ofloxacin were used initially for 3 months. Thereafter isoniazid and rifampicin were continued for additional 6 months.

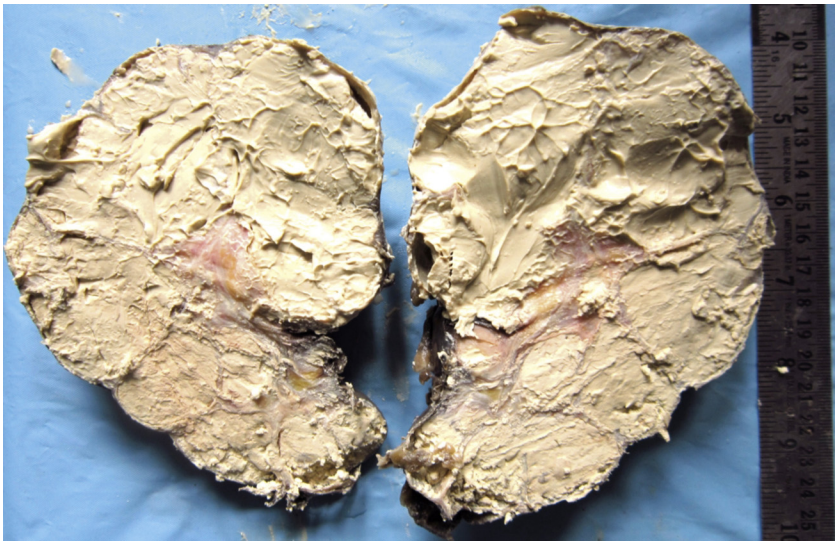

Fig. 2 – (Case 1) Putty kidney: Renal parenchyma and pelvis entirely replaced by multiple caseation-filled caverns delineated by slender septa.

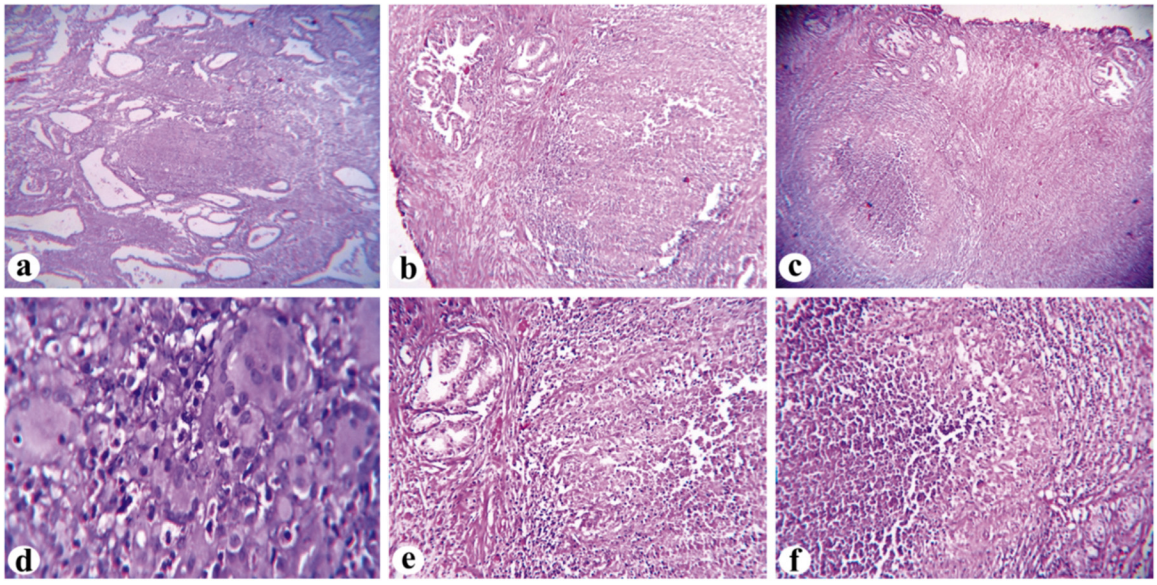

Fig. 3 – (Case 2) PTB with BPH: (a) Dilated glands oriented around non-caseating granuloma, and (b and c) caseating granulomas adjacent to hyperplastic glands [Haematoxylin–Eosin stain, 40×]. (d) Syncitial histiocytic sheets and Langhans giant cells in non-caseating granuloma [Haematoxylin–Eosin stain, 400×], (e and f) caseating granulomas with central amorphous necrosis shrouded by lymphoplasmacytic enclosure [Haematoxylin–Eosin stain, 100×].

