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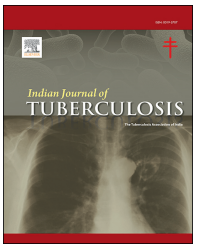

Editorial

Impact of second wave of Covid-19 on tuberculosis control

Second wave of COVID-19 is sweeping across India, resulting in a huge spike in the number of cases. Out of 21 million cases detected so far, 8.3 million have been recorded in the past 30 days. 412,262 new cases were detected on May 6, 2021. Health care providers and facilities are overworked and essential medical supplies like oxygen concentrators and ventilators are decreasing as the cases surge.[1] Two million tests are being conducted on daily basis and this figure has been highly variable across the country, with some regions showing significant declines. As per BBC NEWS, by April 2021, the case numbers had crossed more than 55%, but testing had fallen by 20%, suggesting a much higher underlying level of infections. The World Health Organization's 'End TB' strategy has been hit hard by COVID-19. WHO strongly recommends that TB services are maintained. It also highlights that people suffering from both TB and COVID-19 may have poorer treatment outcomes if TB treatment is interrupted. With lockdowns and the strain on the health services it is challenge to cater to multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB patients. As per reports, COVID-19, TB services were disrupted in India, which accounts for the highest number of TB cases globally.[1] WHO quoted that almost a million people across the globe could not receive TB treatment in 2020 marking a huge setback for national TB control programmes.[2,3]

Both the diseases have the capacity to stress health systems, they are airborne transmissible and can be diagnosed rapidly. They cause social stigma and need public awareness and cooperation for prevention, diagnosis and treatment to be effective. Although surveillance is able to report on TB and viral diseases separately, in various countries, information on COVID-19 is still incomplete and information on TB do not contain many clinical and immunological parameters, which would be useful to better understand the interaction between the two diseases. Moreover COVID-19 pandemic has led to a fall in TB notifications.[4] Determinants of mortality for COVID-19 are age and co-morbidities, including HIV co-infection, poverty, diabetes and malnutrition, all of these also have an impact on TB mortality.[5] TB is curable, while evidence on anti-viral agents or other drugs for COVID-19 is still lacking.[5–7] Research on new vaccines is ongoing for both the diseases. Vaccination for COVID-19 has started whereas for TB various candidates are under evaluation to replace the old BCG.[8] The COVID-19 pandemic has resulted in a health shock as well as economic shock. The lockdown in response to the pandemic can have an adverse epidemiologic impact on TB incidence via its effect on poverty, and dietary intakes.[9] This has further affected GDP growth. During this wave, COVID-19 is occurring in the age group (15—45 years) in which TB is also common so there are more chances of co-infection and mortality. Some of the risk factors like Diabetes, autoimmune diseases, renal transplant cases, patients on steroids and immunosuppressive drugs are also similar to make one prone to both the diseases. Thus the efforts to control both the diseases will require close surveillance among this age group and such high risk individuals. Cross referral of cases between COVID screening centres and TB diagnostic centres and at higher level between TB and other national health programmes would prove beneficial for patients.[10]

TB control programme is under strain due to diversion of resources, constraints due to overutilization of laboratories meant for TB work, issues related to availability of TB care workers, movement restrictions etc. .DR-TB centres are being diverted for COVID related work because of change in the priorities of health care delivery[11,12] During this pandemic, diagnosis and treatment of TB, TB and COVID-19 co-infection may be compromised. Both diseases may cause stigma, discrimination, along with economic impact because of loss of productivity. The symptoms of COVID-19 and TB can be similar such as cough, fever, breathlessness which can create confusion among people. As there is already social stigma associated with TB and is also being observed with COVID-19, the people may be afraid of seeking health care when they have such symptoms which actually result from TB. Based on the decline in TB cases during lockdown period it can be predicted that there might be sudden increase in the cases after the lifting of restrictions for covid-19 leading to additional burden on the already over-burdened existing health care system of the country. As per an article a 25% decrease in global TB case detection over a period of three months of lockdown may lead to an additional 13% increase in deaths in 2020, assuming an absence of a rebound in case detection above values prior to the lockdown.[9]

According to an article, assuming a two month lockdown along with a two month recovery period, a recently released report by the WHO Stop TB Partnership has predicted four percent excess deaths globally and 5.7% excess deaths in India

during 2020–25[13] including excess incident cases to the tune of 3.1% globally and 3.6% in India. The article states that these models do not account for increase in TB incidence and death due to increasing impoverishment arising from economic disruption due to lockdown. In addition to the direct effect of impoverishment on TB severity and death, it could also have an indirect effect through delayed health care seeking.

Various agencies are involved in the relief work being carried out in the country. Global companies world over have increased production and delivery of emergency assistance to help India which is facing a devastating second wave of the coronavirus pandemic. Foreign aid began pouring into India, from countries including the UK and the US, from May 2021.

WHO quotes that the consequence of the COVID-19 pandemic would be a worsening of the TB epidemic globally, for reasons like as added pressures on health systems by COVID-19 resulting in weakening of the National TB programmes[2] and the potential biological effects of the interaction of the two infections like TB and HIV.[5]

A Tuberculosis patient can get infected with Covid-19 infection anytime with worst outcomes for TB. More evidence is needed to understand the potential of COVID-19 to favour reactivation of an existing TB infection. The aspecific signs and symptoms common to COVID-19 and TB may facilitate a rapid access to imaging services which may manifest signs of a pre-existing TB. Use of new technologies like digital tracing apps, monitoring and surveillance of diseases along with use of masks are some of the other key learnings from COVID-19 that can serve the objective of TB elimination. Under Nation Tuberculosis Elimination programme (NTEP) people are now being given a month's supply of TB drugs to decrease visits to health centres, and health workers are monitoring the drug intake on video calls.[3] These measures along with TB centres testing people for both COVID-19 and TB will go a long way in handling both the crises.

There is an urgent need to support investment in research and development in the public sector along with collaborations between various agencies. As per an article COVID-19 has exposed the fragile nature of the current health care systems in India and worldwide.[14] Apart from strengthening health care infrastructure, patient-centric services are needed for achieving better TB control. New manifestations of diseases along with an increasing rate of antimicrobial resistance call for urgent action based on learnings from the COVID-19 pandemic. Overburdened health care system by COVID-19 alongside the economic impact is going to pose a challenge for management of TB.

Conflicts of interest

The authors have none to declare.

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K.K. Chopra, Director, Associate Executive Editor*
New Delhi Tuberculosis Centre, New Delhi, India
Indian Journal of Tuberculosis, India

S. Matta, Epidemiologist
New Delhi Tuberculosis Centre, New Delhi, India

V.K. Arora, Vice Chairman (P&R), Executive Editor
TB Association of India, India
Indian Journal of Tuberculosis, India

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

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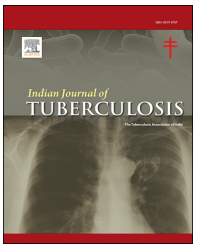

Review article

Neoteric advancements in TB diagnostics and its future frame

Kajal [a], *Diksha Sharma* [b], *Rohit Rai* [a,*]

[a] *Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, 144411, Punjab, India*
[b] *Department of Biotechnology, DAV College, Jalandhar, 144008, Punjab, India*

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ABSTRACT

Tuberculosis (TB) is one of the major infectious disease that causes threat to human health and leads to death in most of the cases. *Mycobacterium tuberculosis* is the causative agent that can affect both pulmonary and extra pulmonary regions of the body. This infection can be presented either as an active or latent form in the patients. Although this disease has been declared curable and preventable by WHO, it still holds its position as a global emergency. Over the past decade many hurdles such as low immunity, co-infections like HIV, autoimmune disorders, poverty, malnutrition and emerging trends in drug resistance patterns are hindering the eradication of this infection. However, many programmes have been launched by WHO with involvement of governments at various level to put a full stop over the disease. Under the Revised National Tuberculosis Control Programme (RNTCP) which was recently renamed as National Tuberculosis Elimination Programme (NTEP), the major focus is on eliminating tuberculosis by the year 2025. The main aim of the programme is to identify feasible quality testing, evaluate through NIKSHYA poshak yozana, restrict through BCG vaccination and assemble with public awareness to eradicate MTB. Numerous novel diagnostic techniques and molecular tools have been developed to elucidate and differentiate report of various suspected and active tuberculosis patients. However, improvements are still required to cut short the duration of the overall process ranging from screening of patients to their successful treatment.

1. Introduction

In the era of high-grade healthcare facilities, with an array of medications and panel of diagnostic tests, *Mycobacterium tuberculosis* (MTB) still remains the leading cause of healthcare disorders and mortality.[1] High rate of mortality worldwide approximating 1.2 million people without HIV (addition of 251,000 people with HIV infection) and morbidity of 10 million people (57% men, 32% women and 11% children age less than 15 years), pushed WHO to declare tuberculosis as a global emergency.[2] Recent WHO report also states that 1.1 million children fell ill with this drastic disease whereas 20,500

* *Corresponding author.*
E-mail address: rohitraisharma44@gmail.com (R. Rai).
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children died because of this infection (data includes HIV coinfected patients too) in the year 2017.[3] Although TB is preventable as well curable with nearly 5.8 million cases saved due to timely diagnosis and treatment between 2000 and 2018, it is still challenging for the healthcare professionals to design a complete elimination strategy for this infection. A highlight of the recent WHO report reflects thirty high TB burden countries that sum up for 87% of the new TB cases.[2] Out of these, two third of the total cases are accounted by eight countries including India on the lead followed by China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and South Africa. Despite numerous strategies and plans being implemented at global platforms, reduction in the incidence of TB infection is hardly 2% per year.[3,4] The trends are highly alarming and become even worse when one looks at the existing conventional methodologies which take up to 3 weeks for accurate MTB diagnosis. This further encourages the emergence of Multidrug Resistant-TB (MDR-TB) and Extensively drug Resistant-TB (XDR-TB)[5] strains. All these challenges have evoked the interests of clinical researchers to focus more on developing biosensors and other point of care techniques that will not only help in rapid detection of MTB but will also mediate plunging of drug resistance in TB strains and elimination of slow expressing and persistent TB infections. Therefore, this article focus on the current developments in the field of MTB diagnostics and the point of care modalities that will prove the techniques of upcoming times.

1.1. *Timeline of tuberculosis diagnosis*

The first step in clinical assessment of tuberculosis is to correlate the signs and symptoms of patients with laboratory findings. The patients with active tuberculosis can be easily recognized and diagnosed by healthcare professional in comparison to the latent tuberculosis and co infection patients. The cases with latent infection are little hard to diagnose, identify and treat as signs and symptoms in these patients are not evident. Hidden infections inside the body can be reactivated and dangerous for the patient and community. General symptoms of tuberculosis primarily include coughing either dry or productive, chills, malaise, body pain, high body temperature and weight loss.[6] Diagnosis of tuberculosis can be bifurcated as direct study i.e., analysis of active bacilli in the biological samples and secondly the indirect response of immune system towards the present bacilli and/or its components. The diagnostic techniques that can be used to identify the cases of tuberculosis comprise radiological studies (chest x-rays, CT scan), microscopic analysis (sputum smear microscopy-acid fast staining/Ziehl Neelsen stain, fluorescent microscopy), culture on solid (Lowenstein Jensen) and liquid media (Middlebrook 7H10/7H11/7H9 broth), drug sensitivity testing (BACTEC MGIT960), molecular diagnosis (Cartridge based-Nucleic acid amplification test using gene expert, line probe assay testing) and lastly serological/immunological response analysis Mantoux tuberculin skin test, interferon-gamma release assay (IGRAs).[7–9] The choice of diagnosis will however depend upon patient's history, availability of the facility and cost effectiveness.

1.2. *Radiological studies*

People suspected for tuberculosis or having prominent symptoms like coughing, fever, chills, sweating, haemoptysis etc., are advised to go for radiological analysis. This is a kind of direct, easy and speedy test for clinician to get an idea. Thorough monitoring, follow up and management of patients are generally done using X rays. This method is beneficial for pre and post analysis of patient treatment. The computed tomography (CT) scan also plays an important role to study the depth of infection for healthcare team by visualising the formation of buds, masses, branching tree appearance etc. In one comparative study of pulmonary tuberculosis with HIV positive and negative patients, it has been reported that nodular detection for coinfected patients using X rays (uncharacterised findings) and CT scan give different sensitivity.[10] One group of researchers found that mediastinal lymph node enlargement is a typical radiological screening in low immune patients along with tuberculosis.[11] According to another study, CT scan is more sensitive than the X ray technique in wide diagnosis and helps the consultants to differentiate between active and latent phase of infections.[12] As per WHO guidelines for early diagnoses of tuberculosis, radiological study in combination with laboratory diagnostic tests can be more helpful. In a retrospective study, it was observed that DL system (deep learning neural networks) could be considered for automated TB diagnosis in future.[13]

1.3. *Microscopic analysis*

In patients with cough persistent for more than 2 weeks, sputum samples are analyzed microscopically along with radiological examination. This method is quick, clear and easy to perform to start treatment of patients. Due to low cost and high sample load, this old technique still works as relic. The staining method includes acid fast staining (ZN staining) which is done in routine cases whereas fluorochrome dye (auramine/rhodamine) is an advanced method for cases where detection of bacilli remains hidden. In a correlative study of patients with a clinical suspicion of tuberculosis (TB) presenting with lymphadenopathy, modified fluorescent method was found to be more advantageous than routine cytology and conventional ZN method, particularly in paucibacillary cases.[14]

To increase the yield of bacilli as well as the sensitivity of staining technique, alternative techniques such as liquification and concentration alteration are used in testing protocols.[15] A study has shown that methods including use of bleach centrifugation and sedimentation technique decrease the test specificity to 2–3% with fix benefits up to 6% and 9%, respectively.[16] The fluorescent microscopy method employing quartz-halogen or high-pressure mercury vapour lamps are costly and essentially require an expert surveillance. To overcome this problem, WHO in 2009 recommended the use of light emitting diode (LED) which was followed by Revised National Tuberculosis Programme (RNTCP) to replace simple staining methods.[17] The classical staining techniques used in routine diagnosis lack the sensitivity and requires bacilli load of approximately 10,000 cells/ml. A research has suggested that LED Fluorescent microscopy can be used as an alternative

to conventional staining method as it facilitates reports of diagnostic services in high burden setting with slight drawback of more expertise, and training requirements.[18]

Idea of microscopy has its own limitations and cannot be used in checking the drug sensitivity pattern and differentiating dead bacilli from the live ones. This drawback open pathway to generate some new technologies to avoid false interpretation of results. Techniques like front load microscopy, sodium hypochlorite microscopy, vital fluorescent staining is being practiced and requires modifications to be established as a standard procedure.[19,20] Work is in progress for the development of automatic microscopic and cell scope technique for making robotic, digital and robust tuberculosis diagnosis.[19,21] Taking into account poor sensitivity of Ziehl Neelsen staining and more than half undiagnosed tuberculosis cases, a battery operated device known as SeeTB was designed. The principle of this portable set up revolves around converting the bright field microscope into fluorescent microscope by equipping the former with an internal reflecting fluorescence excitation system. A study conducted on 237 sputum samples has offered hope for the use of this device in resource deficit sectors owing to its good sensitivity and specificity.[22] To overcome the problems faced in standard microscopy procedures, an operator-independent automatic reading techniques are being designed with focus to accelerate screening of slides in high workload circumstances with reliable results.[23–25] ZEISS Axio Scan. Z1 microscope that is one such technique that is being used for automated detection and enumeration of acid fast bacilli and is proving itself as a tool of recent time.[26] TB diagnosis system TBDx, another computerized microscopy imaging framework with high-resolution power magnifying lens can look up to 200 arranged smears using fluorescent microscopy in a single run.[27,28]

1.4. *Culture method*

The ace of spades or the known gold standard method for isolation and identification of *M. tuberculosis* is culture technique. This method applies the use of both solid and liquid media for the growth of bacilli. On Lowenstein Jensen (LJ) medium, proper colonies of bacteria can be obtained which are used for subculturing and inoculating bacilli in liquid broth (Middlebrook 7H10/7H11). The tubercle bacilli take almost 2–6 weeks to grow, which creates a challenge for scientific laboratory officers to provide fast reports. For a doctor, it takes almost 60–70 days to finally receive all reports along with DST (drug sensitivity testing) to start patient treatment.[29] With the invention of automated culture systems either fully or semi-automated, rapid bacterial cultivation is done using liquid culture media. This revolution began with the introduction of BACTEC TB460, a radiometric operative machine which was best for diagnostic centres but generated large amount of radioactivity. This limitation led to the development of BacT/ALERT MP (bioMerieuxInc, Durham, NC, USA) and BD BACTEC Mycobacterium Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD, USA).[30,31] Recent updates in this method including use of Middlebrook 7H9 liquid culture, BACTEC MGIT 960 (non-radiometric method) have lower down the report updating and turnaround time (TAT). The detection in MGIT (Mycobacterium growth indicator tube) is done by checking the consumption of oxygen level using detectors in presence of UV. Along with the growth of bacteria, drug sensitivity testing can also be performed in this automated system. Till date, this method is highly specific and sensitive as it uses growth control and standards for different batches of tests and chances of contamination being reduced to high extent. Using MGIT tubes, early reports can be issued within 9–16 days for positive patients, 42 days in negative results and DST reports takes up to 14 days.[32] An instrument with inbuilt software can create errors which is a drawback that requires expertise and repeated testing. Limited sets of programmes and commands beyond which machine cannot work and detect the mutations in the pathogenic strains, limits their diagnostic use, necessitating the need to focus on redesigning the current technologies to meet the future needs. To overcome these limitations, nanoscale sensing system also referred to as magnetophoretic immunoassay (MPI) has been introduced. It captures culture filtrate protein (CFP)-10 antigens by using two different nanoparticles (NPs). The results of a comparative study showed that MPI is more robust in terms of sensitivity than MGIT and can therefore be used as an early tool in the diagnosis of TB.[33] Another diagnostic tool, TB-CX employing thin layer agar, MTB culture colour plate with drug susceptibility testing for isoniazid, rifampicin and pyrazinamide has been investigated for sputum examination.[34]

1.5. *Rapid identification tests for* M. tuberculosis

These are also known as one step identification methods that can efficiently address the challenge of differentiating *M. tuberculosis* from Non tuberculous Mycobacteria or NTM. Preliminary identifications can be done based on biochemical analysis of the *Mycobacterium* antigens followed by molecular and immunological response studies. The SD Bioline TB Ag MPT64 Rapid is a commercially available kit used to identify *M. tuberculosis*. Development of this test is based on 33 different proteins secreted by *M. tuberculosis* and the sample can be marked positive or negative within15 min.[35] These tests provide high level of sensitivity and specificity depending upon the kit type used in testing.[36] Despite the promptness of designed technologies, delayed and/or false results and quality of the result interpretation are couple of limiting factors. A group of researchers have designed a sensitive diagnostic test that gives results in 10 minutes without hectic technicalities and processing. The test uses β-lactamase (BlaC; TB specific biomarker) in reporter enzyme fluorescence (REFtb) along with specific fluorogenic substrate, CDG-3.[37] A recent study showed that Patho-tb test is another rapid method that can be used for detection of tubercle bacilli. The non-requirement of skilled technicians, high sensitivity and significant reproducibility grades this technique as an emerging screening tool in TB diagnosis.[23]

1.6. *Molecular methods*

The urge for more sensitive and specific tools in TB diagnosis has shifted paradigm towards molecular approaches. These tools can detect both live and dead bacilli present within the sample from pulmonary as well as extrapulmonary regions.

The nucleic acid amplification test (NAAT) working on the principle of polymerase chain reaction (PCR) detects specific genes and sequences for targeted pathogens. This test can perform both detection and drug resistance for the desired organisms in an accurate manner.[38] Molecular level studies have always established them as a lamp in the dark night. The Cartridge Based-Nucleic acid amplification test (CB-NAAT) has emerged as a potential technique for rapid detection and confirmation of tuberculosis. With the reduction in turnaround time (TAT), this molecular Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA) technique helps in simultaneous detection of TB and resistance to first line drug rifampicin and is proving to be useful in paediatric and extra pulmonary TB.[39–42] The working principle for this technique relies on the amplification of nucleic acid sequence of rpo β genes that are further probed with five molecular beacons (fluorescent probes). The equipment contains inbuilt quality control mechanism consisting of internal probe checking, sample processing controls and quality control-1 and 2. The estimated turnaround time for completion of test and report review is approximately 2–3 hours with analytical limit of 114 CFU/ml.[42] The sample processing cartridges are ready to use and require addition of 2ml suspension (sample plus buffer) for each patient. Further, one-time application and design of cartridge eliminates cross-contamination and enhances sensitivity of detection.[41]

Xpert MTB/RIF Ultra is a new, improved and upgraded tool with high sensitivity over smear-negative, pediatric samples and other paucibacillary specimens including Human Immuno Virus (HIV) positive cases and better rifampicin resistance detection. This transcription-reverse transverse concerted reaction amplification technology remodelling has decreased the processing time i.e. turnaround time (TAT), allows health care professionals to detect 8 samples at once in 30 min by measuring the total fluorescence in real time and start earlier treatment.[43] The LOD of the advanced Ultra technique has been reported to be 15.6 CFU/ml that corresponds to nearly 8% improvement over the previously used Xpert MTB/RIF method.[44]

Truenat, another tool developed by Molbio Diagnostics Pvt. Ltd. Is the first indigenously made Indian machine endorsed both by the Indian Council of Medical Research and by WHO in line with Gene Xpert. It works on chip based nucleic acid amplification testing principle and can be used at peripheral level. It is a small, battery operated, easy to handle device in which extraction of DNA (Trueprep Auto device), amplification (Truenat MTB chip) and detection (TrueLab PCR analyser) of the tubercle bacilli is performed along with detection of rifampicin resistance (Truenat MTB – RIF Dx assay). A comparative study was done to evaluate Truenet (RT Micro PCR device) performance with respect to GeneXpert with 274 sputum samples and the results showed overall good sensitivity and specificity of the former.[45]

Line probe assay testing (LPA) is a drug susceptibility test that uses PCR along with reverse hybridization technique for rapid detection of mutations linked with drug resistance. Hain life sciences Common Mycobacteria (Hain CM) test, Inno Genetics Line Probe Assay (INNO-LiPA) (RIF & MYCOBATCERIA v2) are the commercial kits available in market for the detection and identification of *M. tuberculosis*. To determine multi drug resistant tuberculosis (MDR-TB) strains, MTBDR plus V 2.0 is available.[46] A study of sputum samples collected from 329 suspected drug resistant TB patients showed that LPA has better performance than GeneXpert and can become an alternative of the culture method for detection of RIF resistance. However, major disadvantage of using LPA over Gene Xpert is that it can be performed on smear positive sputum only since its sensitivity is lesser than that of the latter.[47]

1.7. *Immunological diagnosis for latent TB*

These are indirect methods that are used to detect infection and cannot be used as confirmatory tests to diagnose active disease. Two major immunological tests done for TB detection are Mantoux tuberculin skin test (TST) and Interferon -gamma release assays (IGRAs). A study conducted with a group of 1511 pulmonary tuberculosis patients showed that both TST as well as IGRAs (QFT-GIT assay) give a non-significant value for the active tuberculosis infection in the patients. However, ease in the performance of IGRAs and low cost associated with TST makes them a suitable choice for the diagnosis of latent tuberculosis infection.[48] The patients who have previously received BCG vaccine may give a false positive reaction to TST.[49] Alternatively, the prior BCG vaccination does not give any false positives in TB blood test i.e., IGRAs.[50] IGRA is a commercially available kit-based test that works on a principle similar to enzyme linked immune sorbent assay (ELISA). The kit detects presence of MTB infection by measuring immune response towards the tubercle bacilli proteins in whole blood. IGRAs tests can be bifurcated as Quanti FERON-TB gold in tube tests that utilise whole blood and produce interferon gamma in response to MTB antigen; and T-SPOT TB tests which rely upon peripheral blood mononuclear cells (PBMC).[50] The IGRAs testing protocol has its own several disadvantages like requirement of a complex laboratory, sample transportation, high cost, variability of results, etc.,.[51]

C–Tb, a highly specific skin test based on antigens ESAT-6 and CFP-10 addresses several limitations of TST and IGRAs.[52] The recent contact tracing trials including groups of patients with variable degrees of exposure to pulmonary tuberculosis, C–Tb test positivity rates were found in agreement with increasing TB exposure and were strongly in accordance with QFT.[53]

1.8. *Point of care tests*

The major challenge associated with TB diagnostics is collection and transfer of sputum samples from the sample collection centres to the diagnostic labs causing delay or loss of sensitivity resulting in negative results on processing. Additionally, difficulty in sputum production in case of HIV infected ill patients is another limitation of the techniques associated with TB diagnosis due to which approximately 36% of the cases remain undiagnosed and unreported.[2] Therefore, there is a strong need of developing diagnostic tests based on other biological samples. Lateral-flow lipoarabinomannan assay (LF-LAM) is one such technique that processes urine samples of HIV infected patients and is highly sensitive and specific (45% and 92%, respectively) for TB diagnosis.[54] WHO in

2014 has also endorsed the implementation of LAM test to progress towards improving shortcomings of other diagnostics via next-generation assays.[55] *M. tuberculosis* has a unique cell wall with multiple lipid-based molecules (majorly lipoarabinomannan) that create a thick waxy coating upon the cell surface. This LAM acts as an antigen that is detected through the strip based rapid biomarker test using specific target product profile (TPP) within one day.[55] Several assays of LAM have been developed and tested by researchers in the past to obtain desired results with FujiLAM and AlereLAM being extensively used.[56] FujiLAM is a silver based amplification test that offers increased visibility of test and control lines with cut off of approximately 30 picogram per ml. This test has increased detection limits as compared to Alere LAM test with ability to detect nearly 30 folds decreased concentration of LAM in urine.[56]

M. tuberculosis produces volatile organic compounds (VOCs) that can be detected in the breath of infected persons and act as biomarkers for the diagnosis of tuberculosis.[57,58] These compounds are stable (emits odours) and show volatile properties in ambient temperature.[57,58] This non-invasive time saving technique utilizes solid-phase micro extractions, gas chromatography-mass spectrometry, exhaled breathe condensate (EBC), chemiluminescence, optical absorption spectroscopy system, electronic noses or other gaseous sensors.[59] In vitro identification and differentiation of microbes is done through VOC "fingerprinting" or "smell printing" where exhaled breath of presumptive TB patient is blown into the tube of device and MTB specific VOCs bind with titanium oxide compounds to generate electrical impulses which can be read using smart phone applications.[60]

The use of nanoparticles in detection of *Mycobacterium* DNA from clinical samples is emerging as a powerful futuristic tool. The techniques based on nanoparticles are highly sensitive and offer swift and accurate detection of *M. tuberculosis*. Numerous nanoparticles have been engineered over past couple of decades and their potential for clinical purposes has also been explored. GP-1/GP-2 and GP-3/GP-4 probes made up of gold nanoparticles have been reported for the conserved sequences IS6110 and Rv3618, respectively, where former hybridizes with *M. tuberculosis* complex and the latter is specific for *M. tuberculosis* strain[61]. A silver based biosensor (FLAG-C_{60}) was designed for rapid detection of *M. tuberculosis* during early stages of the infection.[62] The work is being done on improving the sensitivity of these biosensors by incorporating several nanoparticles like graphene, nano-beads, etc.

1.9. *Advanced diagnostic techniques*

Next-generation sequencing (NGS) platform can be very helpful in acquiring information regarding molecular epidemiology and drug resistance and susceptibility of TB strains, therefore, effectively changing the global health through better management strategies. Although we have failed to cash the potential of NGS in diagnostics so far but the rapid evolutions and advancements are grooming NGS into a resourceful technology that will provide rapid and precise results for early treatment of the disease [63]. Till date, no NGS based high end facility exists in high burden countries where the need for the same is felt strongly, therefore, development of such technologies will prove a milestone in the TB diagnostics by optimizing patient's treatment, improving treatment outcomes, and reducing the spread of multi drug resistant TB strains.[63] It will also help high burden countries to move towards new strategies where surveillance of drug resistance can be carried out without involving expensive culture facilities and specialized laboratories.[63] Moreover, the introduction of NGS platform will assist low income countries in surveillance and genotypic testing of patients infected with HIV and other infections, a facility that is currently limited to the high income countries.[63]

The mass spectrometry techniques are emerging as mainstream diagnostic methods for identification of various bacterial and fungal strains from the clinical samples viz., application of Matrix-assisted laser desorption/ionization – Time of Flight (MALDI-TOF) for identification of mycobacteria. This technique works by identifying most abundant proteins of the organism in question.[64] Currently, MALDI Biotyper (Bruker Corporation) and the Vitek MS (BioMérieux) are the two MALDI-TOF platforms that have been approved by FDA for the detection and identification of a handful of bacteria. Since, no MALDI-TOF system has yet been approved by FDA for identification of *M. tuberculosis,* therefore, validation and verification of the mycobacteria through this technique requires thorough in depth study. There are several reports in the literature where MALDI-TOF has been employed for accurate identification of mycobacteria.[65–69] This technique offers several advantages over the other techniques like comparatively lower cost of reagents and rapid turn-around-time which may reduce overall patient management cost.[70]

2. Concluding remarks

M. tuberculosis complex is one of the major health challenges across the globe. According to WHO's End TB strategy, the efficient management of tuberculosis is possible only by developing newer technologies and improving the existing tools and techniques available for TB diagnostics. The currently used methodologies for diagnosing TB are slower, expensive and labour intensive. Therefore, novel techniques with increased speed, accuracy and reliability are the need of the hour. In this direction, Next generation sequencers and MALDI-TOF based techniques are being looked as highly sensitive techniques that showcase the potential to revolutionize the field of TB diagnostics. VOC test is another futuristic approach that could be used for diagnosis of children and critically ill patients in a non-invasive and cost efficient manner.

Conflicts of interest

The author has none to declare.

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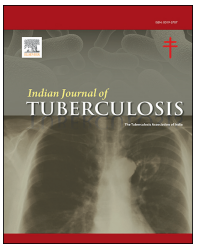

Review article

MicroRNA research: The new dawn of Tuberculosis

Priyanka Mehta [1]

Immunobiology Laboratory, Department of Zoology, University of Delhi, Delhi, 110 007, India

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ABSTRACT

Tuberculosis (TB) is global, one of the most fatal communicable diseases and leading cause of worldwide mortality. One-third of the global population is latently affected by *Mtb* (*Mycobacterium tuberculosis*) due to its ability to circumvent the host's immune response for its own survival. MicroRNAs (miRNAs) are small, non-coding RNAs which function at the post-transcriptional level and are critical in fine-tuning immune responses regulating the repertoire of genes expressed in immune cells. Recent studies have established their crucial role against TB. Furthermore, the differential expression pattern of miRNAs has revealed the potential role of miRNAs as biomarkers which could be utilized to differentiate between healthy controls and active TB patients or between active and latent TB. The recent advancements made in the field of miRNA regulation of the host responses against TB, as well as the potential of miRNAs as biomarkers for TB diagnosis are discussed here in this review.

1. Introduction

Mtb, causative agent of tuberculosis is responsible for worldwide morbidity and mortality. One-third of the global population is latently affected by *Mtb*. Among contagious diseases, it is the second most important, causing 10 million infections with nearly 2 million deaths reportedly in 2018[1,2] gaining notoriety as one of the deadliest pathogen. Despite all the efforts made to stop the spread of TB and availability of effective treatment, the death rate number remains almost unchanged, making it a serious threat to public health in both developing and developed countries.[3] Moreover, the incidence of multidrug-resistant tuberculosis keeps increasing.[4] Macrophages are the major targets for this bacteria.[5] The ability of the intracellular pathogen to survive inside macrophages and form granuloma is majorly responsible for its growth and pathogenesis. Mycobacterial infection is best studied example

Abbreviations: MHC, Major histocompatibility complex; BMDM, Bone marrow derived macrophages; PBMC, Peripheral blood mononuclear cells; IFN-γ, Interferon gamma; TNF-α, Tumor necrosis factor alpha; PFMC, Pleural fluid mononuclear cells; DOTS, Directly observed treatment short-course; MDR, Multi drug resistant; LTBI, Latent tuberculosis infection; NGS, Next generation sequencing; PCR, Polymerase chain reaction; cGAS, Cyclic GMP synthase; ATG, Autophagy related; LAMP1, Lysosomal-associated membrane protein 1; FOXO3a, Forkhead box O3; TFEB, Transcription factor EB; UVRAG, UV Radiation Resistance Associated; DRAM2, Damage regulated autophagy modulator 2; BECN1, Beclin-1; TLR2, Toll like receptor 2; MyD88, Myeloid differentiation factor 88; N-Wasp, Neural Wiskott-Aldrich syndrome protein; PKCδ, Protein kinase C delta; STAT3, Signal transducer and activator of transcription 3; KLF4, Kruppel-like factor 4; Rheb, Ras homologue enriched in brain; AMPK, AMP-activated protein kinase; FSTL1, Follistatin like protein 1; MMP9, Matrix metalloproteinase; TIMP3, Tissue inhibitor of metalloproteinase 3; NLRP3, Nod like receptor protein 3; IL, Interleukin; SNP, Single nucleotide polymorphism.

E-mail address: priyanka.mehta1818@gmail.com.
[1] Tel.: +919971408912.

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of host-pathogen interactions. It is now well established that *Mtb* regulates and modulates cellular processes such as cytokine production, autophagy, apoptosis, MHC class II expression and phagolysosome maturation in macrophages and dendritic cells for its own survival and replication.[6] Also, recent studies have also reported that most of these cellular mediated immune responses are controlled by miRNAs in eukaryotic cells[7,8] and thus it can be said that modulation of miRNAs expression associated with these biological processes is one of the important strategies implemented by *Mtb* to survive inside host immune cells.[9]

miRNAs are class of non coding RNAs of 18–25 nucleotide long that regulate expression of many genes at post-transcriptional level by inhibiting translation or inducing mRNA degradation[10,11] and are known to regulate both adaptive and innate immune responses.[12]

There are more than two thousands potentially functional miRNAs encoded by human genome[13,14] and it is estimated that nearly one-half of all protein-coding transcripts are subjected to miRNA regulation.[15] Each miRNA may suppress multiple genes and one mRNA can be targeted by multiple miRNAs. Moreover, dysregulated miRNA functions have been associated with various diseased conditions. Therefore, disease-associated miRNAs represent a new class of diagnostic markers or therapeutic targets.[16] Upon mycobacterial infection, miRNAs regulate both immune response and inflammatory response pathways[17,18] Furthermore, many research groups have reported that miRNAs were expressed in differential manner when challenged with *Mtb*, suggesting the salient role of these miRNAs in regulating the immune response in TB and presenting the miRNAs as potential biomarkers.[19,20] Thus, due to recent advances made, it has opened a new avenue to exploit miRNAs as biomarkers and to be utilised in host directed therapy. The current review contains the summary of the recent advances made in the understanding of differential expression of miRNAs and their role in TB, and their potential to be used as biomarkers and therapeutic targets for diagnosis and treatment of this deadly disease.

2. miRNA expression profile during *Mtb* infection

Differential expression of miRNAs has a strong association with disease progression in various diseases.[21] Therefore, attempts have been made to determine the effects of *Mtb* infection on the expression of miRNAs in the host.[22] Individuals with *Mtb* infection are usually classified as having Latent TB or active TB. However, there exists a variety of stages from initial exposure to the development of active disease. One of the effective mechanisms employed by macrophages to battle intracellular pathogens like *Mtb* is gene silencing via host miRNAs.[23] However, bacteria can also manipulate host cell pathways by regulating miRNA expression.[24] Therefore, understanding that how miRNAs modulate gene expression upon *Mtb* infection and identifying their target genes is of critical importance. But there are ample of constraints in that direction. In a recent study, BMDMs from mouse were infected with *Mtb* showed up-regulation of miR-155[25] while infection of PBMCs derived macrophages with the same pathogen led to downregulation of miR-155 expression[26] suggesting, that different cell types may respond differently upon infection with *Mtb*. Primary human macrophages infected with avirulent *Mycobacteriumbovis* BCG and *Mtb* H37Rv exhibited differentially expressed miRNAs, including miR- 146a, miR-155, miR-145, miR-222*, miR-27a, and miR-27b.[27] miR-222*, miR-27a, and miR-27b which have been reported to control inflammatory response and lipid metabolism were significantly downregulated.[28] miR-145, which has been reported to induce apoptosis,[29] was also downregulated, which is in concordance with a reduced ability of virulent *Mtb* strain to induce apoptosis.[16] Downregulation of miR-145 also results in overexpression of its targets and inhibition of apoptosis.[30] Macrophages infected with the Beijing/W strain (more virulent) exhibited a repression of 13 miRNAs with respect to macrophages infected with the non-Beijing/W[31] suggesting that virulent characteristics has a role in altering the host immune response. Global miRNA expression profile using microarrays revealed that nine miRNA genes (miR-30a, miR-30e, miR-155, miR-1275, miR-3665, miR-3178, miR-4484, miR-4668-5p, and miR-4497) were differentially expressed in THP-1 cells infected with *Mtb* H37Rv or *Mtb*H37Ra strains.[32]

Two research groups through miRNA profiling have identified a series of miRNA differentially expressed in PBMCs of Chinese patients with pulmonary TB.[17] Studies have also showed that expression of several miRNAs was significantly altered in patients with active TB, with miR-144* being mainly expressed in T cells wherein, miR-144* is responsible for the inhibiting the secretion of two important cytokines, IFN-γ and TNF-α, and also reduces T cell proliferation.[17] Genome-wide miRNA transcriptional responses revealed that the miR-132/212 family is induced in response to *Mtb* infections, in human dendritic cells depending on *Mtb* virulence.[33] In recent years, substantial number of studies have reported the differential expression of miRNAs in different cell types in response to mycobacterial infection.[16]

Hence, these studies suggest that miRNAs might be useful as biomarkers to distinguish between the different stages of TB infection or to study therapy responsiveness. However, none of these miRNAs have been validated in prospective studies and patients with other infections should be included.

3. miRNAs as biomarkers for TB

Considering the fact that the miRNAs display differential expression during the different stages of TB and w.r.t to healthy control and infected patients, they can serve as potential biomarkers and can be used as targets for the treatment.

Global miRNA profiling of PBMCs revealed upregulated expression (miR-424, miR-155 and miR-155*) and downregulated expression (miR-146a) of several miRNAs in TB patients.[17,34,35] In pediatric TB patients miRNA profiling of PBMCs have revealed that miR-1, miR-155, miR-31, miR-146a, miR-10a, miR-125 b and miR-150 are downregulated and miR-29 was upregulated in children with TB.[36] Among them, miR-

31 in PBMCs has been identified as a valuable diagnostic marker in Chinese pediatric TB patients.[37] Notably, miRNA profiling of PBMCs from patients with latent or active TB and healthy controls revealed 7 differently expressed miRNAs (miR-130 b*, miR-21*, miR-223, miR-302a, miR-424, miR-451, miR-486–5p) between active TB and latent TB.[19,38,39] Also, some studies have demonstrated that levels of miR-424 and miR-365 were significantly enhanced in patients with active TB compared to healthy controls.[19] Plasma miRNAs associated with microvesicles or exosomes have been suggested to be effective biomarkers for human diseases.[39]

Serum miRNA profiling studies in healthy controls and TB patients and have identified several candidate miRNA biomarkers in human TB. miR-29a, miR-361–5p, miR-889, miR-576–3p, miR-182 and miR-197 are shown to distinguish TB and healthy controls with sensitivity and specificity.[5,40–43] Also, miR-769–5p, miR-320a and miR-22–3p are suggested to have diagnostic value in TB.[44] In order to address the difficulties in accurately normalizing circulating plasma miRNA levels in different cohorts of TB patients miR-93 has been identified as a suitable reference for normalizing plasma miRNA levels.[45] Total whole-blood miRNA signature in TB patients revealed 3 miRNAs (miR-150 downregulated, miR-21 and miR-29c upregulated) with relatively good sensitivity and specificity in TB diagnosis.[46]

Notably, mononuclear cells obtained from PFMCs of TB patients revealed miRNA expression pattern differed from healthy controls with downregulated expression of miR-223, miR-144* and miR-421 and miR-146a.[35] The level of miR-625–3p in urine was significantly increased in smear-positive than smear-negative patients, indicating its diagnostic value.[47] miRNA profiling in sputum supernatant of patients with active TB revealed overexpression of miR-3179, miR-147 and underexpression of miR-19b-2* in TB group[5,39](Table 1).

4. miRNA response to TB treatment

Treatment for TB disease usually takes a minimum of 6 months on multiple antibiotics. Therefore, monitoring a patient's response to treatment is of prime importance to identify drug resistant *Mtb*. miR-320a is reported to be decreased in drug-resistant TB patients as compared to pan-susceptible TB patients.[44] Levels of microRNA miR-16 and miR-155 were also found to be altered in serum of TB patients and associate with responses to treatment.[49] 37 upregulated (miR-125a-5p was confirmed) and 63 downregulated (miR-21–5p, miR-92a-3p, miR-148 b-3p were confirmed) serum miRNAs were identified in cured TB patients compared to untreated TB patients. DOTS led to up-regulation of miR-155 and down-regulation of miR-326 correlating with diminished Th1 response and increased Th17 response consecutively.[50] miR-155 was found to be decreased in patients with MDR TB, as compared to healthy controls but increased as compared to untreated TB patients. miR-16 levels were lowest in serum of MDR TB patients compared to untreated and treated TB group and healthy controls.[49]

5. Pitfalls of using miRNAs as biomarkers

Despite of several research advancements in past few years, tuberculosis remains an inexplicable infectious disease which is a major threat to mankind. Recently made progress in this direction has led to the identification of involvement of miRNAs in mycobacterial infection that has nurtured our hopes of better understanding of the disease and pathogenesis and also aided in the path of development of sensitive and accurate diagnostic and prognostic biomarkers and possible new therapeutics for tuberculosis.

A very common problem encountered across miRNAs TB biomarkers while studying human samples is that the different stages of TB infection are not always well defined, there is no clear differentiation among recent infection, long-standing LTBI, newly diagnosed active TB, or treated TB groups, which hampers the group comparisons. Also, very often there is no differential analysis between males and females, even though this factor has been reported to define a different profile expression for several miRNAs.[51]

Microarray and NGS are two chiefly studied techniques for miRNA expression profiling, and for the identification of biomarkers.[48,52] However, both these techniques may have their own disadvantages. NGS can introduce bias during PCR amplification and thus can sometimes produce deceiving results, which leads to the misinterpretation of results. Also, during the ligation steps, the use of different adapters and barcodes influences cDNA synthesis efficacy, and the variable specific RNA G/C- content can be associated with unequal PCR amplification efficiency.[53]

On the other hand, microRNA arrays, allows only the study of a large number of miRNAs already identified and the data comparison is impeded due to poorly inter-platform concordance of microRNA expression values.[54]

Real-time validation of miRNA results obtained from NGS and microarray allows miRNA profiling and validation with high sensitivity.[55] However, qPCR data normalization is one of the most taxing steps due to lack of authentic normalizer.[56,57] Currently, small RNAs, like RNU6B, are widely used as normalizer for miRNAs; however it has been shown that it can experience changes in serum samples in a disease-specific manner.[58] Also, these small RNAs do not share the similiar biological and biochemical properties of miRNA molecules in terms of their transcription, processing and tissue-specific expression patterns.[58,59] Therefore, the most accurate reference would be other miRNAs exhibiting stable expression under the same experimental conditions.[60] Alternatively, another strategy could be to use a global mean normalization of a set of reference genes[61,62] by taking the geometric mean of a minimum of three stable housekeeping genes provided a more reliable normalization.[63]

6. miRNAs as regulators of host immune response

It is now well established that miRNAs regulate the various immunological responses exclusively by its own expression

Table 1 – miRNAs as potential biomarkers in Tuberculosis.

Type of cell/tissue	Candidate biomarkers	Reference
PBMCs	7 Differentially expressed (miR-130 b*, miR-21*, miR-223, miR-302a, miR-424, miR-451, miR-486–5p) between active and latent TB	19
PBMCs	7 Differentially expressed miRNAs (miR-144, miR-133a, miR-365, miR-424, miR-500, miR-661,miR-892 b) between active TBand healthy control	19
PBMCs	14 upregulated miRNAs (miR-155 and miR-155*)	34
PBMCs	Downregulated in PFMCs (miR-223, miR-144* and miR-421), downregulated in both PBMCs and PFMCs (miR-146a), upregulated in PBMCs (miR-424)	35
PBMCs	In pediatric patients, 15 upregulated (miR-1, miR-155, miR-31, miR-146a, miR-10a, miR-125 b and miR-150)and 14 downregulated (miR-29)	36
Serum	Upregulated (miR-182 and miR-197)	41
Serum, TB patients before/after therapy	Downregulated (miR-21–5p, miR-92a-3p, miR-148 b-3p, miR-125a-5p) in cured TB patients compared to untreated TB patients	48
Total blood	9 Differentially expressed, downregulated (miR-150), upregulated (miR-21 and miR-29c)	46
Plasma	52 upregulated (miR-3149, miR-147) and 43 downregulated (miR-19b-2*)	5

and transcriptional regulation of target genes. Thus, understanding the role of miRNAs in host-pathogen interactions and offers a new avenue to improve our diagnostic tools and treatments regimes against TB. Among various protective mechanisms utilised by miRNAs of the infected host to limit the outgrowth of *Mtb*, autophagy and apoptosis play a vital roles in the pathogenesis and also in the host defense against *Mtb*.[64]

7. miRNAs: fine players in regulating autophagy

Autophagy helps in eliminating pathogens like mycobacteria, which exploit the cytosolic niche for survival or interfere with phago-lysosome biogenesis.[65] It also facilitates the trafficking of mycobacteria to the lysosome for degradation[65] thus contributing in host immunity by negating the ability of mycobacterium to manipulate phagosome maturation.

Although the autophagic pathways are tightly regulated and well described but the role of miRNAs in regulating autophagy is not well known. However, in recent years many research groups have successfully unveiled the role of several miRNAs in autophagy regulation during *Mtb* infection. The components of autophagy machinery components are important targets of miRNAs during Mtb infection.

During *Mtb* infection, mycobacterial DNA is detected by the cytosolic DNA sensor cGAS. Upon recognition, ATG proteins orchestrate sequential membrane remodeling and trafficking events.[66] Recently, a collaborative role of miRNAs in lipid metabolism and autophagy has been uncovered during *Mtb* infection. *Mtb* induces miR-33 and miR-33* expression, which suppresses autophagy flux through downregulating autophagy effectors (ATG5, ATG12, LC3B and LAMP1) and transcription factors (FOXO3 and TFEB). By repressing autophagy, *Mtb* impairs lipid catabolism and promotes cellular lipid stores for nutrient source through miR-33 and miR-33*, thus favoring its survival. As a result of that, mice with macrophage miR-33 deficiency displayed enhanced *Mtb* clearance during *Mtb* infection *in vivo*.[67] Earlier studies have also shown that *Mtb* infection increases the expression of miR-125a-3p in macrophages, which targets UVRAG to inhibit autophagy activation.[39,68]

Notably, *Mtb* induces the expression of MIR144*/miR-144–5p, which directly targets the 3′-UTR of DRAM2 in human monocytes and macrophages.[69] DRAM2 is well known for its interaction with essential components of the autophagic machinery including BECN1 and UVRAG, and is required for autophagy activation, phagosomal maturation and *Mtb* clearance. As a result, MIR144* induced DRAM2 inhibition is associated with inhibition of autophagy induction.[39]

Mtb infection induces miR-155 which thereby suppresses autolysosome fusion and favours its survival. miR-155 has been shown to target 3′UTR of ATG3, an E2-ubiquitin-like-conjugatingenzyme with an essential role in LC3 lipidation and autophagosome formation.[70] Another study reported that BCG infection increased the levels of miRNA-20a, which inhibits autophagy by targeting ATG7 and ATG16L1 in macrophages.[71] In another study miR-30a has been reported to play a negative role in regulating autophagy in *Mtb* infection and the levels of miR-30a and BECN1 were negatively correlated in *Mtb* patients.[72] In another study, overexpression of miR-23a-5p dramatically prevented *Mtb*-induced activation of autophagy in macrophages by modulating TLR2/MyD88/NF-kB signaling.[73] Similarly, miRNA-20a targets ATG7 and ATG16L1 and is able to inhibit autophagy.[16,71]

miRNAs downregulated by *Mtb* infection are also involved in the modulation of autophagy. For example, *Mtb* infection leads to downregulation of miR-17–5p, which is indicated to be a positive regulator of autophagy through downregulating PKCδ and STAT3 in *Mtb*-infected macrophages.[74] miR-26 targets KLF4, a transcription factor that is responsible for M2 polarization, preventing autophagy and trafficking of *Mtb* to lysosomes.[75] During *Mtb* infection, miR-26a was found to be

down-regulated both *ex vivo* and *in vivo*, leading to upregulation of KLF4, resulting decreased trafficking of *Mtb* to lysosomes. Induction of miR-142-3p upon *Mtb* infection leads to reduced phagocytosis by targeting N-Wasp.[76]

8. miRNAs: fine players in regulating apoptosis

Reportedly, miR-20a-5p functions as a negative regulator of mycobacterial-triggered apoptosis and as a result of that inhibition of miR-20a-5p results in more efficient *Mtb* clearance.[77] miR-155 is also well known to regulate apoptosis by targeting FOXO3a.[78] *Mtb* is also known to secrete a protein called MPT64 which could inhibit apoptosis of RAW264.7 cells through the NF-kB-miRNA21-Bcl-2 pathway.[79] miR-145 modulates apoptosis by targeting TRAF6.[30] Notably, upregulation of miR-29a and let-7e led to sustained inhibition of apoptosis by targeting caspase-7 and caspase-3 respectively.[80] Other studies have also reported the role of miRNAs in apoptosis in *Mtb* infection.[30,80]

Collectively, these findings highlight the importance of miRNAs in regulation of autophagy and apoptosis in TB (Table 2). Suggesting its application as therapeutics to restrain the multiplication and enhance the clearance of *Mtb* from the host cells.

