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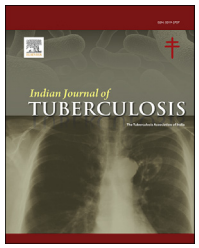

Editorial

Hepatotoxicity associated with first-line antitubercular drugs in children at a tertiary care facility

Dear Editor,

The prevalence of ATT induced hepatotoxicity has been reported earlier in children with limited evaluation of associated risk factors or protocol for testing and evaluating the same in all children.

This cross-sectional study was done in the Department of Paediatrics and Chest Clinic of a large tertiary care hospital in India. All paediatric tuberculosis patients diagnosed as per the Revised National Tuberculosis Control Programme (RNTCP)[1] guidelines, who were attending outpatient, inpatient or Chest clinic and receiving ATT (as per the recommended weight-bands under RNTCP[1] in the form of fixed dose combination (FDC) tablets) were assessed for enrolment over a six months period (August 2019–January 2020). Any patient with multidrug resistant tuberculosis or known history of chronic liver disease, or hepatitis was excluded. The clinical including anthropometry, demographic and treatment related details of enrolled patients were recorded on a predesigned pro-forma. Undernutrition was defined as weight-length SDS ≤ -2 in under-five years old[2] and body mass index-SDS ≤ -2 in older children.[3] Any event of hepatitis developing during the course of therapy was recorded with baseline values, wherever available. Drug induced hepatitis (DIH) was defined as 2.5–3 times elevation of serum transaminases in symptomatic patients, and up to five times of transaminases in asymptomatic patients[4] or a rise in the serum total bilirubin above 1.5mg/dL. The patients who developed hepatitis during the study period were followed up weekly for clinical and laboratory monitoring for adjustment of drug doses as per RNTCP guidelines.[1] No additional investigations were done as part of study protocol.

Statistical analysis was performed on SPSS version 23. Sample size calculation was done using OpenEpi, Version 3, software. The proportion of children who developed ATT induced hepatotoxicity was 6.9% based on an earlier study.[5] The sample size with 95% confidence intervals with design effect of 1 was calculated as 101 patients.

A total of 99 patients were enrolled. Seventy nine (79.8%) had extrapulmonary tuberculosis among which disseminated (25%) and central nervous system (21%) were the commonest, followed by lymph node TB (11%), abdominal (10%), pleural TB (8%) and spinal TB (4%). No patient had hepatic tuberculosis or HIV. The median (minimum–maximum) doses of HRZES consumed during intensive phase were 8.0 (2–13), 13 (4–19), 30 (9–38), 21 (14–34), 20 (15–23) mg/kg respectively; streptomycin was used in 10 children only. Undernourished children received a significantly higher dose of isoniazid and rifampicin than children with normal BMI, $P < 0.05$; Table 1. BMI SDS had a significant negative correlation with doses of rifampicin ($r = -0.31$) and isoniazid ($r = -0.30$); $P = 0.002$.

A total of 99 children (HIV negative) with mean ± SD age 10.0 ± 4.6 years were enrolled. Seventy nine had extrapulmonary tuberculosis. Hepatotoxicity was seen in 21 (21.2%) patients at a median (IQR) period of ATT intake as 17 (8.5, 60) days with earliest onset at 3 days. Jaundice and vomiting were the most common presenting features in 16 (72.7%) and 13 (59%) patients respectively. Baseline LFT records were available only in 6/21 (28.6%) patients which were normal and comparable to those without hepatotoxicity; $P > 0.05$. The odds (OR, 95% CI) of developing hepatotoxicity were higher with undernutrition [1.59 (0.59, 4.3); $P = 0.44$] and concurrent antiepileptic drug intake [1.55 (0.52, 4.6); $P = 0.55$], and lower with pulmonary tuberculosis [0.38 (0.1, 1.4); $P = 0.17$] than extrapulmonary tuberculosis.