3. Discussion

Due to imprecise diagnostic criteria, and lack of prompt, accurate and inexpensive diagnostic measures most cases of UGTB are primordially missed. Additionally its clinical manifestations are nonspecific.[3] Constitutional symptoms are generally absent. Only a third of the patients retain roentgenographic evidence of healed thoracic patch. KTB patients usually present with lower urinary symptoms, back/flank pain and dysuria, which typically suggest bacterial cystitis. Renal colic or haematuria may appear with significant involvement of ureters. TB is suspected on detection of sterile pyuria or intractable urinary symptoms unresponsive to conventional antibacterials.[2,6] The currently reported KTB patient experienced an identical manifestation. Suspicion for TB surfaced with negative urinary culture. Then the diagnosis was confirmed upon isolation of MTB from his urine.

There are 4 stages of KTB. Stage 1: minimal parenchymal destruction, stage 2: TB papillitis, stage 3: cavern formation, and stage 4: polycavernous form.[3] Once renal pelvis gets involved there ureteric stricture occurs. It leads to TB pyelonephritis that detriment onto pyonephrosis-like morphology, called 'cement' or 'putty' kidney, or autonephrectomy. The kidney is occupied by multiple caseation-filled cavities.[3,5] Such cavitation evolves in ~24% cases of KTB. However, the net renal sufficiency may still be adequate. Glomerular filtration is compromised only if the deformity is bilaterally extensive. About 12% patients with cavernous KTB progress to end-stage renal failure.[7] The cavitary forms are incurable with anti-TB therapy only. Surgery is required in majority cases.[3] Symptomatically the discussed patient of case 1 was no different either. His renal function remained unaltered despite there was 'autonephrectomy' on right side. Rest of the urinary system appeared uninvolved. Post-chemotherapy, surgery was hastened in attempt to halt the disease progression.

PTB is a relatively neglected disease, detected post-humously in three-fourth of men succumbing to TB. It is mostly encountered in biopsies from suspected BPH or prostatic cancer.[3] But coexistence of PTB with cancer, or with BPH as in the present report is a rarity. It causes infertility and can transmit sexually. 79% of PTB has simultaneous renal involvement. Only 5% cases are primary.[3,8,9] PTB manifests with perineal pain, dysuria and sometimes with haematospermia. PSA generally remain normal, but may rise marginally as in any prostatitis. Morphologically there is granuloma, caseous necrosis, and in extreme cases cavern formation ensues. *In vivo* diagnosis of PTB is difficult. It requires isolation of MTB by culture or PCR performed with prostatic fluid, ejaculate or biopsy specimen.[4,8] However, no such methodology was considered in the present case of PTB. It was clinico-radiologically adjudged to be BPH. Histology demonstrated the concomitant presence of caseating granuloma, but the AFB was still elusive. Ultimately PCR on the resected tissue confirmed the diagnosis. The infection likely disseminated from the asymptomatic yet dormant lesion of his lung.

UGTB is treated with different drug regimens from that of pulmonary TB. Kulchavenya et al.[3] described 5 different anti-TB regimens according to the stage and form of UGTB. In compliance to the same the presently reported case of stage 4 polycavernous KTB was managed. The therapy of PTB is furthermore problematic. Most antibiotics including the standard anti-TB drugs fail to attain optimum concentrations

at prostate. Only rifampicin and ofloxacin are noted to reach there sufficiently.[9] In the discussed patient with PTB the similar therapy was successfully implemented.

In conclusion, KTB should be suspected early in patients from endemic regions, who suffer from sterile pyuria or urinary symptoms refractory to common antibiotics. Otherwise it may irreversibly progress to autonephrectomy making surgical intervention as a mandatory. PTB is either asymptomatic or simulates prostatic cancer/BPH. Since an utmost level of suspicion can only produce its *in vivo* diagnosis.

Conflicts of interest

The authors have none to declare.