9. miRNAs as therapeutic targets

According to recent findings, it has been entrenched that many miRNAs can be effectively targeted to treat TB. For instance, miR-155 is negatively associated with the TB-suppressing activity of NK cells[84] and also associated with autophagy mediated mycobacterial elimination by targeting Rheb.[81] Studies revealed that miR-23a-5p promotes *Mtb* survival and inhibits autophagy through TLR2/MyD88/NFkB pathway.[73] Silencing of miR- 33 and miR-33* by genetic or pharmacological means enhances autophagy flux by suppressing autophagy effectors and through AMPK-dependent activation of the transcription factors FOXO3 and TFEB.[67] Another study revealed that the induction of miR-32-5p strongly increases the survival rate of *Mtb* by directly targeting FSTL1 through the TLR- 4/miRNA-32-5p/FSTL1 pathway.[85] miR-206 has been suggested to function as an inflammatory regulator leading to the expression of MMP9 by targeting TIMP3 in *Mtb* infection.[86] Similarly, studies demonstrated that miR-20b can alleviate the inflammatory response in TB mice by targeting the NLRP3/caspase-1/IL-1b pathway.[87]

Since one miRNA is known to have multiple targets, therefore, exogenousmiRNA administration might exhibit off-target effects. In reality, the exploitation of miRNAs in therapy is still in its infancy stage but it has opened an exciting avenue for the control and treatment of TB.[16]

10. Limitations of miRNAs as therapeutic agents

One of the limitation of utilising miRNAs as therapeutic agents is that most of the miRNAs are not entirely gene-specific. It is well studied that one miRNA has many targets and it contributes in various biological processes therefore, it could have varying effects in different tissues, host and disease. Thus, exogenous administration might exhibit off target effects[63] or could lead to nuclease mediated degradation before achieving target modulation. To conquer this hindrance, several chemical modifications have been tried such as replacing the phosphodiester group with phosphorothioate and the introduction of a fluoro, an O-methyl group, or a 2-methoxyethyl group.[88,89] However, these chemical modifications may also exhibit off-target effects, such as reduced miRNA activity, and production of toxic metabolites. Thus, all these issues call for a suitable delivery system, which will protect the naked miRNAs from nucleases and conditions *in vivo* and which will provide robust binding.[90]

Therefore, all the above challenges should be kept in mind while taking the initiative for the development of miRNAs targeted/based biomarkers or therapeutics for tuberculosis or other diseases.

Table 2 – miRNA regulation of host immune response in Tuberculosis.

miRNA	Target	Cell type/tissue	Function	Reference
miR-145	TRAF6	Stem cells	Apoptosis	30
let-7e	Caspase-3	Monocyte derived Macrophages	Apoptosis	80
miR-29a	Caspase-7	Monocyte derived Macrophages	Apoptosis	80
miR-155	FOXO3a	Monocytes	Apoptosis	78
miR-20a-5p	JNK-2	Macrophages	Apoptosis	77
miR-21	Bcl-2	RAW 264.7 Macrophages	Apoptosis	79
miR-155	Rheb	RAW 264.7 Macrophages	Autophagy	81
miR-142-3p	N-Wasp	J774A.1 and primary human macrophages	Autophagy	76
miR-33	ATG5, LAMP1	THP-1 and HEK293 cells	Autophagy	67
miR-125a-3p	UVRAG	AW264.7 and J774A.1 macrophages	Autophagy	68
miR-17-5p	ULK-1	RAW 264.7 Macrophages	Autophagy	82
miR-144-3p	ATG4a	RAW 264.7 Macrophages	Autophagy	83
miR-20a	ATG7 and ATG16L1	RAW 264.7 Macrophages	Autophagy	71
miR-23a-5p	TLR2/MyD88/NF-kB	RAW 264.7 Macrophages	Autophagy	73
miR-26a	KLF-4	RAW 264.7 Macrophages	Autophagy	75
miR-17-5p	Mcl-1/STAT3	RAW 264.7 Macrophages	Autophagy	74

11. miRNA polymorphism and TB susceptibility

SNPs are the single nucleotide variations in the genome sequence. Human genetic studies have also shown that it affects the susceptibility towards spectrum of diseases by altering the innate immune response towards a pathogenic challenge and its disease outcome.[50] Due to the regulatory role of miRNAs in immune responses against *Mtb* infection, SNPs within miRNAs may alter their target selection and expression, resulting in functional changes. 2 SNPs, rs2910164 (miR-146a G > C) and rs3746444 (miR-499 T > C), involved in TLR signaling pathway was analyzed in 337 TB cases and 738 healthy controls, including 318 Tibetan and 757 Han individuals. Reportedly, SNP (miR-146a G > C) has been pointed to have an association with PTB risk in both Tibetan and Han populations. An association was observed between miR-499 T > C and TB in the Tibetan population, and individuals carrying the C allele exhibited increased PTB risk.[91] However, neither of these SNPs were associated with TB risk in Iranian population as shown by other studies.[92,93] The miR-499 T > C SNP is in Chinese Uygur population, and the miR-146a C > G, miR-196a2 T > C in Chinese Kazak population, are reported to have an association with TB risk. Contrary to that, two recent meta-analysis studies indicate that the miR-146a G > C and the miR-499 T > C SNPs are not associated with TB.[94,95] Additionally, rs3742330 A > G within miR-632 was associated with a 27% decreased risk for TB (95% CI 0.55–0.97, P = 0.03) in the Chinese Tibetan population.[96]

12. Conclusion

Tuberculosis is a global health problem, one of the highly communicable diseases and difficult to eradicate. Due to the co-evolution of this intracellular pathogen, it has developed strategies to survive under high immune pressure. Macrophages play a central role in the host immune response against *Mtb,* which is tightly governed by multiple factors, including miRNAs. miRNAs have emerged as an important factor regulating innate and adaptive immunity in mycobacterial infection leading to better understanding of host-pathogen interaction. Differential expression of miRNAs, in the diverse stages of TB infection might shed light on the nature of the immune response to this evading pathogen. However, miRNA expression is highly dependent on the experimental model conditions, which leads to variability in its expression pattern and creates difficulties to arrive at any conclusion.

Although there is lot of information available about miRNAs controlling host immune responses in macrophages but there is scarcity of data regarding miRNA expression profile of TB related miRNAs in other immune cells. Furthermore, many studies have revealed the potential role of miRNAs as biomarkers which could be utilized to differentiate between healthy controls and active TB patients or between active and latent TB thus providing insights into pathogenesis in-depth. However, one of probable limitation in the use of miRNAs as biomarkers is attaining gene-specificity because most of the miRNAs are not completely gene-specific. But the emerging role of nanotechnology has opened new avenues for the exploitation of miRNAs for the treatment of TB. This rapid advancement and explosion in miRNA research nurtures our hope for a giant leap in better diagnosis and treatment of contagious diseases like tuberculosis in future.

Consent for publication

Not applicable.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Availability of data and materials

All the information is provided along with the manuscript.

Conflicts of interest

The author has none to declare.

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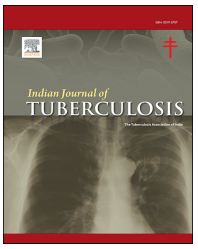

Review article

Post covid 19 pulmonary fibrosis. Is it real threat?

*Deependra Kumar Rai**, *Priya Sharma*, *Rahul Kumar*

Department of Pulmonary Medicine, AIIMS Patna, 801505, India

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ABSTRACT

After the COVID-19 outbreak, increasing number of patients worldwide who have survived COVID-19 continue to battle the symptoms of the illness, long after they have been clinically tested negative for the disease. As we battle through this pandemic, the challenging part is to manage COVID-19 sequelae which may vary from fatigue and body aches to lung fibrosis. This review addresses underlying mechanism, risk factors, course of disease and treatment option for post covid pulmonary fibrosis. Elderly patient who require ICU care and mechanical ventilation are at the highest risk to develop lung fibrosis. Currently, no fully proven options are available for the treatment of post inflammatory COVID 19 pulmonary fibrosis.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The global pandemic began in Wuhan, China, in December 2019, and has since then spread worldwide.[1] As of September 30, 2020, the cases of COVID-19 infection continues to soar worldwide with no peak in sight making total case tally standing at 63,12,585 including 9,40,705 active cases, 52,73,202 cured/discharged/migrated and 98,678 deaths, according to the Ministry of Health and Family Welfare. While whole medical fraternity and researchers across the world continue to learn more about the novel contagion and its bizarre array of symptoms, it is becoming clear that the battle with COVID-19 is not an easy one.

After the COVID-19 outbreak, increasing number of patients worldwide who have survived COVID-19 continue to battle the symptoms of the illness, long after they have been clinically tested negative for the disease. They are called as long – haulers. As we battle through this pandemic, the challenging part is how to manage this COVID-19 Sequelae which may vary from mild in terms of fatigue and body aches to severe forms requiring long term oxygen therapy and lung transplantation due to lung fibrosis, significant cardiac abnormalities and stroke leading to significant impairment in Quality of health. Various studies have reported that around 70–80% of patients who recovered from COVID-19 presents with persistence of at least 1 or more symptoms, even after being declared COVID-free.[2,3]

Considering millions of covid 19 cases worldwide, even small proportion of post covid lung fibrosis is worrisome. Many active clinical trials and studies are underway to know more about the entity post covid pulmonary fibrosis. This narrative review summarizes current clinical evidence regarding post COVID-19 pulmonary fibrosis.

2. Materials & Methods

This review was performed to address following questions for post covid pulmonary fibrosis.

* *Corresponding author*. Tel.: +917764981421.
E-mail address: deependra78@gmail.com (D.K. Rai).
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1. Mechanism
2. Risk factors
3. Clinical course
4. Treatment option

A literature review was performed using different database (PubMed, Scopus, Science Direct, and Google Scholar) to identify relevant English-language articles published through September 25,2020. Search terms included coronavirus, severe acute respiratory syndrome coronavirus 2, COVID-19, Post covid fibrosis, antifibrotic. The search resulted in 2,567 total articles. Due to the lack of RCTs, we have also included case reports, case series, and review articles. The authors independently reviewed the titles and abstracts for inclusion. Additional relevant articles were identified from the review of citations referenced. Active clinical trials were identified using the disease search term coronavirus infection on ClinicalTrials.gov.

2.1. *Mechanism of post COVID pulmonary fibrosis*

Various mechanisms of lung injury in COVID-19 have been described, with both viral and immune-mediated mechanisms being implicated.[4] Pulmonary fibrosis can be either subsequent to chronic inflammation or an idiopathic, genetically influenced and age related fibroproliferative process. Pulmonary fibrosis is a known sequela to ARDS. However, persistent radiological abnormalities after ARDS are of little clinical significance and have dwindled with protective lung ventilation.[5]

It has been found that 40% of patients with COVID-19 develop ARDS, and 20% of ARDS cases are severe.[6] The prevalence of post-COVID-19 fibrosis will become apparent with time, but early analysis from patients with COVID-19 on hospital discharge suggests that more than a third of recovered patients develop fibrotic abnormalities. The pathological feature of ARDS is diffuse alveolar damage (DAD) which is characterized by an initial acute inflammatory exudative phase with hyaline membranes, followed by an organizing phase and fibrotic phase.[7] Previous studies highlight that duration of disease is an important determinant for lung fibrosis post ARDS. This study showed that, 4% of patients with a disease duration of less than 1 week, 24% of patients with a disease duration of between weeks 1 and 3, and 61% of patients with a disease duration of greater than 3 weeks, developed fibrosis.

Cytokine storm caused by an abnormal immune mechanism may lead to initiation and promotion of pulmonary fibrosis. Epithelial and endothelial injury occurs in the inflammatory phase of ARDS due to dysregulated release of matrix metalloproteinases. VEGF and cytokines such as IL-6 and TNFα are also involved in the process of fibrosis. The reason remains unknown as to why certain individuals recover from such an insult, whereas others develop progressive pulmonary fibrosis due to accumulation of fibroblasts and myofibroblasts and excessive deposition of collagen.[8]

Although ARDS seems to be the main predictor of pulmonary fibrosis in COVID-19, several studies showed that covid induced ARDS is different (High and low elastance type) from the classical ARDS. CT findings in many covid cases are also not suggestive of classical ARDS. Along with, abnormal coagulopathy is another pathological feature of this disease. So, mechanism of pulmonary fibrosis in COVID-19 is different from that of IPF and other fibrotic lung diseases, especially with pathological findings pointing to alveolar epithelial cells being the site of injury, and not the endothelial cells.

2.2. *Risk factor*

One of the risk factors for the development of lung fibrosis in COVID-19 is **advanced age** and this finding is same as in MERS and SARS-CoV.[9–11]

Second risk factor is **increased disease severity** which includes comorbidities such as hypertension, diabetes, and coronary artery disease[12] and Lab findings like lymphopenia, leukocytosis, and elevated lactate dehydrogenase (LDH).[7] Serum LDH level has been used as a marker of disease severity following acute lung injury. It is an indicator of pulmonary tissue destruction and correlates with the risk of mortality. According to the World Health Organization, 80% of SARS-CoV-2 infections are mild, 14% develop severe symptoms, and 6% will become critically ill.

Third risk factor is **prolonged ICU stay and duration of mechanical ventilation**. While disease severity is closely related to the length of ICU stay, mechanical ventilation poses an additional risk of ventilator-induced lung injury (VILI). Abnormalities of pressure or volume settings underlie this injury leading to a release of proinflammatory modulators, worsening acute lung injury, and increased mortality or pulmonary fibrosis in survivors.[13]

Smokers are 1.4 times more likely to have severe symptoms of COVID-19 and 2.4 times more likely to need ICU admission and mechanical ventilation or die compared to nonsmokers.[14,15]

The World Health Organization (WHO) and the National Institute on **Alcohol Abuse** and Alcoholism (NIAAA) have issued communications warning people to avoid excessive drinking, saying it may increase COVID-19 susceptibility and severity. Alcohol use disorder increases the risk for complications of COVID-19.[16]

2.3. *Clinical course*

What proportion of covid 19 patients developed lung fibrosis remains speculative and should not be assumed without appropriate prospective study. But we can extract data from SARS and MERS pandemic. Zhanga et al[17] followed 71 SARS patients for 15 years and found 9.4% at beginning of study, 4.6% at one year and 3.2% patients after 15 years had pulmonary lesions visible on CT scans. Similar findings were reported for MERS also. The follow-up of 36 MERS patients for an average of 43 days showed that lung fibrosis developed in a significant number of convalescents, and risk was found highest among patients who were elderly, hospitalised with severe disease in ICU.[18] We have paucity of data for course of post covid pulmonary fibrosis. In one of the study[19] chest CT scan was performed on the last day before discharge, two weeks and four weeks after discharge. Compared with the last CT scan before discharge, the abnormalities (including focal/multiple GGO, consolidation, interlobular septal thickening,

subpleural lines and irregular lines) in lungs were gradually absorbed in the first and second follow-ups after discharge. The lung lesions of 64.7% discharged patients were fully absorbed after 4-week follow-up. It indicated that the damage to lung tissue by COVID-19 could be reversible for the common COVID-19 patients. It also suggested that the prognosis of non-severe patients is favourable, and the clinical intervention should be conducted in time to prevent common COVID-19 patients from worsening to severe patients.

Another study[2] conducted at Italy (between April 2020 to May 2020) assessed persistent symptoms in 143 patients who were discharged from the hospital after recovery from COVID-19. Patients were assessed at a mean of 60.3 days after the initial onset of COVID-19 symptom; at the time of evaluation, only 18 (12.6%) were completely free of any COVID-19–related symptom, while 32% had 1 or 2 symptoms and 55% had 3 or more. None of the patients had fever or any signs or symptoms of acute illness. Worsened quality of life was observed among 44.1% of patients. They also found that most common symptom persistent beyond discharge was fatigue (53.1%), dyspnea (43.4%), joint pain, (27.3%) and chest pain (21.7%).

Another follow up study[20] which studied the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery enrolled 55 patients and found different degrees of radiological abnormalities in 39 patients. Blood Urea nitrogen concentration at admission was associated with the presence of CT abnormalities.

Many studies have shown that most common abnormality of lung function in discharged survivors with COVID-19 is impairment of diffusion capacity, followed by restrictive ventilatory defects, both associated with the severity of the disease[21,22] Both decreased alveolar volume and K_{CO} contribute to the pathogenesis of impaired diffusion capacity.[23] At 3-months after discharge, residual abnormalities of pulmonary function were observed in 25.45% of the cohort which was lower than the abnormal pulmonary function in COVID-19 patients when discharged.[10] Lung function abnormalities were detected in 14 out of 55 patients and the measurement of D-dimer levels at admission may be useful in prediction of impaired diffusion defect.[16]

2.4. *Treatment of post COVID 19 pulmonary fibrosis*

Currently, no fully proven options are available for the treatment of post inflammatory COVID 19 pulmonary fibrosis. Various treatment strategies are under evaluation. It has been proposed that prolonged use of anti-viral, anti-inflammatory and anti-fibrotic drugs diminish the probability of development of lung fibrosis. However, it is yet to be ascertained whether early and prolonged use of antiviral agents may prevent remodeling of lung or which of the available antiviral is more effective. Though risk-benefit ratio should be assessed prior to use, prolonged low dose corticosteroid may prevent remodeling of lung in survivors.[24] Anti-fibrotic drugs, such as pirfenidone and nintedanib, have anti-inflammatory effects as well and thus they may be used even in the acute phase of COVID-19 pneumonia.[25] Pirfenidone exerts anti-fibrotic, antioxidative and anti-inflammatory effects. Pirfenidone could attenuate ARDS induced lung injury as it reduces LPS-induced acute lung injury and subsequent fibrosis by suppressing NLRP3 inflammasome activation.[26] There are few concerns regarding antifibrotic in acute phase. Many covid 19 patients have hepatic dysfunction in the form of raised transaminases and both antifibrotics pirfenidone and Nintedanib cause hepatotoxicity. Nintedanib is associated with increased risk of bleeding as most of the covid 19 patients are on anticoagulant.

Evidence is present regarding use of pirfenidone, azithromycin and prednisolone in the management of pulmonary fibrosis post-H1N1 ARDS, based on data from a case report of three patients.[27] Now the literature supports the use of antifibrotic by the first week of ARDS onset to prevent consequences such as lung fibrosis. Thus, there is urgent need for the identification of biomarkers early in the disease course to identify patients who are likely to progress to pulmonary fibrosis. The rationale for using antifibrotic therapy should be personalized and the role of precision medicine assumes prediction of high-risk population, better understanding of pathophysiology and prevention of disease worsening or/and lung fibrosis development.

Rehabilitation in the acute stage and particularly in the recovery stage is beneficial. It improves respiratory function, exercise endurance, self-care in daily living activities and psychological support.[28] However, scientific research is required for concluding its definite benefits.

3. Conclusion

Considering huge numbers of individuals affected by COVID-19, even rare complications like post covid pulmonary fibrosis will have major health effects at the population level. Elderly patient who require ICU care and mechanical ventilation are the highest risk to develop lung fibrosis. Currently, no fully proven options are available for the treatment of post inflammatory COVID 19 pulmonary fibrosis.

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Conflicts of interest

The authors have none to declare.

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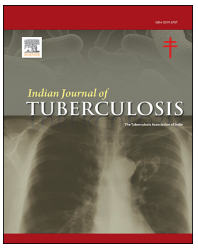

Original article

Status and challenges for tuberculosis control in India – Stakeholders' perspective

Gargi Thakur [a], *Shalvi Thakur* [b], *Harshad Thakur* [c,d,*]

[a] *DAV Public School, Airoli, Navi Mumbai, India*
[b] *Indian Institute of Science, Education and Research, Bhopal, India*
[c] *School of Health Systems Studies, Tata Institute of Social Sciences, Mumbai, India*
[d] *National Institute of Health and Family Welfare, New Delhi, India*

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ABSTRACT

Background: Tuberculosis is one of the ten major causes of mortality worldwide. The trend of increasing TB cases and drug resistance in India is very disturbing. The objectives of the study were to study the perspectives and opinions of different stakeholders on the status, challenges and the ways to tackle the issues of TB in India.

Methods: The online survey was done for the data collection from national and international experts. The data collection took place during October 2017. We received 46 responses.

Results: The experts had varied answers as to the menace of TB in India, effect of TB on individuals, family and society, failure of government plans in India, TB awareness campaign and ways to create awareness. Everyone believed that urgent action needs to be taken against the disease like improving the healthcare infrastructure of the country (improving the quality and quantity of medical facilities and doctors) and creating awareness about the TB.

Conclusion: Government of India is making lot of efforts to bring down the problems associated with TB through. In spite of this, there is a long way to go to achieve significant reduction in high incidence and prevalence of TB in India. Factors like lack of awareness and resources, poor infrastructure, increasing drug resistant cases, poor notification and overall negligence are the major challenges. If we eradicate poverty and undernourishment, educate the masses and eliminate the stigma attached with TB, we can hope for a disease free future.

* *Corresponding author*. School of Health Systems Studies, Tata Institute of Social Sciences, Mumbai, India.
E-mail address: harshad_thakur2000@yahoo.co.in (H. Thakur).

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1. Introduction

Tuberculosis (TB), one of the most ancient diseases of mankind, is one of the ten major causes of mortality worldwide.[1] It is an infectious disease caused by bacteria *Mycobacterium tuberculosis*. It usually affects lungs (Pulmonary TB) but can also affect other organs of the body. Pulmonary TB is an air borne disease. TB can be diagnosed by chest X-ray, sputum and other tests. Combinations of antibiotics are given for more than 6 months as a treatment. Vaccination with BCG (Bacille-Calmette-Guerin), early diagnosis and detection, proper and complete treatment, awareness, etc. can lower the burden of TB.

Poor socioeconomic status and living conditions are considered as strong risk factors linked with Latent Tuberculosis Infection in addition to malnourishment.[2] BCG is the vaccine commonly available against TB. It does offer some protection against serious forms of TB in childhood but its protective effect wanes with age.[1] Latent TB is also becoming a major issue in ageing population.

All countries and age groups are affected by TB but most cases (90%) in 2016 were in adults. Almost two-third was accounted for by eight developing countries with India contributing 27% of 10.4 million cases.[3,4] In 2017, only 64% of the global estimated incident cases of TB were reported, the remaining 36% of 'missing' cases was undiagnosed, untreated or unreported. These 'missing TB cases' have generated much hype for the challenges they present in achieving the End TB Strategy.[5] Many people with TB (or TB symptoms) do not have access to adequate initial diagnosis. In many countries, TB diagnosis is still reliant on sputum microscopy, a test with known limitations.[6]

Wide spread misuse of anti-tubercular drugs has also resulted in emergence of drug resistant TB including Multi Drug Resistant TB (MDR-TB) and Extensively Drug Resistant TB (XDR-TB) globally. India has the highest incidence of new and MDR-TB cases in the world. It is difficult to diagnose MDR-TB and XDR-TB as compared to regular TB.[7,8] TB treatment default, missing medical appointments for two consecutive months or more, is a serious problem not only for individuals but also for societies and health-care systems.[9] An increasing burden of MDR-TB patients, especially in the young population with increased risk of transmission posing a major challenge in achieving TB elimination targets.[10] In India, major challenges to control TB include poor primary health-care infrastructure in rural areas of many states, unregulated private health care, lack of political will and corrupt administration. WHO with its "STOP TB" strategy has given a vision to eliminate TB as a public health problem from the face of this earth by 2050.[11]

Since 2000, there has seen the emergence of new diagnostics and drugs for TB. A new and potent drugs such as Bedaquiline, Delamanid, Teixobactin have been evolved which may serve as a nice step forward, with a better outcome.[12] However, these are yet to reach community, and access remains a major challenge for patients in low and middle income countries.[13]

The National Strategic Plan (2017–25) of India proposes bold strategies with commensurate resources to rapidly decline TB in the country by 2030. This is in line with the Global End TB targets and Sustainable Development Goals to attain the vision of a TB free India. The goal is to achieve a rapid decline in burden of TB, morbidity and mortality while working towards elimination of TB in India by 2025.[14] India achieved the MDG targets of reducing the prevalence by half in 2015. In spite of this the trend of increasing TB cases and drug resistance in India is very disturbing. After collecting preliminary secondary information from the internet, journals, etc. about the disease, it was felt that there is a need to compile opinions of different stakeholders working in the healthcare field especially related to status and challenges of TB in India.

2. Methods

The objectives of the study were to study the perspectives and opinions of different stakeholders on the status, challenges and the ways to tackle the issues of TB in India. We decided to contact the clinicians, policy implementers and academic researchers as they play a pivotal role in controlling and preventing the disease. These stakeholders are also pioneers in reducing TB burden in India and their opinions will be useful in developing effective strategies.

A questionnaire for the survey was prepared and later on data was collected using available modern technology. The questionnaire was created on the online platform of www.surveymonkey.com. This online questionnaire made it easy for the survey to reach national and international experts. Social media (like email, Facebook, WhatsApp, etc) was utilized to send survey all across the world. The survey link (https://www.surveymonkey.com/r/H67S3YV) was sent to more than 1000 national as well as international experts and doctors working in diverse medical fields related to TB (academicians, researchers, clinicians, policymakers, implementers, etc). The online survey fulfilled the purpose of reaching out to many experts with variety of expertise like with the help of limited resources.

The data collection took place during October 2017. The responses were collected over the period of next 7–10 days. A semi-structured interview schedule was used comprising nine questions and mainly focusing on the effects of TB on society, the opinions of experts regarding what they felt was lacking in the country's efforts to reduce TB prevalence and the ways of creating more and better awareness about TB. The consent of each respondent was taken and the confidentiality was maintained as we did not ask them for their personal information.

The data was analyzed manually using MS Excel software. Data analysis largely followed the framework approach. The answers were entered in the worksheet. Data was coded, indexed and charted systematically to seek meaning from all of the data that was available. The data was categorized and sorted into patterns as the primary basis for organizing and reporting the study findings.

We received 46 responses. The respondents consisted of people from all age groups ranging from 24 years to 68 years and belonged to categories like Clinicians, Policy makers/

Table 1 – The category of respondents.

Categories	No. of people
Clinician	12
Policy maker/implementer	9
Academician	12
Researcher	8
Other	5
Total	**46**

implementers, Academic Researchers and others. Table 1 presents number of people belonging to various categories.

Out of the 46 respondents, two were distinguished professors and academic researchers from USA. Three were from The World Health Organization (WHO) – Chief Medical Officer (TB) from an Asian country and two Medical Consultants from WHO–RNTCP (Revised National TB Control Program) India. The response was received from experts and specialists belonging to the Municipal Corporation of Greater Mumbai hospitals, District Health Officers, National Health Mission, The United Nations Children's Fund (UNICEF) and various other national and international organizations working actively in India. Apart from this, others were from diverse fields such as Neurologist, Laryngologist, Obstetrician and Gynecologists, Pathologists, Medical Students, PhD students, etc. from various reputed institutions like King's College (London), Holy Spirit Hospital, Bombay Hospital, Jaslok Hospital, Breach Candy Hospital, SRCC NH Children's Hospital, Wadia Children Hospital and so on.

3. Results

The experts had varied answers as to the menace of TB in India, effect of TB on individuals, family and society, failure of government plans in India, TB awareness campaign and ways to create awareness. The perspectives of policy makers, implementers and clinicians differed from that of the academicians and researchers but they also converged at a lot of points.

The respondents gave various perspectives to answer what made TB a menace all over the country. They mentioned social reasons like overcrowding, urbanization leading to congested cities, social problems like smoking and alcoholism, poor living conditions, unhygienic habits and poor nutrition are the major causes. On the other hand, few respondents stated that there are concerns related to health systems and services and said that poor public health conditions, lack of awareness about the TB amongst the masses, lack of universal access to healthcare, private sector malpractices, poor implementation of government health programs and poor drug supplies are the major problems. Few cited drug resistance as the cause of TB becoming a menace.

Effects of TB could be felt at individual, family, society and country level. Immunity of the person contracted with TB reduces which makes him/her susceptible to other diseases as well and reduces life expectancy. According to the respondents, the main effect of TB on the family was loss of income of the family due to which the family is slipped into poverty and their quality of life is affected. If the sole breadwinner of the family contracts the disease, then the family loses its only source of income and is forced to spend all its meager monetary resources on the treatment of the person. The family of the infected person also is at a high risk of contracting the contagious disease. Also, the social stigma attached to the disease can't be ignored. The effect of TB on society and country is such that it affects the National Economy at the macro level mainly due to decreased workforce. This leads to lower per capita income and a lower GDP.

According to the experts, the TB eradication programme of government has failed because of inadequate budgetary allocation to the programs, lack of proper infrastructure and manpower and poor implementation of programs. The various policies of the government fail to address the root cause of the problem. Also, the corruption in the healthcare system is hindering the policies from reaching the population. The Government policies need proper management. The TB program of the government will sink or swim with the Primary health program. Unless Primary Healthcare is improved and the problem of malnutrition is addressed, the program will fail to make any difference.

The experts say that each and every person in the country needs TB awareness and no section of the population must be exempted. But they feel that a special emphasis must be given on the poor and the marginalized sections of the society as these sections survive in poor and congested living conditions and the rate of malnutrition is high among them. The respondents feel that for the TB Awareness Campaign to reach every nook and corner of the country, innovative and creative methods have to be used so that the campaign catches everyone's attention. Use of mass media and social media will help in reaching the whole country. The use of local language for promoting the campaign will help in reaching out to the remotest places of India. The Awareness Campaign needs to be promoted by a celebrity or a famous personality so that people respond to the campaign. Also, awareness workshops should be held in schools and colleges so that the young generation is well informed about the disease. As one respondent mentioned, *"The government should start an educational series on TB along the lines of "Mann ki Baat" by the Prime Minister of India."*

The additional efforts required to reduce the menace of TB include improving the overall scenario of public and primary healthcare in India. Universal access to healthcare and treating MDR-TB efficiently can substantially reduce the prevalence of TB. Improving the general health facilities, improved standard of living conditions, proper nutrition are some of the ways to tackle this deadly disease. Access to free or cheaper drugs and treatment, usage of quicker and more accurate diagnosis technology, involving private sector in the management of the TB Program will help to improve the existing infrastructure and healthcare services. These are some of the measures which will help tackle the problem of TB in the long run.

Finally, all the experts believe that urgent action needs to be taken against the disease. Improving the healthcare infrastructure of the country (improving the quality and quantity of medical facilities and doctors) should be the main motto the Government. Awareness and mass education about the

various killer diseases is the need of the hour. TB is just like any other communicable disease. It needs to be tackled in a rational and scientific way. This is possible only if the whole country takes part in the campaign against TB menace.

4. Discussion

TB along with Acquired Immune Deficiency Syndrome (AIDS) and malaria rank among the top three fatal infectious diseases which pose threat to global public health, especially in middle and low income countries.[15] Asia has the highest burden of TB in the world. Optimizing the diagnosis and treatment of TB is one of the key strategies for achieving the WHO 'End TB' targets.[16] Majority of TB cases of resource-poor settings experience food insecurity, which impacts treatment adherence and outcomes. Additional food or cash assistance for this subgroup might improve food insecurity and thereby nutritional status.[17] But again, this is a temporary measure. The root causes of TB, like poverty, poor socio-economic conditions, and improper hygienic practices are still neglected. Most of the developed countries have eliminated diseases like TB before the advent of anti-tubercular drugs through socio-economic improvement. India is earmarking funding for prevention and control of TB, but it is still mainly for diagnosis and treatment and not for primary prevention.

A significant proportion of the general population has incomplete knowledge on the routes of the spread of TB infection. Social stigma, such as reluctance to disclose about a family member being infected with the disease to others, also remains high. Imminent need for appropriate policy mechanisms for involving the private sector and raising consciousness through suitable advocacy measures is re-emphasized.[18] Quality of TB care is suboptimal and must urgently be addressed; merely focusing on coverage of TB services is no longer sufficient. While the world awaits revolutionary vaccines, drugs and diagnostics, programmatic data indicate that much can be done to accelerate the decline of TB.[5] Efforts are also being made to understand the genetic/molecular basis of target drug delivery and mechanisms of drug resistance.[19]

TB during childhood is also quite under diagnosed and under reported in India. Increased detection of childhood TB cases is essential to control TB in general population.[20] Social determinants like overcrowding, lack of awareness and knowledge about TB, and malnutrition have to be tackled in order to combat TB. There is urgent need for advocating educational activities among the patients and the more vulnerable population about the cause, transmission, preventive measures, duration and dosage of therapy of TB with the help of DOTS providers and apt IEC (Information Education and Communication) materials. Very few patients i.e., only 3 lakh out of estimated 15 lakh are notified by private sector making the issue underrated.[21] The current National Strategic Plan for TB Elimination (NSP 2017–25) has been worked out to provide nutrition support and reduce out of pocket expenditure of the patients and is aimed at ending TB by 2025.[14,22]

Successful control of TB globally will depend on strengthening TB control programs, wider access to rapid diagnosis and provision of effective treatment. Therefore, political and fund provider commitment is essential to curb the spread of TB.[7] There is a pressing need for systematic monitoring of ongoing TB treatment in the private sector: both to cast light on the true scale of the problem, and to help monitor the progress of interventions currently being planned to address this problem.[23] While transformative tools are being developed, high-burden countries like India will need to improve the efficiency of their health care delivery systems and ensure better uptake of new technologies. National TB programs must scale up the best diagnostics currently available, and use implementation science to get the maximum impact.[6] It has been shown that Active Case Finding (ACF) as compared to Passive case finding significantly averts catastrophic costs due to TB among patients. ACF as a strategy could ensure financial protection of TB patients and limit their risk of poverty.[24] In addition, TB elimination efforts need to focus on all forms of TB, including Extra Pulmonary TB, leaving no one behind, in order to realise the dream of ending TB.[25]

The End TB Strategy by WHO envisions a world free of tuberculosis by 2035. This requires reducing the global

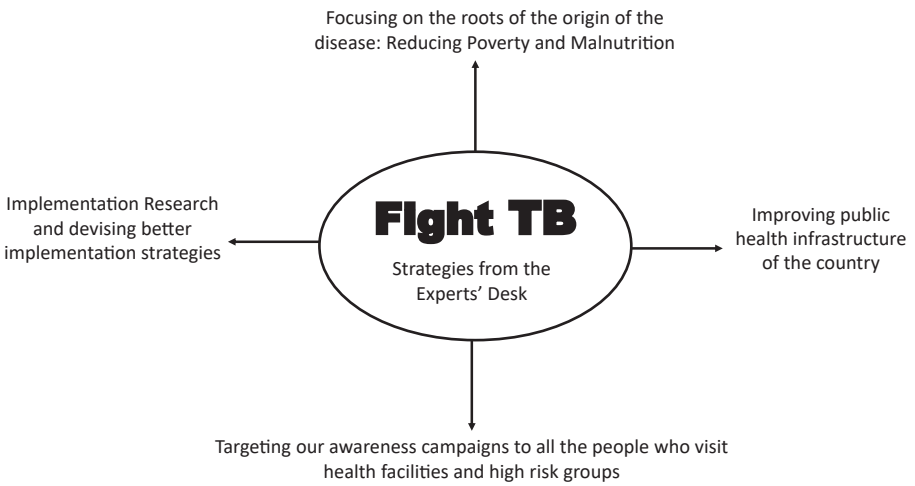

Fig. 1 – Experts' opinion on fight TB in India.

tuberculosis incidence from >125 cases per lakh people to <10 cases per lakh people within the next 15 years, which is quite a herculean task. Expanding testing and treatment of tuberculosis infection is critical to achieving this goal. India will require cost-effective and sustainable interventions aimed at tuberculosis.[26] The WHO End TB Strategy also calls for a global reduction in the case fatality ratio below 5%. India accounts for a third of global TB deaths. Case fatality is a critical measure of the quality of TB care. Increased high-quality reporting on patient outcomes will help improve the evidence base on this topic.[27]

The foundation of end TB strategy includes integrated patient centric care and prevention, bold policies, supportive statement, intensified research and innovation which requires engaging a wide range of collaborators across government, communities and private sector.[28] India needs to sustain the existing DOTS based program while introducing new components including services to address TB/HIV, treatment for MDR-TB, strengthening laboratory services and integrating TB services in both public and private healthcare sectors. Fig. 1 summarizes the strategies given by all the stakeholders/experts to Fight and End TB in India. The effectiveness of the program can be increased with focused efforts undertaken by Government of India in strengthening the primary healthcare system under National Health Mission through careful planning, thorough implementation, stable funding and innovations.

In India, TB is still one of the most commonly prevalent diseases as far as both morbidity and mortality is concerned. The incidence is quite high but it is only the tip of the iceberg. There are many missed cases either due to non-reporting by private sector or due to misdiagnosis. The next issue is inadequate and improper treatment of identified cases leading to increasing burden of drug resistant TB. Availability and affordability of sound diagnostic technology which helps in early diagnosis of TB cases (both non DR-TB and DR-TB cases) is missing from many parts of country. TB has a tremendous effect at individual, family and community level. This way it even affects the economy of a country. Also it is still neglected as India is more concentrating towards other conditions like Non Communicable Diseases and other emerging health issues.

5. Conclusion

Government of India is making lot of efforts to bring down the problems associated with TB through revised plans and their implementation across the country. In spite of this, there is a long way to go to achieve significant reduction in high incidence and prevalence of TB in India. Factors like lack of awareness and resources, poor infrastructure, increasing drug resistant cases (MDR TB and XDR TB), poor notification and overall negligence are the major challenges. Contagious disease like TB can victimize anyone. Even vaccinations do little to reduce its impact. If we eradicate poverty and undernourishment, educate the masses and eliminate the stigma attached with TB, we can hope for a disease free future. The current Coronavirus pandemic in 2020 has also given us excellent opportunity to create awareness about TB in the community at various levels.

Conflicts of interest

The authors have none to declare.

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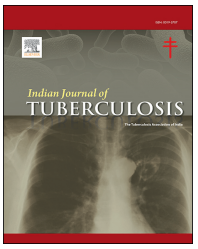

Original article

Sequential Co-infection of *Heligmosomoides polygyrus* and *Mycobacterium tuberculosis* Determine Lung Macrophage Polarization and Histopathological Changes

Laksmi Wulandari [a,b], *Muhammad Amin* [a], *Soedarto* [c], *Gatot Soegiarto* [b,d,*], *Kenji Ishiwata* [e]

[a] *Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*
[b] *Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia*
[c] *Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*
[d] *Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*
[e] *Department of Tropical Medicine, Jikei University School of Medicine, Tokyo, Japan*

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ABSTRACT

Background: Tuberculosis is a chronic infection caused by *Mycobacterium tuberculosis* (M.tb), which needs proper macrophage activation for control. It has been debated whether the co-infection with helminth will affect the immune response to mycobacterial infection.
Objective: To determine the effect of sequential co-infection of *Heligmosomoides polygyrus* (H.pg) nematodes and M.tb on T cell responses, macrophages polarization and lung histopathological changes.
Method: This study used 49 mice divided into 7 treatment groups, with different sequence of infection of M.tb via inhalation and H.pg via oral ingestion for 8 and 16 weeks. T cells response in the lung, intestine, and peripheral blood were determined by flow cytometry. Cytokines (IL-4, IFN-γ, TGB-β1, and IL-10) were measured in peripheral blood using ELISA. Lung macrophage polarization were determined by the expression of iNOS (M1) or Arginase 1 (M2). Mycobacterial count were done in lung tissue. Lung histopathology were measured using Dorman's semiquantitative score assessing peribronchiolitis, perivasculitis, alveolitis, and granuloma formation.
Result: M.tb infection induced Th1 response and M1 macrophage polarization, while H.pg infection induced Th2 and M2 polarization. In sequential co-infection, the final polarization of macrophage was dictated by the sequence of co-infection. However, all groups with M.tb infection showed the same degree of mycobacterial count in lung tissues and lung tissue histopathological changes.
Conclusion: Sequential co-infection of H.pg and M.tb induces different T cell response which leads to different macrophage polarization in lung tissue. Helminth infection induced M2

* *Corresponding author*. Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Academic Hospital, Jl. Mayjen Prof. Dr. Moestopo no. 6-8, Airlangga, Gubeng, Surabaya, East Java, 60286, Indonesia. Tel.: (+62) 8123547784.
E-mail address: gatot_soegiarto@fk.unair.ac.id (G. Soegiarto).
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lung macrophage polarization, but did not cause different mycobacterial count nor lung histopathological changes.

1. Background

Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis* (*M.tb*). According to the World Health Organization (WHO) report in early 2018, it is estimated that 10 million individuals in the world suffer from TB infection, particularly in developing and low-income countries.[1,2] The high incidence of TB in most of those countries was usually associated with the high prevalence of helminth infection and low Bacillus Calmette-Guerin (BCG) vaccination effectiveness.[3,4] There have been debates about the impact of helminth infection on TB infection. Helminth infections has known to cause alteration in the immune response that harms the body's defenses against TB infection.[5–7] The debate about the effect of helminthic infection on the severity of TB needs to be resolved as soon as possible in order to make the right countermeasures. Thus, it will reduce the efforts and costs that have been spent on the prevention and treatment of TB.[1,8]

M.tb is a parasitic facultative intracellular bacillus.[9] The main immune response to eliminate TB is cellular immunity played by macrophages, $CD4^+$ T lymphocytes that secrete IFN-γ, $CD8^+$ T lymphocytes that eliminate mycobacteria-infected macrophages, as well as $\gamma\delta$ T lymphocytes.[10] This response requires a strong Th1 type cytokines. In contrast, helminth infections stimulate the activation of eosinophils, mast cells, basophils, and IgE formation, which are parts of Th2 type immune responses.[11,12] The dominant Th2 type immune response may counteract the Th1 type immune response through suppression by IL-4. Thus, theoretically, helminth infection can suppress the immune response to TB infection,[13] but many previous animal and human studies had yielded contradictory results.[5–7,14] These discrepancies might be due to the difference in mycobacteria strain and helminthes species that were used, the intervals of co-infection, or the duration of the infection.

To solve this problem, it is necessary to conduct a co-infection research of helminthes and tuberculosis sequentially. A study with sequential infection of *M.tb* with the standard model of mice nematode (*Heligmosomoides polygyrus*) was conducted. To ensure the chronicity of nematode infection an interval of at least 8 weeks is required before the mice co-infected with *M.tb*. Chronic nematode infection is recognized to trigger regulatory T cells (Tregs) response.[15,16] Tregs may affect the balance of Th1 and Th2 immune responses. The Th1 – Th2 balance will also affect macrophage function in overcoming the mycobacterial infection.[17,18] If it is proven that chronic infection of nematode stimulates the activity of Tregs that are capable of altering the balance of Th1 – Th2 type immune responses and macrophage functional activity, the debate about the effect of helminth infection on TB infection will be resolved. This study aimed to determine the effect of sequential co-infection of *H. polygyrus* (H.pg) nematodes and M.tb on T cell responses, macrophages activation and lung histopathological changes.

2. Method

2.1. Subjects and infectious agents

The subjects of this study were 8–12 week-old Balb/c male mice (*Mus musculus*) weighted 30–35 grams. All of the mice were purchased from PN Bio Farma (Persero) Bandung and put under pathogen-free environment according the Federation of European Laboratoy Animal Science Associations (FELASA) suggestion. For TB infection we used stock solution of H37Rv strain of M.tb obtained from Bacteriology Laboratory for Tuberculosis Infection, Institute of Tropical Disease, Universitas Airlangga. For nematode infection we used the stage 3 larvae of H.pg obtained from generous donation by Associate Professor Kenji Ishiwata, DVM, PhD, Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan, with signed material transfer agreement and approved by National Institute of Health Research and Development, Indonesian Ministry of Health with decree No. LB.02.01/I.2/14311/2012.

2.2. Study design, allocation to groups of interventions, and ethics

This experimental study was done in the Department of Clinical Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, and the Bacteriology Laboratory for Tuberculosis Infection, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. There were totally 49 mice which were randomly allocated into 7 groups of interventions, i.e.: (1) infected with *M.tb* for 8 weeks (M.tb-8), (2) infected with *M.tb* for 16 weeks (M.tb-16), (3) infected with *H.pg* for 8 weeks (H.pg-8), (4) infected with *H.pg* for 16 weeks (H.pg-16), (5) infected with *H.pg* for the first 8 weeks and then with *M.tb* for the next 8 weeks (H.pg+M.tb), (6) infected with *M.tb* for the first 8 weeks and then with *H.pg* for the next 8 weeks (M.tb+H.pg), and (7) control group without nematode and tuberculosis infection (Control). The ethical clearance for this study was obtained from Animal Care and Use Committee (ACUC) of Veterinary Faculty, Universitas Airlangga, Surabaya, Indonesia No. 151-KE.

2.3. Animal infection procedures

For tuberculosis infection, the mice were exposed to *M.tb* through inhalation using nose-only inhalation system i.e. modified Middlebrook Inhalation Exposure System (Glas-Col,

Terre Haute, IN). Mice were exposed to 10 mL PBS-Tween 80 solution which contained 10^6 bacilli via aerosol nebulization for 30 minutes done in biosafety level 3 laboratory facility.[19] For nematode infection, the mice were inoculated orally using blunt-tipped gavage needle with 100 μL PBS solution containing 2000 L3/mL stage 3 larvae of *H.pg*.[19] The mice were then evaluated according to the groups of intervention procedure mentioned above. After 16 weeks, all the mice were sacrificed.

2.4. *Determination of T cell responses and lung macrophage activation*

Mice were sacrificed using injection of a mixture of Ketamine (100 mg/kg of body weight) and Xylazine (10 mg/kg of body weight) IM on their thigh muscles. Dissection and blood drainage was conducted from the right heart of the mice. The pulmonary veins were perfused with Saline-EDTA to eliminate all blood cells in the pulmonary intravascular.[20] The lung tissues were cut and minced into small pieces according to a protocol detailed elsewhere.[21] The jejunum and ileum tissue segments were taken and processed for the histopathology and immunohistochemical examination.[22] Th1, Th2, and Tregs responses in the peripheral blood serum, lung and intestinal tissues were analyzed using flow-cytometry technique with FACSCalibur using appropriate monoclonal antibodies (BD-Bioscience, Becton Dickinson, San Jose, CA, USA). Cytokines (IL-4, IFN-γ, TGB-β1, and IL-10) were measured in peripheral blood serum using ELISA according to manufacturer's protocol (Boster Immunoleader, CliniSciences, Nanterre, France). Macrophage activation were determined in lung tissue by the expression of iNOS (Thermo Scientific, Freemont, CA, USA) for classically activated macrophage M1, or by the expression of Arginase 1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for alternatively activated macrophage M2. All procedures were done according to the manufacturer's protocol. Observation and quantification was done by 2 independent observers The quantifications were done according to modification of a technique developed by Soini et al,[23] and Pizem & Cor.[24]

2.5. *Quantification of* M. tuberculosis *bacilli and lung histopathology assessment*

Slides of lung tissue were prepared and stained with Ziehl Neelsen (Brightfield). The *M.tb* were quantified using the WHO scale criteria.[25] The degree of histopathological changes in lung tissue were assessed semiquantitatively according to Dormans criteria[26] using a scoring system for 4 types of histopathological parameters i.e. peribronchiolitis, perivasculitis, alveolitis, and granuloma formation, each scored as absent, minimal, slight, moderate, marked or strong, noted as 0, 1, 2, 3, 4, and 5, respectively.