The peak median (Q1,Q3) levels of serum bilirubin, AST, ALT and ALP in patients with hepatitis were 0.8 (0.6,1.6) mg/dL, 238 (135.3, 503) U/L, 208 (130, 301.5) U/L and 203 (134.3, 255.5) U/L respectively. Regular ATT was reinstituted after stopping modified ATT after a median (IQR) period of 30 (19.8, 60) days. In outcome of patients with hepatotoxicity, 1 died from neurological complications of tuberculosis, 4 were improving, the remaining 16 had recovered and none of them relapsed for tuberculosis.

Undernutrition emerged as a risk factor for hepatotoxicity in the present study, as also reported earlier.[6] We suggest the use of WLZ and BMI for paediatric FDC prescriptions instead of weight alone to identify undernutrition.

Table 1 – Comparison of clinical and biochemical parameters between children with normal nutrition and under-nutrition.

Parameter	*Normal nutrition (N=65)*	*Undernutrition (N=34)*	P value
Age (yrs)	11.2 (3.6)	7.8 (5.4)	<0.001
Weight (kg)	29.4 (9.4)	16.9 (8.6)	<0.001
Height (cm)	131.2 (16.0)	114.9 (28.2)	<0.001
BMI (kg/m^2)	16.7 (3.3)	12.0 (1.6)	<0.001
BMI SDS	−0.44 (0.96)	−3.1 (1.2)	<0.001
[a]Tuberculin skin test (mm)	17.0 (10.3)	10.9 (7.8)	0.002
[b]Isoniazid (mg/kg/d)	7.5 (2–13)	9.1 (2–13)	0.008
[b]Rifampicin (mg/kg/d)	12.9 (4–19)	14.5 (9–19)	0.006
[b]Pyrazinamide (mg/kg/d)	29.3 (9–38)	29.4 (15–38)	0.96
[b]Ethambutol (mg/kg/d)	21.7 (17–34)	20.9 (14–34)	0.36
[b,c]Streptomycin (mg/kg/d)	20.60 (16–23)	18.0 (15–23)	0.26

Data expressed as Mean (SD).
[a] n = 60 and 32 in normal nutrition and undernutrition.
[b] Mean (minimum–maximum).
[c] n = 5 in both the groups; P < 0.05 significant.

The small sample size, absence of baseline investigations (LFTs) of all patients, incomplete record retrieval for serially monitored liver function tests in all affected patients, referral bias with higher proportion of patients with extrapulmonary tuberculosis and lack of prospective study design were major limitations of our study.

The time for onset of DIH have been reported between 15 and 60 days,[7,8] with a median time of approximately two weeks in our study; emphasizing the need to monitor vulnerable pediatric patients during early part of disease. Our study, however, highlights a high proportion of ATT induced hepatitis in affected undernourished paediatric population, emphasising the need for a close follow-up for development of hepatitis during the intensive phase of treatment especially among poorly nourished with concomitant antiepileptic therapy.

Authorship

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Conflicts of interest

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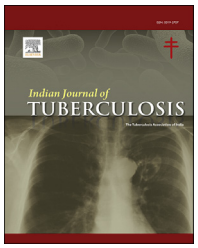

Viewpoint

TB free India: Reaching the unreached tribal population under National Tuberculosis Elimination Programme

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ABSTRACT

India is the highest TB burden country in the world. The burden however is not uniform in different strata including tribal population – one of the key affected populations in the country. As the evidences from tribal population are hardly available, most of the policies and strategies implemented under National Tuberculosis Elimination Programme (NTEP) are usually based on the evidences from general populations. NTEP is continuously taking steps to strengthen TB services in tribal areas. The Social Action Plan including Tribal Action Plan is in place and the appropriate strategies are incorporated in the National Strategic Plan (NSP) to ensure universal access to quality TB services to vulnerable population groups. However, its implementation becomes challenging especially in tribal areas as different tribal groups have their own unique ways of dealing with health issues. These issues are therefore required to be addressed holistically involving all the stakeholders. In view of this a symposium was jointly organized by the Central TB Division (CTD), Govt. of India and ICMR – National Institute of Research in Tribal Health (NIRTH), Jabalpur on 17th and 18th December, 2019 at ICMR – NIRTH, Jabalpur. It provided an excellent platform for all the stakeholders from different parts of the country to share their experiences in tuberculosis particularly among marginalized populations. The recommendations emerged out of this interactive symposium highlight the sincere effort of NTEP to tackle TB situation in tribal population and show the way forward towards India's TB elimination goal by 2025 especially in hard to reach tribal areas.