REFERENCES

[1]. Chandra S, Chandra H, Chauhan N, et al. Male genitourinary tuberculosis—13 years' experience at a tertiary centre in India. *Southeast Asian J Trop Med Public Health*. 2012;43(2):364–369.

[2]. Daher EDF, da Silva Jr GB, Barros EJG. Renal tuberculosis in the modern era. *Am J Trop Med Hyg*. 2013;88:54–64.

[3]. Kulchavenya E, Naber K, Johansen TEB. Urogenital tuberculosis: classification, diagnosis, and treatment. *Eur Urol Suppl*. 2016;15:112–121.

[4]. Ratkal JM. Primary prostatic tuberculosis: a rare form of genitourinary tuberculosis. *Afr J Urol*. 2015;21:142–143.

[5]. Khan R, Quaiser S, Viswanath A. Renal tuberculosis: the enigma continues. *Mycobact Dis*. 2015;5(3):186. http://dx.doi.org/10.4172/2161-1068.1000186.

[6]. Miyake H, Fujisawa M. Tuberculosis in urogenital organs. *Nihon Rinsho*. 2011;69:1417–1421.

[7]. Das P, Ahuja A, Gupta SD. Incidence, etiopathogenesis and pathological aspects of genitourinary tuberculosis in India: a journey revisited. *Indian J Urol*. 2008;24(3):356–361.

[8]. Fazarina M, Tan GH, Badrulhisham B, Shiran MS, Tan GC. Tuberculosis of the prostate presenting as benign prostatic hyperplasia. *Med Health*. 2013;8(2):85–88.

[9]. Kulchavenya E, Brizhatyuk E, Khomyakov V. Diagnosis and therapy for prostate tuberculosis. *Ther Adv Urol*. 2014;6(4):129–134.

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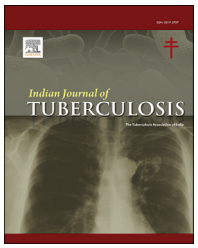

Correspondence

What is the role of "T reg Cells" in tuberculosis pathogenesis?

Dear Editor;

Nowadays, tuberculosis is one of the most important worries in the health and therapeutic aspects, which causes millions of deaths per year in all over the world. According to the reports by the World Health Organization (WHO) in 2016, about 6.3 million new cases were reported for tuberculosis, 1.6 million of them died due to this disease.[1] It has been estimated that about one third of the world's population are affected to the latent tuberculosis infection, 5–10% of which gradually encounter with the tuberculosis clinical symptoms, and these people are affected to the active form of this disease.[2]

In most people and pursuant to the infection with *Mycobacterium tuberculosis*, the host immune system can restrain and phagocyte the tuberculosis bacilli. However, by using virulence genes, *Mtb* prevents the formation of phagolysosomes, remaining inactive in the lung macrophages, and cause the latent tuberculosis infection (LTBI). These people have none of the clinical and radiologic symptoms, but they are the sources for dissemination of *Mtb* to others. Thus, it is essential for them to be rapidly identified and treated; The interferon-gamma (IFN-γ) release assay is one of the most important technics that can be useful in identifying the patients affected to the latent tuberculosis. One of the most interesting questions is what the responsible mechanisms are in reactivation of tuberculosis in those patients. The regulatory T-cells are the most important candidates in that respect (see Fig. 1).[2–4]