2.6. *Statistical analysis*

All variables data were tested for normal distribution using Shapiro–Wilk test. Data with normal distribution and had homogeneity of variance were analyzed with one-way ANOVA. Data with non-homogenous variance were analyzed with Brown–Forsythe test. Analysis for multiple comparation was done using Games–Howell test. The difference in histopathological changes between intervention groups were analyzed with non-parametric statistics Kruskal–Wallis test. The correlation between variables with numerical scale and normal distribution was conducted using Pearson test. For categorical or abnormally distributed variable data, Spearman non-parametric test was used. Path analysis was performed by linear regression test. All statistical calculations were performed using the SPSS-15.0 software for Windows (SPSS Inc., Chicago, IL, USA). The difference was considered significant if the value of p is ≤ 0.05.

3. Results

3.1. *The Th1 lymphocytes response*

The Th1 lymphocytes response were measured as the IFN-γ levels in peripheral blood serum (Table 1). As had been expected, *M.tb* infection stimulated high Th1 lymphocyte response. The highest peripheral blood IFN-γ levels were in the M.tb-8 group (106.48 ± 5.44 pg/mL) compared to the lowest in control group (7.20 ± 2.03 pg/mL) ($p < 0.001$), but it appeared to be somewhat blunted in M.tb-16 group (62.97 ± 7.82 pg/mL). Serum IFN-γ levels were also increased in the groups with *H.pg* and *M.tb* co-infection, but the degree of the increase was dictated by the sequence of the infection (89.92 ± 3.53 pg/mL in H.pg+M.tb group, and 46.16 ± 7.82 pg/mL in M.tb+H.pg group). Group with *M.tb* infection near the end of the study had significantly higher INF-γ levels ($p < 0.001$).

The percentage of $CD4^+$ T lymphocytes that expressed intracellular IFN-γ molecules in lung tissue, intestinal tissue, and peripheral blood was measured by flow cytometry using antibodies to CD4 and INF-γ simultaneously (Fig. 1). The highest expression of Th1 $CD4^+$ lymphocytes in lung tissue was in the M.tb-8 group (4.50 ± 0.94%), compared to the lowest in the control group (0.03 ± 0.01%). The overall results pattern was in parallel with IFN-γ levels pattern in peripheral blood serum. The expression of lung tissue Th1 $CD4^+$ lymphocytes significantly differ in all groups of intervention ($p < 0.001$). In the intestinal tissue, there was no significant difference in Th1 $CD4^+$ expression in all groups of intervention ($p = 0.109$). The expression of Th1 $CD4^+$ in peripheral blood was almost similar as in lung tissue. The highest percentage of Th1 $CD4^+$ expression in peripheral blood serum was in the M.tb-8 group (5.86 ± 0.19%) compared to the lowest values in the control group (0.46 ± 0.18%). There were significant differences of peripheral blood Th1 $CD4^+$ expression in all groups of intervention ($p < 0.001$; Table 1).

3.2. *The Th2 lymphocytes response*

The Th2 lymphocytes response was measured as the IL-4 levels in peripheral blood serum. *M.tb* infection did not stimulate Th2 lymphocytes. In contrast, *H.pg* infection stimulated robust Th2 lymphocytes response as can be seen in Table 1. The highest peripheral blood IL-4 levels were in the H.pg-8 group (93.88 ± 7.27 pg/mL), compared to the lowest in the

Table 1 – Level of peripheral blood cytokines, T cells response in the blood, intestinal or lung tissues, and lung macrophage activity.

Test	H.pg-8	H.pg-16	H.pg+M.tb	M.tb+H.pg	M.tb-16	M.tb-8	Control	p
Peripheral Blood Serum								
IFN-γ (pg/mL)	8.56 ± 1.41	29.46 ± 6.27	89.92 ± 3.53	46.16 ± 7.82	62.97 ± 7.82	106.48 ± 5.44	7.20 ± 2.03	0.000*
IL-4 (pg/mL)	93.88 ± 7.27	78.96 ± 12.37	20.78 ± 4.04	66.62 ± 13.93	16.96 ± 5.20	14.00 ± 4.41	5.01 ± 1.35	0.000*
IL-10 (pg/mL)	54.22 ± 7.18	20.59 ± 5.36	18.53 ± 3.70	18.23 ± 5.05	20.38 ± 5.99	61.62 ± 7.83	4.65 ± 0.57	0.000*
TGF-β (pg/mL)	61.36 ± 8.58	25.34 ± 4.45	17.92 ± 2.58	16.52 ± 3.01	36.70 ± 4.69	72.74 ± 9.14	8.54 ± 1.92	0.000*
$CD4^+$ + IFN-γ (%)	0.52 ± 0.31	1.90 ± 0.33	4.95 ± 0.23	2.42 ± 0.41	3.64 ± 0.54	5.86 ± 0.19	0.46 ± 0.18	0.000*
$CD4^+$ + IL-4 (%)	5.58 ± 0.32	4.66 ± 0.24	1.19 ± 0.55	3.96 ± 0.30	0.96 ± 0.27	0.84 ± 0.17	0.33 ± 0.15	0.000*
Foxp3 + IL-10 (%)	3.26 ± 0.59	1.26 ± 0.27	1.11 ± 0.26	1.03 ± 0.32	1.28 ± 0.28	3.70 ± 0.42	0.26 ± 0.17	0.000*
Foxp3 + TGF-β (%)	3.69 ± 0.43	1.51 ± 0.30	1.07 ± 0.24	0.96 ± 0.16	2.19 ± 0.25	4.35 ± 0.39	0.50 ± 0.21	0.000*
Intestinal Tissue								
$CD4^+$ + IFN-γ (%)	0.04 ± 0.03	0.06 ± 0.04	0.26 ± 0.16	0.21 ± 0.15	0.14 ± 0.05	0.29 ± 0.28	0.13 ± 0.08	0.109
$CD4^+$ + IL-4 (%)	4.27 ± 0.48	3.94 ± 0.23	1.04 ± 0.35	2.71 ± 0.50	0.12 ± 0.08	0.16 ± 0.10	0.19 ± 0.11	0.000*
Foxp3 + IL-10 (%)	3.21 ± 0.42	1.46 ± 0.35	1.14 ± 0.36	1.04 ± 0.25	0.61 ± 0.33	0.70 ± 0.29	0.11 ± 0.10	0.000*
Foxp3 + TGF-β (%)	3.68 ± 0.29	1.63 ± 0.32	1.27 ± 0.17	1.16 ± 0.37	0.70 ± 0.29	0.90 ± 0.20	0.18 ± 0.13	0.000*
Lung Tissue								
$CD4^+$ + IFN-γ (%)	0.13 ± 0.07	1.10 ± 0.47	3.24 ± 0.51	1.27 ± 0.66	2.05 ± 0.84	4.50 ± 0.94	0.03 ± 0.01	0.000*
$CD4^+$ + IL-4 (%)	0.81 ± 0.12	0.82 ± 0.23	0.79 ± 0.22	0.88 ± 0.30	0.92 ± 0.19	0.69 ± 0.18	0.01 ± 0.01	0.000*
Foxp3 + IL-10 (%)	1.13 ± 0.35	0.78 ± 0.14	0.72 ± 0.23	0.73 ± 0.18	0.80 ± 0.25	1.14 ± 0.24	0.33 ± 0.24	0.000*
Foxp3 + TGF-β (%)	1.21 ± 0.28	0.82 ± 0.26	0.75 ± 0.16	0.69 ± 0.20	0.80 ± 0.23	1.20 ± 0.33	0.15 ± 0.09	0.000*
Mϕ iNOS	10.00 ± 1.73	10.80 ± 1.64	21.00 ± 2.35	12.60 ± 2.30	11.40 ± 1.67	26.40 ± 3.29	4.40 ± 1.82	0.000*
Mϕ Arginase 1	19.20 ± 0.45	23.40 ± 1.14	10.00 ± 1.41	25.00 ± 2.00	15.20 ± 1.64	8.80 ± 0.45	3.80 ± 0.45	0.000*

Note: H.pg = *Heligmosomoides polygyrus* infection; M.tb = *Mycobacterium tuberculosis* infection; 8 and 16: denotes infections for 8 and 16 weeks, respectively; H.pg+M.tb = *Heligmosomoides polygyrus* infection followed by *Mycobacterium tuberculosis* infection; M.tb+H.pg = *Mycobacterium tuberculosis* infection followed by *Heligmosomoides polygyrus* infection; Mϕ = macrophage; *significant $p < 0.001$, multiple comparations between groups, one-way ANOVA and Games–Howell test.

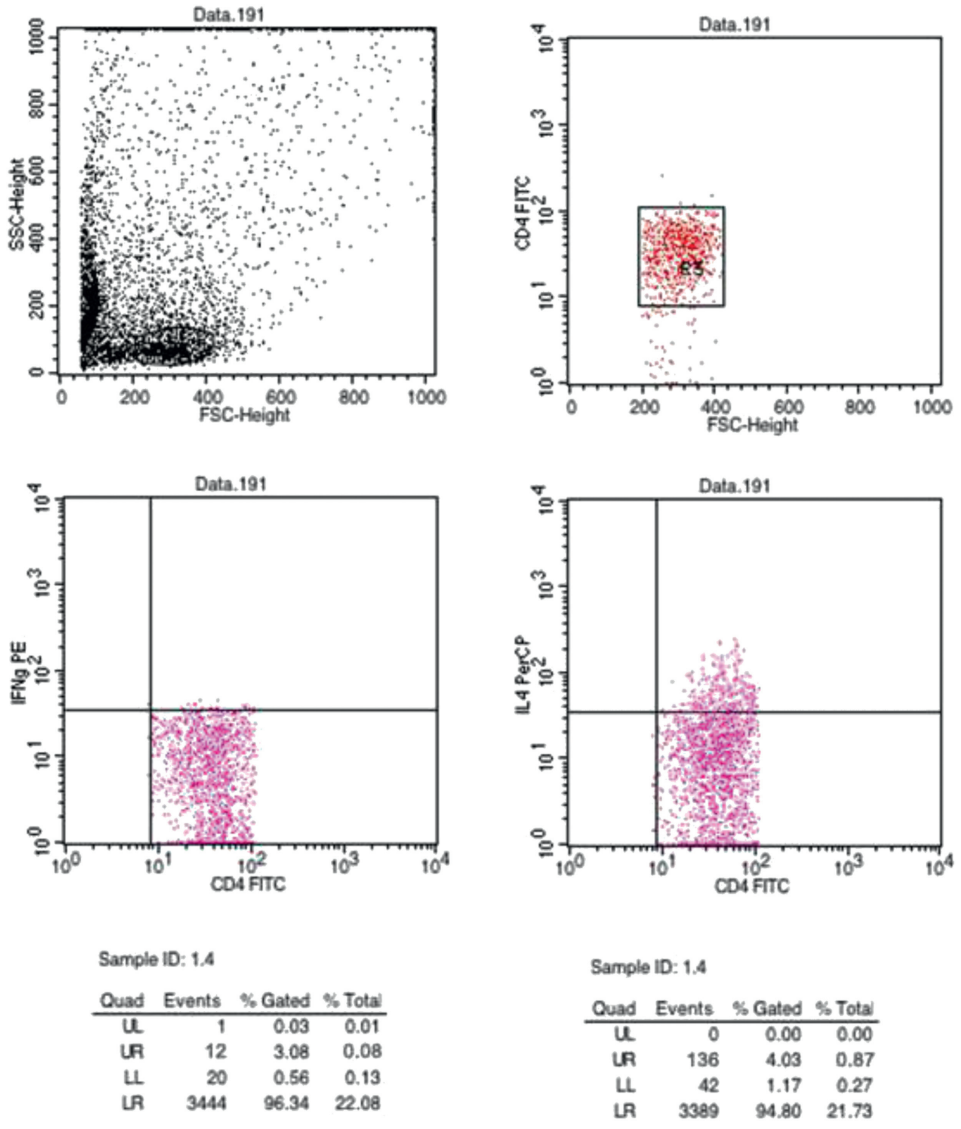
control group (5.01 ± 1.35 pg/mL) ($p < 0.001$). Again, the response seemed to be somewhat blunted in the H.pg-16 group (78.96 ± 12.37 pg/mL). Serum IL-4 levels were also increased in the groups with *H.pg* and *M.tb* co-infection, but again, the degree of the increase was dictated by the sequence of the infection (20.78 ± 4.04 pg/mL in H.pg+M.tb group, and 66.62 ± 13.93 pg/mL in M.tb+H.pg group). Group with *H.pg* infection near the end of the study had significantly higher IL-4 levels ($p < 0.001$). The groups with *M.tb* infection had low IL-4 levels in the peripheral blood (14.00 ± 4.41 pg/mL in M.tb-8 group, and 16.96 ± 5.23 pg/mL in M.tb-16 group; $p = 0.948$ between both of them).

The percentage of $CD4^+$ T lymphocytes that expressed intracellular IL-4 molecules in lung tissue, intestinal tissue, and peripheral blood was measured by flow cytometry using antibodies to CD4 and IL-4 simultaneously (Fig. 1). There were no significant difference of Th2 $CD4^+$ expression in lung tissue lymphocytes between groups of intervention, but each of them significantly differ from the control group ($p < 0.001$). In the intestinal tissues, *H.pg* infection induced the highest increase in the expression of Th2 $CD4^+$ lymphocytes (4.27 ± 0.48% in H.pg-8 group, and 3.94 ± 0.23% in H.pg-16 group, respectively). Groups with *M.tb* infection had very low expression of Th2 $CD4^+$ lymphocyte in the intestine, which were comparable to the control group. In the co-infection groups, *H.pg* infection near the end of the study had significantly higher Th2 $CD4^+$ expression (2.71 ± 0.50%), while *M.tb* infection near the end of the study seemed to dampen the Th2 $CD4^+$ response in the intestine (1.04 ± 0.35%). The expression of Th2 CD4+ lymphocytes in the peripheral blood follow the pattern of peripheral blood IL-4 levels (Table 1).

3.3. *The regulatory T lymphocytes response*

The regulatory T lymphocytes (Tregs) response was measured as the immunoregulatory cytokines levels (IL-10 and TGF-β) in peripheral blood serum. Contrary to our expectation, the groups with longer duration of infection, either with *M.tb* (M.tb-16) or *H.pg* (H.pg-16), do not show the highest levels of IL-10 and TGF-β. Instead, the group infected with *M.tb* or *H.pg* for 8 weeks had the highest levels of IL-10 (61.62 ± 7.83 pg/mL in M.tb-8 group, and 54.22 ± 7.18 pg/mL in H.pg-8 group) and TGF-β (72.74 ± 9.14 pg/mL in M.tb-8 group, and 61.36 ± 8.58 pg/mL in H.pg-8 group), suggesting that the Tregs response wanes with time Table 1.

The percentage of Tregs, defined as $CD4^+$ T lymphocytes that expressed CD25 and Foxp3 molecules and produced IL-10 and/or TGF-β, in lung tissue, intestinal tissue, and peripheral blood was measured by flow cytometry using antibodies to CD25, Foxp3, and IL-10, or TGF-β simultaneously (Fig. 2). Overall, the pattern of those Tregs responses follow that of the IL-10 and TGF-β levels in the peripheral blood serum, except in the intestinal tissue where the infection with *H.pg* for 8 weeks (H.pg-8 group) clearly showed the highest percentage of Tregs response (3.21 ± 0.42% IL-10 producing Tregs, and 3.68 ± 0.29% TGF-β producing Tregs, Table 1). Infection with *H.pg* for 16 weeks (H.pg-16 group) showed a damped Tregs response (1.46 ± 0.35% IL-10 producing Tregs, and 1.63 ± 0.32% TGF-β producing Tregs, Table 1). Interestingly, albeit in low level, infection with *H.pg* for 8 weeks did influence the percentage of



Sample ID: 1.4

Quad	Events	% Gated	% Total
UL	1	0.03	0.01
UR	12	3.08	0.08
LL	20	0.56	0.13
LR	3444	96.34	22.08

Sample ID: 1.4

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	136	4.03	0.87
LL	42	1.17	0.27
LR	3389	94.80	21.73

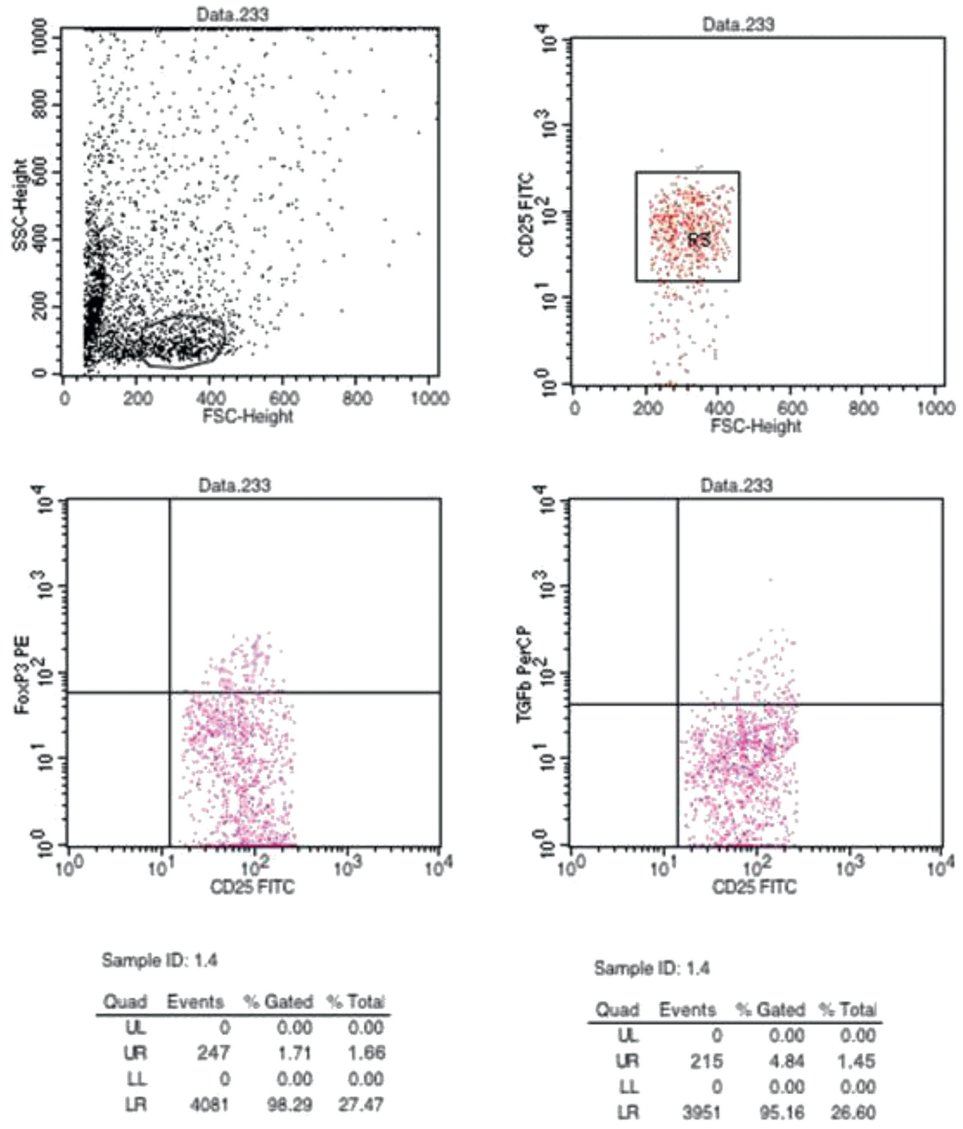
Fig. 1 – Flow cytometry of Th1 and Th2 CD4$^+$ T Lymphocytes in Lung Tissue. The CD4$^+$ T lymphocytes were identified by anti-CD4 antibody conjugated with fluorochrome fluorescein isothiocyanate (FITC). The cells were then permeabilized and marked with anti–IFN–γ antibody conjugated with pycoerythrin (PE) for Th1 lymphocytes or with anti-IL-4 antibody conjugated with peridinin chlorophyll protein (perCP) for Th2 lymphocytes. The percentage of each can be read in the accompanying table as the percentage of event in upper right quadrant (UR). The figure represent the data for subject no 1.4.

Tregs in lung tissues which was comparable to infection with *M.tb* for 8 weeks (1.13 ± 0.35% IL-10 producing Tregs, and 1.21 ± 0.28% TGF-β producing Tregs in H.pg-8 group, compared to 1.14 ± 0.24% IL-10 producing Tregs, and 1.20 ± 0.33% TGF-β producing Tregs in M.tb-8 group, $p > 0.05$, Table 1). For groups with co-infections, the Tregs responses were all in the mid-range, and the sequence of infection did not cause any significant difference.

3.4. *The macrophage activity in lung tissue*

Macrophage activity in lung tissues can be that of classically activated macrophage (M1) which expressed iNOS or that of alternatively activated macrophage (M2) which expressed Arginase 1. The quantification of macrophage activation was carried out by two independent observers which showed a consistent results and good correlation ($p = 0.341$ on paired t test, and $p < 0.001$ on Pearson correlation test).

The highest value of iNOS expression was found in the M.tb-8 group (26.74 ± 3.29), and the lowest was in the control group (4.40 ± 1.82) with a significant comparison between groups ($p < 0.001$; Table 1). The highest value of Arginase1 expression was found in the M.tb+H.pg group (25.00 ± 2.00) and followed by the H.pg-16 group (23.40 ± 1.14), which were significantly differ compared to the control group (3.80 ± 0.45; $p < 0.001$). The duration of *M.tb* infection affected the level of iNOS and Arginase1 expression by macrophages in lung tissue ($p < 0.001$). In contrast, *H.pg* infection in the intestine did not profoundly affect the expression of iNOS by macrophages that



Sample ID: 1.4

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	247	1.71	1.66
LL	0	0.00	0.00
LR	4081	98.29	27.47

Sample ID: 1.4

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	215	4.84	1.45
LL	0	0.00	0.00
LR	3951	95.16	26.60

Fig. 2 – Flow cytometry of $CD4^+CD25^+Foxp3^+$ T Lymphocytes in Lung Tissue. The $CD4^+CD25^+Foxp3^+$ T lymphocytes were identified by anti-CD25 antibody conjugated with fluorochrome fluorescein isothiocyanate (FITC). The cells were then permeabilized and marked with anti-Foxp3 antibody conjugated with pycoerythrin (PE) and with anti-TGF-β antibody conjugated with peridinin chlorophyll protein (perCP) for Th2 lymphocytes. The percentage of each can be read in the accompanying table as the percentage of event in upper right quadrant (UR). The figure represent the data for subject no 1.4.

infiltrated lung tissues, but instead induced increased Arginase 1 expression. The expression level of iNOS and Arginase 1 was affected more by the presence of *M.tb* infection in the lung. The expression level of Arginase 1 was also influenced by the presence of co-infection with *H.pg*.

3.5. *Quantification of* M. tuberculosis *bacilli*

The successful infection of *M.tb* using our modification of Middlebrook Inhalation Exposure System is shown in Fig. 3. With Ziehl–Neelsen staining, the lung tissue slides clearly showed groups of bright red colored acid-fast bacteria in mice infected with *M.tb*. It was also clear that in the group infected only with *H.pg* and in the control group no acid-fast bacteria was found in lung tissue. The quantification of the acid-fast bacteria were done using scoring system according to the Guidelines for Mycobacteriology Service in California developed by California Department of Public Health and California Tuberculosis Controllers Association,[25] which is also still recommended by CDC/ATS and WHO. The results were shown in Table 2.

3.6. *Changes in lung tissue histopathology*

The semiquantitative scoring for histopathological changes in lung tissue as assessed by 4 parameters, i.e. peribronchiolitis, perivasculitis, alveolitis, and granuloma formation[25] in each group of interventions can bee seen in Table 3.

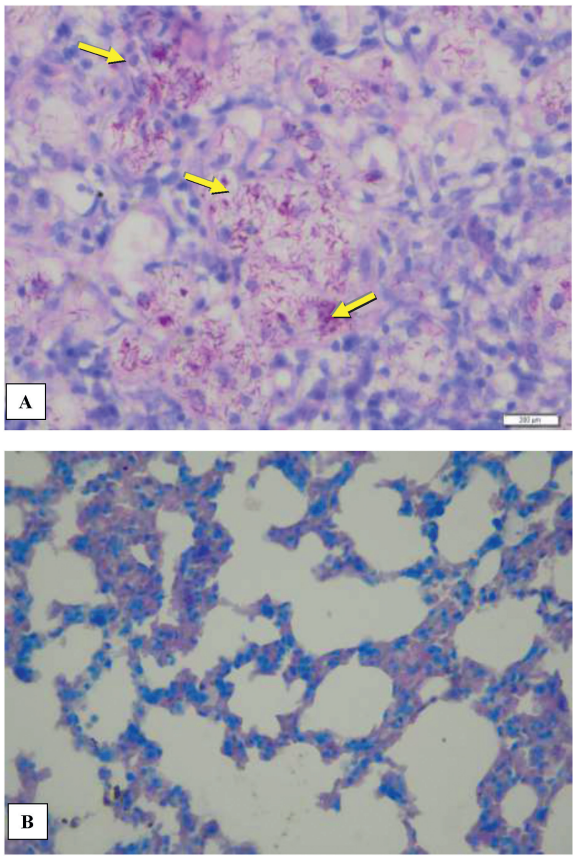


Fig. 3 – Ziehl-Neelsen staining of acid-fast bacteria in lung tissue. The colony of *Mycobacterium tuberculosis* (*M.tb*) is seen as a group of red colored acid-fast bacteria with Ziehl Neelsen staining on lung tissue slides (yellow arrows) which spread widely on the visual field (A). For comparison, also shown lung tissue slides without *M.tb* infection (B). The pictures above are seen with light microscopy with 400× power of magnification.

3.7. *Inter variable correlations*

The percentage of Th1 lymphocytes activity in lung tissue was significantly associated with the percentage of Th1 lymphocytes activity in peripheral blood, and intestinal tissue ($p < 0.001$), while the percentage of Th1 lymphocyte activity in peripheral blood was significantly correlated with peripheral blood IFN-γ levels ($p < 0.001$). The same trends were also observed for Th2 lymphocytes and Tregs lymphocytes (Table 4).

Details of the correlation between iNOS or Arginase 1 expression by lung macrophages and Th1 or Th2 lymphocyte activity were presented in Table 5. The correlation between lung macrophage activity and mycobacterial count and lung histopathological changes could be seen in Table 6.

4. Discussion

Immunity to *M.tb* infections clearly needs the host's ability to mount Th1 immune response, in which several Th1 cytokines production such as interferon-γ (IFN-γ), IL-2, and tumor necrosis factor-α (TNF-α) activate the macrophage to kill the mycobacteria.[27,28] Other subsets of T cells such as γδ T cells and CD1-restricted T cells are also stimulated, and together they induce granuloma formation at the site of infection.[29] Immunity to helminthes such as *H.pg*, on the other hand, need a robust Th2 immune response that produce IL-4, and IL-5, which in turn induce accumulation and activation of eosinophils, mast cells, and the production of IgE, all known to promote nematode expulsion from the intestine.[30,31] There have been conflicting results about the influence of helminth infection on immunity to tuberculosis. Some authors stated that helminth infection had negative impact,[4–7] while others found no effects.[14] The dispute can only be solved by studying the lung macrophage polarization during infection with *M.tb*, *H.pg*, or *M.tb* and *H.pg* co-infection.

There were substantial variation in the susceptibility of different mouse strains to infection with virulent *M.tb* H37Rv. Mouse strains can be divided into clusters of susceptible or resistant strains according to their ability to survive an infection with *M.tb* for more than 300 days. We chose BALB/c mouse strains which is categorized as resistant strain and considered it as appropriate. For aerosol infection of *M.tb* we exposed the mice by nebulizing 10 mL of PB-Tween 80 containing 10^6 bacilli/mL (equivalent to $10^{2.7}$ CFU of *M.tb*) as per protocol suggested by the literatures.[32] One literature stated that inoculation by aerosol route showed a faster rate of bacillary growth in the lungs, so it might be the case in our study. Moreover, the use of semiquantitative scoring system

Table 2 – Quantification of *Mycobacterium tuberculosis* in lung tissue (WHO Scale)[a].

WHO Scale Brightfield (1000× magnification)		Groups of intervention						
Number of acid-fast bacilli (AFB) per field	Notation (score) for report	H.pg-8	H.pg-16	H.pg+M.tb	M.tb+H.pg	M.tb-16	M.tb-8	Control
No AFB	Negative	✓	✓					✓
1-2 AFB per 300 fields	Report number observed							
1-9 AFB per 100 fields	Report number observed							
1-9 AFB per 10 fields	1+							
1-9 AFB per field	2+							
10-99 AFB per field	3+			✓	✓	✓	✓	
>99 AFB per field	3+							

[a] Semi-quantitative reporting of acid-fast specimens using Ziehl-Neelsen staining procedure according to Centers for Disease Control/American Thoracid Society (CDC/ATS) and World Health Organization (WHO) guidelines.

Table 3 – Histopathological changes in lung tissues.

Group of interventions	Score for histopathological changes in lung tissues[a]				
	Peribronchiolitis	Perivasculitis	Alveolitis	Granuloma	Total score
H.pg-8	1	1	1	0	3
H.pg-16	2	1	1	0	4
H.pg+M.tb	4	5	5	4	18
M.tb+H.pg	5	4	5	5	19
M.tb-16	5	5	5	5	20
M.tb-8	5	4	4	5	18
Control	1	1	0	0	2

[a] The score for each group of intervention is expressed as the average score of all members in the particular group. Each parameter of histopathological changes is scored as: absent, minimal, slight, moderate, marked or strong, noted as 0, 1, 2, 3, 4, and 5, respectively, according to Dormans et al[29]

according to the Guidelines for Mycobacteriology Service in California developed by California Department of Public Health and California Tuberculosis Controllers Association to quantify the acid-fast bacilli could be regarded as inappropriate, as this reporting system was originally meant for mycobacterial quantification in the sputum, not in the lung tissues. We should have used the more sophisticated and more accurate methods such as quantitative real-time PCR,[33] nested PCR,[34] or stereological analysis of bacterial load,[35] which we were unaware at the time of our study conception. Dormans criteria for semiquantitative scoring of histopathological changes in lung tissue,[26] was also appeared not sensitive enough to detect the significant difference between the intervention groups. Alternatively, the change in lung histopathology caused by mycobacterial infection is a slowly evolving process, so that 8 or 16 weeks of observation may not be sufficient to detect the evolution of the granuloma and histopathological changes.

Helminth infections induced the appearance of regulatory T lymphocytes ($CD4^+$ $CD25^+$ $Foxp3^+$) in both intestinal tissue, peripheral blood, and lung tissue. Regulatory T cell activity could be assessed by increasing levels of IL-10 and TGF-β cytokines in peripheral blood serum, as well as the percentage of regulatory T lymphocytes in intestinal tissue, lung tissue, and

Table 4 – Association of Th1, Th2, and Tregs lymphocytes activity in various tissues and in peripheral blood.

Inter variable correlations	Pearson's correlation	
	R	p
Th1 type response		
Lung tissue vs. peripheral blood T cells	0.95	<0.001
Lung vs. intestinal tissue T cells	0.55	<0.001
Intestinal tissue vs. peripheral blood T cells	0.52	0.001
Peripheral blood T cells vs. serum IFN-γ level	0.98	<0.001
Th2 type response		
Lung tissue vs. peripheral blood T cells	0.45	<0.01
Lung vs. intestinal tissue T cells	0.40	<0.05
Intestinal tissue vs. peripheral blood T cells	0.96	<0.001
Peripheral blood T cells vs. serum IL-4 level	0.98	<0.001
Tregs response		
Lung tissue vs. peripheral blood Tregs cells	0.82	<0.001
Lung vs. intestinal tissue Tregs cells	0.61	<0.001
Intestinal tissue vs. peripheral blood Tregs cells	0.48	<0.01
Peripheral blood Tregs cells vs. serum IL-10 level	0.97	<0.01
Peripheral blood Tregs cells vs. serum TGF-β level	0.98	<0.001

r: correlation coefficient; p: level of significance.

Table 5 – Association between iNOS and Aginase1 expressions by lung macrophages with Th1 and Th2 lymphocytes activity.

Type of T lymphocyte response	iNOS expression		Arginase 1 expression	
	R	p	r	p
Th1 response				
IFN-γ cytokine levels in peripheral blood serum	0.91	<0.001	0.06	0.714
Percentage of Th1 lymphocyte in peripheral blood	0.90	<0.001	0.03	0.832
Percentage of Th1 lymphocyte in lung tissue	0.90	<0.001	0.09	0.587
Percentage of Th1 lymphocyte in intestinal tissue	0.62	<0.001	−0.07	0.674
Th2 response				
IL-4 cytokine levels in peripheral blood serum	−0.24	0.166	0.60	<0.001
Percentage of Th2 lymphocyte in peripheral blood	−0.26	0.131	0.58	<0.001
Percentage of Th2 lymphocyte in lung tissue	−0.33	0.047	0.71	<0.001
Percentage of Th2 lymphocyte in intestinal tissue	−0.27	0.115	0.35	0.040

r: correlation coefficient; p: level of significance.

Table 6 – Association between lung macrophage activity and mycobacterial count or lung histopathological changes (Dorman's score).

Inter variable correlation	Spearman correlation	
	R	p
iNOS expression vs. mycobacterial count	0.72	<0.001
Arginase 1 expression vs. mycobacterial count	0.23	0.188
iNOS expression vs. Dorman's score	0.52	0.001
Arginase 1 expression vs. Dorman's score	0.31	0.068
Mycobacterial count vs. Dormans score	0.87	<0.001

r: correlation coefficient; p: level of significance.

peripheral blood. Similar to the findings on the immune responses of Th1 and Th2 cells, the regulatory T cell response was most pronounced in the group of helminth infections for up to 8 weeks and then seemed to subside in longer infection period of 16 weeks. We thought that the emergence of regulatory T cells mainly played a role in reducing the excessive inflammatory process that could have pathological effects on the hosts, as also had been suggested by others,[36,37] and had been reviewed extensively elsewhere.[38]

Apparently, the activation of regulatory T cells was also observed in *M.tb* infection, particularly in shorter duration (8 weeks) of infection, and then relatively subdued in longer duration (16 weeks) of infection. Other studies have also reporting the induction of Tregs in tuberculosis infection.[39] The emergence Tregs could be regarded as detrimental in terms of controlling the pathogen. However, we thought that it was more a manifestation of immunoregulation to control the inflammatory response, which if left unchecked might lead to excessive tissue damage to the host. That is why the Tregs activities were more pronounced in the shorter period of infections (either with *H.pg* or *M.tb*), where the inflammation caused by the pathogens were in its peak, and then somewhat damped in the longer period of infections when the inflammation begun to be reduced. Our study also consistent with the result of Leepiyasakulchai et al,[40] who found that in mouse strain that relatively resistant to tuberculosis infection (such as BALB/c mice) Tregs response were increased compared to susceptible mice strain (such as DBA/2 mice).

Our study also demonstrated the plasticity of T cells and macrophage response to different types of pathogens. H.pg infection (H.pg-8, H.pg-16 groups) clearly induced Th2 type immune response in the intestine and peripheral blood (but not in the lung), and it was associated with M2 lung macrophage polarization. M.tb infection (M.tb-8, M.tb-16 groups) induced Th1 type immune response in the lung and peripheral blood (but not in the intestine), and was associated with M1 lung macrophage polarization. While in the M.tb and H.pg co-infection, the Th1 or the Th2 immune response dictated by the sequence of co-infection. All of those responses were believed to involve the role of antigen presenting cells such as dendritic cells. This plasticity was also observed by Cervi et al[41] Both infection with H.pg or M.tb induce Tregs immune response, which serve more as immunoregulation to control the inflammatory response and prevent excessive tissue damage. Tregs response was higher in shorter duration of H.pg or M.tb infection when the inflammation was in its peak.

5. Conclusion

The sequential co-infection of H.pg and M.tb induces different T lymphocyte immune response which leads to different macrophage polarization in lung tissue as measured by different levels of iNOS and Arginase1 expression. Although helminth infection influence the expression of Arginase 1 (M2 macrophage polarization), it did not cause different mycobacterial count nor different levels of lung histopathological changes as measured by Dorman's score.

Conflicts of interest

The authors have none to declare.

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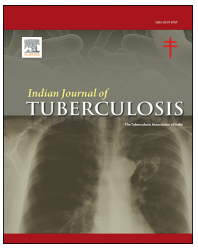

Original article

Prevalence of tuberculosis infection and its relationship to stunting in children (under five years) household contact with new tuberculosis cases

Bs. Titi Haerana [a,b,*], *Nurhayati Adnan Prihartono* [a], *Pandu Riono* [c], *Ratna Djuwita* [a], *Syahrizal Syarif* [a], *Ella Nurlaella Hadi* [d], *Nastiti Kaswandani* [e]

[a] *Department of Epidemiology, Faculty of Public Health, University of Indonesia, Indonesia*
[b] *Department of Public Health, Universitas Islam Negeri Alauddin Makassar, Indonesia*
[c] *Department of Biostatistics, Faculty of Public Health, University of Indonesia, Indonesia*
[d] *Department of Health Education and Behavioral Sciences, University of Indonesia, Indonesia*
[e] *Pediatric Department, RSCM Hospital, Faculty of Medicine, University of Indonesia, Indonesia*

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ABSTRACT

Background: Children who inhabit the same house with tuberculosis (TB) patients are at high risk for infection and illness with TB. Nutritional status (stunting) in children is related to the child's ability to withstand MTB (Mycobacterium Tuberculosis). This study aims to estimated the prevalence of tuberculosis infection and its relationship to stunting in children (under five years) with household contact (HHC) with new TB cases.

Methods: A cross-sectional design was implemented. Conducted in July 2018–April 2019 at 13 Public Health Center in Makassar City. The sample size was calculated using one sample situation-about precision formula. Samples were children under five who had contact with new diagnosed TB cases. Tuberculosis infection was measured by TST (tuberculin skin test). Logistic regression with causal model to examine TB infection relationship with stunting and covariate variable, analyzed using Stata/MP 13.0 software.

Results: One hundred twenty-six (126) eligible children. Prevalence of tuberculosis infection was 38.10%. Frequency of stunted was 31 children (24.60%). Stunted nutritional status (aPR): 2.36, 95% CI 1.60–3.44), boys (aPR: 1.47, 95% CI 0.96–2.25), not getting BCG immunization (aPR: 1.58, 95%) CI 0.89–2.82), and high contact intensity (aPR: 2.62, 95% CI 1.10–6.22) best predicted the tuberculosis infection in children with TB case household contacts with a model contribution of 64%.

Conclusion: Stunted nutritional status (moderate and severe), boys, not getting BCG immunization, and high contact intensity are the determinants of TB infection transmission in children HHC with TB. Children under five years of age who have close contact with TB

* *Corresponding author*. Pesona Prima Griya Cluster Emerald B.23, Kelurahan Bangkala Kecamatan Manggala Kota Makassar Provinsi Sulawesi, Selatan, Indonesia. Tel.: +62 85299689855.
E-mail address: bs.titihaerana@gmail.com (Bs.T. Haerana).
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cases should be targeted for priority interventions to prevent the transmission of TB infection and progressing to TB cases.

1. Introduction

WHO (World Health Organization) measures that globally amongst children under 15 years there are 1,010,000 TB cases and 194,000 TB deaths, respectively 10% and 15% of the global total.[1] Pediatric tuberculosis indicates ongoing transmission. Diagnosis of tuberculosis in children is quite challenging, particularly in countries with limited health resources followed by a high burden of the disease.[2] Case detection discovered only 11% of children <5 years with a TB diagnosis, these data underestimating the actual burden of childhood TB.[3] TB in children is oftentimes not confirmed because it is paucibacillary in nature and it is challenging to get an adequate sputum sample.[4–6] Consequently, reliable identification of MTB infection is crucial to warrant proper prevention. Tuberculin skin test (TST) is considered to assess evidence of tuberculosis infection.[2] WHO commends TST in children in low to middle-income countries because TST is more affordable and shows good sensitivity.[7] Mild side effects of TST such as itching, irritation, pain, red bumps at the injection site and severe side effects are rare.[8]

Very young children usually contract tuberculosis because of the primary infection and the risk of contracting severe TB is higher (miliary or meningitis). If the disease is not detected and untreated or without adequate treatment, the child will be at high risk for death, especially children under five years of age.[9,10] Previous research in Indonesia reported most TB cases in childhood (53–58%) were <5 years old with household exposure.[11,12] A systematic review observed that exposure, social and demographic factors should be weighed in assessing childhood tuberculosis infection and disease.[11] The principal risk factors for children are household contact with pulmonary TB cases (especially smear-positive or culture-positive), age <5 years, HIV infection, BCG immunization, frequency of contact, and malnutrition.[13]

Stunting or growth disturbance is linearly associated due to long-term malnutrition. Global figures report that 149 million (22%) children under the age of 5 are stunted, this figure is beyond the maximum limit set by WHO, which is 20%. WHO has designated Indonesia as a country with a distressing nutritional status with 7.8 million or around 35.6% undergoing stunted.[14] Children <5 years old have immature immunity. So that, stunting conditions worsen the deterioration of immunity which impairs the body's ability to fight against invading bacteria/viruses.[15] Subsequently, children with stunting have a higher risk of transmitting infectious diseases, including TB.[16,17,18]

Several previous studies recorded high evidence of infection/TST positive rates (24%–48%) in children (under five years) HHC with TB (new and old TB cases).[19–21] In this research, we wanted to measure the prevalence of tuberculosis infection in children (under five years) HHC with a focus on new TB cases (intensive treatment). Stunted children who HHC with pulmonary TB cases are at high risk for TB infection and illness, but there is still a gap of knowledge (paucity of research) that stunting creates susceptibility to TB infection. Stunting is a cause in this study because it is a long-standing condition and tuberculosis infection is the consequence/outcome because it is the current condition as a primary infection. This study aims to estimate the prevalence of tuberculosis infection and risk factors for stunting in children with household contacts with new TB cases.

2. Methods

2.1. Study design and setting

This study used a cross-sectional design with a prospective of routinely collected program data and medical records. Contact tracing of children is performed when new cases are diagnosed. The sample in this study were children (<5 years) who resided in the same house with adult TB cases (index cases) that were bacteriologically confirmed (smear-positive and positive expert genes). Inclusion criteria were child contacts of index cases diagnosed <4 weeks (new TB cases, intensive treatment).

The investigation was conducted in July 2018–April 2019. The sample size was calculated using one sample situation-about precision formula assuming 95%CI. Finally, the estimated minimalize sample size was 109 children (<5 years). Samples were children under five who had contact with new diagnosed TB cases. Consecutive sampling procedures were used for enroll every single participant who meets our inclusion criteria.

2.2. Study area

Study conducted at 13 Public Health Center (PHC) in Makassar i.e Tabaringan PHC, Pampang PHC, Karuwisi PHC, Kaluku Bodoa PHC, Pattingalloang PHC, Rappokalling PHC, Mallimongan Baru PHC, Maccini Sawah PHC, Bangkala PHC, Antang PHC, Perumnas Antang PHC, Tamamaung PHC, Batua PHC.

Makassar is the largest city in eastern Indonesia and the capital city of South Sulawesi, overall prevalence of TB were 300/100.000 populations. Pediatric TB proportion was 4.9%. (Baseline health research 2017).

2.3. Data collection

The dependent variable in this study was tuberculosis infection from the measurement results of the tuberculin skin test.

TST measurements are conducted by registered health worker/TB clinic with standard doses and recommended by the mantoux method. TST was administered by intradermal injection of PPD RT 23 2TU/0.1 ml. The result will be recorded after 48–72 hours. The TST category is positive if the induration is ≥ 10 mm and negative if the induration is < 10 mm and the criteria is ≥ 5 mm for severe acute malnutrition (SAM) and HIV status[13,22]

Nutritional status variables based on height/body length versus age. Measurements are executed according to standard procedures. Height-for-age z-scores (HFAz), created using the 2006 World Health Organization (WHO) child growth standard reference, were applied to define normal (HFAz ≥ - 2 SD), moderate stunting (HFAz <−2SD), and severe stunting (HFAz <−3SD).[23]

Data collection by direct interviews with the child's parents/caregivers to obtain information on age, sex, socioeconomic status, contact intensity, and the relationship between index cases and children using a questionnaire instrument. BCG immunization was collected from child immunization data in maternal and child health book. The variable for the level of positivity for index cases was retrieved from medical records.

The house physical environment variables measured were lighting, occupancy density, and ventilation. Lighting based the minimum intensity was 60 lux, occupancy density based on floor area and the number of occupants, ventilation based on the ratio of floor area and ventilation area. All environmental variables are then matched with the requirements for a healthy home by standard of Indonesian Ministry of Health (No. 829/Menkes/SK/VII/1999).

To maintain the quality of the data was conducted training was given for data collectors and perceptual equalization for registered health worker/TB clinic about standard doses and mantoux method.

2.4. Data analysis

Univariate analysis to assess epidemiological measures in the form of prevalence, bivariate analysis to evaluate risk factors, and multivariate analysis with logistic regression analysis with causal model to asses the association between the TB infection variable and the stunting variable concurrently with the covariate variables.

2.5. Statistical tools

Analyzed using statistical tools Stata/MP 13.0 software.

2.6. Ethical clearance

Ethical clearance was obtained from Research and Community Service, Faculty of Public Health, the University of Indonesia with register number WA63/UN2.F10/PPM.00.02/2018. Written informed consent was obtained by each child's parents. Children who positive TST were referred to a pediatrician for treatment decisions. Children who are tuberculin negative are considered for isoniazid preventive treatment (IPT).

3. Results

(Table 1).

3.1. Prevalence of tuberculosis infection

One hundred twenty-six (126) eligible children were involved in this study. We found prevalence of tuberculosis infection/positive TST in children were 38.10% (48/126).

3.2. Characteristic of children

Based on nutritional status, there was a difference in the proportion of tuberculosis infection ($p = 0.000$) with the largest proportion with severe nutritional status (4/5, 80%) and moderate (19/26, 73%).

The characteristics of children revealed that the mean age of children was 34 months ± 16.6 SD. Characteristics of children aged 24–35 months (35%) and 36–59 months (44%) had a higher proportion of children aged 0–11 months (31%) and 12–23 months (25%) to tuberculosis infection. Boys (47%) had the higher proportion of tuberculosis infection than girls (28%) ($p = 0.031$). Only 8% of children did not get BCG immunization and the largest proportion of children who had tuberculosis infection were children who did not get BCG immunization (7/10, 70%) ($p = 0.030$).

3.3. Characteristic of index case

Children who had household contacts of tuberculosis infection with male index cases (40%) had a higher proportion than females (35%), this proportion difference was not significant ($p = 0.56$). There was no difference in tuberculosis infection based on bacteriological positivity, and the proportion of 3+ is the highest (13/28, 46%). The child's parents (father and mother) as the index case had the highest proportion (50%, 42% respectively) of tuberculosis infection. Children in contact with high intensity of tuberculosis infection, namely ≥8 hours/day had the highest proportion of 49% (42/86) and the lowest <8 hours/day (6/40, 15%), the difference in the proportion was significant ($p = 0.001$).