1. Background

Tuberculosis (TB) remains a major public health Problem globally, with an estimated 10 million cases and 1.2 million deaths in 2018.[1] India has the highest number of TB cases in the world with an estimated 26.9 lakh new cases in 2019.[2] The burden however is higher in many settings in the country, especially in key affected populations. The tribal population is one of the key

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affected populations in the country having poor access to the health care delivery systems.[3] As per 2011 census, Scheduled Tribes account for over 104 million people, representing 8.6% of India's population. Although tribes of different ethnic origin are present in most of the states of India, Jharkhand, Orissa, Madhya Pradesh, Chhattisgarh and North eastern states are some of the states where the tribal population comprises a significant portion of the community. A total of 705 communities have been notified as Scheduled Tribes in 30 states/UTs. Of these, 75 tribal groups are notified as Particularly Vulnerable Tribal Groups (PVTGs) based on the low level of literacy, declining or stagnant population, and pre-agricultural level of technology. Remoteness, unique beliefs and practices, illiteracy, poverty etc increases their risk for many health problems.[4] Though research studies on TB among tribal population are very few, available literature indicates a wide variation in TB prevalence rates in different tribal groups.[5] A meta-analysis of existing studies estimated TB prevalence of 703 per 100,000 population in tribal population of the country.[6] However, studies by the ICMR – National Institute of Research in Tribal Health (NIRTH), Jabalpur showed an alarmingly high prevalence ranging from 1500 to 3294 per 100,000 population amongst the Saharia tribe of central India.[7,8] In spite of the vast resources invested by the Government for welfare of tribal population of the country, TB remains a huge challenge mainly due to the structural and unique cultural barriers faced by tribal populations.[9] As the evidences from tribal population are hardly available, most of the policies and strategies implemented under National Tuberculosis Elimination Programme (NTEP) are usually based on the evidences from general populations. The commissioned study from the Central TB Division identified gaps in the provision of TB services to the tribal population, *viz.*, access to services and the awareness among the community. This led to the development of tribal action plan in 2005[3] which calls for strengthening and expansion of additional health facilities in tribal areas of the country. As the information on its implementation in various states was not fully available, another assessment was done in 2011 to understand the specific issues among theses populations. The report revealed limited improvement in bridging the gaps and found that insufficient community engagement, non – involvement of traditional healers, remoteness of the tribal populations from the health services, and lack of appropriate awareness building measures, etc. as some of its major impediments in delay and incomplete access to the TB services in tribal areas. Based on these findings, the Social Action Plan including the Tribal Action Plan (TAP) was developed in 2013[10] and is incorporated in the National Strategic Plan (NSP)[11] to ensure universal access to quality TB services to vulnerable population groups.

The Government of India is committed to "TB Free India" by 2025. The strategies under the National Strategic Plan (NSP) along with the National TB Elimination Program (NTEP) are accordingly designed to achieve this goal. The Social Action Plan including the Tribal Action Plan (TAP) is in place. However, its implementation and performance in tribal areas need to be monitored to achieve the goal set under National Strategic Plan. This becomes especially challenging as the tribal population in the country is not a homogenous group and different groups have their own unique ways of defining health and disease, and also dealing with health issues.[12] The issues among tribal population are therefore required to be addressed holistically involving all the stakeholders including policy makers, programme managers, health officials, community representatives and non-governmental organizations (NGOs). Keeping this in mind, a symposium on "TB Free India: Tribal perspective" was jointly organized by the Central TB Division (CTD), Govt. of India and ICMR – National Institute of Research in Tribal Health (NIRTH), Jabalpur on 17th and 18th December, 2019 at ICMR – NIRTH, Jabalpur. It was attended by more than 60 participants including officials from Central TB Division (CTD), Govt. of India, State TB officials from various tribal dominating states, district TB officers, researchers in the field, faculty from medical colleges, non-governmental organizations and members of the tribal community which comprised of *Sarpanch* (village head), *ASHA* (Accredited Social Health Activist-a frontline health worker in the village), traditional healer (a person whom tribal people approach first in case of health issues) and TB Champions (cured TB patients) belonging to the tribal community. The symposium provided an excellent platform for all the stakeholders from different parts of the country to interact with each other and share their experiences in tuberculosis particularly among marginalized populations.