After the entrance of *Mycobacterium tuberculosis* to the patient's lung, the person will be exposed to the polluted aerosols by alveolar macrophages, dendritic cells, and surrounding neutrophils and hence, the tuberculosis bacilli are ingested by phagocytes. In this regard, the dendritic cells, as the most important antigen-presenting cells (APC), ingest the antigenic peptides, and consequently, the immunogenic peptides are supplied via MHC I & MHC II to CD4+ and CD8+ lymphocytes, causing the activation of the cells. Finally, by producing IFN-γ, the activated lymphocytes lead to destruction of *mycobacterium tuberculosis* (*M. tuberculosis*).[5] While this response prevents the reproduction of the bacteria and distribution of *Mtb* in the body and on the other hand damages the body tissues, a balance between these reactions should be established to maintain the normal body homeostasis.[6] Treg cells are considered as one of the most important subgroups of T cells CD4+ lymphocytes that express the markers such as CD25 and Foxp3. These cells are capable of producing the regulating IL-10 and TGF-β1 cytokines that can restrain the TH_1 activity and hence, stopping the production of IFN-γ.[2,6] In the study by Gerosa et al, they succeeded for the first time to separate CD4+ T cells, the producers of IL-10 and IFN-γ from the BAL sample (Broncho alveolar lavage) taken from the active tuberculosis patients.[7] It was determined in the next studies that some of the *Mtb* strains (including CDC1551, H37Rv, CH and HN878 strains) can induce the production of IL-10 via producing Treg cells. Moreover, the contaminated macrophages to *Mtb* can produce TGF-β1, and the cytokines also influence in production of active Treg cells.[2,6] It has been observed in the recent studies that mRNA expression of Foxp3 gene in the patients affected to active TB (active tuberculosis) is higher in comparison with the healthy people and the ones affected to latent tuberculosis. Thus it can be concluded that the rate of Treg lymphocytes in the patients affected to active TB is higher than the normal range. This phenomenon caused the researchers to have studies about the effect of Treg in the pathogenesis trend of the tuberculosis bacteria.[6,8] In another study, Sharma et al found that Treg cells separated from BAL sample of the patients affected to active tuberculosis continuously produce IL-10, which prevents the activities and reproduction of activated TH_1 with *Mycobacterium tuberculosis* antigens.[4] In a study, Burl et al compared the rate of Treg cells of two populations with active and latent tuberculosis. According to the findings of this study, the rate of Treg in the patents affected to latent tuberculosis is less than that in the patients affected to the active form of the disease.[9] Moreover, Revol et al depleted the CD4+ and CD25 high T cells of the peripheral blood of the patients affected to TB, and observed that the T cells producing activated IFN-γ increased by the *Mtb* antigens. It was also found in this study that the mRNA Foxp3 expression (resulting in increasing the production of Treg cells) in the patients affected to extrapulmonary tuberculosis is much higher than other types of tuberculosis.[6] One of the most important observations of this study was that the highest rate of Treg was found in the tissues contaminated by these bacteria. It was proved in the previous studies that BAL cells express a great amount of IL-

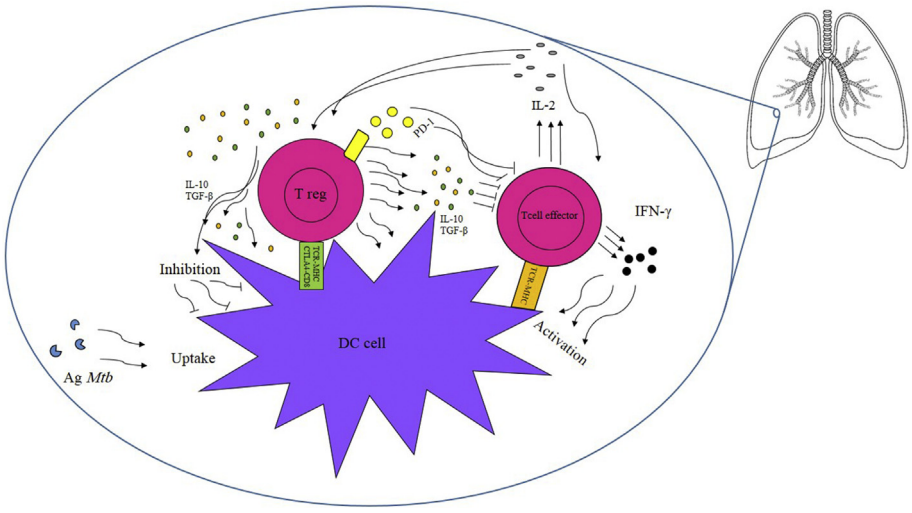

Fig. 1 – Mechanisms of action of Treg cells in pathogenesis of tuberculosis.