3.4. Characteristic of physical environment

Amongst children with tuberculosis infection who lived in a physical environment with inadequate ventilation and occupancy density had the same proportion (38%). The disparity in the proportion of the two is not significant. There was no difference in the proportion of inadequate lighting compared to adequate lighting on tuberculosis infection (42% vs. 35% $p = 0.44$).

3.5. Multivariate final model

In the causal model analysis, confounding testing is carried out on all covariate variables, the covariate variable which is the confounder is maintained in the model, while the covariate variable which is not a potential confounder is excluded in the model. So that the final model is obtained as follows:

Table 1 – Tuberculosis infection based characteristics of children, index case, and physical environment — Makassar, Indonesia, 2019.

Characteristics	Tuberculosis infection				Crude PR 95% CI	P-value	Multivariate model	
	Positive (n = 48)		Negative (n = 78)				Adjusted PR 95% CI	P-value
	n	%	n	%				
Characteristics of children								
Nutritional status								
Normal	25	26	70	74	**1**	**0.000***	**1**	**0.00***
Moderate stunting	19	73	7	27	**2.81 (1.6–3.44)**		**2.36 (1.60–3.44)**	
Severe stunting	4	80	1	20				
Age								
0–11 month	4	31	9	69	0.78 (0.36–1.70)	0.392	–	–
12–23 month	5	25	15	75	0.56 (0.21–1.45)			
24–35 month	8	35	15	65	0.69 (0.24–1.96)			
36–59 month	31	44	39	56	1			
Sex								
Girls	17	28	43	72	**1**	**0.031***	1	0.05
Boys	31	47	35	53	**1.65 (0.91–2.99)**		1.47 (0.96–2.25)	
BCG status								
Yes	41	35	75	65	**1**	**0.030***	1	0.11
No	7	70	3	30	**1.98 (0.88–4.41)**		1.58 (0.89–2.82)	
SES								
Low	24	41	35	59	1.1 (0.50–2.48)	0.853	–	–
Intermediate	16	36	29	64	0.97 (0.41–2.28)			
High	8	36	14	64	1			
Characteristics of index case								
Sex								
Male	29	40	43	60	1.14 (0.64–2.04)	0.560	–	–
Female	19	35	35	65				
Positivity								
3+	13	46	15	54	1.21 (0.50–2.94)	0.561	–	–
2+	8	29	20	71	0.75 (0.28–1.99)			
1+	19	39	30	61	1.01 (0.44–2.32)			
Gen expert	8	38	13	62	1			
Relationship with child								
Mother	8	42	11	58	1.68 (0.58–4.85)	0.361	–	–
Father	15	50	15	50	2 (0.77–5.15)			
Grandparents	19	37	33	63	1.46 (0.58–3.65)			
Sibling	0	0	1	100	1.26 (–)			
Uncle/Auntie	6	25	18	75	1			
Contact intensity								
<5 hours/day	0	0	5	100	**1**	**0.001***	**1**	**0.03***
5–7 hours/day	6	17	29	83	**2.38 (–)**		**2.62 (1.10–6.22)**	
≥8 hours/day	42	49	44	51	**6.79 (1.38–7.65)**			
Characteristics of physical environment								
Lighting								
Adequate	25	35	46	65	0.84 (0.47–1.48)	0.449	–	–
Inadequate	23	42	32	58				
Occupancy density								
Adequate	27	39	43	61	1.02 (0.57–1.79)	0.902	–	–
Inadequate	21	38	35	62				
Ventilation								
Adequate	25	38	40	62	1.02 (0.57–1.79)	0.930	–	–
Inadequate	23	38	38	62				

Note: *(statistically significant).

After adjusting for nutritional status, age, sex, BCG status, socioeconomic status, index case sex, positivity, relationship with child, contact intensity, lighting, occupancy density, and ventilation, the following results were obtained:

The final model find out that stunted (moderate and severe) nutritional status, boy, did not get BCG immunization, and high contact intensity best predicted the incidence of tuberculosis infection in children who had household contacts with TB cases with a model contribution of 64%.

The risks of tuberculosis infection were 2.36 folds higher in stunted (moderate and severe) nutritional status (aPR: 2.36 [95% CI: 1.60–3.44]). Boys increases the risks of tuberculosis

infection by 1.47 folds higher (aPR: 1.47 [95%CI: 0.96–2.25]. The risks of tuberculosis infection were 1.58 folds higher among child who get BCG immunization (aPR: 1.58 [95% CI: 0.89–2.82]). Contact intensity increases the risk of tuberculosis infection by 2.62 folds higher (aPR: 2.62 [95%CI: 1.10–6.22]).

The stratification analysis with the homogeneity test was carried out to see the interaction between two independent variables in a substance that has the potential for interaction. Based on the analysis carried out on the variables age, sex, and socio-economic data, there was no modification effect ($p \geq 0.05$) on the involvement of the independent variable with tuberculosis infection.

4. Discussion

To our understanding, this is the first study to assess the prevalence of TB infection in children (under five years) with new TB cases (diagnosed <4 weeks, intensive treatment). Therefore, this study ascertains proof that newly diagnosed index cases are able of transmitting to vulnerable populations, namely children living in the same house. The limitation of this study is that it is likely that this result is underestimated because it cannot detect whether the child is in the incubation period so that the TST result is negative and is unable to assess the likelihood of exposure from TB patients who are not household/neighbour.

This study observed the prevalence of tuberculosis infection at 38.09% of the total number of 126 respondents. Our research contributes proof that TB patients who newly diagnosis had already transmitted it to children under five who lived at home. This prevalence will be higher if TST is measured at the end of intensive treatment, where index cases are still infectious so that TST-negative children have the potential to be infected. Previous study in Yogyakarta, Indonesia which evaluated the contact of children with index cases overall (new and old TB cases) report prevalence of tuberculosis infection at 38%.[24] This prevalence is a sizeable figure when compared with the evidence of infection rates in several countries. Studies establishing positive TST as induration ≥10 mm report the proportion of TB infections ranging from 24% to 48% in children (under five years).[19–21]

Tuberculosis infection can be limited by reducing the risk factors for transmission. Among other things, by maintaining nutritional status in normal conditions by eating a balanced nutritious diet that can improve endurance. Improve the socioeconomic status and maintain the distance of children to index cases, act hygienically, and adjustment of the home environment since tuberculosis was mainly contracted through airborne droplets, it follows that transmission of MTB was more likely if there is bad lighting, overcrowding and poor ventilation.[25,26]

We observed stunting to be a predictor of tuberculosis infection. A study in India explicated a dose–response relationship with an increase in the diameter size of induration of TST results with an increase in malnutrition status.[19] This was because children with growth disorders are more susceptible to the risk of infection by its negative impact on the epithelial barrier function dan from altered immune responses. Therefore stunted children increased susceptibility to MTB infection and a subsequent increase in positive TST.[27,28,29] Conversely, infection also affects growth disorders, forming a vicious circle that points to growth defects.[30]

We find out that children who did not receive BCG immunization were proved to be more likely to contract tuberculosis infection. BCG immunization can protect against tuberculosis, where the effect will diminish over time and the best effectiveness at <9 years old is 67%.[31] TST reactivity after BCG immunization cannot be associated if TST> 10 mm. Consequently, BCG immunization has no remarkable effect on the interpretation of TST reactivity.[32]

Boys have a higher chance of contracting tuberculosis infection. These results also found in previous studies that boys, younger age, contact with TB patients, and stunting significantly affect TST positive results.[33] If it is associated with impaired nutritional status, the study has found that girls experience more nutritional status maladies. Furthermore, in the general population, it was also found that male gender was associated with tuberculosis infection.[34] Nonetheless, differences in risk levels based on the sex of the child still need to be studied more intensely in terms of gender and physiological roles.

This study determined that the contact intensity of ≥8 hours has a higher chance than <8 hours. It is also reported in recent studies that the increasing prevalence of tuberculosis infection increases with the duration of exposure.[34] Increased risk of high-intensity exposure to TB infection due to exposure to repeated doses of the same inoculum or a single larger infectious inoculum.[35] A single infectious unit is enough to initiate the primary TB infection, this is reported by a recent outbreak in children <5 years of age where a short contact with an infectious case was enough to produce TB infection.[36]

This prompted to us suggest recommendation that 1) do screening TST on the contact the child as soon as possible, so that children get early treatment decision. 2) Need intervention to all of members household (especially, caregivers and index case) to collaborate prevent transmission to children HHC with TB. 3) Temporal relationship was not clear evidence in cross sectional design, need future study to explain this issue.

5. Conclusion

Our research contributes proof that TB patients who newly diagnosis had already transmitted it to children under five who lived at home with prevalence of TB infection was 38.10%. It can be predicted if the children (<5 years) was stunted, boys, did not receive BCG immunization, and had high contact intensity in index cases. All of this can contribute to predicting as much as 64%. This figure is quite good as a basis for consideration in translating health programs that require a multidimensional approach in preventing tuberculosis and stunting in children.

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Conflicts of interest

The authors have none to declare.

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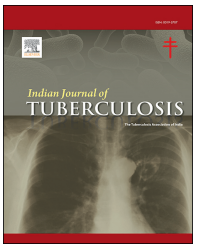

Original article

Revisions in TB programme - boon or bane? A qualitative study exploring barriers and facilitators among health care workers in private and public sector, Kerala

Geethu Mathew [a,*], *Sruthy C.S. Kumar* [a], *Koshy M. Cherian* [a], *Nidhish Issac* [b], *Anoop I. Benjamin* [a]

[a] *Department of Community Medicine, Believers Church Medical College, Thiruvalla, Kerala, 689103, India*
[b] *District Tuberculosis Office, Pathanamthitta, Kerala, India*

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ABSTRACT

Background: Despite many serious and organized efforts worldwide, Tuberculosis (TB) remains one of the major public health concerns in many countries. India accounts for more than one quarter of global TB cases and deaths each year. India's National Tuberculosis Elimination Programme (NTEP) is the largest TB control program in the world, placing more than 100,000 patients on treatment every month. There have been so many revisions in the programme guidelines in the last 5 years. As we are gearing up for TB elimination in India, knowledge regarding the barriers is very crucial in the successful undertaking of these revised guidelines. Exploring perceptions of health care workers, both from the private and public sector will help to design appropriate strategies at the field level.

Objective: To explore the barriers and facilitators among health care workers in the implementation of revised NTEP guidelines in a selected district of central Kerala.

Methodology: This qualitative study was conducted among health care workers from all levels involved in the implementation of NTEP from private and public sector. Qualitative data was collected through Focus Group Discussions (FGD) and Key Informant Interviews using a topic guide till data saturation. All discussions were audio recorded with the consent of participants. Sociogram was plotted to confirm equal participation of interviewees. A total of 4 FGDs (2 from each sector) and 12 Key informant interviews (7 from public sector and 5 from private sector) were conducted after obtaining written consent from the participants.

Results: Overall awareness about revisions was found to be good. However, the study identified a "Gap between planners and implementers". Frequent nature of revisions without understanding the practical difficulties in the field, additional job responsibilities, inadequate knowledge among grass root level workers/private practioners in small clinics and increased side effects were the major barriers identified. In addition to that, insufficient logistics, not enthusiastic in learning revisions, fear of losing patients, delay in communication, decreased compliance with new regimen, increased out of pocket

* *Corresponding author.*
E-mail address: matgeet@gmail.com (G. Mathew).
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expenditure and grey areas in the current guidelines were also adversely affecting the successful implementation At the same time, facilitators like positive attitude and commitment of health care workers, introduction of M-health technology, strong public private partnership, inclusion of costly investigations in the revised guidelines, good administrative support, financial assistance, innovative initiatives like Treatment Support Groups (TSGs) and concept of Family Directly Observed Treatment Short-Course increased the effectiveness of the programme to a large extent.

Conclusion: The study identified gaps in knowledge, attitude and practice of revised guidelines at the field level. Gap between 'Planners and implementers could impede the successful implementation of TB Elimination programme and needs to be addressed.

1. Background

Despite many serious and organized efforts worldwide, TB remains one of the major public health concerns, more so in South-East Asia and African countries.[1] Tuberculosis (TB) imposes a significant impediment to social and economic development in India, the country with the greatest epidemiological burden of TB in the world.[2] More than one quarter of global TB cases and TB-related deaths occur in India each year.[3] National Tuberculosis Elimination Programme (NTEP) is the largest TB control program in the world, placing more than 100,000 patients on treatment every month. Launched in 1997, the NTEP is based on the World Health Organization (WHO)-advised Directly Observed Treatment Short-Course (DOTS) strategy.[4] India has a public sector DOTS programme and a large private sector catering to the needs of the patients[2,5] In India the program is well supported by proactive political commitment, improved funding, regular monitoring, quality drugs and direct observation. Although DOTS has been in existence in India for close to two decades, TB continues to be a leading cause of morbidity as well as mortality in the country. The India has also witnessed a change in the disease epidemiology, with the emergence of higher rates of drug -resistant TB, a rising burden of TB among persons infected with HIV and the persistence of poor social determinants that put higher numbers of people at risk of acquiring TB infection.[4] India has attained the Millennium Development Goals targets for TB; but a lot has to be done if we are to meet the year 2030 targets of Sustainable Development Goals and year 2035 targets of the End TB strategy.[1] The WHO recommendations issued in 2010 highlight the importance of daily dosing of drugs.[4] There have been so many revisions in the NTEP guidelines since last 5 years which include switch to daily regimen, revision of categories and drug regimen, introduction of weight band based treatment, change in organizational structure of NTEP, operational definitions, diagnostic algorithm, clinical and long term follow up.[6]

For NTEP to be successful, it is imperative to understand the factors that facilitate and those that impede the implementation of these revisions at the ground level. Health workers are an important bridge for NTEP to reach the unreached. Exploring the perceptions of health care workers both from the private and public sector is very crucial to design appropriate strategies for the successful undertaking of the revisions of the programme. It is equally important that we should be aware about the perceived facilitators and barriers in the implementation of these guidelines in the field. As we are geering up for TB elimination in India, knowledge regarding the barriers are very crucial and no existing literature exploring this aspect is available in Kerala.

The study was undertaken with the following objectives.

Primary objective – To find out the barriers among health care workers in the implementation of revised NTEP guidelines at the field level in a selected district from central Kerala.

Secondary objective – To explore the facilitators among health care workers in the implementation of revised NTEP guidelines in the field.

2. Methodology

This qualitative study was carried out among health care workers involved in the implementation of NTEP from both private and public sector. Permission to conduct the study was obtained from District Tuberculosis office. The Institute Research Board and Institute Ethics Committee approval was obtained for the study. Health care workers from public and private sector involved in the NTEP and who were willing to participate in the study were identified with the help of District Tuberculosis Officer (DTO) from public sector and nodal officers of NTEP in private sector. Later snow ball sampling technique was used to identify key informants for the study. This included health care workers involved in the planning, implementation, treating doctors, nurses, ASHA workers, DOTS providers, treatment support group (TSG) members, laboratory staff, pharmacists etc. Qualitative methods such as Focus Group Discussions (FGD) and Key Informant Interviews were used to obtain data. A topic guide was prepared and validated with the guidance of professionals in this area. Probes for discussion were built into the topic guides to allow for thorough understanding of the topic. Topic guide contained questions on information on factors that facilitate or impede the implementation of revised guidelines in the field. The objective of the study and implication were explained at the start of the data collection. Participant information sheet was given to each participant

and purpose of the study, benefits of participating, procedure of maintaining confidentiality and right to not participate was made clear to the participants. Written informed consent was obtained from all participants before data collection. FGD and key informant interviews was conducted with the help of topic guide till data saturation. Each discussion lasted for 30–60 minutes. The number of participants in the FGD ranged from 8 to 12. All the discussions were moderated by one of the researchers and notes was written down during the discussions. Sociogram was plotted to confirm equal participation of interviewees and moderator ensured that all participants were given a chance to express their views fully. All discussions were audio recorded with the consent of participants. A total of 4 FGD (2 from each sector) and 12 Key informant interviews (7 from public sector and 5 from private sector) was conducted.

2.1. Details of key informant interview

Public sector – 7 Interviews were conducted with DTO, Pulmonologist in District Tuberculosis center, Medical officers in charge of Tuberculosis Elimination, PHC Medical officers and DOTS providers.

Private sector – Medical officer in charge of DOTS clinic, Lab technician who is also a DOTS provider, treating physician, treating pulmonologist, Nodal officer of NTEP.

2.2. Details of FGD

Public sector – 2 FGDs were conducted with Nurses, pharmacists, lab techinician, Senior Treatment supervisors, Senior TB laboratory supervisors, NGO representatives involved in TB elimination in the district, DOTS providers, TSG members, Junior Health Inspectors and ASHA workers.

Private sector – 2 FGDs were conducted with treating physicians, pulmonologists representatives from Management involved in decision making, Doctors and staff from Microbiology and Community Medicine Department.

The audio recording of the collected data was transcribed into the local language Malayalam and was later translated into English by a transcriber fluent in both languages and not a part of this study. Using Grounded Theory, thematic analysis of data sources namely the transcribed notes from the audio recordings and written field notes was done. Data triangulation was done by 3 researchers independently to improve data quality. Important verbatims are quoted in the results.

3. Results

A total of 12 Key Informant Interviews and 4 FGDs were conducted.

Findings of the analysis are put under these themes.

1. General awareness regarding revisions in guidelines
2. Barriers in the successful implementation of revised guidelines
3. Facilitators in the successful implementation of revised guidelines

3.1. General awareness about recent revisions

Most of the participants were aware that guidelines have changed. However their knowledge about the revisions was not complete. Majority of them were aware about the switch to daily regimen, weight band treatment and changes in investigation protocols. Some knew about the introduction of fixed drug combination regimen and treatment duration in the new guidelines. Especially in the private sector, only doctors are aware of the recent changes. Health care workers in public sector are more aware compared to private sector because they get more correct and prompt information about the revisions. There is a delay in information reaching the private sector.

> "Awareness is mainly given only to for people who work directly in DOTS. Other doctors and staff are not updated regularly about revisions".

Some of them are unaware about the reasons for changing the guidelines often. They feel that it would be good if the need for revisions is explained properly during training. Overall they believe that all revisions in the guidelines are necessary and based on research

> "Revisions are based on scientific studies on effectiveness of various drugs and research. It will help to reduce treatment failures, death rate and improve the cure rate"

3.2. Barriers in the implementation of revisions

We observed barriers in many levels. Various themes and subthemes identified is summarized in Fig. 1.

3.3. Inadequate awareness

Awareness levels regarding recent revisions are inadequate among grassroot level health care workers and private practioners especially those who work in small clinics. Many of them told that frequent changes in the guidelines are confusing and difficult to learn and memorise for field level workers. Some said that reasons for revisions are not properly explained to health care workers. Training and dissemination of information is not complete among Health Care Workers of private sector. There are many small private clinics where General Practioners treat patients taking shifts. So it is difficult to create awareness among all private practitioners about revisions. Some of the health care workers are not adequately trained in using electronic tablets.

> "By the time a revision is implemented in the field, the guidelines will be revised again and it is becoming

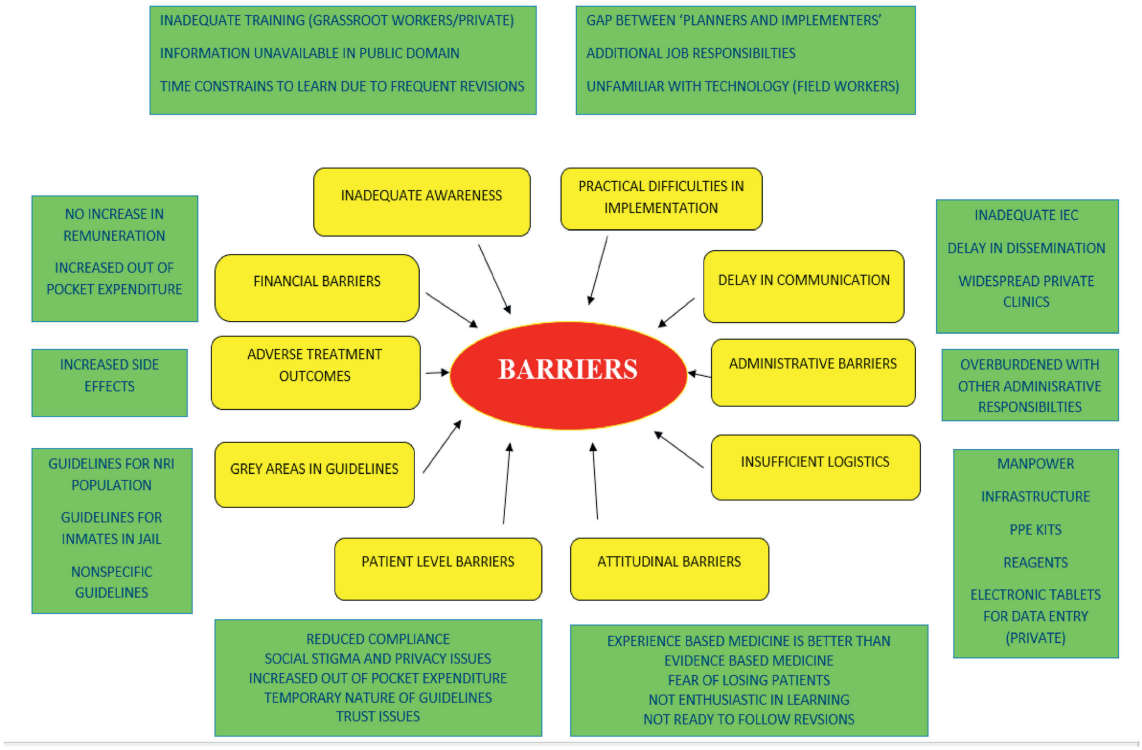

Fig. 1 – Barriers for implementation of revisions at various levels.

extremely difficult for us" "Information about recent revisions is not available in internet also"

3.4. *Attitudinal barriers*

Some are of the opinion that revisions are not needed and there were good results with old regime. Many Dots Providers admitted that were comfortable with "box system" (One box per patient). Few of them especially old private practitioners having long years of experience are not ready to follow the revised guidelines because they believe in their clinical experience. Some participants told that they are not enthusiastic to learn changes completely as they feel that this also may change in short period.

> " What is the point in learning a revision … soon another revision will come and we will have to learn that …."

Private practitioners don't want to lose their patients and few of them feel that quality of treatment provided in the programme is not good as it's given free of cost.

3.5. *Practical difficulties*

Most of them opined that there is a huge gap between "planners and implementers".

> "Policy makers are not always aware about the field realities as they are not working in the field. They make changes based on data to make the programme successful. But most of the times its practically very difficult to implement these changes"

> "It will be good if all revisions are done at the same time than changing it frequently. Very difficult to implement frequent changes both for trainers as well as for the practioners"

Many said that implementation of revisions needs extra effort from Health care workers involved in the TB elimination who are already overburdened with additional duties.

> "All grassroot level health workers are not very young and not very familiar with this technology. So they won't be able to manage when they encounter any technical problems."

3.6. *Barriers in communication*

Most of the times the updates regarding revisions are communicated to doctors and there will be communication gap between them and field workers resulting in unnecessary confusion. Sometimes representatives from private sector who were not directly involved in DOTS attend the monthly meeting of NTEP and there used to be so much delay in disseminating information to concerned people/staff involved in TB care. Few told that the updates regarding revisions should be communicated through proper channels (Hierarchy). Most of the times juniors in the administrative posts are

informed directly first about the changes before seniors and this affects smooth implementation

> "All the private hospitals are not getting the communication regarding revisions. Medical colleges with DOTS clinic and Community Medicine Departments will get timely updates regarding revisions "

> "Sometimes even medical officers come to know about the revisions late. So we won't be able to pass the information on time to field workers"

3.7. Logistics

Some participants told that there is lack of adequate manpower and infrastructure to implement revisions. PPE kits are unavailable for some workers at the grassroot level from both sectors. In addition to that some of the participants from private sector informed that there is insufficient supply of reagents and shortage of loose medications to give for patients with side effects. No separate IP facility for TB patients in DTC will eventually affect the programme. In addition to that most of the centers in both sectors donot have a separate sputum collection areas/counselling areas/cough corners/waiting areas for TB patients. Electronic tablets for data entry in NIKSHAY is provided only for health care workers from public sector.

> "Only one CBNAAT testing center is there for the entire district. Frequent power failures along with large number of specimens result in huge delay in getting the reports of CBNAAT/LPA on time".

3.8. Administrative barriers

Health care workers in administrative posts have additional responsibilities other than implementation of NTEP which makes it difficult for them to manage. "MOTC has to look after many PHCS. So they won't have much time to spend and care for the patients".

3.9. Grey areas in current guidelines

Some participants are worried about the unavailability of specific guidelines for NRI population, who starts treatment here.

> "They are forced to come back after 2 months of IP to continue CP which makes it difficult for them. Some of them do not undergo prescribed investigations and/or send the results".

A few health workers expressed their difficulty in providing medications to TB patients who are arrested/jailed. Contact tracing (Under-five prophylaxis) and proper follow up is not done with patients who take treatment in private clinics. Many opined that it is extremely difficult for the system to trace these patients. Default patients are renamed as "Lost to follow up' according to new guidelines and this creates mental stress for the DOTS providers.

" Sometimes after starting treatment based on a particular weight band, patients weight will change … Even doctors are confused how and when to change the treatment … should we change it that month itself or next month?

> "INH prophylaxis is for children below 6 years. Still only tablets are available in the programme, not syrups for kids. Very difficult to implement it"

> "So many false negatives results in CBNAAT … In addition to that atypical and uncommon presentations on TB is not clearly mentioned in guidelines …. So clinical suspicion should be given as importance as investigations in the guidelines"

3.10. Financial barriers

Some of the participants feel that DOTS providers have to put more effort for the implementation of new regimes but the remuneration is still the same which makes them less productive. Out of pocket expenditure incurred for patients has also increased while following new regimen.

3.11. Barriers due to adverse treatment outcomes

Many of the health care workers mentioned about the increase in theincidence of side effects after switching to daily regimen, which makes patients less complaint to the new regime. Due to the fixed drug combination regimen, it is not possible to withdraw a particular drug which seems to be Resistant/ADR/Side effects.

> "Side effects have increased a lot after taking daily medications … most commonly seen side effects are hepatitis,visual impairement and skin reactions"

3.12. Patient level barrier – health workers perspective

Even though the objective of the project was only to explore the barriers among healthcare workers, some of them mentioned about the barriers faced by patients which indirectly affected the successful implementation of the project.

Many health workers mentioned that the patients were finding it difficult to comply with daily regimen as the number of tablets were more. In addition to that, many patients were worried about side effects and many patients are losing trust in the new regimen due to side effects. Patients are not able to identify drugs causing ADR as it is a fixed drug combination. Patients feel more comfortable with old regimen as it is intermittent.

"Already BCG vaccination is given for children … then why to take INH prophylaxis" some mothers are asking health care workers

"Weight band treatment guidelines changed twice in a short span which created too much confusion for patients and health care workers"

Due to social stigma and privacy issues, patients do not prefer to go and take treatment from public sector.

3.13. *Facilitators*

Factors that facilitate the successful implementation of revisions in guidelines are concised in Table 1.

4. Discussion

The study attempted to find out various barriers and facilitators in the implementation of revisions made in the TB Programme from the perspective of health care workers working in both private and public sector in Pathanamthitta district, Kerala. This study has helped to develop an insight towards the ground realities in the field in relation to the revisions of guidelines. The study has found that there are barriers in knowledge, attitude and practical difficulties in successful implementation of revisions in the field. The study identified a gap between the "Planners and Implementers". Even though efforts have been made to provide adequate knowledge, many a times it is not reaching the grassroot workers and many private practioners due to the scattered nature, shift system and temporary nature of employees working in clinics. This same observation was made in a similar study done in Kerala as a barrier for practicing public private partnership in TB programme.[7] Considering that more than 70% of the patients are catered by private sector, efforts should be made to sensitize and update the private sector equally as public sector. The unavailability of revised guidelines freely on the internet makes the situation worse. The overall attitude of the health workers towards revisions was good. However, health workers are generally dissatisfied with the frequent nature of revisions which does not give time to understand, learn, memorize and plan local strategies. Frequent nature of revisions without understanding the practical difficulties in the implementation in the field may impede the success of the programme. Revised guidelines demand extra effort from health workers who are already burdened with additional responsibilities. This was identified as a major barrier to accept daily regimen for TB in a similar study done in India.[4] In addition to the gap in knowledge, attitude and communication, financial difficulties and treatment outcomes also emerged as an important sub themes in the study. Treatment adherence is a critical determinant of treatment outcomes. Poor outcome and emergence of drug resistance are mainly due to irregular and incomplete treatment.[8] Increased incidence of side effects and out of pocket expenditure with daily regimen can reduce the compliance of the patients which inturn affect the effectiveness of the programme. A similar observation was made in an Indian study where loss of income, family responsibilities and increase in side effects resulted in decreased compliance to treatment.[4]

ACSM (Advocacy, communication and social mobilization) strategy at all levels was proposed to mainstream several revisions and to increase the acceptability.[1,4] An important element of the patient centered strategy is to assess and promote adherence to the treatment regimen and to address poor adherence when it occurs. These measures should be tailored based on the patient's clinical and social history. It also should be mutually acceptable to the patient and the provider.[8] However, to what extent we have succeeded in implementing this strategy at the field level needs to be examined thoroughly. Available literature suggests that we need to conduct studies to cast light over the preferences, adherence, and felt problems of the people involved in the programme. The introduction of M Health technology assisted strategies, inclusion of new investigations like CBNAAT in the revised guidelines along with good support from private sector and administration were the major facilitators. However the current infrastructure needs to be strengthened to provide quality services to all patients. Accumulating evidence has pointed to the effectiveness of a wide variety of approaches including community and family-centered DOTS which is more achievable for most developing healthcare systems.[4] This study showed DOTS is no longer directly observed and current guideline is promoting concept of Family DOTS. In a review article published in 2018, it was pointed out that without studying the felt needs of beneficiaries, the decision to modify a strategy may not be a correct strategy. The results of our study also share the same thought of understanding the felt needs before making proposed revisions on a larger scale for success of the programme.

Table 1 – Facilitators in the implementation of revisions in NTEP guidelines.

Sl No	Facilitators
1	Positive attitude and commitment of Health Care Workers
2	M-health technology
3	Strong public private partnership
4	Financial assistance through DBT (Direct Bank Transfer)
5	Good administrative support
6	Inclusion of costly investigations in the revised guidelines
7	Innovative initiatives in the revisions (JEET/TSG/STEPS)
8	Good rapport with the community
9	Family DOTS

4.1. *Strengths*

- First and one of its kind study from Kerala exploring the barriers and facilitators in the implementation of revised guidelines in the field
- Study included participants from both private and public institutions which helped us to explore perspectives of health care workers from different settings
- We have obtained inputs from participants across all levels of health system in the district (top level to the grassroot level) which helped us to understand barriers among

planners, implementers and to some extend patient level barriers (from perspective of health care workers)

4.2. *Limitations*

- Only one district was involved in the study.

5. Conclusion

The study identified gaps in knowledge, attitude and practice of revised guidelines at the field level. Gap between 'Planners and implementers 'could impede the successful implementation of any programme and needs to be addressed.

Conflicts of interest

All authors have none to declare.

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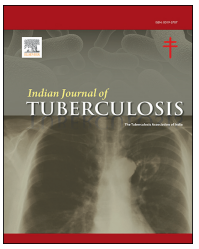

Original article

Pathways to diagnosis of pediatric TB patients: A mixed methods study from India

Neeraj Raizada [a], *Andrew McDowell* [b], *Debadutta Parija* [a], *K.S. Sachdeva* [c], *Sunil D. Khaparde* [c], *Raghuram Rao* [c], *T.N. Pavani* [a], *S. Sudha* [a], *Himshweta Tyagi* [a], *Y. Mary Rebecca* [a], *Sophie Huddart* [d], *Virender Singh Salhotra* [c], *Sreenivas Achuthan Nair* [e], *Claudia M. Denkinger* [f], *Sarabjit Singh Chadha* [a], *Sanjay Sarin* [a], *Aakshi Kalra* [a,*]

[a] *Foundation for Innovative New Diagnostics, New Delhi, India*
[b] *Tulane University, New Orleans, LA, USA*
[c] *Central TB Division, Government of India, New Delhi, India*
[d] *McGill University, Montreal, Canada*
[e] *Stop TB Partnership, Geneva, Switzerland*
[f] *Foundation for Innovative New Diagnostics, Geneva, Switzerland*

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ABSTRACT

Background: A significant proportion of pediatric tuberculosis (TB) patients go unnotified due to the challenges in diagnosis of TB among children. The experiences of this vulnerable group while going through the TB care cascade remain largely undocumented. The aim of this study was to explore the experiences of pediatric TB patients and families along the pathway to TB diagnosis and appropriate treatment in four cities of India.

Methods: The study used a mixed methods, single phased, embedded design. The primary qualitative and secondary quantitative data were collected simultaneously by interviewing families of 100 randomly selected Xpert MTB/RIF positive pediatric TB patients, under the pediatric TB project, in 4 Indian cities using a semi-structured questionnaire. The qualitative component was analyzed to deduce patterns and themes on the patient and family experiences. Descriptive statistics were used to quantify various events along the TB care pathway including various delays (patient, diagnosis and total) and number of providers visited by patients during the diagnostic process.

Results: The median patient, diagnostic and total delays were 3 (IQR: 2,5), 39 (IQR: 23, 91) and 43 days (IQR: 28.5, 98.5), respectively. Patients visited a median of 3 (IQR: 2,4) providers before accessing Xpert MTB/RIF testing. On an average, 68.4% of physicians ordered any test most of them being irrelevant for TB diagnosis. Qualitative data showed considerable suffering for children and their families before and after TB diagnosis including serious concerns of stigma, disruption in education and social life and recurrence of the disease.

Conclusion: Our study highlights the significant physical and social distress that the children with TB and their families undergo along the TB care pathway. It also shows

* *Corresponding author.*
E-mail address: draakshikalra@gmail.com (A. Kalra).
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diagnostic delay in excess of a month during which multiple providers were met and the patients underwent several diagnostic tests, most of them being inappropriate. Efforts to make Xpert MTB/RIF testing more accessible and part of physicians' toolkit will be of considerable value to ease the complexity of TB diagnosis in children. In addition, communication strategy needs to be developed and implemented to generate awareness among general population around pediatric TB and its management.

1. Introduction

Tuberculosis (TB) in India presents a significant challenge to public health with the country bearing the highest burden of TB and multi-drug resistant TB (MDR-TB) globally.[1] Studies show that children constitute about 15% of the TB patients in high burden countries.[2,3] In 2019, a little over 0.15 million childhood TB cases were notified, accounting for only 6% of the total notified TB cases in India.[4] Significant proportion of missed cases among this vulnerable population raise concerns related to delay in disease management and quality of TB care in India.[3,5]

The existing literature on patient pathways in India are mainly focused on adults,[6–9] with limited data on pediatric pathways.[10,11] Studies on adults indicate significant delays associated with the patients (from symptoms onset to initiation of care seeking) and the health system (delays associated with diagnosis after initial care seeking from any provider).[7,9,12,13] Relative to adults, diagnosis of TB in children is complex due to limited accessibility to highly sensitive rapid diagnostic tools, difficulty in obtaining quality specimen and paucibacillary nature of disease.[3] Furthermore, pediatric TB raises important questions among parents about their child's life in terms of survival, extent of suffering, future health and social prospects, which need to be considered when making policy decisions about pediatric TB management.[2,14] Accordingly, better understanding of events leading to pediatric TB diagnosis may help understand the effects of TB on children and their families[15] and various challenges related to diagnosis. These may further guide TB control strategies aimed at increasing case detection, improving quality of care, and reducing the risk of acquired resistance in this particularly vulnerable group.

Under the guidance of the National TB Elimination Program (NTEP) (erstwhile Revised National TB Control Program (RNTCP)), Foundation for Innovative New Diagnostics (FIND) implemented a novel initiative in 2014 which offered free of cost upfront Xpert MTB/RIF testing for TB diagnosis in pediatric populations in four Indian cities. The project has been successful in increasing TB and rifampicin resistance detection rates, and in engaging a large number of providers in each city.[16–18] However, among the cases diagnosed under the project between April 2014 to June 2016, 6% of the patients died with over half of them between the period of specimen collection and 15 days of treatment.[18] This suggests advanced disease at diagnosis due to possible delayed diagnosis. Based on this background and through this study, we aimed to explore the experiences of pediatric TB patients and families along the pathway to bacteriological confirmation of TB using Xpert MTB/RIF and appropriate treatment within this project in four cities of India. To seek additional insights, we determined the duration and nature of delays (patient, diagnosis and total delays) faced by the pediatric TB patients as well as the care seeking behavior including events, diagnostic tests and treatment preceding the diagnosis of TB.

2. Methods

Data for this paper were drawn from the pediatric TB project implemented by FIND, in collaboration with NTEP in four major cities of India; Delhi, Kolkata, Chennai and Hyderabad. The details of the project implementation have been described elsewhere.[16–18]

2.1. Study design, setting, and sampling

This research aimed to attain its objectives using a program-based, retrospective, single-phased, embedded mixed-methods design with a primary qualitative component. The study sample consisted of 25 Xpert MTB/RIF positive pediatric TB patients randomly selected per city among the pool of each city's Xpert MTB/RIF-positive patients detected under the project between April 2014 to June 2016 (total N = 100), irrespective of the sector (public or private).

2.2. Data collection

Qualitative and quantitative data were collected simultaneously through interviews with the parents/guardians of the sampled patients conducted in health facilities or at the patient's residence by a team of two trained interviewers per city using a semi structured questionnaire. The interviews were conducted during routine programme monitoring interactions with patients in line with NTEP guidance on patient follow-up in the local language, understood and preferred by the interviewees. The semi-structured questionnaire, which included few close ended questions eliciting quantitative information, was modeled on the community-based patient pathway to TB care study conducted by Mistry et al.[9] Interviewers were encouraged to be flexible with the order of questions to best fit with parent/guardians' narrative. Interviews were audio recorded. Available supporting documents, including test results and prescriptions, were photographed whenever available to cross-check dates of

testing and consultation. Following an interview, the interviewers filled an interview report which solicited additional quantitative data, translating direct quotes from patients to Hindi or English, information about Xpert MTB/RIF testing experience, and removed identifiers. Interviewers were also asked to write an open-ended narrative of the interview providing detailed information and context. Complex narratives were transcribed in full. These reports were then checked and cross-checked by team leaders for uniformity and validity. Following completion of 100 interviews, interview reports from each city were cross-checked by interviewers from every other participating city in person with real time feedback.

2.3. Data analysis

The objective of the overall pediatric TB project was to improve access to upfront Xpert MTB/RIF. Hence this was taken as end point for calculating *diagnostic delay* even if the patient had been diagnosed with TB prior to prescription of Xpert MTB/RIF test. However, no patient in the study had a microbiologically confirmed diagnosis prior to testing by Xpert MTB/RIF. Descriptive statistics (numbers, proportions and inter quartile range (IQR)) were used to quantify duration of '*diagnostic delay*' in days operationally defined in the literature as time elapsed between first care seeking and TB diagnosis (here, Xpert MTB/RIF).[6] Additionally, the duration of associated '*patient delay*' (time elapsed between first recognition of symptoms and seeking a medical consultation from formal or informal healthcare provider), and '*total delay*' (time elapsed between onset of symptoms and initiation of TB treatment) were also calculated.[6] We also investigated the type of providers consulted by the patients along the care pathway, diagnostic tests ordered for the patients, and treatment status. Analysis was performed using "R" and a complete case analysis was conducted (missing data <5%).

Qualitative data were subjected to a thematic content analysis. Data reports, narratives, and recordings were reviewed for trends firstly within each city and then across cities. They were also reviewed for statements made by patient families that linked TB duration and pathway delay to future of children. After developing initial qualitative hypotheses, the data were re-read for fit with these hypotheses. Any hypothesis that did not fit the data was discarded. Remaining hypotheses were checked against quantitative results to ensure coordination between the two forms of data. Finally, a set of qualitative results were generated.

2.4. Ethical considerations

Written consent forms were obtained from all participants. Non-consenting respondents were not included. For privacy reasons, a structured record of those who declined to participate was not maintained. The current project was undertaken by FIND, after approval from and in collaboration with NTEP. Through the results, we document our experience of implementing approved interventions in a programmatic setting within the existing accredited NTEP TB diagnostic lab network, which in turn is a part of Standard of TB care in India. Hence, separate ethical clearance was not required (Communication from Central TB Division, Ministry of Health and Family Welfare, Government of India is available).

3. Results

3.1. Quantitative results

The average age of patients included in this analysis was 10 years (range: 0.1–14 years) and two-thirds (n = 67) were females. The median patient, diagnostic and total delays were 3 (IQR: 2,5), 39 (IQR: 23, 91) and 43 days (IQR: 28.5, 98.5), respectively. Half of the respondents, (51.0%) had a family history of TB but this did not affect the 'patient' (median 3 days for both, with and without history of TB), or 'total' delay (median 37 and 44 days with history and without history of TB respectively, Wilcoxon Rank Sum test (p = 0.919)). Median delay up to diagnosis using Xpert MTB/RIF was 43 days irrespective of the type of sample tested.

Prior to prescription of Xpert MTB/RIF testing, patients visited a median of 3 providers (IQR: 2,4; range: 1,7) with one-fourth visiting 4 or more and 10% visiting 5 or more providers. A total number of 304 physicians were consulted by the 100 pediatric TB cases prior to diagnosis (Fig. 1).

Patients visited a median of 1 (IQR: 1,2) public sector provider and 2 (IQR: 1,3) private sector providers. A median of 1 (IQR 0,1) doctor per care pathway gave symptomatic treatment alone, while 2 (IQR: 1,3) prescribed any test; 1 (IQR: 0,1) per pathway gave a referral, and 1.5 (IQR: 1,3) prescribed an antibiotic not used in drug sensitive TB treatment. On an average, 68.4% (N = 208) of physicians ordered any test (TB or otherwise) and 25% of patients had 3 or more physicians order at least one test. At some point in time along the multiple care pathways identified (Fig. 2), 56.0% of the children were hospitalized.

The interviewees reported 27 different symptoms as reasons for first care seeking. Most (79%) had fever and other reported symptoms were weight-loss (38%), cough (35%), loss of appetite (33%), vomiting (19%), diarrhea (16%), swollen nodes (16%) and hemoptysis (9%).

A total of 205 non-Xpert MTB/RIF tests were ordered for the 100 patients. The most common was sputum smear (n = 77),

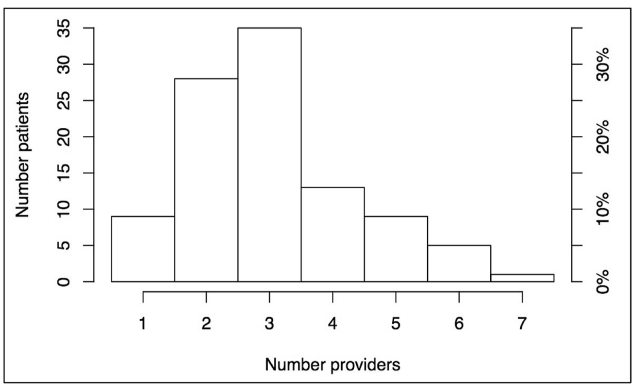

Fig. 1 – A histogram of the number of providers seen and the patient frequency for the respective number of physicians.

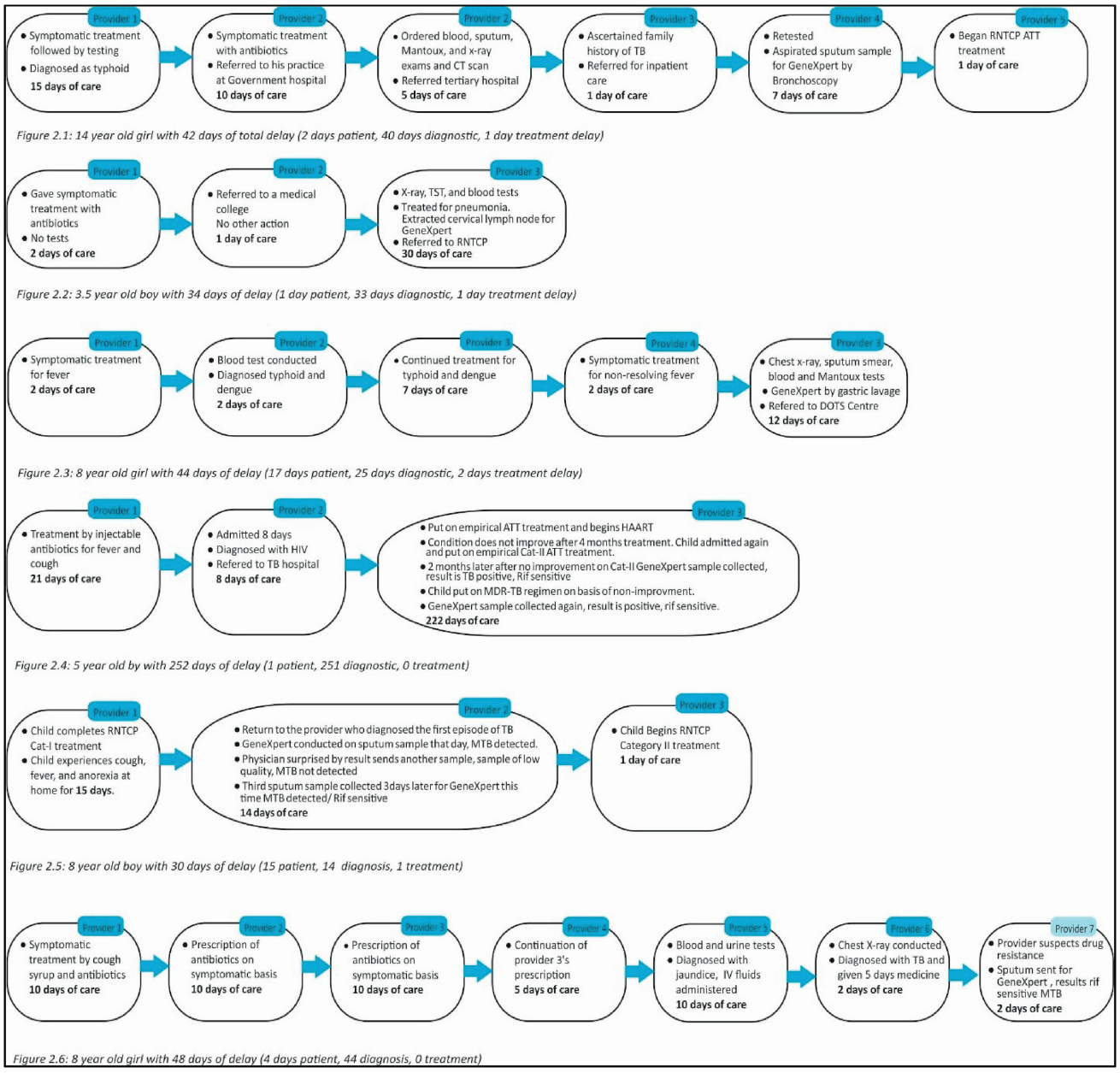

Fig. 2 – 6 randomly selected patient pathways to treatment on the basis of microbiological confirmation with critical events attached to each physician visited. Endpoints for these pathways, like all endpoints presented here, are treatment on the basis of microbiological confirmation rather than treatment alone.

followed by x-rays (n = 64) (Table 1). As details of individual blood tests prescribed were not available, they were clubbed under the category of 'blood test'. Hence, the table probably underestimates the diversity of blood tests undertaken by the patients.

More than three-fourth of the patients (79%) were prescribed a Xpert MTB/RIF test by physicians from the public sector. Approximately 40% of families of those interviewed, reported difficulty in sample collection. Of all patients, 17% received other diagnoses (single or multiple) before being diagnosed with TB which included typhoid (N = 11), pneumonia (N = 3), dengue (N = 2), HIV (N = 1), malaria (N = 1), and

Table 1 – Summary of tests performed before Xpert testing.

Tests	Number of patients
Sputum smear	77
X-ray	64
Mantoux test	19
Blood test	17
CT Scan	12
Urine test	10
Lymph node fine needle aspiration	6

jaundice (N = 1) among others (N = 5). Eighty percent of school going children missed school for over a week.