The technical session highlighted India's overall scenario on TB with focus on tribal population and the national program's response. The session revealed varying burden of tuberculosis in different tribal groups in the country including associated factors and highlighted the need for suitable interventions for control of TB in tribal population. This was followed by the state-wise presentations by various tribal dominated states *viz.* Chhattisgarh, Gujarat, Jharkhand, Madhya Pradesh and Odisha. The state representatives presented overview of the TB situation in tribal population along with the implementation status of tribal action plan in their respective states and also highlighted some state level initiatives in tribal areas. An interactive session was also held with the members of the tribal community comprising of *Sarpanch*, *ASHA*, traditional healer and TB Champions. The session revealed that there is a need to implement TB control activities ensuring active involvement of the tribal communities. It was emphasized that the community involvement should be ensured in making awareness raising activities culturally acceptable in tribal areas. A need was also felt to acknowledge and integrate the services of the traditional healers in TB control activities for effective results. The TB champions gave testimonials on how they adhered to treatment and managed to become TB free. They also shared various schemes and provisions they have accessed as part of the support available for TB patients under TB control programme. The participants felt that the TB Champions belonging to the tribal community along with the traditional healers can act as agents for community mobilization thus bridging the gap between the community aspirations and the programme implementation in tribal areas.

2. Thematic group work

The detailed discussions were held on three major themes related to tribal TB through three thematic groups *viz.* Improving the Coverage & Case Detection Rate; Linkages with

Social Schemes and Interventions in Tribal Action Plan. The groups performed brain storming sessions in their respective groups and made presentations on the recommendations next day.

2.1. *Group 1: improving the coverage & case detection Rate*

The group presented the proposed mechanism vis-a-vis the existing mechanisms. The group felt a need to have a Designated Microscopy Centre (DMC) per 20,000 population against the existing 50,000 population and recommended that all the Primary Health Centres (PHCs) in tribal areas should have a DMC under the TB control programme. The group emphasized the need for community driven approach including involvement of Accredited Social Health Activist (ASHA) and suggested to work towards "Arogya Mitra" through identifying one dedicated volunteer from each village. The group also recommended to ensure involvement of NGOs, private practitioners/traditional healers and others working in tribal areas, schools, Village Gram Sabha for monitoring of TB control activities. The group recommended that family health card for tribal population may be introduced. The group also stressed a need to strengthen inter district/state meetings/ collaboration for better outcome.

2.2. *Group 2: linkages with social schemes*

The group stressed a need for distributing high-protein diet/ nutrition basket during treatment and recommended that TB patients be linked to public distribution system (PDS) as a measure of food security during the treatment. The group felt that there should be active collaboration with other departments such as tribal welfare, women & child welfare, agriculture, rural employment, education *etc.* to have favourable results of TB control activities. This can be done through district level meetings headed by the District Collector. The group also suggested for Skill Development and Social entrepreneurship development in the areas of poultry, Promotion of kitchen garden in the household, skill development programmes of Entrepreneurship Development Cell (EDC) or Industrial Promotion Office (IPO) and training of individuals/ groups as per skill/interest and qualification/experience etc.