2Rα. Revol et al believe that perhaps this phenomenon is due to production of Treg cells in this sits.[6] In their study regarding the immunization of active and latent tuberculosis by using HBHA (heparin-binding hemagglutinin) antigen, Mascart et al showed that this antigen can generate an effective immune response in the patients with latent tuberculosis. In comparison with the latent form, this antigen cannot provide immunization in the patients affected to the active form of this disease.[8] HBHA antigen increases IFN-γ, and this phenomenon can be considered as a diagnosing marker in the cases affected to the latent form. The CD4+ and CD25 high T cells of the patients affected to active tuberculosis were depleted by laboratory technics and the observations showed that the rate of IFN-γ was increased considerably due to HBHA injection.[8] Furthermore, by studying on the patients affected to extrapulmonary tuberculosis, it was observed that the rate of Foxp3+ Treg cells in these patients was more than the patients affected to other forms of the disease. Therefore, the hypothesis regarding the disseminated tuberculosis successive to the activities of T reg lymphocytes was considered.[4,10] According to the observations by Mitra et al about the effect of appropriate treatment of the patients with tuberculosis on the Treg cell changes, it was found that the rates of Treg cells and IL-10 in the treated patients are reduced. It was observed in this study that a considerable rate of Treg cells existed in the peripheral blood samples of the multi-drug resistant patients affected to mycobacterium tuberculosis, which can be considered as the diagnosis marker for the multi-drug resistant strains. By studying on the PD-1/PD-L1 route, these researchers found that by using that route, the Treg cells can deactivate the activated Treg cells by *Mtb* antigens and consequently refrain from increasing IFN-γ.[11]

In summary, regarding the fulfilled studies, it can be found that by using their exclusive mechanisms, Treg cells can probably reduce the response as compared to M. Tuberculosis antigens. It is possible that the microbial pathogens, such as the bacteria causing tuberculosis, use this route in order to get away from the immune system. Thus, regarding the fulfilled studies about the effect of Treg cells on the pathogenesis of tuberculosis, it is suggested to have more profound studies in order to determine the precise role of Treg cells in the tuberculosis pathogenesis.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. World Health Organization (WHO). *Global Tuberculosis Report*; 2017. Available at: http://www.who.int/tb/publications/global_report/en/.
2. Redford PS, Murray PJ, O'garra A. The role of IL-10 in immune regulation during *M. tuberculosis* infection. *Mucosal Immunol*. 2011;4(3):261–270.
3. Barry 3rd CE, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol*. 2009;7(12):845–855.
4. Sharma PK, Saha PK, Singh A, et al. FoxP3+ regulatory T cells suppress effector T-cell function at pathologic site in miliary tuberculosis. *Am J Respir Crit Care Med*. 2009;179(11):1061–1070.
5. Babaki MK, Soleimanpour S, Rezaee SA. Antigen 85 complex as a powerful *Mycobacterium tuberculosis* immunogene: biology, immune-pathogenicity, applications in diagnosis, and vaccine design. *Microb Pathog*. 2017;112:20–29.
6. Guyot-Revol V, Innes JA, Hackforth S, Hinks T, Lalvani A. Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. *Am J Respir Crit Care Med*. 2006;173(7):803–810.
7. Gerosa F, Nisii C, Righetti S, et al. CD4+ T cell clones producing both interferon-γ and interleukin-10 predominate in bronchoalveolar lavages of active pulmonary tuberculosis patients. *Clin Immunol*. 1999;92(3):224–234.
8. Hougardy JM, Place S, Hildebrand M, et al. Regulatory T cells depress immune responses to protective antigens in active tuberculosis. *Am J Respir Crit Care Med*. 2007;176(4):409–416.
9. Burl S, Hill PC, Jeffries DJ, et al. FOXP3 gene expression in a tuberculosis case contact study. *Clin Exp Immunol*. 2007;149(1):117–122.

10. Whittaker E, Nicol M, Zar HJ, Kampmann B. Regulatory T cells and pro-inflammatory responses predominate in children with tuberculosis. *Front Immunol*. 2017;8:448.
11. Singh A, Dey AB, Mohan A, Sharma PK, Mitra DK. Foxp3+ regulatory T cells among tuberculosis patients: impact on prognosis and restoration of antigen specific IFN-γ producing T cells. *PLoS One*. 2012;7(9), e44728.

Masoud Keikha
Mehdi Shabani
Sepehr Navid
Bahar Sadegh Ehdaei
Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Mohsen Karbalaei Zadeh Babaki*
Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Antimicrobial Resistance Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

**Corresponding author.*
E-mail address: KarbalaeiZM931@mums.ac.ir

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