Twenty-nine percent had a history of anti-TB treatment at the time of Xpert MTB/RIF testing (median days of treatment = 180 (IQR: 30, 193)). A history of TB treatment was documented in 23.5% of the 85 children diagnosed with drug sensitive TB (median days of treatment = 82.5 days (IQR: 16.5, 180)) and 60% of the 15 RIF resistant (RR) TB patients (median days of treatment = 180 days (IQR: 150, 210)).

3.2. *Qualitative results*

Very few families expressed a concern about the expenditure caused by TB. Instead, they focused on their willingness to provide the needful treatment despite considerable inconvenience irrespective of sex and age of child (Fig. 3).

Parents often reported taking steps to separate their children from others as a social responsibility (e.g., removing the child from school and limiting children's interactions with neighbors) despite resistance from children. Parents were

As my husband has died, I have to take care of my children. If they are not well there is no reason for me to live on in this world. So, whatever happens, I must make sure my daughter is cured completely ***(Mother of a 10-year-old girl)***

Her condition was really bad. She was really weak, like she was sick. She did not look good. She would get up, bathe and then go back to sleep again. She would eat ice cream and then cough, so we would worry because she started to cough again. But then when it did not go away, we wondered. It's a TB symptom, right, when there is two weeks of cough so we started to wonder. ***(Mother of a 13-year-old girl)***

From here to there, the doctors sent us everywhere. From there to here again we ran around in the sun for several days. ***(Father of a 13-year-old girl)***

She was hospitalized for two weeks and we spent 25,000 rupees (500 USD) on her treatment. They treated her for typhoid and all kinds of things. Then when she felt better, they discharged her, but after a few days she still did not improve and finally we went to another doctor. This time the test was free and the admission was less expensive. ***(Father of a 5-year-old girl)***

I felt very bad that my child had TB at such a young age. We do not have any family history of TB but I was worried about a swelling in my son's neck. It was diagnosed as TB which is curable. We thought that it might be cancer. He does well in school and I think a lot about his future. How he will continue his study? He left the school for a month but he has scored 474 marks out of 500 on the 10th grade exam. If he was not sick maybe he would have scored more. He is cured now, and did not miss much school because he was sickest during the summer vacation. I am still worried about his future, perhaps we will get TB again someday. This scares me. ***(Father of a 14-year-old boy)***

Fig. 3 – Willingness of families to provide treatment to children with TB.

distressed by their felt necessity of limiting their child's movement (Fig. 4).

Families of children suffering from TB expressed considerable distress on receiving a TB diagnosis (Fig. 5). Mostly, this stemmed from concern for the immediate well-being, as well as the future of their child. The latter included worry about success in school, lifelong physical well-being, prospects of marriage, and a later recurrence of TB. Alongside stigma, they expressed a concern both for the effects of the disease and its treatment on their children's wellbeing regardless of sex or age.

Many parents reported confusion about the source of their child's illness, and this had serious social repercussions for the children and their families (Fig. 6). Some parents reported suspecting their family members, themselves, classmates or neighbors as the source of their child's illness.

All my family members said, "Keep her far from other children. Don't send them out too much together. Don't let them eat from her plate. Use separate plates and utensils for her. Feed her separately." ***(Mother of a 10-year-old girl)***

My daughter is only 6 months old. How she can get TB at this age? In my family no one had TB. How she could get TB? At first I was really sad. Even now I do not tell anyone about her illness. They will ignore her and in our circle they will not let their children play with her. ***(Mother of a 6-month-old girl)***

We thought that perhaps she could spread the TB infection to everyone in the family. The doctor explained to us that this is not the case and that TB is curable. Still I instructed my wife to keep her away from her siblings. She has to eat and sleep separately. We keep separate utensils aside for her. ***(Father of a 9-year-old girl)***

The neighbors avoided him, yes, but we didn't worry about the neighbors. The teacher at school told him 'every time you cough use your hand kerchief so it will not spread to the other children.' One incident happened in school: One time when he did not take the tablets in the morning meaning he forgot to take morning dose and went to school. I realized and I went to school and gave him the medicine there, but the teacher was upset and really scolded me, 'don't give medicine in the school and keep it in the school. If you want to treat him, then take a month of leave for him and keep him at home. Come back after the full treatment.' So from the next day onwards, we made sure to give him the medicine only at home. ***(Mother of a 7-year-old boy)***

I told her to refuse to get too close other children because I know she has TB but other people don't know about her illness. When I kept her from playing with her friends sometimes, she used to cry and say 'why are you also avoiding me like that mom". So in these cases I tell her, 'only we know that you have TB. They don't know. Because of that I am telling you to avoid them. Don't cry because when you are cured I will allow you to play with them again.' ***(Mother of a 13 year-old-girl)***

Fig. 4 – Stigma related experiences including isolation.

I am worried about her future. She is among the best students in her school but due to her sickness she cannot concentrate on her studies. She does not play with anyone. She always seems depressed so we try to involve her in other things hoping that she will feel good. I always tell her to go and play but she is not able to go. ***(Father of a 14-year-old girl)***

I am very worried about her marriage. She is a girl, who will marry her if they know about TB? It can spoil her marriage, her education, and her future, but I have faith in my daughter. She will be fine and live her life like before. ***(Mother of an 11-year-old girl)***

I was very worried about his health, studies, and future. I am afraid that TB might affect his children too. He is short for his age. We are worried that this will affect his growth, but he is better now. He is gaining weight. ***(Father of a 3.5-year-old boy)***

All the members of our family and relatives were worried when they heard about the TB diagnosis. They are all anxious about what might happen with her. Will she be fine or not? Will she survive or not? We are all praying for her. I am also worried about her education, due to TB she cannot start school. I want her to start school soon but I am delaying her admission because I do not want to expose the other children. ***(Father of 3.5-year-old girl)***

Fig. 5 – Distress experienced by families of children with TB.

Parents who had been successfully treated for TB were less fearful of their children's ability to complete treatment and live a full life (Fig. 7). Most parents reported some concern about sample collection, particularly related to collection procedures of non-sputum samples (e.g., gastric lavage). However, these concerns were largely overshadowed by a general concern for the child's health, anxiety about the child's future and contemporary health status.

4. Discussion

In this manuscript, using a mixed methods approach, we investigated several themes that characterize pediatric TB patient care pathways before being prescribed Xpert MTB/RIF testing. Our findings indicate the significant physical and social distress that pediatric TB patients and their families undergo during the TB care pathway, and bottlenecks such as large time delay between first care seeking and TB diagnosis.

Literature shows that pathways to TB care among adults are characterized by significant patient and diagnostic delay.[6,7,19] However, our analysis documents notably lesser '*patient delay*' and longer '*diagnostic delay*', associated with pediatric TB care pathways relative to adults. A recent study conducted in Delhi on pediatric TB patients documented similar findings.[10] Pediatric patients presenting with non-specific symptoms could be a key factor contributing to the longer diagnostic delay.[20,21,22] Also, the patterns of resort in India differ with age, with children being taken to a physician more quickly than adults.[23,24]

Approximately 29% of children were prescribed Xpert MTB/RIF after treatment initiation, suggesting potential drug resistance or a desire to arrive at microbiological confirmation by physicians. Our findings suggest that apart from approaching several physicians for correct diagnosis, considerable number of diagnostic tests and empirical drug exposure occur in the course of seeking care. The similar duration of

Why it is happening to my child? It's my fate and that's why the child has TB. My heart is breaking. I'm worried about the child's marriage. How will she find a husband or have children? ***(Mother of a 14-year-old girl)***

The landlord had TB. They live in the same complex. I think maybe it spread from him because he used to spit in bathroom and in the courtyard. We share the same bathroom maybe that is one of the reasons it spread. We began planning to move after she was diagnosed with TB. ***(Mother of a 12-year-old girl)***

Maybe my daughter got TB in school. Maybe she drank water from other children's bottles. Perhaps that is why she now has TB. ***(Father of a 9-year-old girl)***

We lived as a joint family with my mother-in-law, nephews and everyone. I suspect it came to him when we all lived together, maybe. Now we have moved to live alone and he never goes there. ***(Mother of a 10-year-old boy)***

I was most worried about what would happen when the treatment starts. How will she take medicines for TB? She is too young. It is my fault. My daughter got TB just because of me. I had TB when she was of only a few months old and nobody instructed me how I could prevent spreading TB to my children. My son also gets fever frequently, maybe he also has TB. My children are suffering just because of me. ***(Father of a 1-year-old girl)***

Fig. 6 – Perspectives of families' on how their children got infected with TB.

diagnostic delay among patients with and without family history of TB suggests that physicians may have incorrectly appreciated the risk of TB among these patients or the utility of Xpert MTB/RIF as a diagnostic aid. Since diagnosing physicians have accessed Xpert MTB/RIF and received a definitive diagnosis of TB, it is unknown how much longer any physician may have waited before empirically diagnosing TB[25] or using a standardized diagnostic algorithm if they did not refer for/have access to Xpert MTB/RIF testing.[14,26,27] These findings reiterate the challenges in diagnosing TB among children, necessitating availability of upfront access to quality and affordable diagnostics and need to generate awareness among providers.[28]

Despite families visiting two private providers for every one public sector provider, samples for Xpert MTB/RIF testing were mostly sent by public sector physicians. This finding is consistent with studies that show low sputum test utilization by private providers.[29,30] Referrals to hospitals and subsequent admissions by physicians capable of collecting non-sputum samples like broncho-alveolar lavage (BAL) and gastric lavage were an additional source of delay. Cost of sample collection also led patients to public sector hospitals where the diagnostic algorithm was reinitiated leading to further delay in diagnosis. This reinforces the need to help patients' access facilities with superior sample collection capabilities and avoid costly hospitalizations.

We also documented a high rate of diagnoses other than TB which suggests that either children were ill with diseases like typhoid or dengue along with TB, or the treating providers made incorrect diagnosis in favor of diseases they felt might correspond to the child's state of health. The physical and social impact prior to the delayed microbiological confirmation of TB was considerable and can be mitigated by upfront access to Xpert MTB/RIF testing.

Because we have had TB in the family I was not so scared for my daughter. We know it can be cured, but for some time she was too weak to digest the medicines and she would vomit. We had to forcefully give her the medicines and she told us that she would commit suicide because of the disease. Before that we were not worried for her but now we are afraid. She is ready to take injections only and we have no problem with that. At least she is taking something otherwise she will not take injections too. Everyone is tired of explaining all the things to her but she doesn't want to understand anything. She thinks only about her studies. When she was taking medicines, she forgot everything even who is she or where she is. After vomiting, however, she became ok. Maybe those were side-effects of TB because she was too weak to digest the medicines. ***(Father of a 14-year-old girl)***

I heard the news that she had TB, but I was not scared. I already knew about TB and its treatment, that its curable. I was diagnosed with TB 8 or 9 years ago when she was only 2 years old. So I am sure that after taking the proper and complete treatment, she will be alright. But still I did not disclose the news of TB to anyone in my community because I think that it can create a major problem at the time of her marriage. When the DOTS center staff asked for a home visit I asked them not to say anything to the neighbors. ***(Father of an 11-year-old girl)***

I was treated for TB so I know that she can get better, but I also know how difficult the medicines were for me. So I am worried more about what will happen to her now with the medicines. They are so many and she is just small. I am not really worried about her future. ***(Mother of a 14-year-old girl)***

It cannot feel good. After the biopsy at the time we were already suffering and then having heard the diagnosis I was out of my mind. TB medicines are so hard. I thought about what would happen to her after she eats them? Just thinking about that I was really disturbed. ***(Mother of a 12-year-old girl)***

Fig. 7 – Outlook of parents with history of TB on their child's TB treatment.

Both qualitative and quantitate data suggest that increased awareness and access to Xpert MTB/RIF among physicians and patients is necessary. Xpert MTB/RIF is an important arbiter when other tests remain inconclusive or with history of TB treatment.[31] According to patient narratives, physicians resorted to Xpert MTB/RIF testing when a) they suspected TB but had inconclusive x-ray, sputum, or tuberculin skin test, or b), they observed little or no improvement after starting empirical TB treatment which may include suspicion of RR TB, or c) children had symptoms of extra-pulmonary TB. The pediatric pathways show that Xpert MTB/RIF ended the diagnostic process and provided certainty to physicians and family to initiate/continue TB treatment. The lengthy diagnostic delays combined with no significant difference in time to Xpert referral between patients with and without family history show that even when available free of cost, upfront Xpert MTB/RIF testing for pediatric presumptive TB cases appears to be a test of last resort.

Qualitative data show that parents were not as concerned about the difficulty of sample collection as they were about

the wellbeing of their children. We documented high levels of perceived stress TB diagnosis puts on families through risk of stigma, disruption of education, concerns for the child's future health and social status. Hence, early diagnosis and prompt linkage to treatment and appropriate communication strategies for increasing awareness about pediatric TB and destigmatizing it become even more essential.

4.1. *Limitations*

Our study had few limitations. First, we enrolled only children diagnosed on the basis of Xpert MTB/RIF. Sample was selected from those diagnosed under the project which facilitated upfront Xpert testing. Hence, it is not a representative of the larger group with limited access to Xpert MTB/RIF. Hence, the delays may be underestimated. However, there is limited existing data on pediatric pathways to compare. By sampling from Xpert MTB/RIF confirmed patients, our study can be certain that previously undocumented and extensive suffering experienced by sampled patients' illness is most likely caused by TB. Nevertheless, we limit our claims to longer diagnostic delay. Also, the turnaround time for Xpert MTB/RIF could not be compared with smear microscopy as this data was not collected for samples sent for microscopy. Second, it is impossible to know if diagnoses other than TB were earlier misdiagnoses or actual comorbidities. However, they reveal the diagnostic challenges posed by pediatric TB and efforts undertaken by physicians and patients to obtain a diagnosis. Finally, recall bias and self-censoring may reduce "patient delay" and distort the events that occurred in the patient pathway to present parents in a positive light. Further studies are needed to corroborate our findings.

5. Conclusion

Our findings suggest that care seeking behavior on the patient's side is a less significant obstacle than sensitizing physicians to recommend Xpert MTB/RIF test alongside or before other tests, particularly in children with history of TB contact. Despite the provision of free of cost Xpert MTB/RIF testing for all presumptive pediatric TB cases in the public sector and access at reduced priced of Xpert in the private sector,[32] physicians, irrespective of the sector, are less aligned towards this best practice. Accordingly, efforts to make Xpert MTB/RIF testing more accessible will be of considerable value despite the complexity of TB diagnosis in children. Second, providers need to be sensitized to ensure that Xpert MTB/RIF is seen as a part of their toolkit for diagnosing TB in children. This is further evidenced by the fact that enrollment in the current project was achieved only after increased interventions like Continued Medical Education sessions, individual sensitization of physicians, provision of sample transport options, and quick reporting. Lastly, based on the findings of qualitative analysis, communication strategy needs to be developed and implemented to generate awareness among the general population to demystify the apprehensions around diagnosis and treatment of pediatric TB and destigmatize it. Overall, need of the hour is that the TB control strategies look beyond policy to provide high quality holistic care to pediatric patients.

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Conflicts of interest

The authors have none to declare

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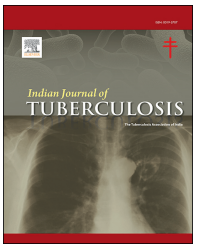

Original article

Study to identify incidence and risk factors associated Residual pleural opacity in tubercular pleural effusion

*Deependra Kumar Rai**, *Somesh Thakur*

Department of Pulmonary Medicine, AIIMS, Patna, 801505, India

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ABSTRACT

Introduction: Residual pleural opacity (RPO) is a common radiographic sequela in patients with tubercular pleural effusion at the end of the treatment. This study was designed to find out the risk factors associated with residual pleural opacity (RPO).

Materials & methods: This was a prospective longitudinal study performed to analyse data of 56 patients (46 males & 10 females) who were diagnosed as tubercular pleural effusion and treated for the same between 1st Jan 2019 to 30th March 2020. Chest X-ray posteroanterior & Lateral view was done (performed) at 0 and 6 months of treatment to quantify the amount of pleural effusion and measured the residual pleural opacity at the end of the treatment. RPO included both non resolving pleural effusion as well as residual pleural thickening (RPT). All statistical analysis was done using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Multivariate logistic regression was performed to explore the association of risk factors and Residual pleural opacity. The statistical significance level was set at 0.05 (two-tailed).

Results: The incidence of Residual pleural opacity (RPO) at the end of 6 months of antituberculosis treatment was 53.57% (30/56)). The study patients were divided into RPO and non- RPO group. Male gender had significantly higher incidence of RPO (93.3% vs 69.2% $P = 0.01$)). Patients with RPO group had significantly more cough and weight loss as compared to non RPO group (96.6% vs 65.3% $P = 0.002$ and 60% vs 23% $P = 0.005$). The proportion of patients who underwent therapeutic aspiration and gained weight of more than 5kg during treatment (19.5% vs 7.6% $P = 0.02$ & 46.6% vs 7.6% $P = 0.001$) was significantly higher in RPO group. A significantly lower protein, glucose and higher LDH level in pleural fluid was observed in the RPO group compared to non-RPO group ($P = 0.006$, $P = 0.01$, $P = 0.001$)). No significant difference was found in the pleural fluid ADA, lymphocyte, neutrophil levels between the two groups ($p > 0.05$). Logistic regression analysis showed that the male gender, low pleural fluid glucose, presence of cough and weight loss were associated with significantly increased risk of residual pleural opacity and thickening ($p < 0.05$).

* *Corresponding author*. Tel.: +91 7764981421.
E-mail address: deependra78@gmail.com (D. Kumar Rai).
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Conclusion: Tubercular pleural effusion is associated with residual pleural opacity in more than half of the patients. Male gender and low glucose levels in pleural fluid was associated with increased risk of residual pleural opacity.

1. Introduction

Tuberculous pleural effusion is the second most common form of extrapulmonary tuberculosis after lymph node TB and is the most common cause of pleural effusion in areas where tuberculosis is endemic.[1] The gold standard for the diagnosis of tubercular pleural effusion is demonstration of *Mycobacterium tuberculosis* in pleural fluid, or pleural biopsy specimens, either by microscopy, molecular test and/or culture, or demonstration of caseating granulomas in histopathological examination of pleura along with acid fast bacilli (AFB). However, in high burden countries like India, the diagnosis is mostly made on the basis of exudative nature, lymphocytic predominance and high adenosine deaminase (ADA) levels in the pleural fluid. Anti-tubercular treatment is the same as in pulmonary tuberculosis. Residual pleural opacity (RPO) is a common radiographic sequela in patients with tubercular pleural effusion after completion of treatment. Recent study[2] shows that 23.59% of Pleural effusion patients developed residual pleural lesions, of which 21.2% developed pleural thickening, and 2.4% developed pleural calcification. There are few studies which have assessed the factors associated with residual pleural thickening.[3,4] This study was designed to find out the incidence and factors associated with residual pleural opacity (RPO) in tubercular pleural effusion.

1.1. Objective

1. Proportion of tubercular pleural effusion developed residual pleural opacity at end of six month of treatment
2. Factors associated with Residual pleural opacity

2. Material & methods

Type of study: Prospective Longitudinal study.

Sample size: All newly diagnosed tubercular pleural effusion patients who have completed treatment between 1st Jan 2019 to 30 March 2020 were enrolled.

2.1. Inclusion criteria

1. Age − 18 years or more
2. Patients with newly diagnosed Tubercular Pleural effusion
3. Presence of tubercular evidence in the form of at least one
   a. Pleural fluid ADA>45 U
   b. Acid fast bacilli demonstrated in ZN stain or Pleural fluid culture.
   c. Pleural biopsy showing granuloma.

2.2. Exclusion criteria

1. Patients with Pleural effusion who are already on treatment
2. Those with Concomitant pulmonary tuberculosis
3. HIV positive patients
4. Patient who have received Antitubercular treatment in the past for Pulmonary tuberculosis

All newly diagnosed tubercular pleural effusion patients were included in this study. All Patients underwent (go) routine blood investigations, Pleural fluid analysis was done for protein, glucose, lactate dehydrogenase (LDH), adenosine deaminase (ADA), cytology, GeneXpert and Acid-fast bacilli (AFB) smear examination. Patients were treated with standard antitubercular treatment provided by the Government of India in fixed dose combination (FDC) for six months.

Chest X-ray posteroanterior & Lateral view was done (performed) at 0 and 6 months of treatment. We quantified the amount of effusion in chest x-ray as small (less than one 1/3rd), moderate (between 1/3rd to 2/3rd) and large (more than >2/3rd of hemithorax).[4] All patients with pleural effusion of more than 1/3rd of hemithorax were advised for therapeutic pleurocentesis. The presence or absence of RPO was determined by the pleural based opacity on chest radiographs, which consisted of nonresolving pleural effusion as well as true pleural thickening.[6] Pleural thickening was measured in the posteroanterior view of chest radiograph. We measured the distance from the lateral chest wall to the innermost margin of the opacity at the level of the highest point of the hemidiaphragm. Patients with opacity of more than 2mm of pleural thickness as measured by scale at 24 weeks were defined to have residual pleural thickening.[4,5]

Residual pleural opacity (RPO) = non resolving effusion + residual pleural thickening (RPT)

Types of outcome measures.

1. Proportion of patients who developed Residual pleural thickening (RPO)
2. Risk factors associated with Residual pleural opacity

2.3. Statistical analysis

Continuous data was presented as mean ± SD and categorical variables were presented in relative frequencies, and percentages. All statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Comparisons were made by t-test or Mann–Whitney U-test for continuous variables and Chi-square test or Fisher exact test for categorical variables. Univariate and multivariate logistic regression

analysis was performed to explore the association of risk factors and residual pleural opacity. The statistical significance level was set at 0.05 (two-tailed).

3. Results

A total of 74 patients were diagnosed as tubercular pleural effusion in the defined period. 18 patients were excluded as they were found to have (with) concomitant pulmonary tuberculosis (n = 11) or lost to follow-up (n = 7) (Fig. 1). Consequently 56 patients (46 males & 10 females) were available for final analysis. Baseline characteristics are summarised in Table 1. The incidence of Residual pleural opacity (RPO) at the end of 6 months of anti-tubercular treatment was 53.57% (30/56) (Fig. 1). 9 patients were found to have residual pleural effusion for which antitubercular treatment was extended for another 2 months. The study patients were divided into RPO and non RPO group. Male gender had significantly higher incidence of RPO (93.3% vs 69.2% P = 0.01). Patients with RPO group also had significantly more cough and weight loss as compared to non RPO group (96.6% vs 65.3% P = 0.002 and 60% vs 23% P = 0.005). There was also a higher proportion of patients in RPO group who underwent therapeutic aspiration and had a weight gain of more than 5kg during treatment (30% vs 7.6% P = 0.02 & 46.6% vs 7.6% P = 0.001). In the remaining variables, no significant difference was observed between the two groups.

A significantly lower protein, glucose and higher LDH level in pleural fluid was observed in the RPO group as compared to (in)non-RPO group (P = 0.006, P = 0.01, P = 0.001) Table 2. No significant difference was found in the ADA, lymphocyte, neutrophil levels of pleural fluid between the two groups (p > 0.05).

Multiple logistic regression analysis demonstrated that the presence of male gender (odds ratio [OR], 6.22; 95% confidence interval [CI], 1.18 to 32.68; p = 0.03), presence of cough (odds ratio [OR], 15.35; 95% confidence interval [CI], 1.78 to 131.93; p = 0.01), Weight loss (odds ratio [OR], 5.00; 95% confidence interval [CI], 1.5538 to 16.08; p = 0.007) and Glucose less than 60mg/dl in pleural fluid (odds ratio [OR], 4.81; 95% confidence interval [CI], 1.33 to 17.38; p = 0.01) were the predictors of residual pleural opacity (RPO) on chest X ray in those with tubercular pleural effusion [Table 3].

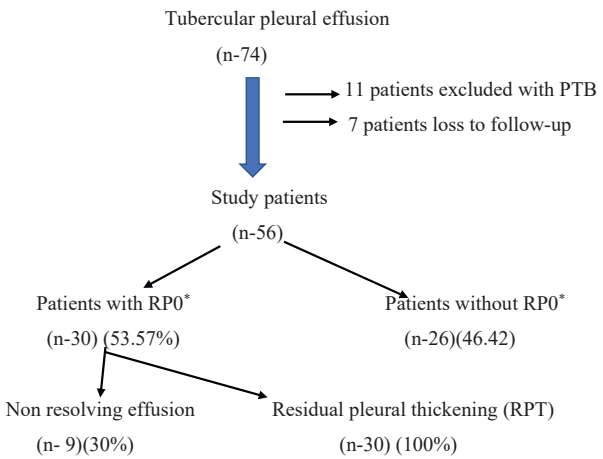

Fig. 1 – Flow diagram of study patients (* RPO- Residual pleural opacity).

4. Discussion

This study showed that more than half of the patients (53.7%) had residual pleural opacity (RPO) and a similar proportion of patients also had residual pleural thickening (RPT) at the end of the treatment. Incidence of RPT varies across studies depending upon cut off level used to define pleural thickening. One of the studies[7] showed an incidence of 37%, but in most other studies it exceeded 40% when a cut-off of 2 mm is used.[3,8] Our study also had a higher incidence as lower cut off (2 mm) was used to define RPT. One recent Indian study which measured pleural sequelae radiologically showed residual pleural thickening in 23.59% of patients.[2]

A significant difference was seen in cough, weight loss and weight gain between RPO and non RPO group (p < 0.05). History of therapeutic aspiration was associated more with the RPO group when compared to the non RPO group and this could be due to a greater number of patients with larger effusion in the former group. However, the influence of therapeutic aspiration in the development of RPT is not known as per several studies.[4,9] One study showed that complete removal of pleural fluid does not appear to reduce the amount of residual pleural thickening.[10]

There was significantly lower protein, glucose and higher LDH level in pleural fluid observed in the RPO group as compared with the non-RPO group (p < 0.05)). Although no significant difference was found in ADA, lymphocyte, or neutrophil levels of pleural fluid between the two groups. Our study as supported by previous studies which showed that lower pleural fluid glucose and higher LDH levels was associated with increased risk of residual pleural thickening.[6,11,12] Corticosteroid is also used in treatment of tubercular pleural effusion as adjunctive therapy. It could reduce the duration or severity of symptoms in the short term, and also reduce the risk of tissue damage leading to lung impairment in the long term. In a Cochrane systematic review, Engel et al[13] found that corticosteroid use had no appreciable effect on the resolution of pleural effusion at eight weeks and development of pleural adhesions. Although our study was not primarily design to assess the effect of corticosteroid on residual pleural opacity, we found in patients who received oral corticosteroid (n-12,21.4%), residual pleural opacity observed in 4 patients (30.7%). Proportion of patients received oral corticosteroid was higher in Non RPO group comparing RPO group but statistically not significant (30.7% vs 13.3% P > 0.05).

Logistic regression analysis showed that male gender, low glucose, presence of cough and weight loss was associated with significantly increased risk of residual pleural opacity and thickening. Many other studies have showed that lower pleural fluid glucose was associated with residual pleural thickening.[8]

The present study had several limitations. The incidence of RPO in this study was higher because a lower cut off was used for residual pleural thickening although selection bias could

Table 1 – Baseline characteristics of study patients.

Variables	Total (n-56)	RPO group (n-30)	Non RPO group (n-26)	P value
Age	37.14 ± 16.91	38.40 ± 16.79	35.69 ± 16.91	0.5
Male (%)	46 (82.1)	28 (93.3)	18 (69.2)	**0.01**
Presenting symptoms				
Fever	40 (71.4)	22 (73.3)	18 (69.2)	0.7
Cough	46 (82.1)	29 (96.6)	17 (65.3)	**0.002**
Dyspnoea	30 (53.5)	18 (60)	12 (46.1)	0.2
Chest pain	44 (78.5)	26 (86.6)	22 (84.6)	0.8
Anorexia	36 (64.2)	22 (73.3)	14 (53.8)	0.1
Weight loss[a]	24 (42.8)	18 (60)	06 (23)	**0.005**
Chest radiograph finding				
Amount				
Small	20 (35.7)	10 (33.3)	13 (50)	0.2
Moderate	28 (50)	14 (46.6)	12 (46.1)	0.9
Large	08 (14.28)	06 (20)	01 (3.8)	0.06
Right: Left	34:22 (60.7: 39.2)	18:12 (60:40)	16:10 (61.5:38.4)	0.9
Weight gain ≥ 5kg	16 (28.5)	14 (46.6)	02 (7.6)	**0.001**
Therapeutic aspiration	11 (19.6))	09 (30)	02 (7.6)	**0.02**
OCS[b]	12 (21.4)	04 (13.3)	08 (30.7)	0.1
Diabetes	03 (5.3)	02 (6.66)	01 (3.84)	0.6

[a] History of >5kg weight loss in last 3 months).
[b] History of oral corticosteroids for at least 3 weeks, Number in bracket showing proportion, P value in dark colour means significant ($p < 0.05$).

Table 2 – Comparison of pleural fluid characteristics.

Plural fluid characteristics	Total (n-28)	RPT (n-15)	Non RPT (n-13)	P value
Total protein (g/L)	5.01 ± 1.18	4.85 ± 1.42	5.19 ± 1.18	**0.006**
Glucose (mg/dl)	76.81 ± 31.19	63.49 ± 31.29	91.24 ± 31.19	**0.001**
LDH (U/L)	655.80 ± 392.03	752.69 ± 392.45	517.02 ± 337.64	**0.01**
Lymphocyte (%)	79.36 ± 11.11	77.2 ± 11.26	82.6 ± 11.11	0.9
Neutrophil (%)	22.15 ± 14.49	22.13 ± 15.14	22.18 ± 14.49	1.0
ADA (IU/L)	61.01 ± 39.57	57.01 ± 41.33	65.31 ± 39.57	0.4

P value in dark colour means significant ($p < 0.05$).

Table 3 – Logistic regression analysis for association.

Variables	OR (95% confidence intervals)	P value
Male gender	6.22 (1.18–32.68)	**0.03**
Cough	15.35 (1.78–131.93)	**0.01**
Weight loss	5.0 (1.5538–16.08)	**0.007**
History of therapeutic aspiration	5.14 (0.99–26.52)	0.05
Protein (<5 gm/L)	2.22 (0.69–7.15)	0.1
Glucose < 60 mg/dl	4.81 (1.33–17.38)	**0.01**

P value in dark colour means significant ($p < 0.05$).

not be ruled out. Second limitation would be of small sample size from which generalisation cannot be made. The diagnosis of tubercular pleural effusion in this study was mostly made on clinical and biochemical analysis of pleural fluid, which is not a gold standard and based on which (possibility of) other possible aetiology cannot be ruled out. There was no measurement of the degree of residual pleural thickening which could have more clinical significance. CT chest would have be a better modality to identify and characterise radiological sequelae, which was not performed in this study. Lastly, pulmonary function test was not done in the present study and hence functional limitation could not be estimated.

5. Conclusion

Tubercular pleural effusion was associated with residual pleural opacity in more than half of the patients in our study. Male gender (patients) and pleural fluid with low glucose level were two most common factors associated with residual pleural opacity.

Conflicts of interest

The authors have none to declare.

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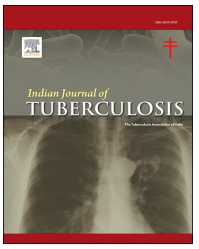

Original article

Study of treatment outcomes of multidrug-resistant tuberculosis under programmatic conditions and factors influencing the outcomes in Hyderabad District

Subhakar Kandi [a], *Tilak Kumar K* [b,*], *Shravika Reddy Kandi* [c], *Neeta Mathur* [d], *Challa Devi D* [e], *Rajesham Adepu* [f]

[a] *Department of Respiratory Medicine, Kamineni Academy of Medical Sciences & Research Centre, Hyderabad, Telangana, India*
[b] *Department of Respiratory Medicine, Government General & Chest Hospital, Osmania Medical College, Hyderabad, Telangana, India*
[c] *Mallareddy Institute of Medical Sciences, Hyderabad, Telangana, India*
[d] *Department of Community Medicine, Apollo Institute of Medical Sciences and Research, Hyderabad, Telangana, India*
[e] *District TB Control Officer, Hyderabad, Telangana, India*
[f] *Joint Director of Tuberculosis, Hyderabad, Telangana, India*

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ABSTRACT

Background: Treatment outcomes for Multidrug-Resistant Tuberculosis (MDR TB) is generally poor. The study aims to know about the treatment outcomes of MDR-TB under programmatic conditions in Hyderabad District and to analyze the factors influencing the treatment outcomes.

Methods: This is a retrospective study in which 377 patients of Hyderabad district, Telangana state who were diagnosed with MDR TB and registered at Drug Resistance TB Treatment site of Government General & Chest Hospital, Hyderabad from 4th quarter 2008 to 4th quarter 2013 were included in the study. Impact of Demographic factors (age, sex; Nutritional status (BMI); Co-morbid condition (Diabetes, HIV, Hypothyroidism); Programmatic factors (time delay in the initiation of treatment); Initial Resistance pattern on the outcomes were studied and analyzed.

Results: The treatment outcomes of Multidrug-Resistant Tuberculosis under Programmatic Conditions were: 57% cured, 21.8% died, 19.6% defaulted, 1.1% failed and 0.5% switched to XDR. Age, Sex, BMI had a statistically significant impact on treatment outcomes. Hypothyroidism and Delay in the initiation of treatment >1 a month had an impact on the outcomes though not statistically significant. NO impact on treatment outcomes was found when Rifampicin resistance & INH sensitive patients were compared with those resistant to both INH and Rifampicin.

Conclusion: To reduce MDR-TB transmission in the community, improvement of treatment outcomes, via ensuring adherence, paying special attention to elderly patients is required.

* *Corresponding author*. Petunia 1, Nectar Gardens, Madhapur Hyderabad, 500081, India.
E-mail addresses: subhakar_kandi@yahoo.co.in, drsubhakar@gmail.com (T.K. K).
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The Programmatic Management of Drug Resistance Tuberculosis (PMDT) should seriously think of providing Nutritional support to patients with low BMI to improve outcomes. In the programmatic conditions if we could address the problems like delay in initiation of treatment and proper management of comorbidities like HIV, Diabetes, Hypothyroidism would definitely improve the treatment outcomes.

1. Introduction

India (27%) shares the largest global burden of Multidrug-resistant (MDR) tuberculosis followed by China (14%) and the Russian Federation (9%). [1] Globally, 3.4% of new TB cases and 18% of previously treated cases had multidrug-resistant TB or rifampicin-resistant TB (MDR/RR-TB). [1] The global proportion of RR-TB cases estimated to have MDR-TB was 78%. [1] In India, the estimated incidence of MDR/RR TB in 2018 is 130 per lakh population with 69% of RR-TB with MDR-TB. [1] The proportions of new and previously treated TB cases with MDR/RR-TB in India are 2.8% and 14% respectively..[1]

Globally, the latest treatment outcome data has shown success rates of 85% for TB, 75% for HIV-associated TB, 56% for MDR/RR-TB, and 39% for extensively drug-resistant TB. [1] In a pooled analysis on patients with Drug-Resistant TB 65% [95% CI 59–71%] had a successful outcome, 15% [95% CI 12–19%] defaulted, 13% [95% CI 9–18%] died, and 6% [95% CI 3–11%] failed treatment for a total of 35% [95% CI 29–41%] with unsuccessful treatment outcome.[2]

Factors associated with worse outcome included male sex 0.61 (OR for successful outcome) [0.46–0.82], alcohol abuse 0.49 [0.39–0.63], low BMI 0.41 [0.23–0.72], smear positivity at diagnosis 0.53 [0.31–0.91], fluoroquinolone resistance 0.45 [0.22–0.91] and the presence of an XDR resistance pattern 0.57 [0.41–0.80].[3]

In view of the magnitude of the MDR problem and increased mortality both globally and in India, we made an attempt in the present study to systemically evaluate reasons for poor treatment outcomes under programmatic settings at our DOTS-Plus site.

2. Methods

This is a retrospective study. The study setting is a DOTS-plus center, Government General and Chest Hospital, Hyderabad, Telangana. It is a 670-bed hospital providing out-patient and in-patient care for TB and chest diseases. This tertiary hospital follows the WHO recommended guidelines for the management of TB patients.

In this study 377 patients of Hyderabad district, Telangana state who were diagnosed with MDRTB and registered at DOTS PLUS site from 4th quarter 2008 to 4th quarter 2013 were included in the study.

INCLUSION CRITERIA: MDR-TB patients belonging to Hyderabad District who is diagnosed according to PMDT guidelines and enrolled at DOTS PLUS site at G.G.C.H from 4th quarter of 2008 to the 4th quarter of 2013 and initiated on treatment.

EXCLUSION CRITERIA: 1. MDR-TB patients of other districts. 2. Privately treated MDR-TB patients.

Treatment outcomes and Impact of Demographic factors (age, sex); Nutritional status (BMI); Co-morbid condition (Diabetes, HIV, Hypothyroidism); Programmatic factors (time delay in the initiation of treatment); Initial Resistance pattern on the outcomes were studied and analyzed.

2.1. *Definitions*

FAVOURABLE OUTCOME is defined as Cured and Treatment completed and UNFAVOURABLE OUTCOME as died during treatment, lost to follow-up, treatment failure, Switched to XDR .[4,5.]

BODY MASS INDEX (kg/m2) were classified [6] as Underweight <18.5, Healthy weight 18.5–24.9, Overweight 25.0–29.9, Obesity Class I 30.0–34.9, Obesity Class II 35.0–39.9, Extreme obesity Class III $\geq$ 40.

THYROID STIMULATING HORMONE (thyrotropin): TSH levels 0.34–4.25 mIU/L is taken as the Euthyroid and patients with TSH >4.25 mIU/L were grouped into Hypothyroid and TSH < 0.34 mIU/L were Hyperthyroid.

DIABETES: In the present study diabetes was based on the treatment history and patients were grouped into Diabetics and Non Diabetics.

Delay in treatment initiation after diagnosis: Patients were divided on the basis of delay in initiation of treatment after diagnosis into those who were initiated on treatment in <1 month duration and >1 month duration.

All statistical analyses were performed using SPSS 16.0 software, version 16.0 (SPSS, Chicago). Comparisons of categorical variables were performed using the Pearson Chi-square tests to compare different groups.

3. Results

The treatment outcomes of Multidrug-Resistant Tuberculosis under Programmatic Conditions were: 57% cured, 21.8% died, 19.6% defaulted, 1.1% failed and 0.5% switched to XDR (Table 1).

3.1. *Factors influencing the treatment outcomes of MDR TB (Table 2)*

Based on the Age, patients were divided into <50 years group and >50 years group. Patients aged exactly 50 years old come in the >50 years group. They were analyzed statistically, which showed that patients within the age group <50 years

Table 1 – Treatment outcomes of patients with Multidrug-Resistant Tuberculosis under Programmatic Conditions.

	Number	Percent
Died	82	21.8
Lost to Follow up	74	19.6
Cured	215	57.0
Failed	4	1.1
Switched to XDR	2	0.5
Total Sample Size	377	100.0

had more Favourable outcomes (58.8% vs 42.5%) compared to those > 50 years with statistical significance of p = 0.037.

In the present study females had more favourable outcomes (65.9% vs 49.3%) and males had more unfavourable outcomes (50.7% vs 34.1%) with a statistical significance of p = 0.001.

After getting a statistical significance of the association of sex with treatment outcomes an attempt was made to look into the statistical significance of each the individual outcome with sex which gave the following results: Death rate and Lost to follow up rates were less and Cure rates were high in females with a statistical significance of the p-value of 0.016.

This analysis showed that patients in the underweight category that is with low BMI had less favourable (47.6%) and more unfavourable (52.6%) outcomes and also patients with normal BMI that is Healthy weight category had more favourable (64.4%) and less unfavourable (35.6%) outcomes with a statistical significance of p = 0.005.

In the present study Diabetes had no impact on treatment outcomes. In HIV Reactive patients both favourable and

Table 2 – Factors influencing the treatment outcomes of patients with Multidrug-Resistant Tuberculosis.

Factors	Favourable Outcomes	Unfavourable Outcomes	p value
Male (53.30%)	99(49.3%)	102(50.7%)	0.001*
Female (46.70%)	116(65.9%)	60(34.1%)	
Under weight (43.77%)	78(47.6%)	86(52.4%)	0.005*
Healthy weight (46.93%)	114(64.4%)	63(35.6%)	
Overweight (8.22%)			
Obese (1.06%)			
Age<50	198(58.8%)	139(41.2%)	0.037*
Age>50	17(42.5%)	23(57.5%)	
Non-Diabetic (91.77%)	195(56.4%)	151(43.6%)	0.451
Diabetic (8.23%)	20(64.5%)	11(35.5%)	
Hypothyroid (24.1%)	167(52.7%)	119(47.3%)	0.395
Euthyroid (75.9%)	48 (58.4%)	43(41.6%)	
HIV Reactive (3.7%)	7(50%)	7(50%)	0.595
HIV Non-Reactive (96.3%)	208(57.3%)	155(42.7%)	
Treatment Initiation< month (87.8%)	191(57.7%)	140(42.3%)	0.290
Treatment Initiation>1month (12.2%)	24(52.2%)	22(47.8%)	
R- Resistant (56.50%)	121 (56.8%)	92 (43.2%)	0.990
R+H Resistant (43.50%)	94 (57.3%)	70 (42.7%)	
* = Significant Statistically			

unfavourable outcomes were equal (50%) however in HIV Non-Reactive patients favourable outcomes were more (57.3% vs 50%) and unfavourable outcomes were less (42.7% vs 50%) compared to HIV Reactive patients though not statistically significant. Euthyroid patients had more favourable (58.4% vs 52.7%) and less unfavourable outcomes (41.6% vs 47.3%) compared to hypothyroid though not statistically significant.

The favourable outcomes were less (52.2% vs 57.7%) and unfavourable outcomes were more (47.8% vs 42.3%) in those patients were in treatment initiation was delayed by > 1 month though not statistically significant. No impact on treatment outcomes were found when Rifampicin mono resistance patients were compared with those resistant to both INH and Rifampicin.

4. Discussion

In the present study with 377 patients with MDR TB registered in the PMDT programme, the outcomes are: 57% were cured, 21.8% died, 19.6% defaulted, 1.1% failed and 0.5% switched to XDR. Overall, the proportion of MDR/RR-TB patients in the 2013 cohort who successfully completed treatment (i.e. cured or treatment completed) was 52%: 17% died, 15% were lost to follow-up, 9% were determined to be treatment failure, and 7% had no outcome information. [7] So in comparison to global data, the present study had almost similar outcomes but a little higher cure, death, lost to follow-up rates but treatment failure rates were low. The high proportion of patients who died (22%) can be due to the tertiary center of the state for MDR TB and may be due to adverse drug effects. The high proportion of patients who were lost to follow-up (20%) can be due to the long duration of treatment.

Examples of high MDR-TB burden countries with better treatment success rates (>70%) are Bangladesh, Ethiopia, Kazakhstan, and Myanmar. [1] As per the TB INDIA 2016 RNTCP ANNUAL STATUS REPORT: Indian RNTCP is the world's largest DOTS program achieving global targets of case finding and treatment success rate but the same success has not been achieved with PMDT.[7]

In a study done by Khan et al [8] in 179 patients with MDR TB, 133 (74.3%) were declared cured, 34 (19%) patients died during treatment, 10 (5.6%) were classified as treatment failures, and 2 (1.1%) patients defaulted during treatment. In a study done by L F Anderson et al [9] a total of 70.6% (144/204) of patients successfully completed treatment at 24 months or more. For those with unsuccessful outcomes 6.9% (14/204) had their treatment stopped, 6.4% (13/204) died, 7.8% (16/204) were lost to follow up, 2.9% (6/204) completed treatment within 12 months and 0.5% (1/204) completed treatment but relapsed. Ten of the 204 (4.9%) patients had neutral outcomes. In a meta-analysis by Pamela Weiss et al [2] 10 studies reporting outcomes on 1288 patients with Drug-Resistant TB were included for analysis. Of this population, 65% of patients had a successful outcome, 15% defaulted, 13% died, and 6% failed treatment for a total of 35% with unsuccessful treatment outcome.

In the present study Mean Age of the patients is 32 years which was similar to the study done by Ebru Unsal et al [10] In a study done by Shenjie Tang et al [5] mean age was 44 years. In a study by Khan et al [8] mean age is less compared to other studies probably that was the reason for more favourable outcomes compared to other studies. Hence the most productive age group is affected in the present study and other studies which show that MDRTB results in serious loss to their family in turn to the society and country.

In, the present study females had more favourable outcomes and males had more unfavourable outcomes. In a study done by Elliot et al [4] Male sex was associated with unfavourable outcomes as in the present study. In studies done by Shenjie Tang et a, [5] Ebru Unsal et al [10], Khan et al [8] sex had no statistically significant association with treatment outcomes in contradiction to the present study. In a systematic review and meta-analysis of various studies by James C. Johnston et al [3] showed that the Male sex had unfavourable outcomes as in the present study.

As per the TB INDIA 2016 RNTCP Annual Status Report [7]: Lower BMI is the major attributable factors observed for poor treatment outcomes in the country. Hence the finding in the study coincides with the national data. In a study done by Elliot et al [4] BMI was not recorded but Weight < 45 kg was associated with unfavourable outcomes. In a study done by Shenjie Tang et al [5] BMI less than 18.5 kg/m2 was associated with unfavourable outcomes. A systematic review and meta-analysis of various studies by James C. Johnston et al [3] showed that Low BMI had unfavourable outcomes as in the present study. The present study as in other studies has clearly shown that Low BMI is associated with poor treatment outcomes. It has also shown that MDR patients with normal BMI had good outcomes emphasizing the role of nutrition in the treatment outcomes of MDR patients.

In the present study Diabetes had no impact on treatment outcomes as the numbers were not comparable that is diabetics were less in the study compared to Non-diabetics. In a study done by Shenjie Tang et al [5] Diabetes was associated with unfavourable outcomes.

Analysis of the present study shows that in HIV Reactive patients both favourable and unfavourable outcomes were equal (50%) however in HIV Non-Reactive patient's favourable outcomes were more (57.3% vs 50%) and unfavourable outcomes were less (42.7% vs 50%) compared to HIV Reactive patients though not statistically significant. In a study by Mugabo.P et al [11] which was exclusively done on whether HIV infection and antiretroviral therapy influence multidrug-resistant tuberculosis treatment outcomes: HIV-infection and antiretroviral therapy did not influence MDR-TB treatment outcomes. Probably because of better HIV TB coordination and early initiation of ART as per new NACO guidelines the treatment outcomes in HIV Reactive group are on par with Non-Reactive group.

Delay in the initiation of treatment can be from programme perspective and also from a patient perspective. PMDT programme should work on appointing counselors as this would improve immediate treatment initiation and good adherence to treatment as it's seen in the ART programme for treating HIV patients. Proper counselling in patients treated for MDR would definitely improve treatment outcomes. Even though there is no statistical significance for the Impact of delay in initiation of treatment on treatment outcomes, early treatment initiation gives better results and prevents the spread of MDRTB in the community. So prompt diagnosis and

early initiation of treatment is the need of the hour to combat MDRTB and to achieve better treatment outcomes.

No impact on treatment outcomes was found when Rifampicin resistance & INH sensitive patients were compared with those resistant to both INH and Rifampicin. The influence of Polyresistance could not be evaluated because of the paucity of data on the initial resistance of the other drugs. The major attributable factors observed for poor treatment outcomes in the country data are resistance to FLQ, Ethambutol, lower BMI, and previous treatment episodes. [7] Hence in the country data Resistance to Fluoroquinolones and Ethambutol were associated with poor outcomes that were not taken in the present study. In a study was done by Khan et al [8] resistance to second-line drugs (SLD) and resistance to Ofloxacin was associated with poor outcomes. In a systematic review and meta-analysis of various studies by James C. Johnston et al [3] showed that fluoroquinolone resistance was associated with poor outcomes.

It is a retrospective study being carried out at a single center is the main limitation of the study. It may not be a true replica of all the districts. This study did not find out possible factors for lost to follow-up of treatment. Influence of Polyresistance could not be evaluated because of the paucity of data on the initial resistance of the other drugs.