2.3. *Group 3: interventions in tribal action plan*

The group acknowledged the provisions made under the tribal action plan and recommended additional components that may be included in Tribal action Plan. These are volunteer at the village level, Active Case Finding (ACF) quarterly and involvement of NGOs, PRIs. These measures would be helpful in achieving TB control through increase in case notification including prompt and complete treatment through involvement of tribal community. The group felt that the definition of tribal blocks should be broadened and the schemes which are being implemented in tribal blocks, should also be extended to blocks having tribal population of 50,000 or more. The group also recommended that the laboratory assistants may be trained from local schools which has been done earlier under Malaria and Kala Azar programmes.

3. Key recommendations/actionable points

Keeping in mind the goal of TB elimination by 2025 in general and to strengthen TB control activities in tribal areas to achieve this goal in particular, the following key recommendations were made during the two days symposium through interactive sessions involving all the delegates -

- The NTEP should consider signing MoU with other relevant ministries such as Ministry of Tribal Affairs (MoTA), Ministry of Housing etc. for holistic approach for TB control in tribal areas.
- For tribal population, there is a need to analyse active case finding data in 'Nikshay', a web enabled patient management system under NTEP.
- Central TB Division/States should consider using Tribal Department's radio channels for awareness generation campaign in tribal populations.
- States having tribal populations should focus on involvement of NGOs through existing partnership schemes.
- ICMR – NIRTH to spearhead multicentric Operational Research protocol in Tribal population and lead an Expert Group for development of a Revised Tribal Action Plan as part of NSP for Ending TB in tribal population.

The recommendations emerged out of this interactive symposium highlight the sincere effort of NTEP to tackle TB situation in tribal population and show the way forward towards TB elimination in the country especially in hard to reach tribal areas.

Author's contribution

All the authors were actively involved in organizing this symposium and writing the manuscript. All the authors read and edited the manuscript.

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Conflicts of interest

The authors have none to declare.

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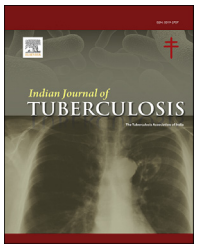

Viewpoint

Tuberculosis with discordant drug resistance patterns- A diagnostic dilemma

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ABSTRACT

Programmatic management of drug-resistant tuberculosis (PMDT) guidelines in India specify the use of cartridge based nucleic acid amplification test (CBNAAT) and Line probe assay (LPA) for early diagnosis of drug-resistant Tuberculosis. However, discrepancy among these genotypic tests (CBNAAT and LPA) or with the phenotypic DST in real practice poses a clinical dilemma. The usual solutions are to rely on methods with short turnaround times like CBNAAT and LPA to start an initial regimen. The culture and DST results, that are typically available after at least a few weeks, are used to modify the regimen if required. This practice is based on the fact that culture and DST based sensitivity patterns are considered the gold standard for diagnosing and drug resistance. DNA sequencing by pyrosequencing, Sanger sequencing and next generation sequencing (NGS) are being evaluated; their future availability may help in early clarifications in discordant drug resistance patterns. Such tests are costly and have limited availability, however, in view of immense benefit to detect TB Drug-resistant phenotypes, national guidelines plan to scale up their use in national and well-performing intermediate TB reference laboratories.

1. Introduction

Despite introducing universal drug sensitivity testing in India, diagnosing and treating Drug resistant tuberculosis (DR-TB) is a public health challenge. Programmatic management of drug-resistant tuberculosis (PMDT) guidelines in India specify the use of cartridge based nucleic acid amplification test (CBNAAT) and Line probe assay (LPA) for early diagnosis and subsequent appropriate therapy.[1] Both these are genotypic polymerase chain reaction (PCR) based tests which give quick and reliable results. CBNAAT is a *Mycobacterium tuberculosis*-specific automated assay providing results within 100 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the rpoB gene of *M. tuberculosis*, which is the critical gene associated with rifampicin resistance.[2] If rifampicin resistance is indeterminate in CBNAAT, it is repeated again and if still indeterminate, is tested by line probe assay or liquid culture followed by

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Table 1 – Review of literature of studies with discordant drug sensitivity tests.

S no	Title and authors	Discordant results	Follow up/treatment	Possible explanation
1	Does Xpert® MTB/RIF assay give rifampicin resistance results without identified mutation? Review of cases from Addis Ababa, Ethiopia (Alemu et al)[10]	Six out of 100 samples tested by Xpert MTB/RIF assay were reported as **Rifampicin resistant although no mutations were observed in Probes A to E**; delta Ct max was >4.3	Treated with standard therapy	Authors reasoned that delta Ct values were greater than 4
2	Clinical implications of discrepant results between genotypic MTBDR *plus* and phenotypic Löwenstein-Jensen method for isoniazid or rifampicin drug susceptibility tests in tuberculosis patients (Kang et al)[11]	1069 TB patients, 63 (5.9%) had discrepant results for the 2 DSTs. Of the 57 MDR-TB cases diagnosed by either DST, 18 (31.6%) showed discordant results for INH or RIF. The **most frequent** pattern of discordance was **genotypic susceptibility with phenotypic resistance to INH**.	Forty-five of the 54 patients (83.3%) had a favorable outcome with a mean treatment of 14 months.	Some noncanonical *rpoB* mutations confer low-level resistance which may lead to adverse treatment outcomes
3	False-positive rifampicin resistance on Xpert® MTB/RIF caused by a silent mutation in the rpoB gene (Mathys et al)[12]	A case of smear-positive pulmonary tuberculosis that was **CBNAAT-resistant but phenotypically susceptible** to RMP	Treated with standard therapy	Complementary investigations (repeat Xpert, Genotype®MTBDRplus assay and sequencing of the rpoB gene) revealed the presence of a silent mutation in the rpoB gene, leading to the conclusion of a false-positive Xpert result
4	Mixed *Mycobacterium tuberculosis* Complex Infections and False-Negative Results for Rifampin Resistance by GeneXpert MTB/RIF Are Associated with Poor Clinical Outcomes (Zetola et al)[13]	Rifampin resistance was detected by the Xpert assay in 52 (14.1%) and by phenotypic DST in 55 (14.9%) patients. Mixed MTB infections were identified in 37 (10.0%) patients. The Xpert assay was 92.7% (95% confidence interval [CI], 82.4%–97.9%) sensitive for detecting rifampin resistance and 99.7% (95% CI, 98.3%–99.9%) specific	When restricted to patients with mixed MTBC infections, Xpert sensitivity was 80.0% (95% CI, 56.3–94.3%). False-negative Xpert results and mixed MTB infections were strongly associated with poor clinical outcome.	The Xpert assay failed to detect rifampin resistance *in vitro* when <90% of the organisms in the sample were rifampin resistant.
5	Comparison of Xpert MTB/RIF Assay and GenoType MTBDRplus DNA Probes for Detection of Mutations Associated with Rifampicin Resistance in *Mycobacterium tuberculosis* (Rahman et al)[14]	Six samples were detected to be rifampicin resistant by Xpert but sensitive by DRplus assay. Two samples were reported as rifampicin resistant by DRplus but sensitive by gene sequencing. Further, one isolate was found to be rifampicin resistant by CBNAAT but sensitive when analysed by gene sequencing, and in LJ medium based DST.	Seven out of eight isolates were from retreatment cases.	DRplus failed to detect certain types of mutations across multiple locations. It was postulated that since isolates from retreatment cases can have mixed populations, the samples obtained from them can also show heteroresistance. The mutation picked by CBNAAT was inferred as a silent mutation upon analysis of gene sequencing and DST results.