5. Conclusion

In the programmatic conditions if we could address the problems like low BMI with good nutritional supplementation, treatment initiation without any delay after diagnosis, proper management of comorbidities like HIV, Diabetes, and hypothyroidism would definitely improve the treatment outcomes.

Credit author statement

Subhakar Kandi: conceptualized and designed and supervised the study. Tilak Kumar K: involved in Data Curation and writing original draft. Shravika Reddy Kandi: Actively involved in Visualization, software validation and writing. Challa Devi D: critical suggestions and inputs. Neeta Mathur: Reviewed and Edited the Manuscript. Rajesham Adepu: helped in logistic support and resources from program.

Conflicts of interest

All authors have none to declare.

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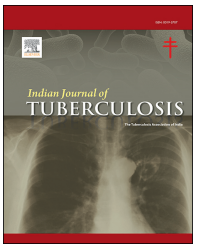

Original article

A study to assess the clinico-radiological presentation and outcome predictors in cases of tubercular meningitis

*Priya Jadaun, Rajesh Patil, Sharmila Ramteke, Manjusha Goel**

Department of Pediatrics, Gandhi Medical College, Bhopal, India

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ABSTRACT

Introduction: Tubercular bacterial meningitis continues to be an important cause of morbidity (especially neurologic handicap) in children from resource-poor countries. The present study was planned to assess the clinical and radiological presentation in cases of tubercular meningitis as well as to study the factors associated with mortality.

Methodology: This study was done over a period of 12 months on children between 5 years and 13 years with suspected TBM. Staging of tubercular meningitis was done according to **RNTCP Pediatric TB guideline 2019**.

Result: The study was conducted on a total of 47 pediatric patients with TBM. Mean age of children in present study was 8.77 ± 2.5 years. Our study documented male preponderance for TBM. Severe thinness was observed in 38.3% patients with TBM. Only 59.6% patients were immunized against tuberculosis and history of contact was documented in 40.5% patients. Maximum children belonged to stage I of TBM (59.6%) followed by stage III and stage II in 34% and 6.4% patients respectively. Montoux test positivity was observed in 14.9% patients only. CSF CBNAAT was positive in 6.4% patients. The most common finding was meningeal enhancement seen in 27.7% of patients followed by tuberculomas in 10.6%.Chest X ray was abnormal in 44.7% patients. In present study mortality was observed in 11 (23.4%) cases. Out of various risk factors, mortality was significantly associated with nutritional status and stage of TBM ($p < 0.01$).

Conclusion: TBM is associated with high morbidity and mortality in children especially in India where Burden of TB is high. Our study emphasized on the risk factors associated with mortality in children with TBM and need for early diagnosis and appropriate treatment.

* *Corresponding author.*
E-mail address: manjushagoel@rediffmail.com (M. Goel).

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1. Introduction

Infections of the central nervous system (CNS) pose a various challenges to the physicians, due to both the potential morbidity and mortality that they cause as well as the inherent difficulties involved in their treatment.[1] According to WHO global report 2019, an estimated 10.0 million (range, 9.0–11.1 million) people fell ill with TB in 2018 and of them 11% of cases were in children (aged <15 years).[2]

According to WHO[2] extra pulmonary TB account for 15% cases of overall tuberculosis cases.Tubercular meningitis contribute to one of the important cause of extrapulmonary TB among children in resource-poor countries. WHO estimated that about 25% of pediatric TB cases are extrapulmonary, with tuberculous meningitis (TBM) being the most severe form of TB and accounting for majority of the deaths due to TB.[2]The estimated mortality due to TBM in India is 1.5 per 100,000 population.[3]

The diagnosis of TBM require a detailed history and relevant investigations i.e. biochemical, immunological as well as radiological. The present study was thus planned to assess the clinical and radiological presentation in cases of tubercular meningitis as well as to study the factors associated with mortality.

2. Objective

1. To study the clinical as well as radiological presentation in cases of childhood tuberculous meningitis (TBM).
2. To study the factors associated with mortality in childhood TBM cases.

3. Methodology

The present study was conducted as a cross sectional study over a period of 12 months i.e. from 1st March 2018 to 28st Feb 2019.All the children in the age group of 5 years–13 years with suspected TBM, admitted at a tertiary care centre, Bhopal were enrolled. TBM was suspected based on following features: Fever and/or a cough for ≥2 weeks, neurological symptoms (irritability, refusal to feed, headache, vomiting, altered sensorium, abnormal movements or seizure) with weight loss or poor weight gain. TBM cases on treatment, those with malignancy or HIV and those on immunosuppressive therapy were excluded from the study. Staging of tubercular meningitis was done according RNTCP Pediatric TB guideline 2019.

3.1. *RNTCP pediatric TB guideline 2019*[4]

Stage I: Lasting for 1–2 weeks, with non specific symptoms such as fever, headache irritability/drowsiness, malaise, anorexia, inadequate weight gain or weight loss, stagnation or regression of development milestones.

Stage II: Begins abruptly and is characterized by increased intracranial pressure, meningeal irritability and vasculitis without marked changes in sensorium. Clinical constellation of lethargy, nuchal rigidity, Kerning and Brudzinski signs, seizures, hypertonia, vomiting, cranial nerve palsies with basal meningitis and other focal neurological deficits.

Stage III: Coma, hemiplegia or paraplegia, decerebrate posturing, deterioration in Vital signs.

After obtaining written consent from the parents/guardians of the patients, a detailed history regarding socio demographic profile, onset of symptoms, immunization and history of contact was obtained and entered in proforma. Apart from this, all the patients were examined clinically and findings were documented in proforma. The level of consciousness was assessed using modified Glasgow coma scale, signs of raised intracranial pressure (ICP) and meningeal irritation were also noted. All the children were further subjected to complete blood examination, Chest X ray, montoux test. Cerebrospinal fluid (CSF) analysis was done to determine the presence of AFB.

Table 1 – Distribution according to Baseline characteristics.

Baseline characteristics		Number	Percentage
Age group (years)	5 to 7	17	36.2
	8 to 10	19	40.4
	11 to 13	11	23.4
Gender	Male	31	66.0
	Female	16	34.0
Residence	Rural	17	36.2
	Suburban	16	34.0
	Urban	14	29.8
SES	I	4	8.5
	II	6	12.8
	III	17	36.2
	IV	15	31.9
	V	5	10.6
BMI	Normal	13	27.7
	Thinness	16	34
	Severe thinness	18	38.3
BCG vaccination		28	59.6
History of contact		19	40.5
Symptoms	Cough	17	36.2
	Fever	11	23.4
	Headache	17	36.2
	Vomiting	23	48.9
	Seizures	40	85.1
	Altered consciousness	33	70.2
Signs	Hemiparesis	9	19.1
	Cranial nerve palsy	6	12.8
	Meningeal irritation	32	68.1
	Raised Intracranial tension	25	53.2
GCS	Mild	14	29.8
	Moderate	23	48.9
	Severe	10	21.3
Stage of TBM	I	28	59.6
	II	3	6.4
	III	16	34.0

Table 2 – Findings of investigations in children with tuberculous meningitis.

Investigations		Number	Percentage
Montoux test		7	14.9
CSF appearance	Clear	26	55.3
	Turbid	21	44.7
CSF Protein (mg/dl)	40–400	46	97.9
	>400	1	2.1
CSF cell count (/mm^3)	<10	7	14.9
	10–100	31	65.9
	101–400	9	19.2
CSF Glucose (mg/dl)	<20	4	8.5
	20–60	34	72.3
	>60	9	19.1
Lymphocyte count (%)	<20	11	23.4
	20–80	23	48.9
	>80	13	27.7
CSF CBNAAT	Positive	3	6.4
	Negative	44	93.6
Chest X ray	Normal	26	55.3
	Abnormal	21	44.7
Neuroimaging	Hydrocephalus	2	4.3
	Meningeal enhancement	13	27.7
	Hydrocephalus with Periventricular edema and infarct	9	9.1
	Meningeal enhancement and Hydrocephalus	1	2.1
	Meningeal enhancement with infarct and hemorrhage	1	2.1
	Hydrocephalus with Periventricular edema and meningeal enhancement	2	4.3
	Hydrocephalus with Periventricular edema, infarct and meningeal enhancement	1	2.1
	Tuberculoma	5	10.6
	None	13	27.7

Statistical analysis- Data was compiled using Ms Excel and analysed using SPSS 20 software. Chi square test was applied to assess the association of mortality with various risk factors. $P < 0.05$ was considered statistically significant.

4. Result

The study was conducted on a total of 47 pediatric cases with TBM.

Mean age of children in present study was 8.77 ± 2.5 years and majority of children belonged to 8–10 years of age (40.4%) followed by 36.2% children in the age range of 5–7 years. Our study documented male preponderance 66% for TBM and rural population constituted 36.2%. About 36.2% patients belonged to socioeconomic status III followed by 31.9% patients belonging to SES IV.

Severe thinness was observed in 38.3% patients with TBM. Only 59.6% patients were immunized against tuberculosis and history of contact was documented in 40.5% patients.

Seizures and altered sensorium were the most common symptoms observed in 85.1% and 70.2% patients respectively. Signs of meningeal irritation were seen in 68.1% and raised ICT was observed in 53.2% patients with TBM. GCS was moderate in 48.9% patients whereas it was severe in 21.3% children. Maximum children belonged to stage I of TBM (59.6%) followed by stage III and stage II in 34% and 6.4% patients respectively.

Montoux test positive was observed in 14.9% patients only. CSF was turbid in 44.7% whereas raised CSF protein was observed in all patients. CSF cell counts were in the range of 10–100/mm^3 in 65.9% patients and CSF glucose for majority of patients was in the range of 20–60 mg/dl. CSF CBNAAT was present in 6.4% patients. Chest X ray was abnormal in 44.7% patients. Most common neuro imaging finding was meningeal enhancement observed in 27.7% cases.

In present study mortality was observed in 11 (23.4%) cases. Out of various risk factors, mortality was significantly associated with nutritional status and stage of TB ($p < 0.01$).(Tables 1–4).

For multivariate analysis, backward selection of variables was done. On analysis, none of the clinical variables were associated with poor outcome ($p > 0.05$).

5. Discussion

Our study aimed to assess the clinical and radiological presentation of childhood tubercular meningitis cases. Also we assessed various risk factors associated with mortality. Non specific symptoms of TBM in childhood pose a major challenge in its diagnosis. TBM needs to be diagnosed and treated

Table 3 – Factors associated with mortality in TBM cases.

Risk factors		Outcome		Chi sq value	P value
		Survived	Death		
Age group (years)	5–7	13	4	1.65	0.44
	8–10	16	3		
	11–13	7	4		
Gender	Male	26	5	2.69	0.10
	Female	10	6		
Residence	Rural	14	3	2.74	0.25
	Suburban	10	6		
	Urban	12	2		
SES	I	3	1	3.04	0.55
	II	5	1		
	III	15	2		
	IV	10	5		
	V	3	2		
BMI	Normal	13	0	23.2	0.001
	Thinness	16	0		
	Severe thin	7	11		
BCG		23	5	1.19	0.28
Contact History		16	3	1.34	0.51
Raised ICT		21	4	1.63	0.20
GCS	Mild	12	2	2.22	0.33
	Moderate	18	5		
	Severe	6	4		
Montoux	Present	5	2	0.12	0.73
	Absent	31	9		
TBM Stage	I	26	2	14.68	0.001
	II	3	0		
	III	7	9		

early as it is associated with high mortality and severe neurological sequelae especially in endemic countries like India.[5]

Most common age group affected in present study was 8–10 years (40.4%) followed children in 5–7 years. These findings were contrasting to study by Israni AV et al in which the authors documented most common age group with TBM as<3 years (60%) followed by 3–6 years (16%).[6] This observed discrepancy between present study and reference study could be explained by difference in inclusion criteria. Our study included children between age group of 5–13 years whereas the reference study involved children with age group of up to 14 years of age group/Globally, children <5 years of age have been found to be most vulnerable.[7,8]The male preponderance (66%) observed in the our study was consistent with earlier pediatric studies.[6,9]

Majority of patients with TBM were belonging to rural area and low socioeconomic class. Poor nutritional status i.e. severe thinness was observed in 38.3% children. Some of the major risk factors for increased risk of meningitis are poor socio-economic condition, overcrowding, recent colonization with pathogenic bacteria, close contact with patients, splenic dysfunction, and cerebrospinal fluid (CSF) communications (congenital or acquired) across the mucocutaneous barrier.[10] Israni AV et al observed majority of children were from lower socioeconomic status (85%) and were malnourished (76.6%).[6]

Though BCG vaccination is included under the universal immunization programme since its inception, but in present study only 59.6% children were immunized against tuberculosis.BCG vaccination have been known to be protective against severe from of TB (e.g., TBM, and disseminated or milliary TB), a lower vaccination coverage could explain a higher prevalence of severe form of TB (e.g., TBM) in the present study.[11] Various literature clearly documented the efficacy of BCG vaccine in reducing mortality from TBM.[12,13] Chiang SS et al in their systematic review emphasized the role of BCG vaccination and recommended it as an low cost preventive measure against TBM.[13]

History of contact with tubercular patients is the most important risk factor for tuberculosis especially in children.

Table 4 – Multivariate logistic regression of clinical variables for prediction of outcome.

Variables	Regression coefficient	SE	P value	OR	95%CI
Age <10 yrs	2.39	0.59	0.48	2.01	0.67–4.44
Female	3.34	0.98	0.59	2.09	0.78–4.87
Residence (Rural and suburban)	2.26	1.13	0.52	1.34	0.04–2.98
SES (IV,V)	2.78	2.09	0.67	1.02	0.07–2.78
BMI (Thin and severe thin)	32.29	27.3	0.99	26.8	20.2–33.4
BCG	−2.25	17.1	1.0	1.11	0.01–2.21
Contact history	−8.4	1.9	1.0	1.30	0.02–3.34
Raised ICT	−46.2	25.9	0.99	8.1	7.1–9.1
GCS	−30.3	15.3	0.99	7.3	6.9–7.7
Montoux Test	23.1	7.2	1.0	1.0	0.5–1.5
Stage III	13.34	3.4	0.67	2.2	0.03–5.45
Cough	13.9	2.9	1.0	1.2	1.0–1.4
Fever	34.04	0.0	0.87	2.0	1.8–2.2
Headache	−0.19	2.4	1	1.83	0.33–3.33
Vomiting	28.7	2.16	0.99	2.8	2.4–3.2
Seizure	22.2	0.0	0.89	3.6	3.3–3.9
Altered consciousness	−20.6	3.3	0.99	1.1	0.6–2.2
Hemiparesis	15.8	2.7	1.0	7.1	6.0–8.2
Nerve palsy	11.1	3.8	1.0	6.8	5.6–8.0
Meningeal irritation	7.6	3.1	1.0	2.1	0.9–3.3
Abnormal chest Xray	12.22	3.3	0.69	3.44	0.34–7.77

Contact with TB was documented in 40.5% patients in present study.

Seizures and altered sensorium were the most common symptoms whereas the most common sign were meningeal irritation and raised ICT in present study. GCS was moderate in 48.9% patients. These findings were supported by Israni AV et al in which the authors reported fever, altered sensorium, and seizure to be the most common symptoms and the most common sign were that of meningeal irritation.[6]

Maximum children in our study were observed in stage I, however these findings were in contrast to study by Israni AV et al in which maximum patients were identified in stage 3 (61.7%).[6]

Our study documented raised CSF protein in all patients with TBM (>40mg/dl). CSF CBNAAT was present in 6.4% patients. CSF findings in tubercular meningitis are characterized by low glucose, high protein.[14] Marx GE et al mentioned characteristic cerebrospinal fluid (CSF) findings of TBM include a lymphocytic-predominant pleocytosis, elevated protein, and low glucose.[15] In another study by Siddiqui Z et al, an increase in CSF lactate and CSF ADA levels were observed to be associated with increase in severity of clinical stage of TBM.[16] However we could not test CSF lactate, ADA because of resource limitation.

Chest X ray was abnormal in 44.7% patients in our study comparable to Israni AV et al in 38% of patients.[6] As children present with non specific symptoms and collection of sputum is difficult, diagnosis of TB in children is mainly based upon Chest X ray.

The overall mortality in our study was observed in 11 (23.4%) cases. Various studies have reported the mortality in the range of 9–23.4%.[6,17,18] In present study, mortality was significantly associated with poor nutritional status and higher stage of TB ($p < 0.01$). These findings were supported by Israni AV et al in which the authors documented significant association of mortality with nutritional status and stage of TB.[6] Van Well GT suggested advanced stage has been found to be the single most important factor associated with poor outcome.[8]

6. Conclusion

TBM is associated with high morbidity and mortality in children especially in India where Burden of TB is high. Though technology and investigative modality have advanced in urban area, TBM is still prevalent in rural and suburban area. Our study emphasized on the risk factors associated with mortality in children with TBM and need for early diagnosis and appropriate treatment.

Authors contribution

Priya Jadaun: Data collection and analysis. Rajesh Patil: Drafting the manuscript, Sharmila Ramteke: Concept and designing, Manjusha Goel: Concept and designing, final critical review of manuscript, We state that all authors contributed equally and significantly in the study.

Conflict of interest

All authors have none to declare.

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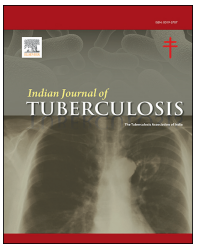

Original article

Sharma's parachute sign a new laparoscopic sign in abdomino pelvic tuberculosis

*Jai Bhagwan Sharma**

Department of Obstetrics and Gynaecology, All India Institute of Medical Science, New Delhi-110029, India

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ABSTRACT

Aims: To demonstrate a new laparoscopic sign "Sharma's Parachute sign" in abdominopelvic tuberculosis in women with infertility.

Methods: A total of 104 women who were diagnosed to have abdominopelvic tuberculosis, on endometrial sampling or on laparoscopy were enrolled in this ongoing study on tuberculosis in infertility. A new laparoscopic "Sharma's parachute sign" was looked for in these cases on laparoscopy.

Results: The mean age, pairty and duration of infertility was 27.6 years, 0.58 and 4.1 years respectively. Menstrual dysfuction were common especially hypomenorrhoea (34.61%), oligomenorrhoea (36.53%) along with constitutional symptoms and abdomino pelvic pain or lump. Diagnosis of abdominopelvic tuberculosis was made by identification of acid fast bacilli (AFB) on microscopy or culture of endometrial aspirate or peritoneal biopsy or positive gene Xpert or positive polymerase chain reaction (PCR) or histopathological demonstration of epithelioid granuloma on endometrial or peritoneal biopsy, various laparoscopic findings on pelvic and abdominal organs were tubercles and shaggy areas (white deposits, caseous nodules encysted ascites, abdominal and pelvic adhesions, tubal findings (hydrosalpinx, pyosalpinx, beaded or calcified tubes). A new "Sharma's parachute sign"in which ascending colon was totally adherent to anterior abdominal wall with its mesocolon looking like an open parachute with small caseous nodule was seen in 11 (10.5%) cases.

Conclusion: Diagnostic laparoscopy is an important investigation for abdominopelvic tuberculosis showing various adhesions including new parachute sign.

1. Introduction

Tuberculosis continues to remain a public health problem all over the world wide 10 million new cases annually with 1.33 million deaths.[1] Most cases occure in Africa and Asia especially in reproductive age group. World health organization started directly observed treatment short course (DOTS) strategy in 1993 to control the disease globally.[2] The revised National Tuberculosis Control Program (RNTCP) of India diagnose 71% cases with a cure rate of 87% by using DOTS strategy.[3]

* *Corresponding author*. Tel.: +11 26589665.
E-mail address: jbsharma2000@gmail.com.

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Abdominopelvic tuberculosis is an extrapulmonary tuberculosis causing significant morbidity in the form of menstrual dysfunction especially oligomenorrhea and hypomenorrhoea, infertility, abdominal pain, chronic pelvic pain, abdominal and pelvic lumps with or without constitutional symptoms like pyrexia, loss of appetite, loss of weight and malaise.[4–6] Abdominopelvic tuberculosis is an important cause of infertility (both primary and secondary) in India through tubal factors (blockage), peritubal and paraovarian adhesions, ovarian factors, endometrial atrophy and adhesions (Asherman's syndrome).[5–10] Abdominopelvic TB is particularly notorious to make vascular and thick adhesions in peritoneal cavity especially perihepatic adhesions (Fitz-Hugh-Curtis syndrome) with Sharma's hanging gall bladder sign, Sharma's ascending colonic adhesions and tubal adhesions including Sharma's kissing fallopian tubes sign.[11–14] Abdominopelvic TB can also mimick like ovarian cancer producing ascites and abdominal masses necessitating unnecessary laparotomy.[15] It can make fallopian tubes fibrosed and calcified looking like dried tree branches (Sharma'sdried tree branch sign).[16]

Diagnosis of abdominopelvic tuberculosis by endometrial and peritoneal biopsy for demonstration of acid fast bacilli (AFB) on microscopy or culture, polymerase chain reaction (PCR), gene Xpert, histopathological demonstration of epithelioid granuloma or laparoscopic findings of abdomnopelvic tuberculosis.[5,7,8] Laparoscopy is the most reliable diagnostic modality for abdominopelvic tuberculosis especially for tubal, ovarian, peritoneal and intestinal disease.[17,18] Various findings of TB on laparoscopy on various pelvic and abdominal organs can be tubercles, shaggy areas (white deposits), straw coloured fluid in pouch of Douglos, encysted ascites, pelvic or abdominal adhesions, beading, convolution or calcification of fallopian tubes.[17] We present our findings on new type of adhesion "Sharma's parachute sign" on ascending colon in which ascending colon is totally adherent to anterior abdominal wall with its mesocolon looking like an open parachute with small caseous nodule shining in it.

2. Material and methods

A total of 104 women of infertility diagnosed to have abdominopelvic tuberculosis on demonstration of AFB on microscopy or culture of endometrial aspirate or biopsy or peritoneal biopsy or demonstration of epithelioid granuloma on endometrial or peritoneal biopsy and or laparoscopic findings of abdomino pelvic tuberculosis were included in this prospective study. The study was part of our large ongoing tuberculosis project which was ethically cleared by our Institute's Ethical Committee. The study was performed in a tertiary referral centre in Northern India with patients coming from all over the country between June 2011 to March 2019.

Informed written consent was taken from all the study participants. Detailed clinical history including past history of tuberculosis or antitubercular drug therapy or family history of tuberculosis was taken from all patients. All patients underwent endometrial aspirate or biopsy in premenstrual phase as a part of their infertility work up and endometrial sample was divided in parts. One part in saline was sent for AFB microscopy, AFB culture, gene Xpert (CB-NAAT [Cartridge based nucleic acid amplification test]) and polymerase chain reaction (PCR) while the other part was sent in formalin for histopathological demonstration of epithelioid granuloma. Diagnostic laparoscopy with or without laparoscopy was performed in most cases. During laparoscopy, careful inspection of whole pelvis and abdominal cavity was made by rotating the laparoscope by 360° for any tubercular lesions and abdominal and pelvic adhesions. All tuberculous lesions like tubercles, shaggy areas, caseous nodules, findings in fallopian tubes like beading of tubes, convoluted tubes, hydrosalpinx, pyosalpinx, hyperemia and adhesions of tubes and patency of tubes were carefully observed and recorded in all the cases. Whole of abdominal and pelvic cavity was carefully visualized for adhesions and their details. Particular attention was paid to adhesions of ascending colon and its mesocolon to anterior abdominal wall making an open parachute sign.

All women diagnosed to have abdominopelvic TB were given free antitubercular therapy as per World Health Organization's Directly Observed Treatment Short Course (DOTS) strategy under Revised National Tuberculosis Control Programme (RNTCP) of India using 4 drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) daily for 2 months (Intensive phase) followed by three drugs (Rifampicin, Isoniazid and Ethambutol) daily for 4 months (Continuation phase). All women were regularly followed up for any complication and adverse events. Liver function tests were done when clinically indicated and pyridoxine was only prescribed if any symptom of peripheral neuritis.

3. Statistical analysis

Descriptive statistics such as mean, median, standard deviation and range values were calculated for study characteristics like age, body mass index and duration of infertility. Continuous variables were tested for normality assumptions using Kolmogrov Smirnov tests, Chi square test or Fisher's exact test was used for frequencies of categorical variations. A two tailed probability level with $p < 0.05$ was considered for statistical significance. All data analysis were carried out using IBM SPSS statistics for windows version 19.0 New York IBM Corp.

4. Results

The characteristics of women in the study are depicted in Table 1. The mean age ranged from 19 to 45 years with mean being 27.6 ± 3.26 yrs. Mean body mass index (BMI) was 22.1 ± 2.18 Kg/m^2 while mean parity was 0.58. Infertility was seen in all 104 (100%) cases with mean duration of infertility being 4.1 years. Most (78.8%) women had primary infertility while 21.2% had secondary infertility. The symptoms and signs of patients are shown in Table 2. The constitutional symptoms were pyrexia (21.15%), loss of appetite (35.65%), loss of weight and (35.57%), malaise (39.42%).

Menstrual dysfunctions were very common in abdominopelvic TB with only 28 (26.92%) women having normal menstrual pattern. Heavy menstrual bleeding was seen in only 3 (2.88%) women while hypomenorrhoea, oligomenorrhoea, primary amenorrhoea and secondary dysmenorrhea

Table 1 – Characteristics of women (n = 104).

S.No.	Characteristics	Range	Mean
1.	Age (Years)	19–45	27.6 ± 3.26
2.	BMI(Kg/m^2)	16.9–31.6	22.1 ± 2.18
3.	Parity	0–4	0.58
4.	Past history of Tuberculosis	69	66.3%
5.	Duration of Infertility (Years)	1–13	4.1
6.(i)	Type of infertility- Primary	82	78.8%
6.(ii)	Type of infections- Secondary	22	21.2%

BMI: Body mass index.

Table 2 – Symptoms and signs (symptomatology) in study (n = 104).

S.No.	Symptomatology	No. of women	Percentage
1.	Pyrexia	22	21.15
2.	Loss of appetite	35	33.65
3.	Loss of weight	37	35.57
4.	Malaise	41	39.42
5.	Normal menstrual pattern	28	26.92
6.	Hypomenorrhoea	36	34.61
7.	Ologomenorrhoea	38	36.53
8.	Primary amenorrhoea	2	1.92
9.	Secondary amenorrhoea	11	10.57
10.	Heavy menstrual bleeding	3	2.88
11.	Abdominal pain	12	11.53
12.	Chronic pelvic pain	37	35.57
13.	Abdominal lump	11	10.57
14.	Pelvic or adenexal mass	39	37.5

X: Some patients had more than 1 symptom.

were seen in 36 (34.61%), 38 (36.53%), 2 (1.92%) and 11 (10.57%) patients respectively. Abdominal pain, chronic pelvic pain, abdominal lumps and pelvic or adenexal mass were seen in 12 (11.53%), 37 (35.57%), 11 (10.57%) and 39 (37.5%) patients respectively with some patients having more than one symptom or sign.

Various diagnostic modalities used to make diagnosis of abdominopelvic TB are shown in Table 3. The commonest finding was positive polymerase chain reaction (PCR) in 97 (93.26%) women on endometrial aspirate or biopsy, positive AFB (acid fast bacilli) on microscopy of endometrial aspirate in 12 (11.53%) cases, positive AFB culture on endometrial aspirate in 14 (13.46%) cases, positive AFB on microscopy or culture on peritoneal biopsy in 8 (7.69%) cases, epithelioid granuloma or chronic granulomatous endometrium on endometrial biopsy in 26 (25%) cases and epithelioid granuloma on peritoneal or caseous nodule biopsy in 12 (11.53%) cases, positive CB-NAAT (cartridge based nuclic acid amplification test) or gene xpert in 12 (11.53%) cases. Laparoscopy was performed in most cases. Definite finding of tuberculosis were seen in 42 (40.38%) cases while probable findings of abdominopelvic TB were seen in 47 (45.19%) cases.

Various laparoscopic findings of abdominopelvic TB are shown In Table 4. Tubercles on genital organs were seen in 49 (47.11%) cases, on general peritoneum and omentum or bowel in 15 (14.42%) cases while shaggy areas (white deposits) were observed in 47 (45.19%) cases on internal genital organs and in 17 (16.34%) cases on general peritoneum, bowel or liver surface (Fig. 1). Caseous nodules were seen in 31 (29.8%) cases in pelvis (Fig. 2) and in 22 (21.15%) cases in upper abdomen. Other findings of abdominopelvic TB on laparoscopy were encysted ascites (16.34%), fluid filled pockets (21.15%), abdominal adhesions (37.5%), pelvic adhesions (41.34%) (Fig. 3), hydrosalpinx (21.15%), pyosalpinx (4.80%), beaded or convoluted tubes (6.73%), calcified and rigid tubes (10.57%). A new laparoscopic sign "Sharma's parachute sign" was adhesion of ascending colon to the anterior abdominal wall on the right side of abdomen with its mesocolon spreading like a parachute (Figs. 4–6) was seen in 11 (10.57%) of cases. It makes the right side of abdomen unsafe for putting the port on right side of abdomen due to risk of injury to ascending colon. Small caseous nodule can be seen in the mesocolon of ascending colon as glistening white pearls (Figs. 5–6) in the background of clear peritoneum confirming it to abdominopelvic TB. Performing laparoscopy in abdominopelvic TB needs expertise and is associated with difficulties and increased complications as shown in Table-5.

Various difficulties and complications were difficult. Pneumoperitoneum in 23 (22.11%) cases, difficulty in insertion of trocar and cannula in 13 (12.5%) cases, difficulty in visualization of pelvic organs due to adhesions in 17 (16.34%) cases, excessive bleeding in 9 (8.65%) cases, failed or abandoned

Table 3 – Methods of diagnosis of abdominopelvic tuberculosis (n = 104).

S.No.	Diagnostic modality	No. of women	Percentage
1.	Positive AFB on microscopy of endometrial aspirate or biopsy	12	11.53
2.	Positive AFB on culture of endometrial aspirate or biopsy	14	13.46
3.	Positive AFB on microscopy or culture on peritoneal biopsy	8	7.69
4.	Epitheloid granuloma or chronic granulomatous endometrium on histopathology of endometrial biopsy	26	25.0
5.	Epitheloid granuloma on histopathology of biopsy from peritoneal tubercle or caseous nodule	12	11.53
6.	Positive CB-NAAT or gene expert on endometrial or peritoneal biopsy	12	11.53
7.	Positive polymerase chain reaction on endometrial aspirate or peritoneal fluid	97	93.26
8.	Definite findings of abdominopelvic tuberculosis on laparoscopy	42	40.38
9.	Probable findings of abdominopelvic tuberculosis on laparoscopy	47	45.19

X: Some patients had more than one finding.
AFB: Acid Fast Bacilli, CB-NAAT: Cartridge based nucleic acid amplification test.

Table 4 – Laparoscopic findings in abdomino-pelvic tuberculosis (n = 104).

S. No.	Findings on laparoscopy	No. of women	Percentage
1.	Tubercles on fallopian tube, uterus, ovaries, pelvic peritoneum, pouch of Douglas	49	47.11
2.	Tubercles on omentum, general peritoneum, bowel	15	14.42
3.	Shaggy areas (white deposits) on uterus, tube, pelvic peritoneum	47	45.19
4.	Shaggy areas (large white deposits) on general peritoneum, bowel, liver surface	17	16.34
5.	Caseous nodules on tubes, pelvic peritoneum or pouch of Douglas	31	29.80
6.	Caseous nodule on general peritoneum, bowel, omentum	22	21.15
7.	Encysted ascites	17	16.34
8.	Fluid filled pockets in pelvis or abdomen	22	21.15
9.	Abdominal adhesions	39	37.50
10.	Pelvic adhesions	43	41.34
11.	Hydrosalpinx	22	21.15
12.	Pyosalpinx	5	4.80
13.	Beaded or convoluted tubes	11	10.57
14.	Calcified and rigid tubes	7	6.73
15.	Parachute sign (Ascending colon adhesion to anterior abdominal wall with mesocolon spreading like an open parachute)	11	10.57

procedure due to dense adhesions at umbilicus in 2 (1.92%) cases, bowel injury in 2 (1.92%) cases, subacute intestinal obstruction in 9 (8.65%) cases and flare up of tuberculosis in 7 (6.73%) cases. In 2 cases of bowel injury, there was injury to ileum in one case detected at the time of laparoscopy for which surgeon was called who did resection of injured ileum (4cm) and end to end anastomosis. In another case, there was trocar injury to sigmoid colon which was not detected at the time of laparoscopy. Patient presented 36 hours later with the abdominal distension. Laparotomy, repair of sigmoid injury and colostomy were performed at 48 hours and patient recovered well.

All patients were given category I of antitubercular therapy under Directly Observed Treatment Short Course (DOTS) strategy of Revised National Tuberculosis Control Program (RNTCP) of India using rifampicin, isoniazid, pyrazinamide and ethambutol daily for 2 months followed by rifampicin, isoniazid and ethambutol daily for next 4 months. Most patients tolerated drugs well. However adverse events were seen in some cases as shown in Table 6. Commonest side effects were nausea in 22.11% cases, vomiting in 14.42% cases, derangement of liver function test in 11.53% cases, drug induced hepatitis in 1.92% cases and peripheral neuritis in 0.98% cases. Most cases of nausea and vomiting could be controlled by use of metoclopromide tablet while derangement of liver function tests settled down with time. However, drugs need to be stopped and restarted in consultation with chest physician in case of drug induced hepatitis and peripheral neuritis.

5. Discussion

Tuberculosis continued to be a major health problem globally.[1,2] Abdominopelvic TB is a type of extrapulmonary tuberculosis causing significant morbidity and sequalae particularly menstrual dysfunction especially oligomenorrhoea and hypomenorrhoea, abdominal and pelvic pain and

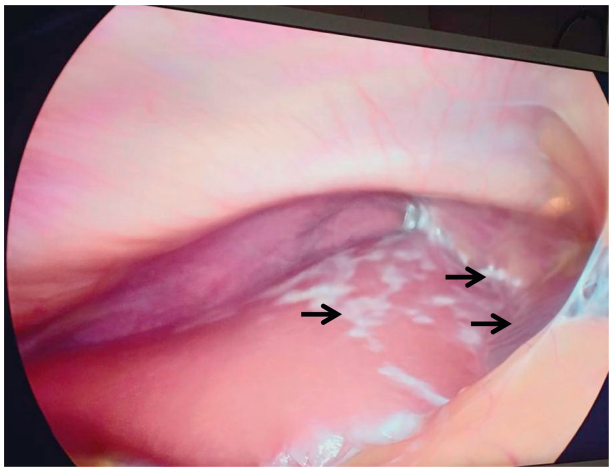

Fig. 1 – Laparoscopy showing shaggy areas on liver (arrow) and perihepatic adhesions (double arrow) in a case of abdomino-pelvic TB.

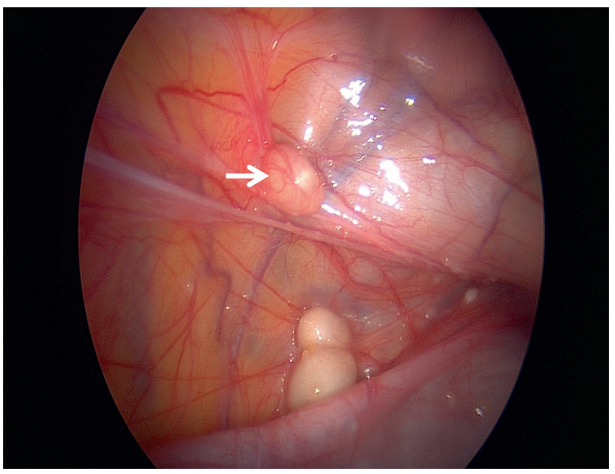

Fig. 2 – Laparoscopy showing caseous nodules (arrow) in pouch of Douglas in a case of abdominopelvic TB.

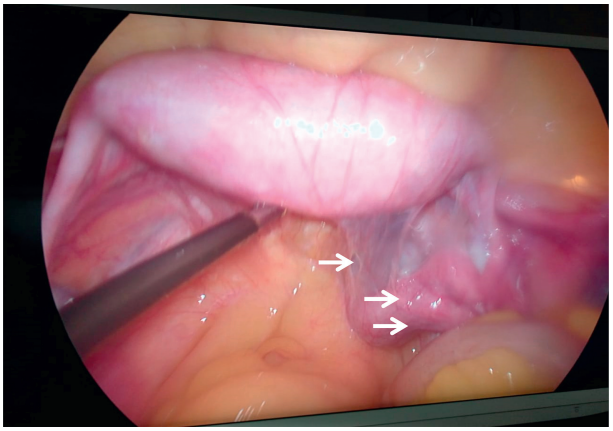

Fig. 3 – laparoscopy showing pelvic adhesions (single arrow) and hypermeic tube (double arrow)in a case of abdominopelvic TB.

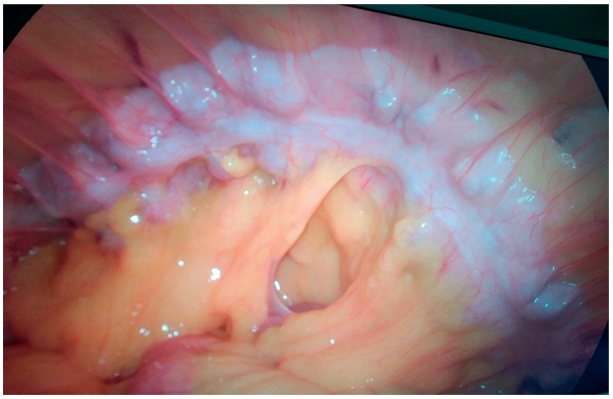

Fig. 4 – Laparoscopy showing "Sharma's parachute sign" with ascending colon adherent to anterior abdominal wall along with its mesocolon resembling an open parachute in a case of abdominopelvic TB.

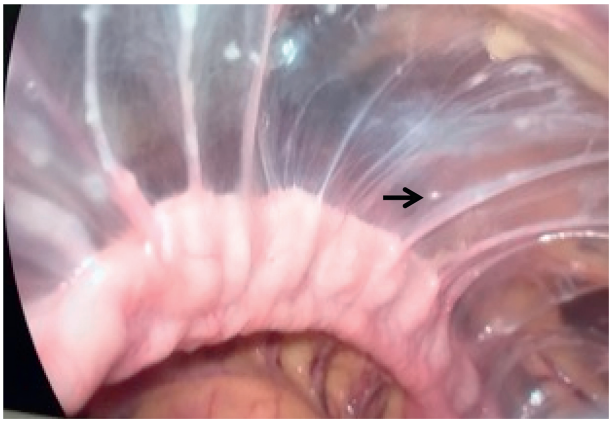

Fig. 5 – Laparoscopy showing closer view of "Sharma's parachute sign" showing glistening white caseous nodules in mesocolon of ascending colon confirming it to be abdominopelvic TB.

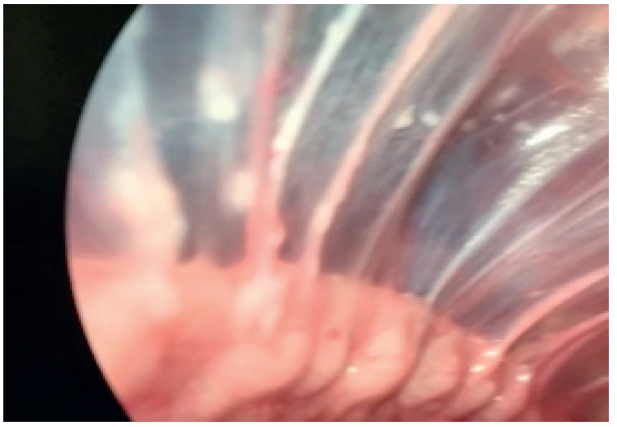

Fig. 6 – Laparoscopy showing "Sharma's parachute sign" showing adherent ascending colon to anterior-abdominal wall with glistening white caseous nodules in mesocolon in a case of abdominopelvic TB.

lumps and above all infertility which can be both primary or secondary infertility.[4–8] Fallopian tubes are involved in majority (90–100%) cases followed by uterus (70%) ovaries (20–30%), cervix (5–15%) and rarely vulva and vagina (1%).[5,7,8] Abdominopelvic tuberculosis is an important cause of infertility in India affecting about 10% cases but the incidence is higher up to 18–26% in tertiary hospitals and in women seeking assisted reproduction (up to 48%)[6,19]. Infertility in tuberculosis is due to involvement of tubes (tubal blockage and peritubal adhesions), endometrial atrophy (Ashermann's syndrome) and ovarian involvement.[5,9,10] Abdominopelvic tuberculosis causes pelvic adhesions and abdominal and pelvic lumps and may mimick ovarian cancer as has been reported by us.[15] It can also cause ectopic pregnancy through peritubal adhesions and tubal damage.[20] It can even coexist with endometriosis or malignancy as has seen our experience.[21] Diagnosis of abdominopelvic and female genital TB is difficult due to its paucibacillary nature. Though endometrial aspirate or biopsy or peritoneal biopsy from lesions has been used to detect AFB by microscopy, culture, gene xpert and histopathology they may miss the diagnosis in many cases.[5,7,8,22] Polymerase chain reaction though highly sensitive, has high false positive rate and alone is insufficient to start treatment.[23]

World Health Organization has recommended use of cartridge based nucleic acid amplification (CB-NAAT) also called gene Xpert for diagnosis of pulmonary and extra-pulmonary TB. We reported 35% sensitivity and 100% specificity of gene Xpert in our study on female genital TB.[24] Radiological methods like ultrasound, CT scan, Magnetic Resonance Imaging and Positron Emission Tomography (PET) have been used especially in cases of abdominopelvic masses and to differentiate between ovarian cancer and tuberculosis.[25–27] Hysterosalpingography is usually avoided in suspected female genital TB but is often done as a part of infertility management in unsuspected cases and may show tubal pathology like tubal blockage, hydrosalpinx and various appearances of tubes like maltese cross appearance, tobacco pouch appearance.[28]

Table 5 – Difficulties and complications during laparoscopy in abdominopelvic tuberculosis (n = 104).

S. No.	Difficulty or complications	No. of women	Percentage
1.	Difficult Pneumoperitoneum	23	22.11
2.	Difficulty in insertion of trocar and cannula	13	12.5
3.	Difficulty in visualization of pelvic organs due to adhesions	17	16.34
4.	Excessive bleeding (controlled by bipolar cautery)	9	8.65
5.	Failed or abandoned procedure (due to dense adhesions at umbilicus)	2	1.92
6.	Bowel injury	2	1.92
(i)	Small bowel injury detected at the time of surgery and repaired by General Surgeon	1	0.96
(ii)	Sigmoid injury detected after 48 hrs. needing colostomy	1	0.96
7.	Subacute intestinal obstruction (managed conservatively)	9	8.65
8.	Flare up of tuberculosis	7	6.73

Table 6 – Complications of antitubercular treatment (n = 104).

S.No.	Complications	No. of women	Percentage
1.	Nausea	23	22.11
2.	Vomiting	15	14.42
3.	Derangements of liver function tests (increased ALT and ALP)	12	11.53
4.	Drug induced hepatitis	2	1.92
5.	Peripheral neuritis	1	0.96

Treatment of abdominopelvic tuberculosis is by use of primary drugs (rifampicin, isoniazide, pyrazinamide and ethambutol) daily for 2 months followed by three drugs (rifampicin, pyrazinamide and ethambutol) daily for 4 months.[1,2,29] However, rarely primary or secondary multidrug resistant (MDR) female genital TB can also be there as detected on gene Xpert or drug sensitivity testing which needs use of reserved drugs for 18–24 months as has been our experience.[30]

Abdominopelvic TB is notorious to cause various abdominal or pelvic adhesions which are very vascular and dense making surgery (both laparoscopy and laparotomy) difficult and hazardous as has been our experience.[31,32] We have reported various types of abdominal and pelvic adhesions in abdominopelvic and female genital tuberculosis like Sharma's hanging gall bladder sign[12] and Sharma's ascending colonic adhesion at junction of lower 2/3rd and upper 1/3rd of ascending colon.[13] We have also reported new laparoscopic signs in female genital infertility like Sharma's kissing fallopian tubes sign[14] with adhesion of fimbrial ends of both fallopian tubes, Sharma's blue python sign[33] (bluish coloration and alternate constriction and dilatation of fallopian tubes on instillation of dye) and calcification of fallopian tubes (Sharma's dried tree branch sign)[16] in female genital TB.

In the present study we observed cases a new laparoscopic sign (Sharma's parachute sign) in 11 (10.57%) which ascending colon with its mesocolon is totally adherent to anterior abdominal wall resembling an open parachute with small caseous white lesion glistening in clear background of mesocolon to confirm it to be of tuberculous pathology (Figs. 4 and 5). The finding is significant as putting a second port of laparoscope on right side can cause injury to the ascending colon. Hence second and third port during laparoscopy in suspected abdominopelvic tuberculosis should be placed on left side under vision to avoid bowel injury.

6. Conclusion

The new sign "Sharma's parachute sign" appears to be a useful laparoscopic sign in abdominopelvic TB. However large prospective studies are recommended before its routine recommendation in clinical practice.

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Conflicts of interest

The author has none to declare.

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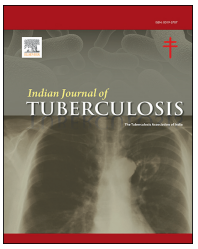

Perspective

Does active case finding for tuberculosis generate more false-positives compared to passive case finding in India?

Keywords:
TB case finding
Predictive value
Yield
False positive
Bacteriological confirmation

1. Background

We read with interest the review article by Chadha VK and Praseeja P in the 66th issue of Indian Journal of Tuberculosis (2019) *"Active tuberculosis case finding in India – The way forward"*.[1] In this article, the authors have argued that active case finding (**ACF**) **algorithm one** (symptom screen using >2 weeks cough in the community followed by sputum microscopy at a health facility) in populations with TB prevalence less than 2.5% will result in unacceptably high levels of false-positives and will not be cost-effective. The authors considered a positive predictive value (PPV) ≥90% as acceptable. The cost-effectiveness argument was based on an arbitrary cut-off of less than INR 5000 (≈67 US$) per ACF-detected case.[1]

World Health Organization recommends that *"systematic screening for active TB* [Tuberculosis] *may be considered for geographically defined subpopulations with extremely high levels of undetected TB (1% prevalence or higher) and for other subpopulations that have very poor access to health care"*.[2]

We debate whether ACF algorithm one generates more false-positives compared to passive case finding (PCF) and if the cost cut-off used is appropriate. We then discuss the implications of using validity estimates from TB prevalence surveys in ACF settings. We also calculate the PPV if sputum microscopy is replaced by cartridge-based nucleic acid amplification test, also widely known as Xpert MTB/RIF assay® (Cepheid Sunnyvale USA). This was not assessed by Chadha VK and Praseeja P.[1] This is relevant because the national guidelines in India recommend the use of Xpert MTB/RIF for diagnosis of TB among vulnerable groups and those who are screen-positive during ACF.[3]

2. Sensitivity and specificity assumptions

For symptom screen (>2 weeks cough), Chadha VK and Praseeja P used a sensitivity of 56.2% and a specificity of 95.3%.[1] For sputum microscopy among screen-positive during ACF, they used a sensitivity of 46.2% and specificity of 99.3%.[1] These estimates were based on sub-national prevalence surveys from India.[4,5]

3. PPV for PCF and ACF algorithm one involving sputum microscopy

While Chadha VK and Praseeja P seem to suggest that ACF generates more false-positives than PCF, they do not present any calculations of PCF algorithms in support of this. They chose an arbitrary PPV cut-off of ≥90% for ACF, but do not seem to use the same cut-off to evaluate PCF.[1] We are unclear why. One of the challenges here relates to the limited information on the prevalence of TB in the hospital-attending population and sensitivity of screening criteria in this population. In the absence of this information, we took the sputum positivity rate among presumptive TB patients reported by India's national TB programme (ranging from 5% to 15%) and back-calculated the actual TB prevalence (which ranged from

5% to 22%) among them using Bayes theorem. We then calculated the PPV in PCF, which ranged from 62% to 90% (Table 1). The PPV remained <90% even when the prevalence of TB among screen-positive (during PCF) was as high as 15%. The PPV values of ACF algorithm one calculated by Chadha VK and Praseeja P ranged from 80% (0.5% prevalence in population where ACF is conducted) to 89% (1% prevalence) to more than 90% (≥1.5% prevalence) (Table 1). These results show that ACF algorithm one does not generate more false-positives compared to PCF in similar settings.