MTB-Mycobacterium tuberculosis, Ct-cycle threshold, MDR-TB- multidrug resistant tuberculosis, DST-drug sensitivity testing, RIF- rifampicin, INH- isoniazid, CBNAAT-cartridge based nucleic acid amplification test, DR-drug resistant.

phenotypic drug sensitivity testing (DST).[1] False positive detection of rifampicin resistance has been documented in rare situations when *M. tuberculosis* (Mtb) is detected very low. In such situations, it is often safer to repeat the CBNAAT or do an LPA if possible, to confirm the resistance before proceeding with treatment for DRTB.[3] First line and 2nd line LPA are done according to whether CBNAAT detects rifampicin resistance. First line LPA detects resistance to isoniazid and rifampicin. More importantly, it differentiates between katG and inhA mutations for isoniazid resistance helping in deciding whether the drug regimen can include high dose isoniazid (inhA mutation) or not (katG mutation). Second line LPA detects resistance against fluoroquinolones and second line injectables. There is no genotypic test at present to detect resistance to ethambutol and pyrazinamide.[4]

2. Discordant DST results

Discrepancy among these genotypic tests (CBNAAT and LPA) or with the phenotypic DST in real practice poses a clinical dilemma. If the genotypic DST is a false positive (false resistant), the patient would be prescribed an inappropriate drug for a long time, which increases the risk of side effects and lowers the efficacy of the regimen. Similarly, if the susceptibility shown by the genotypic DST is a false negative (false susceptible), the patient would also be prescribed a drug regimen, which would be ineffective and lead to treatment failure. This, eventually increases the chances of further drug-resistant forms of tuberculosis.

3. Discordance between CBNAAT and LPA

It is uncommon and can have multiple plausible mechanisms including contamination of the sample during collection, transportation or analysis, low bacterial load in the sample, mixed organisms and lastly, silent mutations. The current guidelines do mention to conduct a third rifampicin resistance testing by LPA if rifampicin resistance is indeterminate by CBNAAT twice. However, in discordant CBNAAT and LPA results, it is advised in the latest national guidelines released on 24 march 2021 to do a third text by CBNAAT and follow the results of two concordant results among the three.[4] A phenotypic drug sensitivity testing in such cases can be helpful but are time-consuming and unhelpful in starting the initial regimen. However, the phenotypic test in culture must be sent in such situations and regimen can be modified later if required according to results.

4. Discordance between genotypic (CBNAAT and LPA) and phenotypic tests

Reported literature suggests that genotypic sensitive and eventually phenotypic resistant reports are common which if undiagnosed lead to treatment failure. All the more, in such studies, it has been shown that LPA and CBNAAT have a high level of coherence.[5] A study from India used pyrosequencing at a reference laboratory in discordant isolates between genotypic and phenotypic tests; and identified pyrosequencing to have a definite role to detect the synonymous (silent) mutations that are detected as rifampicin resistant by CBNAAT but do not translate to amino acid modification.[6,7] However, studies have also reported that some isolates with rifampicin resistance detected by CBNAAT but negative phenotypic DST (generally considered as CBNAAT false positives) in fact have mutations[8] associated with a RIF-resistant phenotype suggesting that some supposedly false-positive CBNAAT results may be accurate and actually reflect insufficient phenotypic DST sensitivity.[9]

5. Solutions

The usual practice is to rely on methods with short turnaround times like CBNAAT and LPA to start an initial regimen. The culture and DST results, that are typically available after at least a few weeks, are used to modify the regimen if required. This practice is based on the fact that culture and DST based sensitivity patterns are considered the gold standard for diagnosing and drug resistance. Culture and DST have the advantage of showing sensitivity to a drug rather than merely ruling out resistance based on a few genes. Even though gene sequencing from the sample may be able to rule out more drug resistance-genes, it is not practical to subject every sample to this technique. Finally, even culture and DST provide in-vitro sensitivity reports and not in-vivo effectiveness, which can be affected by not just mycobacterial genes but also the host individual's genetic pattern and pharmacodynamics. Mycobacteria Growth Indicator Tube (MGIT) with phenotypic DST may miss rifampicin resistance and hence is not a gold-standard. World health organisation also in February 2021 suggested lowering critical concentration from 1μg/ml to 0.5μg/ml to decrease discordance between genotypic and phenotypic test results.[4] It may therefore be prudent to make clinical follow up as the most important criterion to determine the drug regimen and overall management for the individual. A summary of studies having discordant drug resistance tests for tuberculosis has been presented in Table 1.