There are two issues with the use of validity estimates from prevalence surveys to assess ACF algorithms.

First, prevalence surveys are done in general population while ACF is done in high risk population. The prevalence of TB among screen-positive will be higher in ACF setting,

Table 1 – Positive Predictive Value (PPV) of PCF and ACF-detected sputum smear-positive TB in a population of 0.1 million, India.

Screening			Diagnosis				
TB prevalence per 0.1 million	TP	TP + FP (Screen-positive)	TB in screen-positive	TP	TP + FP (Sputum positive TB)	PPV for sputum positive TB	TB in population detected assuming half of screen-positive identified during ACF are tested
(a)	(b)	(c)	(b/c)	(d)	(e)	(d/e)	[(d/a)/2]
N	N	N	(%)	N	N	(%)	(%)
ACF – Screening using >2 week cough (Sens 56.2%, Spec 95.3%)[4a] followed by sputum microscopy among screen-positive (Sens 46.2%, Spec 99.3%)[4a]							
500	281	4958	5.7	130	163	79.8	13
1000	562	5215	10.8	260	292	88.9	
1500	843	5473	15.4	389	422	92.2	
2000	1124	5730	19.6	519	552	94.0	
2500	1405	5988	23.5	649	681	95.3	
ACF – Screening using >2 week cough (Sens 56.2%, Spec 95.3%)[4a] followed by sputum microscopy among symptom screen-positive (Sens 63.6% Spec 98.5%)[b]							
500	281	4958	5.7	179	249	71.9	18
1000	562	5215	10.8	357	427	83.6	
1500	843	5473	15.4	536	606	88.4	
2000	1124	5730	19.6	715	784	91.2	
2500	1405	5988	23.5	894	962	92.9	
ACF – Screening using >2 week cough (Sens 56.2%, Spec 95.3%)[4a] followed by Xpert MTB/RIF among screen-positive (Sens 92%, Spec 99%)[2c]							
500	281	4958	5.7	259	305	84.9	26
1000	562	5215	10.8	517	564	91.7	
1500	843	5473	15.4	776	822	94.4	
2000	1124	5730	19.6	1034	1080	95.7	
2500	1405	5988	23.5	1293	1338	96.6	
ACF – Screening using >2 week cough (Sens 56.2%, Spec 95.3%)[4a] followed by Xpert MTB/RIF among screen-positive (Sens 69.7%, Spec 98.5%)[10c]							
500	281	4958	5.7	196	266	73.7	20
1000	562	5215	10.8	392	462	84.9	
1500	843	5473	15.4	588	657	89.5	
2000	1124	5730	19.6	783	853	91.8	
2500	1405	5988	23.5	979	1048	93.4	
PCF using sputum microscopy (Sens 61%, Spec 98%)[2]							
–	–	–	5.0[@]	–	–	61.6	–
–	–	–	10.0[@]	–	–	77.2	–
–	–	–	15.0[@]	–	–	84.3	–
–	–	–	22.0[@]	–	–	89.6	–
PCF using Xpert MTB/RIF (Sens 92%, Spec 99%)[2]							
–	–	–	5.0[@]	–	–	82.9	–
–	–	–	10.0[@]	–	–	91.1	–
–	–	–	15.0[@]	–	–	94.2	–
–	–	–	22.0[@]	–	–	96.3	–

TB – tuberculosis, ACF – active case finding, PCF – passive case finding, TP – true positive, FP – false positive.

[a] Sensitivity and specificity figures of symptom screen and sputum microscopy among screen-positive during ACF obtained from sub-national prevalence surveys. For PCF, we used sensitivity and specificity reported by WHO using facility-based data (61%).

[b] Calculated using TB prevalence survey data from Gujrat.

[c] For Xpert MTB/RIF among screen-positive during ACF, we did two separate calculations i) assuming similar sensitivity (92%) as in PCF setting and ii) assuming 69.7% sensitivity from Philippines national TB survey [@]TB prevalence among PCF-detected presumptive TB = (test positivity proportion – (1 – specificity))/(sensitivity – (1 – specificity)).

therefore we hypothesize that the PPV will be higher than what was reported by Chadha VK and Praseeja P.[2,4]

Second, the definition of 'screen-positives' is different in prevalence surveys (symptomatic or chest radiograph positive) and ACF algorithm one.[4,5] The same diagnostic tool will yield different sensitivity and specificity in different populations, due to spectrum differences.[6] Therefore, sensitivity and specificity of sputum microscopy among screen-positive from prevalence surveys cannot be extrapolated to ACF setting. To examine this hypothesis, we requested for disaggregated data from Gujarat prevalence survey and found that the sensitivity and specificity of sputum microscopy was 63.6% and 98.5% among people with symptoms while they were respectively 46.8% and 99.2% for people without symptoms. Using 63.6% sensitivity and 98.5% specificity, we obtained precise estimates of PPV for ACF algorithm one (Table 1). The PPV ranged from 72% (0.5% prevalence in population where ACF is conducted) to 84–89% (1–1.5% prevalence) to more than 90% (≥2% prevalence). These precise PPV estimates also show that ACF algorithm one does not generate more false-positives compared to PCF in similar settings.

4. Cost-effectiveness of ACF algorithm one

It is also unclear why Chadha VK and Praseeja P used a cut-off of <INR 5000 (≈66 US$) to make a judgement about cost-effectiveness of ACF. In Cambodia, additional costs documented per ACF case was 249–316 US$.[7] Modelling studies have revealed that ACF in India is a highly cost-effective strategy, even at US$ 1200 per additional case.[8] Hence, we do not agree with the arbitrary cut-off of INR 5000. Chadha VK and Praseeja P calculated that ACF algorithm one in a population with 1% prevalence detects one case at a cost of INR 10 444 (≈136 US$).[1] This appears to be cost-effective when compared to the modelling data (cost effective at US$ 1200).[8] We think this needs further study.

5. PPV for PCF and ACF algorithm one involving Xpert MTB/RIF

Xpert MTB/RIF has sensitivity of 92% and specificity of 99% among screen-positive in PCF settings.[2] For smear-negative culture-positive pulmonary TB, the pooled sensitivity of Xpert MTB/RIF was 68%.[9] To our knowledge, there is no data about validity of Xpert MTB/RIF among people with cough >2 weeks or symptom screen-positive identified in a community setting. The data from the prevalence survey in Philippines (sensitivity of Xpert MTB/RIF among screen-positive was 69.7%),[10] cannot be extrapolated to ACF setting, because of the reasons mentioned previously. This is especially so in Philippines TB prevalence survey, where people with symptoms accounted for just 15% of all those who underwent sputum examination.[10] With Xpert MTB/RIF as the diagnostic test (sensitivity 92%), the PPVs were consistently above 90% for PCF if TB prevalence among symptom screen-positive was ≥10% as well as for ACF if done in a population with ≥1% TB prevalence (Table 1).

We have also presented the yield (TB detected as a percentage of total TB in the population) of ACF algorithm one involving sputum microscopy and Xpert MTB/RIF in Table 1.

6. Conclusion

There are three key points. First, the prevalence of TB among screen-positive in ACF and PCF settings is similar (10–15%). Second, the PPV of ACF-detected sputum positive TB is not lower than PCF. Hence, ACF does not wrongly put more people on anti-tuberculosis treatment when compared to PCF. Third, if we replace sputum microscopy with Xpert MTB/RIF, the PPV is consistently ≥90% irrespective of ACF or PCF assuming similar sensitivity of Xpert MTB/RIF in PCF and ACF setting.

We recommend studies from India assessing the validity estimates for screening and diagnostic tests (sputum microscopy and Xpert MTB/RIF) in an ACF setting. This will give more precise estimates of PPV for ACF algorithm one. We also recommend systematic studies before coming to a conclusion about cost-effectiveness.

Disclaimer

The contents of this paper do not necessarily reflect the views of the organizations the authors are affiliated to.

Data availability statement

The Microsoft Excel sheet used to make these calculations has been shared as a supplementary annex (S1 Annex).

Conflicts of interest

The authors, HDS, SS and AMVK have published research manuscripts assessing the individual level and community level benefits of ACF when compared to PCF in marginalised and vulnerable populations of India (see link).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2020.09.012.

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Hemant Deepak Shewade*
Srinath Satyanarayana
International Union Against Tuberculosis and Lung Disease (The Union), Paris, France
The Union South-East Asia (USEA) Office, New Delhi, India

Ajay MV. Kumar
International Union Against Tuberculosis and Lung Disease (The Union), Paris, France
The Union South-East Asia (USEA) Office, New Delhi, India
Yenepoya Medical College, Mangaluru, India

**Corresponding author*. The Union South East Asia, New Delhi, 110016, India.
E-mail address: hemantjipmer@gmail.com (H.D. Shewade)

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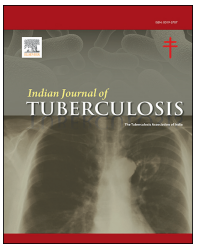

Correspondence

Sharma's Parachute Sign in abdomino-pelvic TB

I read with the great interest the excellent article entitled "Sharma's Parachute Sign in abdomino-pelvic TB" by Prof. J B Sharma from AIIMS Delhi published in Indian Journal of Tuberculosis.[1] I am a general surgeon and have performed laparotomy and laparoscopy on many patients of abdomino-pelvic tuberculosis. I fully agree with Prof. Sharma that at surgery is very difficult and hazardous in abdomino-pelvic TB as there are often dense adhesions between anterior abdominal wall and bowel and omentum. Many times there may be frozen pelvis or inability to see pelvic structures because of thick bowel and omental adhesions. The adhesions can occur in any part of abdomen but are more on right side and also in the upper abdomen on right side. Perihepatic adhesions are common in abdomino-pelvic TB as has been our experience and observational data and by other authors.[2] In case of abdomino-pelvic TB, I have also observed adherent ascending colon to anterior abdominal wall. Hence, the surgeon should be very careful during laparoscopy and laparotomy to avoid injury to it.[3] On the other hand, descending colon is usually not adherent to anterior abdominal wall making left side as a better site for putting ports of laparoscopy. The reason of higher incidence of adherent ascending colon to abdominal wall as compared to descending colon could be due to the fact that ileo-caecal TB is the commonest site of abdominal TB from where infection goes easily to ascending colonbut not to the descending colon by which time disease regresses.

I also want to point out that one should not make it a prestige point to do surgery in abdomino-pelvic TB as performing surgery is hazardous in TB and can cause bowel injury and may not be needed as patient will ultimately require full course of anti-tuberculous therapy.

Hence, it is better in such cases to note down the findings of abdominal TB (tubercles, caseous nodules, adhesions, etc), take a biopsy from representative area and to close the abdomen and give full 6 months course of anti-tubercular therapy with rifampicin, isoniazid, ethambutol and pyrazinamide for 2 months (intensive phase) followed by 4 months of rifampicin, isoniazid and ethambutol (continuation phase) as has been recommended by various authors[4] World Health Organization (WHO)[5] and Revised National tuberculosis Control Program of India (RNTCP).[6]

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Ram Gopal Sharma
Department of Surgery, Maharishi Markandeshwar(Deemed to Be University), Mullana, Ambala, Haryana, India
E-mail address: drramgopa9@gmail.com

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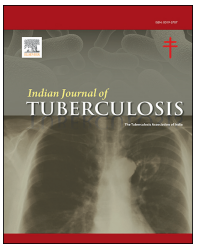

Short communication

Is the tuberculosis vaccine BCG an alternative weapon for developing countries to defeat COVID-19?

*Wenping Gong, Xueqiong Wu**

Army Tuberculosis Prevention and Control Key Laboratory, Beijing Key Laboratory of New Techniques of Tuberculosis Diagnosis and Treatment, Institute for Tuberculosis Research, The 8th Medical Center of Chinese PLA General Hospital, Beijing, 100091, China

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ABSTRACT

Backgroud: Coronavirus disease (COVID-19) is a new respiratory infectious disease, and there is no vaccine currently. Previous studies have found that BCG vaccination can provide extensive protection against respiratory infectious diseases.

Methods: Herein, we obtained the latest data from the World Health Organization (WHO) as of August 12, 2020, and determined the relationship between three parameters (including the BCG vaccination coverage, human development index (HDI), and transmission classifications) and the incidence rate and mortality of COVID-19.

Results: The results showed that the morbidity and mortality of COVID-19 in countries with BCG vaccination recommendation were significantly lower than these in countries without BCG vaccination recommendation, and countries with lower HDI have lower morbidity and mortality. In addition, we also found that the mode of virus transmission is also related to the morbidity and mortality of COVID-19.

Conclusions: Although our data supports the hypothesis that BCG vaccination is beneficial in reducing the morbidity and mortality of COVID-19, the data supporting this result may be inaccurate due to many confounders such as PCR testing rate, population characteristics, and protection strategies, the reliability of this result still needs to be verified by clinical trials.

Abbreviations: BCG, Bacille Calmette-Guérin vaccine; COVID-19, coronavirus disease; HDI, human development index; IL, interleukin; LDCs, least developed countries; LLDCs, landlocked developing countries; PCR, polymerase chain reaction; SIDs, small island developing states; TB, tuberculosis; TNF, tumor necrosis factor; WHO, World Health Organization.

* *Corresponding author*. Army Tuberculosis Prevention and Control Key Laboratory, Beijing Key Laboratory of New Techniques of Tuberculosis Diagnosis and Treatment, Institute for Tuberculosis Research, the 8th Medical Center of Chinese PLA General Hospital, 17# Heishanhu Road, Haidian District, Beijing, 100091, China. Fax: +008610 80115555.

E-mail address: xueqiongwu@139.com (X. Wu).

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1. Background

The coronavirus disease (COVID-19) has been spread globally for eight months, people's lives and health are under unprecedented threat. Vaccination is considered to be the most effective way to stop the spread of the pandemic. As of 3 Septermber 2020, there are 321 candidate vaccines for COVID-19 in preclinical evaluation, and 33 candidate vaccines for COVID-19 in clinical trials.[1] Of the 33 COVID-19 vaccines in clinical trials, six vaccines have been in phase III clinical trials.[1] The protection efficiency and safety of these vaccines are still under investigation. Recently, the Russian Ministry of Health has announced the approval of the world's first COVID-19 vaccine, but its safety and effectiveness have been questioned because the vaccine skipped Phase III clinical trials (http://www.chinadaily.com.cn/a/202008/11/WS5f325c7aa31083481725fa58.html). Besides, the pandemic has severely resulted in a shortage of personal protective equipment, biomedical equipment, and diagnostic reagents in more and more countries. Developing countries, especially the least developed countries (LDCs), landlocked developing countries (LLDCs), and small island developing states (SIDS) are at an absolute disadvantage in this vaccine race and the battle for medical supplies. Those countries are suffering from long-term impediments to sustainable development.

Fortunately, most developing countries have carried out a wide range of Bacille Calmette-Guérin vaccine (BCG) vaccination strategies. Studies have found that the prevalence of COVID-19 is relatively mild in these countries,[2,3] which may be due to the trained immunity induced by the BCG vaccine[4], such as enhanced production of tumor necrosis factor (TNF), interleukin 1β (IL-1β), and IL-6.[3] However, there are contradictory reports on whether BCG can effectively prevent COVID-19.[5,6] In May 2020, Dr. Matteo Riccò et al claimed that BCG might not avoid SARS-CoV-2 infection based on a meta-analysis including 13 studies (12 papers are preprints that have not been peer-reviewed).[6] Therefore, it is very urgent to clarify the effect of BCG vaccination on COVID-19 prevention by analyzing the latest data of confirmed cases and deaths of COVID-19 in various countries, which will provide a reference for formulating efficient and scientific prevention strategies.

2. Main text

2.1. *The BCG coverage and human development index (HDI) are associated with the morbidity and mortality of COVID-19*

Herein, we obtained the global COVID-19 pandemic data from the official website of the World Health Organization (WHO) on 12 August 2020. According to the coverage rate of BCG, all countries were divided into three levels. We found that the COVID-19 incidence rate of countries with BCG recommendation was significantly lower than that of countries without BCG recommendation (Fig. 1A), and the COVID-19 mortality rate of countries with BCG coverage of $\geq$90 was also significantly lower (Fig. 1B). These data indicated that higher BCG coverage might have a significant effect on reducing the incidence rate and mortality of COVID-19. According to the data released by WHO, most countries nationally recommend BCG vaccination are LDCs, LLDCs, and SIDs, but the HDI of these countries is generally low. In order to verify the relationship between HDI and the incidence rate and mortality of COVID-19, we divided all the countries into four levels according to the Human Development Report 2019 released by the United Nations Development Programme. The data indicated that countries with a lower HDI had lower COVID-19 incidence (Fig. 1C) and mortality (Fig. 1D). Countries with low HDI are mainly concentrated in LDCs, while the BCG vaccination rate in LDCs is generally higher than 90%. This result once again shows that increasing the coverage of BCG can effectively reduce the morbidity and mortality of COVID-19.

2.2. *Transmission classifications have a influence on the development of the COVID-19*

Furthermore, the transmission classifications play an important role in affecting the development direction of the COVID-19 pandemic, which reflects the quality of a country's prevention strategy for COVID-19. We proposed a hypothesis that the transmission of COVID-19 is mainly classified as clustered or sporadic cases in countries with good prevention strategies, while the transmission is classified as community transmission in countries with poor prevention strategies. To test this hypothesis, we analyzed the impact of different transmission classifications on the incidence rate and mortality of COVID-19. Our analysis showed that the COVID-19 morbidity (Fig. 1E) and mortality (Fig. 1F) of countries with clustered or sporadic cases were significantly lower than those of countries with community transmission.

2.3. *Data and reality: confounders that cannot be ignored*

It should be pointed out that although the current data seem to support the hypothesis that the BCG vaccine induced protection from COVID-19 infection, some confounding factors behind the data may affect the accuracy of the data. First, international comparisons of COVID-19 epidemiology are difficult because the ways in which countries record COVID-19 cases and deaths differ depending on the polymerase chain reaction (PCR) results or clinical judgment.[7] Second, population characteristics of countries such as population density, median age and urban population, and mainly SARS-CoV-2 test rates are the main confounders that can lead to a misinterpretation of the BCG vaccination policy as protective against COVID-19. Test rates are low in less developed countries, which also affects the incidence of COVID-19.

2.4. *Developing countries are facing severe challenges*

Although our data support the hypothesis that BCG vaccination can reduce the morbidity and mortality of COVID-19, this hypothesis needs more clinical trials to verify. It is encouraging that at least five clinical trials are currently evaluating the role of BCG in the prevention and treatment of COVID-19

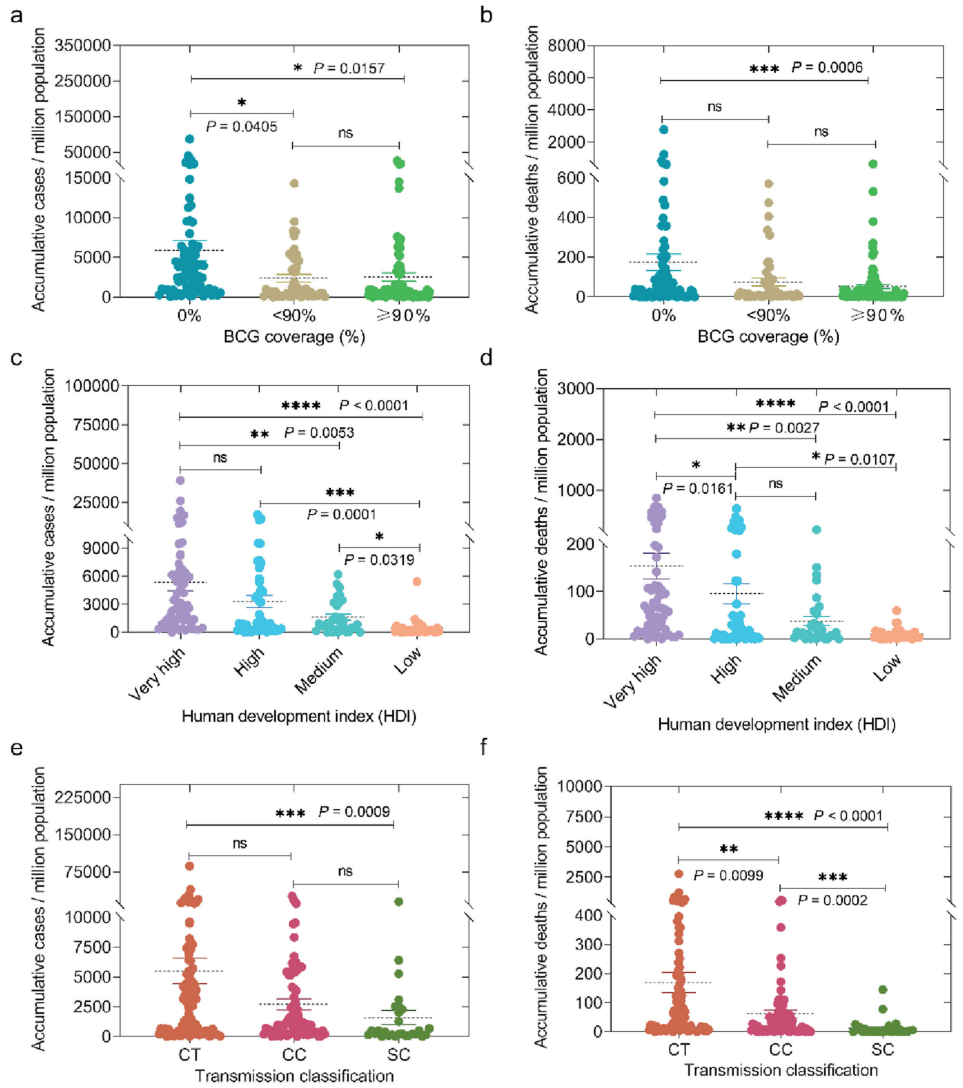

Fig. 1 – The relationships between BCG coverage (a and b), HDI (c and d), or transmission classification (e and f) and incidence rate and mortality of COVID-19. All of the results in this study were performed by using a GraphPad Prism 8 software (San Diego, CA, USA). The data were expressed as confirmed cases or deaths per 1 million population and compared with one-way analysis of variance (ANOVA) or Kruskal–Wallis test according to the data normality and homogeneity of variances. All data were shown as mean +SEM ($n = 6$ or 7). $P < 0.05$ was considered significantly different. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, no significance. CT, community transmission; CC, clusters of cases; SC, sporadic cases. All the original data used in this study can be obtained from the Supplementary Material Table S1.

in different countries.[8] However, the advantages of widespread BCG vaccination will not allow developing countries to achieve a decisive victory in this epidemic. Even more worryingly, an editorial comment published in the journal Nature warned that COVID-19 might accelerate the spread of tuberculosis (TB) in developing countries, especially in LDCs.[9] The population of 47 LDCs accounted for 12% of the world's total population, but their total GDP accounted for only 2% of the world.[10] These countries are not only suffering from infectious diseases such as COVID-19, tuberculosis, and AIDS, but are also facing the risk of food shortages and humanitarian crises.

2.5. *Open and cooperation are the magic weapon to defeat the COVID-19 pandemic*

It is therefore our hope that this study will give people in a gloomy world a ray of light and confidence in defeating the epidemic, provide an alternative vaccine for high-risk population (such as health care workers, the elderly, etc.), and win time for the development of COVID-19 vaccines. Moreover, routine preventive measures such as keeping social distance, wearing masks, and washing hands frequently should be strengthened to prevent the spread of COVID-19. Here, we recommend that more countries disclose their vaccines as

public products, which will not only help developing countries (especially LDCs, LLDCs, and SIDs) overcome the COVID-19, but will also lay the foundation for the global eradication of the SARS-CoV-2 virus.

3. Conclusions

In countries where the government recommends BCG, the high coverage of the BCG vaccination reduces the morbidity and mortality of COVID-19. However, this result may be inaccurate due to many confounders such as PCR testing rate, population characteristics, and protection strategies. The role of BCG in COVID-19 should be confiermed by clinical trials. Furthermore, we still need to increase investment in human, material, and financial resources to accelerate the development of effective and safe COVID-19 vaccines.

Ethics approval and consent to participate

Not applicable.

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Authors' contributions

Conceptualization: XQW and WPG; Data curation: WPG; Formal analysis: WPG; Funding acquisition: WPG; Methodology: WPG; Software: WPG; Writing - original draft: WPG; Writing - review & editing: WPG and XQW.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

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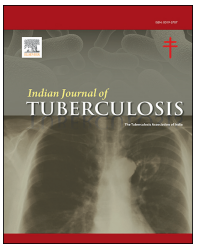

Case report

Addison's disease as a primary manifestation of extrapulmonary tuberculosis; A case report

Nithin Ranawaka[*], *N.H. Welikumbura*

Postgraduate Institute of Medicine, University of Colombo, Sri Lanka

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ABSTRACT

Tuberculosis remains an important public health problem globally. Addison's disease due to bilateral adrenal Tuberculosis as the primary manifestation of Extrapulmonary Tuberculosis is a very rare clinical entity.

Previously healthy 52 years old male presented with increasing darkening of the skin, dizziness, loss of weight, loss of appetite, generalized weakness for one year and diarrhoea, vomiting for 3 months. Patient did not have any history of exposure to Tuberculosis. Physical examination revealed a hyposthenic man with generalized hyperpigmentation especially on the face, oral mucosa, palmer crease, and knuckles. Investigations revealed high erythrocyte sedimentation rate, persistent hyponatremia, and strongly positive mantoux test. Short Synacthen test confirmed the adrenal insufficiency. Ultrasound scan of the abdomen found to have bilaterally enlarged adrenal glands. Contrast-Enhanced Computed Tomography of abdomen confirmed the bilaterally enlarged adrenal glands. Magnetic resonance imaging brain has done, it was normal with no evidence of pituitary masses. Then Computed Tomography guided biopsy has done from left adrenal gland. Histology of biopsy report was compatible with Tuberculosis. With the evidence of above finding this patient diagnosed to have Addison's disease due to tuberculosis of bilateral adrenal glands.

Anti-Tuberculosis Treatment started and continued for six months. Hydrocortisone and Fludrocortisone started. When there is an adrenal insufficiency, it should be always considered the possibility of existence of TB even failure to isolate bacillus Mycobacterium, failure to identify epidemiological exposure.

1. Introduction

Tuberculosis (TB) is an airborne infectious disease caused by bacillus *Mycobacterium tuberculosis* and rarely by *Mycobacterium bovis* and *mycobacterium africanum*. TB primarily affects lung parenchyma, nevertheless can affect any other organ of the body.[1] Adrenal insufficiency can be due to primary or secondary causes. Addison's disease (AD) is another name for primary adrenal insufficiency.[2] AD is a rare disorder. 75–80% caused by autoimmune destruction of adrenal gland and TB account for 7–20% of Addison's Disease.[3] TB can be present

* *Corresponding author*. Nithin Ranawaka. No 123, Andadola, Wathugedara, Sri Lanka.
E-mail address: ranawakanithin@yahoo.com (N. Ranawaka).
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with wide variety of symptoms with or without complications. Symptoms of AD is a rare way of presentation TB and most of heath care workers often missed until late a stage. The aim of this case presentation is to make healthcare workers aware of AD as a way of presenting TB.

2. Case report

A 52 years old Sri Lankan Sinhalese male presented to District General Hospital Kalutara, Sri Lanka, complaining of increasing darkening of skin, dizziness, loss of weight, loss of appetite, generalized weakness for one year and diarrhoea, vomiting for 3 months. He noticed that his skin was increasing darkening over last one year. Darkening initially noticed in lips and extensor surfaces of arms then progressively increased to buccal mucosa, palmer creases, face, neck, and the body. Weight loss was 8kg over last year. Later, his bowel habits started to get altered and developed loose motion about three to four times per day. It was watery type stools. There was no history of passing tarry black stools or bleeding per rectum. At the same time, he developed vomiting about one to two times per day. For the above symptoms, he had taken treatments several times from a general practitioner but did not get better. He was advised to get hospitalised for further investigations. He did not have any cough, difficulty in breathing or fever. Urinary habits were normal. There was no joint pain, skin rash, muscle pain, or weakness. He did not have any hearing impairment, loss of taste, numbness of face, or difficulty in speech.

He had no contact history with TB patient. He did not have any history of tuberculosis, diabetes mellitus, hypertension, Ischaemic Heart Disease or bronchial asthma. He was not on any medication or ayurvedic treatments up to the date. He had not undergone surgeries in the past. There was no history of allergies to food or medicine. There was no significant family history of illness including carcinoma, vitiligo, Diabetes Mellitus. He is a businessman who owned a Grocery. He is a smoker (Fifteen pack years) and a social drinker.

On examination, he looked emaciated and dark pigmented. He was ill look, pale, dehydrated. He was not dyspnoeic. There was no icterus, cyanosis, Lymphadenopathy, clubbing, skin rashes or ankle oedema. His weight was 44kg and his height was 161cm. Body Mass Index was 16.97. He is tachycardic and normotensive. On auscultation no murmurers detected, no abnormal breathing sound heard. Abdomen was soft and non-tender. There was no hepatosplenomegaly or any other detectable abdominal masses. Neurological examination was clinically normal.

Laboratory investigations on admission revealed normochromic, normocytic anaemia, high erythrocyte sedimentation rate, hyponatremia. His renal functions, liver functions, thyroxine level, urine full report, urine culture, screening test for human immunodeficiency viruses were normal. Chest x-ray on admission did not revealed any abnormality. Sputum for acid fast bacilli (AFB) was negative. Ultrasound scan of abdomen revealed 2nd degree fatty liver and bilaterally enlarged adrenal gland. Consultant radiologist suggested the Contrast-Enhanced Computed Tomography (CECT) of abdomen to exclude the adrenal carcinoma. CECT was performed and confirmed the Bilaterally enlarged adrenal glands (3 cm × 3.3 cm on left, 2.5 × 2.1 cm on right) without enlargement of any lymph nodes. Short Tetracosactide (Synacthen) test was positive (Basal cortisol level - 09 μg/dL, Cortisol level after 30min–12 μg/dL) and adrenal insufficiency was confirmed. Magnetic resonance imaging brain has done, it was normal with no evidence of pituitary masses. Secondary causes for adrenal insufficiency were excluded. Mantoux test was performed and was strongly positive with 20mm in 73 hours. Then Computed Tomography guided biopsy has done from left adrenal gland. Histology report reviewed adrenal tissue with extensive caseation necrosis, many epithelioid cells and langahan's type giant cells which strongly suggestive of tuberculosis in adrenal glands. Considering all symptoms, physical examinations and investigations established the diagnosis as AD due to tuberculosis of bilateral adrenal glands.

He was informed about his condition and registered in District TB Register as a case of extra pulmonary TB, TB Addison's Disease. The file was opened, District TB number has given, TB treatment card filled in duplicate and arranged Directly observed treatment, short course (DOTS) from Base Hospital Panadura, Sri Lanka. Category 1 treatment regime was started. As his weight was 44kg, Isoniazid + Rifampicin + Pyrazinamide + Ethambutol (HRZE), Fixed Dose Combination 3 tablets daily started for the intensive phase. Health education was given to the patient and family members. Patient has seen by endocrinologist. Oral hydrocortisone 10mg morning, 5mg noon and 10mg night and oral fludrocortisone 100mg daily started. After the treatment patient feels better and diarrhoea and vomiting were subsided within 2 days. Serum sodium level reached to the normal level on third day. The patient had good compliance with the treatment. After two months of treatment we have reviewed the patient and his condition was markedly improved. His weight was increased by three kilograms. ESR decreased up to 32 1st/h. Appetite also markedly improved. At the end of the intensive phase, Anti TB treatment was changed to the continuation phase. Isoniazid + Rifampicin (HR) fixed dose combination three tablets daily for continuation phase. The continuation phase continued for four months. TB treatment was successfully completed. As the patient is a heavy smoker, we have counselled him for smoking cessation.

3. Discussion

AD (primary hypoadrenalism) is a rare endocrine disease. Incidence of AD is 3–4/million/year and prevalence is 40–60/million.[4] AD occurs when there is destruction of the adrenal cortex and when it does not produce enough hormones, specially cortisol and aldosterone.[4] Endocrinological derangements are very rare in TB. AD usually result from chronic infection of adrenal gland, which may result from early haematogenous spread.[5] Most probably TB infection begins from lungs and spread to adrenal gland by haematogenous way (6). Our patient did not have history of exposure to TB. His lungs were clear and x ray was normal. Sputum examination negative for AFB. Rout of spread TB to the adrenal gland was unknown. Negative result from x ray of lung, sputum for AFB cannot exclude the existence of TB. Positive tuberculin skin test, histopathological findings aid for the diagnosis of TB.

There were rare cases published on primary adrenal insufficiency due to TB adrenalitis in a patient without active pulmonary TB.[6] Even though there are possibilities of recovery of adrenal functions after anti TB treatment, our patient did not regain adrenal functions. Therefore, recommended for life long hormonal therapy.

4. Conclusion

When there is an adrenal insufficiency, it should be always considered the possibility of existence of TB even failure to isolate bacillus Mycobacterium, failure to identify epidemiological exposure. Positive Tuberculin test, positive histopathology collaboration with clinical finding often enough to start Anti TB therapy.

Consent

Written informed consent was taken from the patient.

Conflicts of interest

All authors have none to declare.

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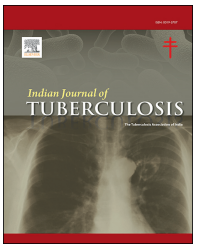

Case report

"Neuroimaging in ethambutol induced optic neuropathy: MRI in time can save the vision"

Vivek S. Murumkar [a], *Shamick Biswas* [a,*], *Jitender S. Saini* [a], *A.R. Prabhuraj* [b]

[a] *Department of Neuroimaging and Interventional Radiology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, 560029, India*

[b] *Department of Neurosurgery, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India*

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ABSTRACT

Ethambutol is an integral part of Antitubercular therapy (ATT) and is often associated with optic neuropathy, However, neuroimaging of ethambutol induced optic neuropathy has been sparsely reported in the literature. We describe the case of a 45-year male patient, diagnosed as Tuberculous spondylodiscitis and was on ATT. Four months after ATT initiation, he presented with visual blurring in both the eyes with bitemporal hemianopia and central scotomas. Visual evoked potential (VEP) revealed prolonged latencies in N75 and P100 waveforms bilaterally. Magnetic Resonance Imaging (MRI) showed optic chiasma and bilateral optic tract hyperintensities on 3D Fluid Attenuated Inversion Recovery (FLAIR) and 3D Double Inversion Recovery (DIR) sequences. Ethambutol was discontinued immediately. On follow-up after 8 weeks, visual acuity reversed back to normal in both eyes.

Key Messages: Every patient with ethambutol therapy should be monitored for visual symptoms and if found symptomatic, prompt withdrawal of ethambutol may reverse the symptoms. Advanced neuroimaging may serve as a problem-solving tool in unusual case scenarios.

1. Introduction

Ethambutol induced optic neuropathy is one of the dreadful complications associated with ATT and warrants prompt withdrawal. The involvement of optic chiasm, optic tracts, and optic nerves have been shown in previous animal studies.[1] However, MRI in only two case reports has shown optic chiasm and optic tract involvement.[1,2] In the index case presented here, we exploited advanced neuroimaging sequences (3D FLAIR and 3D DIR), both of which have superior spatial resolution to demarcate optic pathway lesions. Our case report is the first showing 3D DIR signal abnormality of the optic chiasm and optic tracts in ethambutol induced optic neuropathy.

* *Corresponding author*. Department of Neuroimaging and Interventional Radiology, National Institute of Mental Health and Neurosciences (NIMHANS), Room no 123, New Kabini Hostel, NIMHANS Hospital Campus, Hosur Road, Bangalore, 560029, India. Tel.: +919945345845.

E-mail address: biswasshamick1@gmail.com (S. Biswas).

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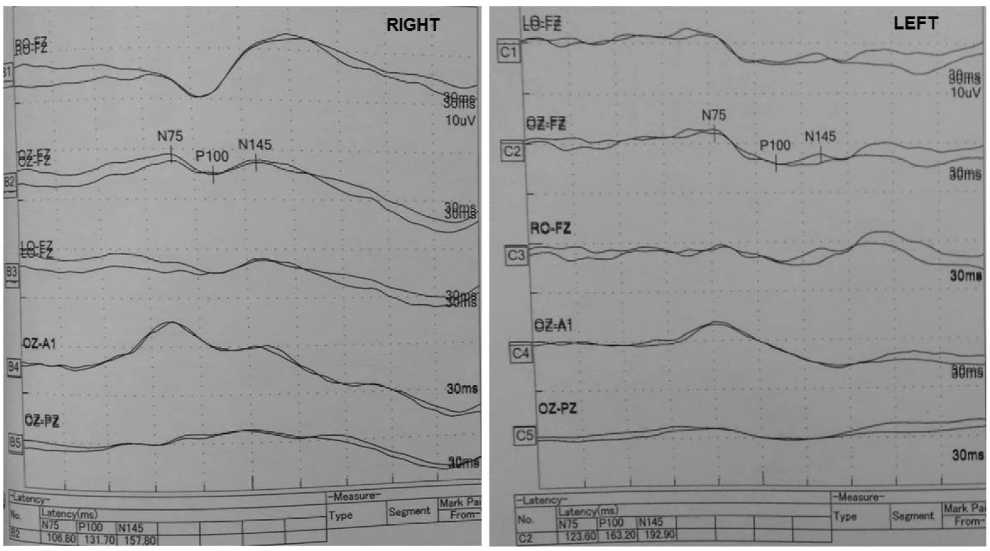

Fig. 1 – Visual Evoked Potential showing prolonged latencies in N75 and P100 waveforms bilaterally.

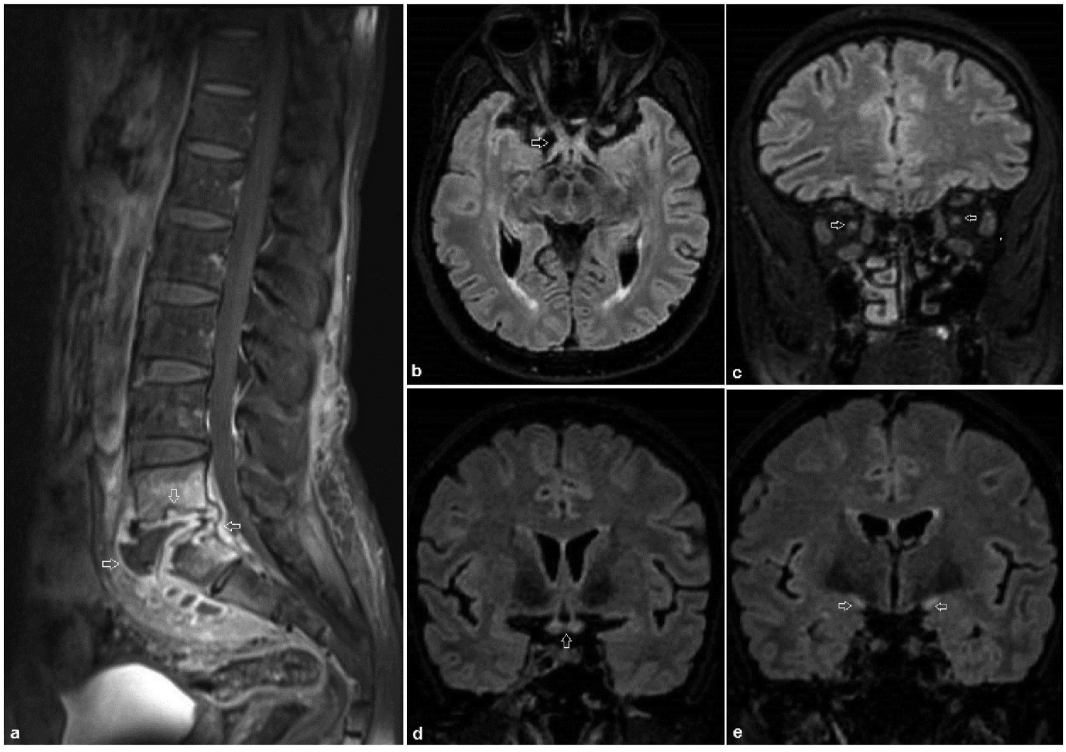

Fig. 2 – (a) Post-contrast Sagittal T1 fat-saturated image of the Lumbosacral spine showing spondylodiscitis involving the L5 and S1 vertebrae with prevertebral and anterior epidural soft tissue component (arrows) - s/o Tubercular etiology, (b) Axial 3D FLAIR image showing hyperintensity in the optic chiasm and proximal portion of both the optic tracts (arrows), (c) Coronal 3D FLAIR image showing normal signal intensity of both the optic nerves (arrows), (d and e) Coronal 3D FLAIR image revealing hyperintense signal intensity of the optic chiasm (d) and bilateral optic tracts (e) (arrows).

2. Case history

A forty-five-year-old male patient, diagnosed case of tuberculous spondylodiscitis (Fig. 1) and was on treatment with ATT [initial 2 months of Isoniazid (10mg/kg), Rifampicin (5mg/kg), Pyrazinamide (25mg/kg) and Ethambutol (15mg/kg) followed by the three drugs - Isoniazid, Rifampicin and Ethambutol in the maintenance phase]. Four months after initiation of ATT, he presented with complaints of visual blurring in

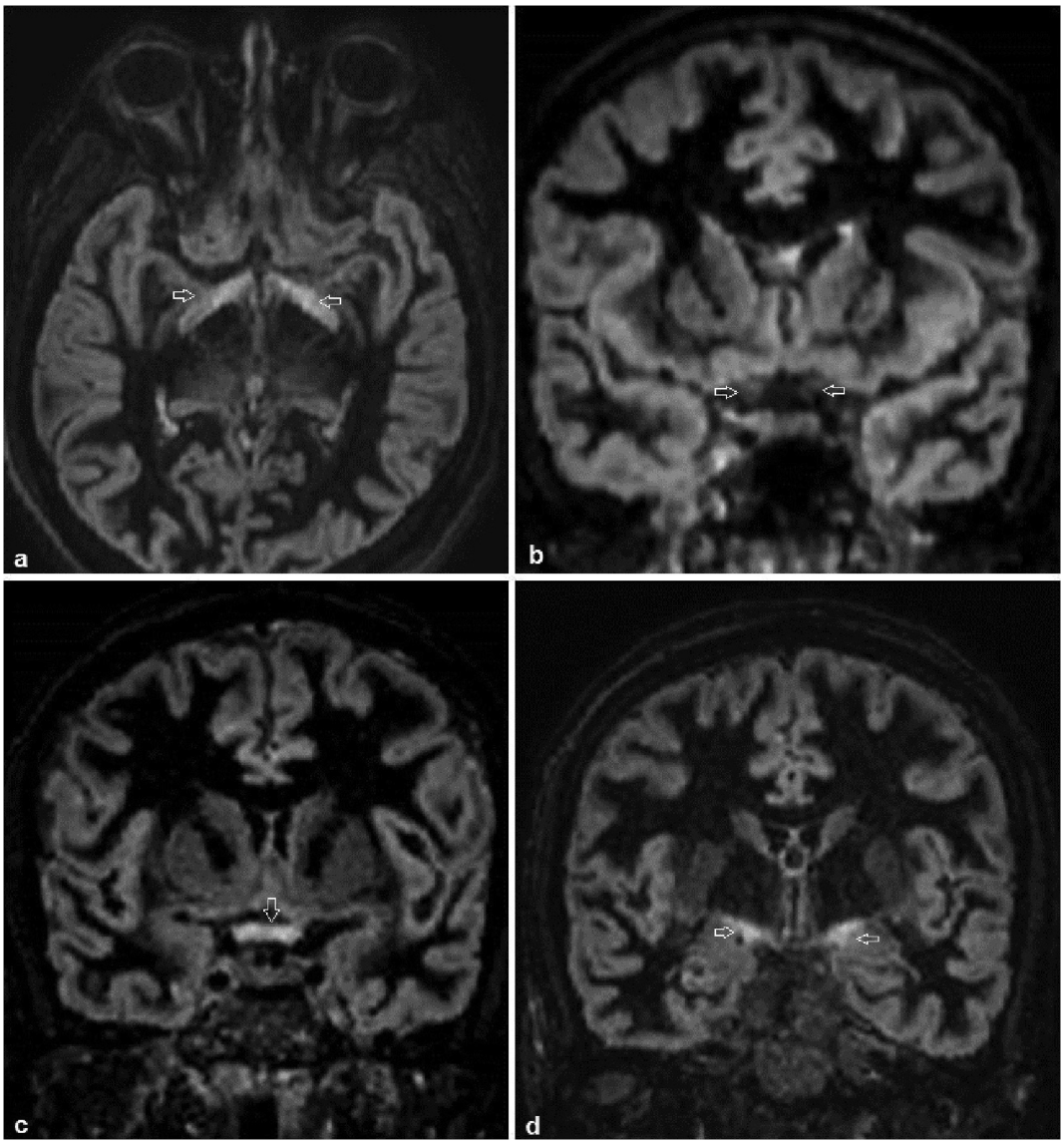

Fig. 3 – (a) Axial 3D DIR image of brain showing marked hyperintensity along the bilateral optic tracts (arrows), (b) Coronal 3D DIR image showing normal signal intensity of both the optic nerves in the cisternal segments (arrows), (c and d) Coronal 3D DIR image revealing hyperintense signal intensity of the optic chiasm (c) and bilateral optic tracts (d) (arrows).

both the eyes. On examination, his visual acuity in the right eye was 20/40 and that on the left side was 20/100. Colour vision was normal in both eyes. Perimetry showed bitemporal visual field defects and bilateral central scotomas. VEP revealed prolonged latencies in N75 and P100 waveforms in both the eyes (Fig. 1). Symptoms despite being treated with a lower dose of ethambutol and preserved colour vision in our case questioned the diagnosis of the ethambutol induced optic neuropathy. To solve the diagnostic dilemma, neuroimaging with dedicated optic pathway imaging was performed which revealed abnormal FLAIR and DIR hyperintense signal intesnity involving the optic chiasma and both the optic tracts with sparing of bilateral optic nerves (Figs. 2 and 3) confirming the diagnosis of ethambutol induced optic neuropathy. After discontinuation of ethambutol, the patient's vision improved gradually over the next eight weeks to 20/20 on both sides. Visual field defects were also reversed. Since the patient improved clinically, repeat neuroimaging was not performed.

3. Discussion

Ethambutol is one of the first-line drugs for the treatment of tuberculosis and is bacteriostatic by nature. Its mechanism of action is poorly understood; it is postulated to act by disrupting one of the metal-containing enzymes of mycobacterial nucleic acid metabolism pathway.[3] Its toxicity can be attributed to a similar mechanism although it is largely unknown. One of the major adverse effects of ethambutol toxicity is optic neuropathy. Various theories have been advocated such as the depletion of intracellular zinc and copper of retinal ganglion cells due to the chelating action of ethambutol.[1] Other proposed mechanism includes

glutamate-induced excitotoxicity causing mitochondrial dysfunction.[1]

It has been postulated that ocular toxicity of ethambutol is dose and duration related. Dose more than 15–25 mg/kg has been reported to cause optic neuropathy.[4] Although reversibility after discontinuation of treatment remains controversial, recent research has shown reversibility in a majority of the cases.[4,5] One of the early manifestations of ethambutol induced optic neuropathy is dyschromatopsia; blue-yellow colour changes are most common.[4] To our surprise, our index case had preserved colour vision. In animal models, the involvement of the optic nerve, optic chiasm, and optic tracts have been demonstrated in ethambutol treated animals. Bitemporal hemianopia and central scotomas are frequent findings on perimetry and can be explained on an anatomical basis.

Neuroimaging for diagnosis of ethambutol induced optic neuropathy has been very rarely performed. To the best of our knowledge and literature search, only two previous case reports have shown optic chiasm signal abnormalities in humans.[1,2,6] Our patient also had bitemporal hemianopia with bilateral central scotomas on perimetry. Imaging was performed in our case to rule out structural lesion and it revealed abnormal hyperintensity involving the optic chiasm and both the optic tracts on 3D DIR and 3D FLAIR. There was relative sparing of the optic nerves. No diffusion restriction was noted. We chose to perform 3D DIR as it causes suppression of CSF as well as normal-appearing white matter by using two inversion pulses. Optic nerve being a continuation of the brain white matter appears as a hypointense structure while its meningeal sheath appears isointense thus providing inherent contrast to detect subtle lesions.[7] 3D DIR has been proved to be better than routine T2 coronal fat-saturated images in the diagnosis of acute optic neuritis.[8] Thus our case is an illustration of the prototype neuroimaging of ethambutol toxicity on advanced MRI sequences with excellent clinico–neuroradiological correlation.

We acknowledge the non-availability of follow-up neuroimaging as a limitation of our report. The patient however showed complete resolution of symptoms with normalization of the visual acuity and reversal of the visual field defect on perimetry.

4. Conclusion

Signal intensity alteration on MRI in the optic pathway, predominantly in its posterior aspect in a patient who has received or is on ATT should alert us to the possible diagnosis of Ethambutol induced optic neuropathy. The patient should be thoroughly evaluated for visual symptoms and if symptomatic, immediate cessation of ethambutol may help in reversing the visual symptoms.

Conflicts of interest

The authors have none to declare.

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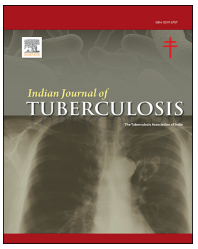

Case report

Case reports of chronic myeloid leukemia and tuberculosis: Is imatinib the link between the two?

*Shailendra Prasad verma**, *Anil Kumar Tripathi, Nidhish Kumar, Suneel Kumar Gupta*

Department of Clinical Hematology, King George's Medical University, Lucknow, Pin-22603, India

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ABSTRACT

Current standard of care for treatment of CML is based on tyrosine kinase inhibitors (TKI's). Imatinib is most frequently used first line tyrosine kinase inhibitor. Various side effects of TKI's are known, but some may still be unknown. We are reporting three cases of CML who developed tuberculosis while on treatment with imatinib or dasatinib. Two cases developed CNS tuberculosis and other one was tubercular pleural effusion. These cases indicate that imatinib and other TKI's probably interfere with immunological functions and predispose patients for tuberculosis.

1. Introduction

Chronic myeloid leukemia is the most common myeloproliferative neoplasm. Treatment of CML with TKI's is currently the standard of care.[1] Literature reveals case reports of occurrence of tuberculosis in patients with CML on treatment with imatinib. Along with inhibition of bcr-abl tyrosine kinase activity, imatinib probably also affects cell mediated immunity.[2–4] Long term treatment with imatinib probably leads to prolonged immunosuppression.[5] We report three cases of CML on tyrosine kinase inhibitor treatment who developed tuberculosis during treatment and showed good response to anti tubercular treatment. Informed consent was taken from all the patients.

2. Case 1

This 28 year young male presented with history of left hypochondriac pain and heaviness, lethargy and early satiety of 2 months duration. There was no history of jaundice, bleeding manifestations or fever. Patient did not have any significant comorbidities like diabetes mellitus, hypertension, COPD or tuberculosis in past. On examination he was found to have mild pallor and massive splenomegaly. His hemogram and peripheral blood examination revealed Hb-81gm/L, TLC-125 $\times$ 10^9/L and platelet count of 400 $\times$ 10^9/L. Peripheral smear examination revealed very high WBC count with preponderance of myelocytes, metamyelocytes and bands with normal platelet counts. BCR-ABL transcript was detected in peripheral blood by qualitative PCR. He was started on imatinib 400 mg

* *Corresponding author.*
E-mail address: drspkgmu@rediffmail.com (S.P. verma).
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once daily. At 3 month he achieved complete hematological response and bcr-abl transcript level was <10% on International scale (IS).

After 10 months on treatment patient presented with diffuse headache, off and on vomiting, and moderate fever of 3 week duration. He developed altered behavior 2 days before admission. On examination he had neck rigidity but no focal deficits. His hemogram was within normal limits and spleen was nonpalpable. CSF examination revealed total cells 5500 cells/mm,[3] and differential of neutrophils 5% and lymphocytes 95%.CSF sugar was 25.9 mg/dl with normal blood sugars. CSF adenosine deaminase level was 13.4 IU. Antigen test and India ink was negative for Cryptococcus. CSF-TB PCR was negative. CSF PCR for HSV, VZV and JE was negative. Considering the possibility of tuberculosis patient was started on anti-tubercular treatment along with steroids. Patient's consciousness improved day 3 onwards and his symptoms improved in 3 week period. Currently he has completed anti-tubercular treatment course of 1 year duration and doing well.

3. Case 2

This 27 year male presented with abdominal pain, lethargy and hepatosplenomegaly of 1 year duration. Hemogram showed moderate anemia and elevated counts with left shift. BCR-ABL was positive and he was diagnosed as a case of CML. He was on Imatinib and was doing well till June 2015 when he developed rising counts and increasing spleen size. Mutation analysis revealed tyrosine kinase mutation F359V sensitive to dasatinib. Patient was not affordable for dasatinib so he was shifted to 600 mg of imatinib. One month after this, patient developed fever, exertional breathlessness and left sided chest pain of subacute onset. Chest X ray revealed moderate pleural effusion. Pleural fluid ADA was 61.77 U/L and TB-PCR for tuberculosis was positive. He was started on 4 drug ATT and his chest X-ray at the end of 2 months intensive treatment showed complete resolution of effusion. His counts were elevated after 6 months of starting higher doses of imatinib.

4. Case 3

This 55 year old gentleman was diagnosed as a case of CML in January 2015. He was started on Imatinib 400 mg OD but he could not achieve hematological response at the end of 3 months. Imatinib resistance mutation analysis did not reveal any tyrosine kinase domain mutation. He was shifted to dasatinib and tolerated well. At the end of 1 year he achieved a major molecular response. He presented with mild diffuse headache with occasional vomiting of 2 week duration and altered sensorium for 1 day. His vitals were stable and he had neck rigidity. His CSF examination revealed total cell count of 350 cell/mm^3 and lymphocytic predominance. CSF protein was 300 mg/dl and sugar 30 mg/dl (corresponding blood sugar- 140 mg/dl). CSF –PCR for tuberculosis was positive, India ink preparation and antigen test for Cryptococcus was negative. CSF-VDRL was also negative. There was no evidence of malignant cells in CSF. This patient was started on steroid and ATT with rifampicin replaced with levofloxacin. He recovered in 2 weeks but again developed altered sensorium after 1 month. CT did not show any radiological changes. He was managed conservatively and improved. He is in follow up currently doing well on dasatinib.

5. Discussion

CML is the most common myeloproliferative neoplasm. Imatinib is one of the tyrosine kinase inhibitors, used as targeted therapy for CML. Although there is no proven association of imatinib treatment and occurrence of tuberculosis, various CML and non CML conditions have been reported where tuberculosis occurred during imatinib treatment. There is always a concern if imatinib treatment increases incidence of tuberculosis. Several other anti-cancer drugs like steroids, rituximab and TNF alfa inhibitors are already known for predisposing patients for tuberculosis.

A review of all confirmed cases of tuberculosis by Kamboj and Sepkowitz at Memorial Sloan Kettering Cancer Centre (New York) identified 290 cases. This review included patients of all hematological disorders between year 1980–2004 and the calculated incidence was 50 per 100,000 persons. TB occurred most frequently in non-hodgkin lymphoma, hodgkin lymphoma and allogeneic stem cell transplant patients.[6] Silva et al has done a retrospective review of 917 cases with various hematological malignancies.[7] Diagnosis of tuberculosis was made in 24 cases and most of them had non-Hodgkin's lymphoma or chronic lymphocytic leukemia. Out of 45 patients of CML only one developed tuberculosis. These reviews summarize that although association between lymphoid malignancies and TB has been described, this is probably not the case for CML.

Patients with CML are routinely treated with tyrosine kinase inhibitors like imatinib. There are several reports of infections with varicella zoster virus infection, herpes zoster infections and reactivation of hepatitis B virus activations in literature.[8–10] This suggests that Imatinib might impair the immune system more likely the cell mediated immunity. Daniels et al. has reported 3 cases of tuberculosis in patients with CML on imatinib treatment. One patient had spinal tuberculosis while other two had pulmonary tuberculosis. Duration of treatment with Imatinib varied from 1 to 4 years before development of tuberculosis. He also suggested that Imatinib may be affecting the immune system predisposing patients for tuberculosis.[11]

Agaimya A et al has reported occurrence of tubercular lymphadenitis in a patient with metastatic GIST who was put on palliative care with imatinib.[12] Yagmour reported a 66 year old case of CML on imatinib who developed left supraclavicular node with lung opacities proven to be tubercular later on. Author explained this as a reactivation of tuberculosis and suggested if we should go for screening of latent tuberculosis in all our patient before starting TKIs.[13]

Takashima et al. has reported a case of metastatic gastrointestinal stromal tumor of small intestine who developed pulmonary tuberculosis after a few months of starting imatinib. Patient developed tuberculosis when he was having neutropenia due to imatinib treatment.[14]

Tubercular bacilli is primarily contained by antigen specific T-cells and macrophases. T cell receptors play an important role in all these process. T cell receptor signal transduction may be affected by use of imatinib. T cell response is important in handling viral and some chronic bacterial infections. Dendritic cell generation from CD34 positive peripheral blood progenitor cells is affected by imatinib mesylate is proven in vitro model.[15] These dendritic cells have reduced capacity to induce cytotoxic T cell response. It has also been postulated that Imatinib may reduce T cell receptor mediated T cell proliferation and activation in a dose dependent manner.[2–4] In vivo studies have also demonstrated reversible and dose dependent hypogammaglobulinemia, lymphopenia and impaired T cell responses indicating imatinib action on various arms of immunity simultaneously.[16–18] Broadly treatment with TKI s may be considered as a state of immunosuppression.

One third of world's population is estimated to be infected with mycobacterium tuberculosis. The prevalence of latent tuberculosis infection ranges from 9 to 80% in various populations in India. On an average the lifetime risk of development of active tuberculosis in individuals with latent tuberculosis infection is 5–10%. This risk further increases if patient becomes immunocompromised.[19]

There is also a concern of interaction of anti-tubercular drugs and tyrosine kinase inhibitors. Rifampicin is an enzyme inducer and can have interaction with TKI s. Bhatnagar et al has reported a case of peritoneal tuberculosis with chronic myeloid leukemia where modified ATT (rifampicin was replaced by moxifloxacin) was used to successfully treat tuberculosis with dasatinib for CML.[20] Anti-tubercular drugs and TKI's may have significant interactions. Most important one being increased metabolism and reduced levels of imatinib, nilotinib and dasatinib, when co-prescribed with rifampicin as it is a strong cytochrome P450 enzyme inducer.[21] CML patients on TKI's need replacement of rifampicin with some other antitubercular drug. Similarly isoniazid is a CYP3A4 inhibitor and can lead to impaired metabolism of dasatinib and needs monitoring.

6. Conclusions

This case series indicates that there is a need for close monitoring of CML patients on tyrosine kinase inhibitors. High incidence of latent tuberculosis in India makes it even more crucial. A baseline tuberculin test may be advised to patient of CML who are going to be started on imatinib or other TKI,s. A nation wise data will be more comprehensive and useful in further decision making. Extra pulmonary tuberculosis may be more common in these patients and antitubercular drug interaction with TKI,s should be kept in mind so that the disease outcome is not compromised.

Conflicts of interest

The authors have none to declare.

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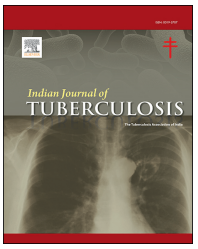

Case report

Myocardial tuberculosis and beyond: A rare form of extra pulmonary TB in a young boy

Bashir Ahmed, Professor [a], *Md. Mamunur Rashid, Assistant Professor* [a,*], *Md. Mahbubur Rahman, Assistant Professor* [b], *S.M. Lutfor Rahman, Assistant Professor* [a], *Shah Md. Saifur Rahman, Assistant Professor* [a], *Pulok Kumar Dey, Assistant Professor* [a], *Md. Abdul Momen, Associate Professor* [c], *Mohammed Shahedur Rahman Khan, Director cum Professor* [a]

[a] *Pulmonology, National Institute of Diseases of Chest and Hospital (NIDCH), Dhaka, Bangladesh*
[b] *Pulmonology, Faridpur Medical College, Faridpur, Bangladesh*
[c] *Cardiology, National Institute of cardiovascular Disease, Dhaka, Bangladesh*

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ABSTRACT

Myocardial tuberculosis is an exceptionally rare form of extra-pulmonary TB. Few cases were reported world-wide. Here a young snake charmer who had skin tuberculosis 5 yrs back admitted into National institute of diseases of Chest and hospital (NIDCH), Dhaka with the complaints of cough, palpitation and breathlessness for 2 months. He had right axillary firm matted lymphadenopathy, left sided large pleural effusion, left ventricular and septal hypertrophy with band and mass inside the ventricle (evident on CT scan of heart and echocardiography). His ESR was 95 mm in1st hr, Mantaux test was 15mm, Pleural fluid was exudative lymphocyte predominant with adenosin deaminase (ADA) 68.6 U/L. Fine needle aspirates from right axillary LNs showed *Mycobacterium tuberculosis* on GeneXpert for MTB/RIF testing and caseous granuloma on cytopathological study. Whole Body F18 FDG PET-CT revealed numerous low FDG avid size significant lymph nodes in right side of neck, mediastinum and right axilla with cardiomegaly with focal FDG avid within the left ventricular cavity likely to be prominent papillary muscle. MRI of heart or Myocardial biopsy for histology was not done due to their cost and invasiveness and also for that there was sufficient evidence of having tuberculosis in lymph node, pleura nas myocardium. This patient was treated with anti tubercular medications (3HRZE2S/5HRE) with prednisolone for six months. After treatment, myocardial lesions, pleural effusion and lymphadenopathy were found resolved. Thus a case of fatal and serious tuberculosis was explored and managed successfully.

* *Corresponding author*. National Institute of Diseases of the Chest and Hospital (NIDCH), TB Gate, Mohakhali, Dhaka, Bangladesh. Tel.: +88017111903377.
E-mail address: dr.mamun98@yahoo.com (Md.M. Rashid).
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1. Introduction

Despite recent medical advances, tuberculosis still remains as a deadly disease with the ability to invade almost any organ of the body.[1] Diversities in clinical presentations of these extra-pulmonary tuberculosis make difficulties in diagnosis. Cardiac involvement occurs in approximately 1–2% of TB patients and of them, most common site of involvement is the pericardium.[2] Myocardial involvement is extremely rare and estimated to be responsible for <0.1% of TB-related deaths. Usually Myocardial TB presents with acute fulminant myocarditis, sudden cardiac death or brady–or tachy arrhythmias[2,3] and diagnosis made on autopsy.[4] Treatment with anti-tubercular drugs has been shown to have better clinical outcome in TB myocarditis. Therefore, awareness of clinicians and prompt diagnosis will ensure better outcomes of this rare form of TB.[5]

2. Case report

Master X, 14 years male, snake charmer hailing from Boktarpur, Savar, Dhaka was admitted into National Institute of Diseases of the Chest and Hospital (NIDCH) with the complaints of Fever, cough and Palpitation for 2 months; Breathlessness and chest pain for 20 days. He had lost 5 kg in the meantime. He had skin TB successfully treated 7 yrs back with complete recovery. He was non-smoker with no co-morbidity. H was Ill-looking, anemic, under-weight, malnourished. He had enlarged right axillary lymph node which was 2 cm in diameter, single, mobile, non-tender, firm without any discharging sinus. There was a blackish scar mark over the flexor aspect of left mid arm (scar of previous healed skin TB). BCG vaccination mark was absent. There was left sided pleural effusion. Heart rate was 92 beat/min (Figs. 1 and 2).

His ESR was 95 mm in 1st hr. MT was 15 mm in 72 hours. Pleural fluid was straw color; protein 4.4 g/dl, sugar 64 mg/dl, adenosin deaminase (ADA) 68.6 U/L, lymphocyte 80%, malignant cell absent. Histopathology of Pleural tissue revealed chronic pleuritis. Fine needle aspirates (FNA) from right axillary lymph node revealed chronic caseous granuloma and geneXpert for MTB/RIF revealed Mycobacterium Tuberculosis which was Rif Sensitive. CT scan of chest (post aspiration) revealed encysted minimal pleural effusion and minimal hydro-pneumothorax with mild pleural thickening, cardiomegaly mainly enlarged left atrium and left ventricle (Fig. 3).

Electrocardiography (ECG) revealed T inversion in Lead III and aVF, left ventricular and septal hypertrophy. Echocardiography revealed an echogenic mass within the dilated left ventricle. It showed enlarged left atrium and ventricle with irregular filling defect due to eccentric hypertrophied ventricular muscle. Whole Body FDG PET-CT was done that revealed numerous low FDG avid size significant lymph nodes in right side of neck, mediastinum and right axilla suggestive of active stage of chronic granulomatous inflammation of lymph nodes. There was cardiomegaly with focal FDG avid within the left ventricular cavity likely to be prominent papillary muscle (Figs. 4 and 5).

In this way, Myocardial TB along with TB lymphadenitis and Pleural TB was explored. He was treated with category II anti-TB drugs (3HRZE2S/5HRE) with prednisolon (40 mg once daily for 2 months followed by tapering doses) and was followed up for 8 months. At the end, patient was found free from fever, cough, breathlessness and palpitation with no pleural effusion and lymphadenopathy. He gained weight. There was no intra-cardiac mass which was replaced by calcification and fibrous tissue seen on Echocardiography.

3. Discussion

Myocardial tuberculosis (TB) was responsible for 0.14% of tuberculosis-related deaths evident on post-portem studies.[6] The First report of myocardial TB was done in 1664 by Maurocordat, followed by in 1761 by Morgagni.[4] The myocardium can be affected by direct extension, secondary to hematogeneous spread from a remote tuberculous focus, retrograde lymphatic drainage from mediastinal nodes, and

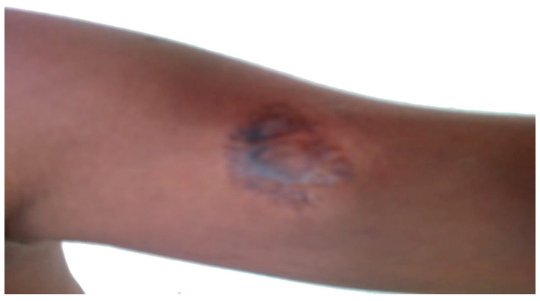


Fig. 1 – A well circumscribed blackish scar mark over the flexor surface of left arm representing previously healed cutaneous tubercular lesion.

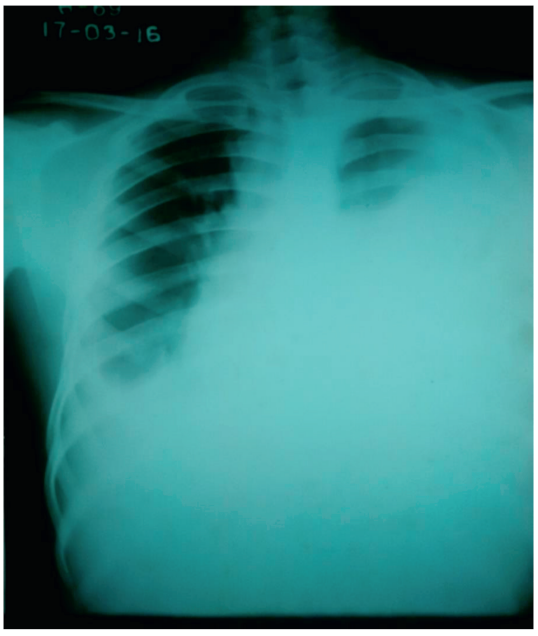


Fig. 2 – X-ray Chest P/A View revealed left sided large pleural effusion that obscured heart and left cardiophrenic and costophrenic angle.

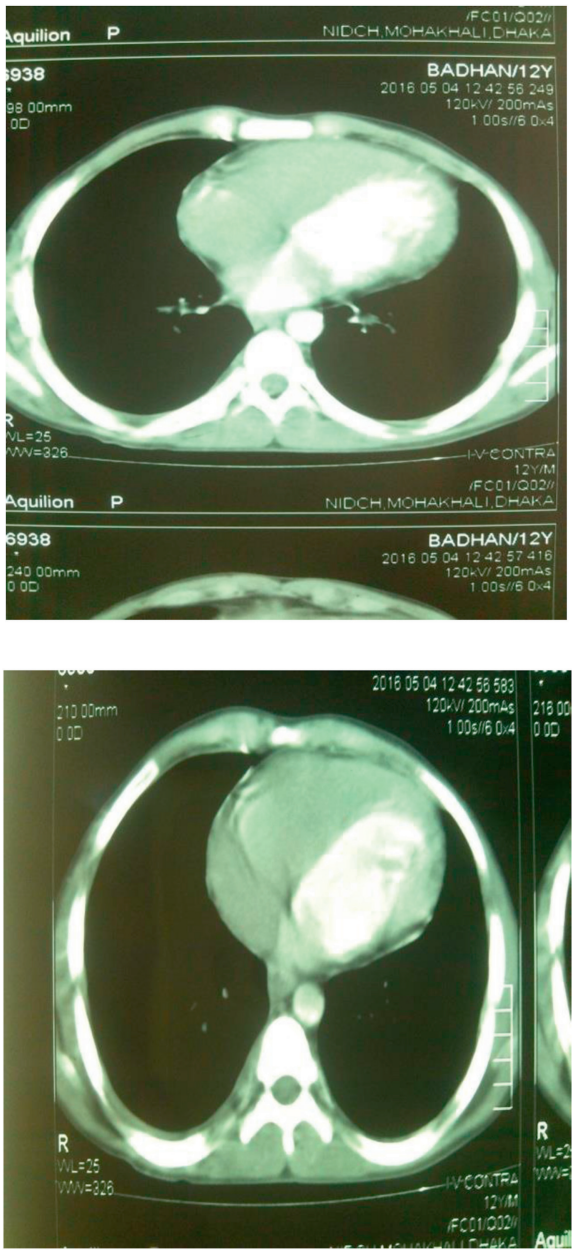


Fig. 3 – CT scan of heart showed cardiomegaly (with enlarged left atrium & left ventricle) with irregular filling defect due to hypertrophied ventricular muscle (tuberculoma).

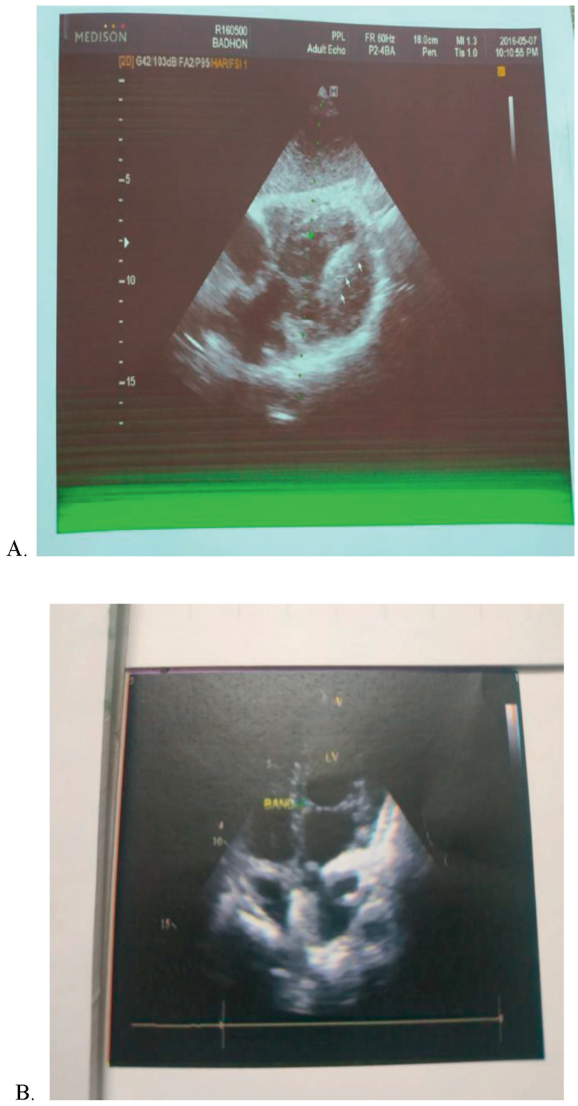
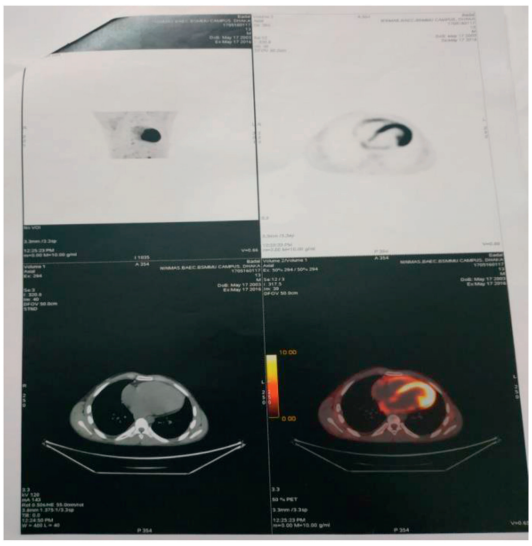


Fig. 4 – Color Doppler Echocardiography revealed dilated left ventricle, band inside left ventricle, intra cardiac mass (Tuberculoma).

direct spread from tuberculous pericarditis.[5] Myocardial involvement have been described in the form of (a) Tuberculomas of the myocardium with central caseation, (b) Milliary tubercles of the myocardium complicating generalized milliary disease (c) Uncommon diffuse infiltrative type associated with tuberculous pericarditis.[7] Here in this case, there was focal aggregation of tuberculoma in papillary muscles and left ventricle. The right heart, particularly the right atrium, is most often affected, probably because of the frequent involvement of the right mediastinal lymph nodes.[8] In this young boy left atrium and ventricle was affected probably extending from left mediastinal lymph nodes or left sided pleural TB.

Cardiac arrhythmias are common in myocardial TB that includes supra-ventricular arrhythmias, ventricular arrhythmias, conduction blocks, sudden cardiac death[3,4, 5,6,11]. This patient had right bundle branch block and non-specific T-wave changes. Echogenic mass in myocardium is typical in myocardial TB that is revealed on echocardiography in this patient who had left ventricular dysfunction as well.[8] PET/CT of the chest with 18-fluorodeoxyglucose (FDG) reveal increased FDG uptake in the myocardium and lymph nodes.[8] Cardiac MRI, a recent diagnostic tool had characteristic appearance on T2W images a) A central iso-intense core, corresponds to central caseation, b) A hypo-intense rim, corresponds to the fibrous capsule, c) A thin hyper-intense line, and corresponds with an inflammatory cellular infiltrate.[9] Though it's invasive one, endomyocardial biopsy is a good tool for diagnosis.[10] In this young man, we didn't go for myocardial biopsy or cardiac MRI considering their invasiveness or cost and also for that



A.

B.

Fig. 5 – Whole Body F18 FDG PET-CT: A. Cardiomegaly with focal FDG avid within the left ventricular cavity likely to be prominent papillary muscle B. Numerous low FDG avid size significant lymph nodes in right side of neck, mediastinum and right axilla that suggest active stage of chronic granulomatous inflammation of lymph nodes.

demonstration of mycobacterium bacilli and caseous granuloma in lymph nodes aspirates establish the diagnosis. Left sided Pleural fluid studies had the evidences of tubercular origin. Myocardial mass was also resolved with anti-TB treatment.

Treatment of myocardial TB with anti-TB drugs is very effective that was seen in this patient.[11] We used drugs for left heart failure temporarily. Antiarrhythmic drugs or implantable cardioverter-defibrillator is recommended in ventricular tachycardia. Radiofrequency catheter ablation is useful for incessant ventricular tachycardia. This patient don't need these. Follow-up is crucial in myocardial TB, especially in ventricular tachycardias. Clinical, radiology and imaging techniques are useful tools for follow-up. This patient improved clinically and there was resolution of echogenic myocardial mass, pleural effusion and lymphadenopathy uneventfully.[5]

4. Conclusion

During evaluation of disseminated TB especially when intrathoracic lymph node involvement occurs, cardiac TB should also be a suspicion. Though extremely rare, myocardial tuberculosis should also be kept in mind especially if patient present with palpitation, unexplained rhythm disturbances or unexplained ventricular dysfunction, even patients may not have constitutional symptoms. An early diagnosis of myocardial TB and its appropriate treatment leads to complete recovery and better outcome.

Conflicts of interest

The authors have none to declare.

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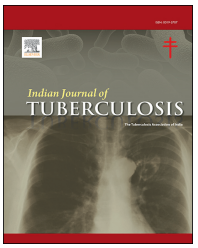

Case report

Opaque hemithorax - An interesting case

Rakesh K. Chawla [a,*], *Aditya K. Chawla* [b], *Gaurav Chaudhary* [a], *Madhav K. Chawla* [c], *Manoj Sareen* [a]

[a] *Jaipur Golden Hospital, Delhi, India*
[b] *Saroj Superspeciality Hospital, Delhi, India*
[c] *Baba Saheb Ambedkar Hospital, Delhi, India*

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ABSTRACT

Objective: To present an interesting case of left opaque hemithorax in an adult female and discuss its assessment and management.
Methods: **Design:** Case Report. **Setting:** Tertiary care hospital. **Patient:** One.
Results: 44yrs retropositive female admitted with complaints of acute onset dry cough since 15–20 days, sudden breathlessness since 5 days which was progressive in nature, left sided heaviness in chest since 5 days. CECT Thorax showed complete collapse of left lung with cut off of left main bronchus while video bronchoscopy showed left main bronchus completely blocked with very thick necrotic mass and was difficult to dislodge. Debulking with cryo probe was done and left main bronchus was completely cleared off. Allergen panel showed very high serum IgE, high S.IgE against aspergillus and high specific S.IgG against aspergillus. Patient and her Chest X-ray showed significant improvement post cryo debulking and was discharged satisfactorily on oral voriconazole therapy.
Conclusion: Endobronchial aspergillosis is characterized by massive intrabronchial overgrowth of the aspergillus species, mainly aspergillus fumigatus. Most patients with chronic pulmonary aspergillosis, including those with simple aspergillomas and Aspergillus nodules, have positive Aspergillus IgG antibodies in the blood. We hereby present a case of 44 yrs female presenting with complaints of dry cough and dyspnea and was diagnosed with endobronchial aspergillosis with complete obliteration of left main bronchus by fungal debris in which cryo debulking was done which relieved the symptoms significantly and was discharged in satisfactory condition on oral voriconazole therapy.

1. Introduction

Aspergillosis refers to the illness caused by a species of the fungus, Aspergillus. A broad range of manifestations can be seen in Aspergillus infection, including allergic disease, semi-invasive, and invasive disease.

Endobronchial aspergilloma sometimes mimic as an endobronchial carcinoid or a lung cancer.[1] It usually occurs in immunocompromised individuals and in patients with

* *Corresponding author*. Department of Respiratory Medicine, Saroj Superspeciality Hospital & Senior Consultant, Jaipur Golden Hospital, 36, Pocket: E-3, Sector-3, Rohini, New Delhi, 110085, India. Tel: 9810072860.
E-mail address: rakeshchawla8888@gmail.com (R.K. Chawla).
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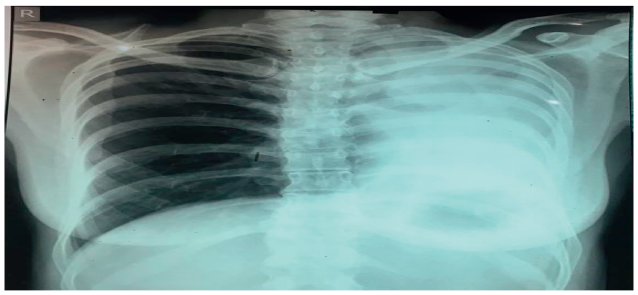

Fig. 1 – Chest skiagram showing complete opacification of left hemi thorax.

underlying lung disease but can also occur otherwise. Bronchoscopy is the tool for diagnosis and it can be confirmed by biopsy.

2. Case history

44yrs female presented to opd with complaints of acute onset dry cough since 15–20 days, sudden breathlesness since 5 days which was progressive in nature, left sided heaviness in chest since 5 days. Patient is a retropositive individual and on ART since 10yrs. There is no other significant past history or family history. No history of DM, HTN, Cardiac diseases, Thyroid disorders or any other systemic illness. On examination, General condition stable, No pallor, no icterus, no cyanosis, clubbing, pedal edema and lymphadenopathy. Vitals – all within normal limits, CVS – S1 S2 heard, no murmurs, P/A-soft, non tender, bowel sounds present, CNS-Conscious, oriented to time, place and person, No neurological deficit, Respiratory examination – Intensity of breath sounds decreased on left side. Routine investigations were within normal limits. Chest skiagram showed complete opacification of left hemi thorax (Fig. 1). CECT chest done showed complete collapse of left lung with cut off of left main bronchus with compensatory hyperinflation of the right lung, post contrast study showed enhancement of collapsed left lung parenchyma (Fig. 2). Video bronchoscopy was done which showed left main bronchus completely blocked with necrotic mass, it was difficult to dislodge, thinking as malignancy bronchial biopsy and cryo biopsy was done. We started debulking with cryo probe and as debulking proceeded, endobronchial lumen started to become visible, we continued with good suction. The muck started to clear, of course with difficulty, bronchial secretions were taken which were positive for fungal hyphae. Cytology reports were suggestive of large no. of neutrophils, eosinophils, alveolar macrophages and scattered bronchial epithelial cells in a mucoid background with fungal hyphae and charcot leyden crystals (Fig. 3) Cryo lung biopsy showed mucus plugs with entangled dense neutrophilic infiltrate along with eosinophils, a few charcot leyden crystals and septate fungal hyphae (Fig. 4). Post procedure skiagram showed partial clearance of opacification (Fig. 5). Bronchial

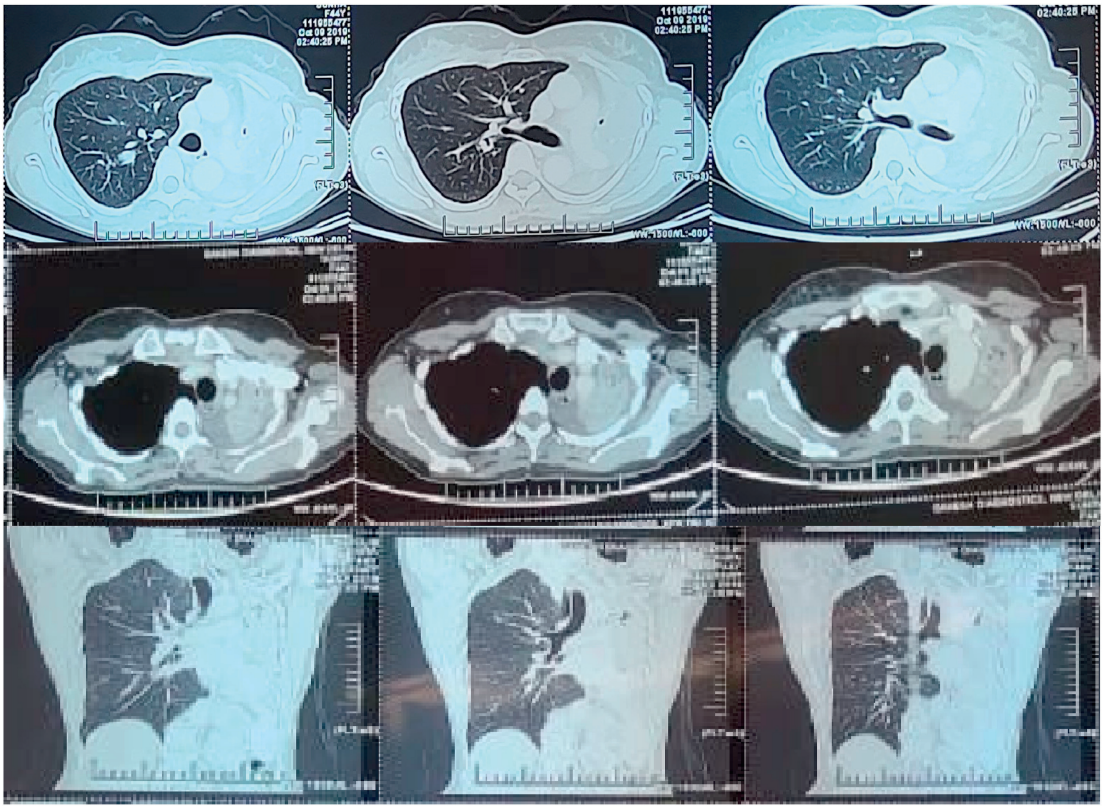

Fig. 2 – CECT chest showing complete collapse of left lung with cut off of left main bronchus with compensatory hyperinflation of the right lung.

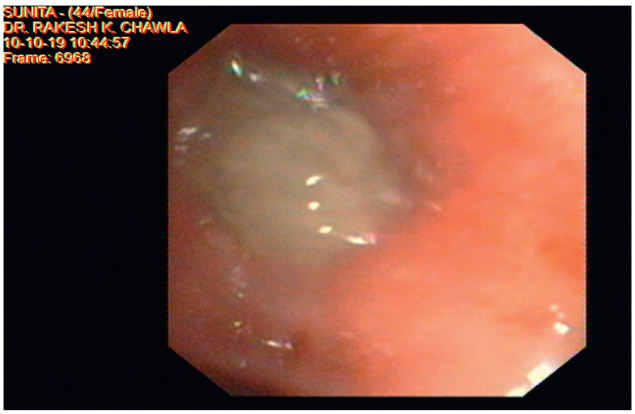

Fig. 3 – Bronchial secretions for cytology showed neutrophilia, eosinophils, alveolar macrophages in a mucoid background with fungal hyphae and charcot leyden crystals.

secretions for fungal culture showed aspergillus fumigatus (Fig. 6). Bronchial biopsy reports showed respiratory lining with underlying dense eosinophilic infiltrate in the subepithelial connective tissue stroma along with a few lymphoplasma cells with marked hyperemia and edema with no evidence of any invading fungal hyphae (Fig. 7). Patient required second fibreoptic bronchoscopy to clear the remaining fungal debris, post procedure chest skiagram showed complete clearance of opacification (Fig. 8). Allergen panel showed very high serum IgE(10,189 kU/l), high S.IgE against aspergillus (22.4 kUA/l) and high specific S.IgG against aspergillus (194 mgA/l). Patient was started on Oral voriconazole 200 mg twice a day with anti-retroviral therapy. Patient is doing well on regular follow-up.

3. Discussion

Aspergillus species are ubiquitous fungi acquired by inhalation of airborne spores and may cause life threatening infections especially in immunocompromised hosts. Aspergillus species are commonly isolated from the soil, plant debris, and the indoor environment, including hospitals. Pulmonary disease caused by Aspergillus, mainly *Aspergillus fumigatus*, presents with a spectrum of clinical syndromes in the lung.[2] Chronic necrotising aspergillosis (CNA) is locally invasive and is seen mainly in patients with mild immunodeficiency or with a chronic lung disease.

Traditionally, invasive aspergillosis has been seen in HIV-infected patients with very low CD4 counts (eg, <50 cells/microL). However, with the introduction of potent antiretroviral therapy, aspergillosis has been seen in those with higher CD4 counts as well. Our case was also a retropositive individual and on anti-retroviral therapy.

The most common symptoms include fever, cough, dyspnea, pleuritic chest pain, generalized malaise, weight loss, anorexia and CNS symptoms, hemoptysis is uncommon and only reported in 5 percent of patients.[3–5] In our case, patient presented with acute onset dry cough, breathlessness and left sided chest heaviness since 3–4 days.

Chest radiographic findings in HIV-infected patients with aspergillosis are similar to those seen in HIV-negative immunocompromised hosts, with the exception of the classic "halo" or "air crescent" signs. Chest radiography is of little use in the early stages of disease because the incidence of nonspecific changes is high. Usual findings include rounded densities, pleural-based infiltrates suggestive of pulmonary infarctions, and cavitations. Pleural effusions are uncommon.[6,7] Chest computed tomography (CT), especially when combined with high-resolution images (HRCT), is much more useful. Typical chest CT scan findings in patients suspected to have IPA include multiple nodules and the halo sign, which is mainly seen in neutropenic patients early in the course of infection (usually in the first week) and appears as a zone of low attenuation due to haemorrhage surrounding the pulmonary nodule. Another late radiological sign is the air crescent sign, which appears as a crescent-shaped lucency in the region of the original nodule secondary to necrosis.[7,8] In our case, Chest skiagram showed complete opacification of left hemithorax.

The cardinal test for chronic pulmonary aspergillosis is a positive *Aspergillus* immunoglobulin (Ig)G antibody test from the serum.[9–12] Most patients with chronic cavitary pulmonary

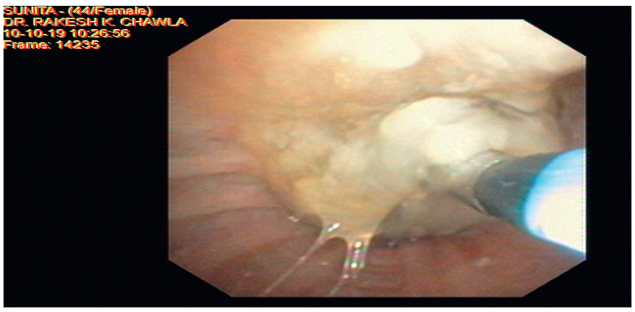

Fig. 4 – Cryobiopsy being taken from mucus plugs with simultaneous debulking being done.

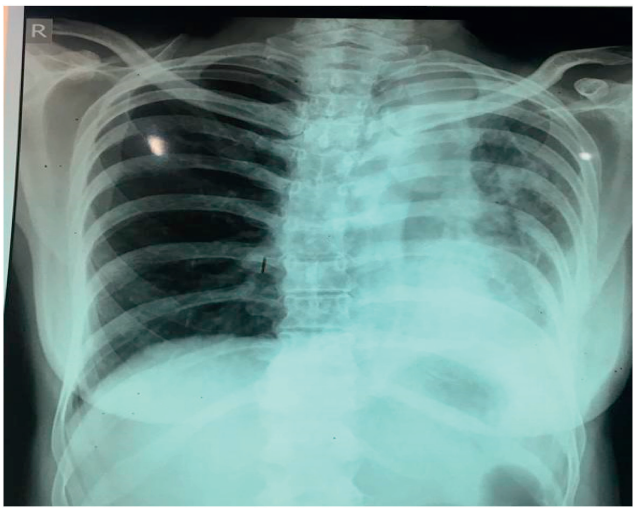

Fig. 5 – Post procedure skiagram showing partial clearance of opacification.

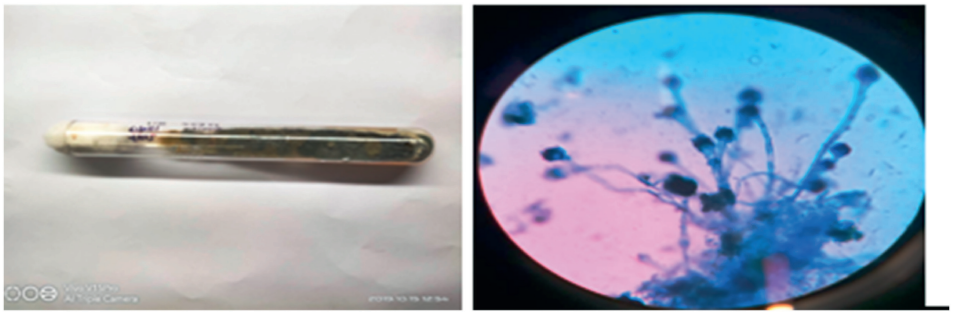

Fig. 6 – Fungal culture showed aspergillus fumigatus growth.

aspergillosis, including those with simple aspergillomas and *Aspergillus nodules, have positive Aspergillus IgG* antibodies in the blood.[13–16] A minority of patients have positive cultures of *A. fumigatus* (or rarely other Aspergillus species) in their sputum.[17] In our case, it showed very high serum IgE(10,189 kU/l), high S.IgE against aspergillus (22.4 kUA/l) and high specific S.IgG against aspergillus (194 mgA/l). Based upon these, our case was diagnosed as seropositive endobronchial aspergillosis.

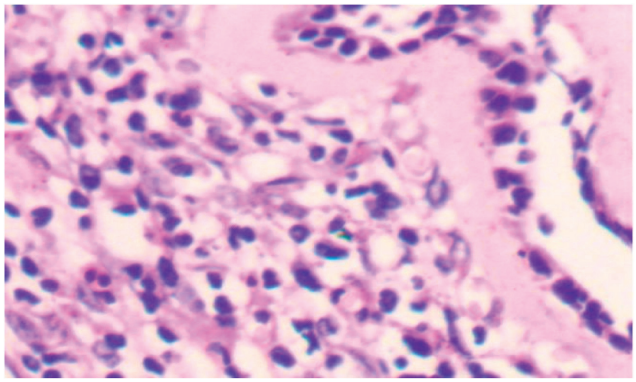

Fig. 7 – Bronchial biopsy HPE picture showing respiratory lining with underlying dense eosinophilic infiltrate in the subepithelial connective tissue stroma along with a few lymphoplasma cells with marked hyperemia and edema with no evidence of any invading fungal hyphae.

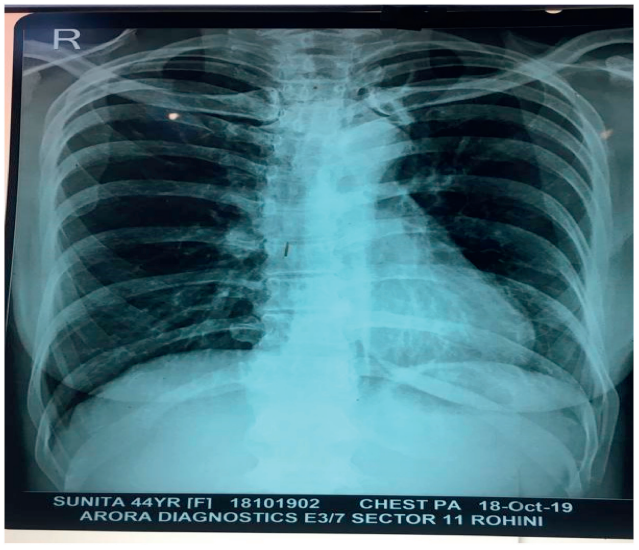

Fig. 8 – Post second bronchoscopy chest skiagram showing complete clearance of opacification.

Oral antifungal therapy is indicated, either itraconazole (200 mg twice daily) or voriconazole (200 mg twice daily) as first-line agents are used.[18,19] In severely ill patients or those infected with pan-azole–resistant isolates, intravenous (IV) therapy is indicated.

Voriconazole (4 mg/kg twice daily), posaconazole (300 mg IV once daily), micafungin (150 mg IV daily), or amphotericin B can be used, in a less nephrotoxic lipid formulation (liposomal amphotericin B at 3 mg/kg IV daily or amphotericin B lipid complex at 5 mg/kg IV daily).[20] Six months of antifungal therapy is often adequate for patients with subacute invasive pulmonary aspergillosis, but a longer duration is necessary in those with ongoing immunosuppression or a poor response to therapy.[18]

4. Conclusion

Endobronchial aspergillosis is characterized by massive intrabronchial overgrowth of the aspergillus species, mainly aspergillus fumigatus. Most patients with chronic pulmonary aspergillosis, including those with simple aspergillomas and Aspergillus nodules, have positive Aspergillus IgG antibodies in the blood. In our case, patient was diagnosed with endobronchial aspergillosis with complete obliteration of left main bronchus by fungal debris with very high serum IgE(10,189 kU/l), high S.IgE against aspergillus (22.4 kUA/l) and high specific S.IgG against aspergillus (194 mgA/l). Cryo debulking was done which relieved the symptoms significantly. Patient was discharged in satisfactory condition on oral voriconazole therapy.

Conflicts of interest

Dr. Chawla has nothing to disclose.

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