6. The path ahead

DNA sequencing by pyrosequencing, Sanger sequencing and next generation sequencing (NGS) are being evaluated; their future availability may help in early clarifications in discordant drug resistance patterns.[10] Such tests are costly and have limited availability, however, in view of immense benefit to detect TB Drug-resistant phenotypes, national guidelines plan to scale up their use in national and well-performing intermediate TB reference laboratories.[4]

7. Conclusion

Discordant genotypic and phenotypic drug resistance must be interpreted with caution taking all investigations and clinical conditions into focus. False positive rifampicin resistance detection by CBNAAT is rare but reported.[11–14]

Starting therapy based on genotypic results and monitoring patients with clinical follow-up and subsequent phenotypic drug sensitivity reports can help attain favourable results. This can help prevent the delay in starting therapy for drug-resistant tuberculosis which in turn further prevents spread of such infections.[15]

Contributions

All the 2 authors contributed to –Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; & Drafting the work or revising it critically for important intellectual content; & Final approval of the version to be published; & Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of interest

The authors have none to declare.

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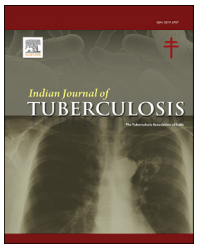

Review article

Evolution of semi-rigid thoracoscopy

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ABSTRACT

Pleural effusions despite being so common, there is no much literature available regarding definite diagnosis for pleural effusions. Application of Light's criteria changed the approach to pleural effusion and till date remains a very useful step in the diagnosis of pleural effusions. Pleural fluid biochemistry and adenosine deaminase (ADA) enzyme levels play a significant role in the diagnosis of tubercular effusion. Studies have shown that levels of ADA are more often higher in tubercular effusion than in any other cause for it. But ADA levels can also be elevated in other types of parapneumonic effusions (PPEs), especially complicated PPEs. Hence it is difficult to distinguish a tubercular pleural effusion (TPE) from other PPEs based on pleural fluid ADA levels alone. LDH/ADA ratio as an indicator for ruling out tuberculosis was analyzed in few studies with high sensitivity and specificity. The pleural fluid cytology has a varying sensitivity, with a maximum of only 60% and it may increase with subsequent tapping. Closed pleural biopsy using a Cope or Abrams needle has a sensitivity up to 80% in cases of tuberculous effusion and 40%–73% in cases of Malignancies.

Semi-rigid thoracoscopy not only allows for visualization of the pleura but also helps in procuring the biopsies under direct visualization from the abnormal looking areas. In cases of primary pleural malignancies like mesothelioma, pleurodesis can also be done in the same setting after taking the biopsy, hence reducing the number of procedures. Limitation of the semi-rigid thoracoscopy is smaller sample size and more superficial sampling of the pleura. Cryobiopsy and Electrocautery guided pleural biopsy using the IT knife are the modifications in the semi-rigid thoracoscopy to overcome the drawback of smaller sample size. While navigation band image guided pleuroscopy helps in better visualization of the vasculature of pleura during the biopsy.

Management of pleural effusions has evolved over a period of time. Starting with a single criterion based on pleural fluid proteins to semi-rigid thoracoscopy. The inexhaustible research in this field suggests the desperate need for a gold standard procedure with cost effectiveness in the management of undiagnosed pleural effusions. Semi-rigid thoracoscopy has revolutionized the management of undiagnosed pleural effusions, but

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it has its own limitations. Various modifications have been proposed and tried to overcome the limitations to make it a cost-effective procedure.

1. Introduction

Pleural effusions are not that rare as we expect, it is one of the most common cause for consultation in respirology clinic.[1] Pleural space involvement is well known in different diseased conditions involving various organ systems in our body like lungs, heart, liver, kidney and musculoskeletal system. Pleural effusions despite being so common, there is no much literature available regarding definite diagnosis for pleural effusions. A good clinical history clinical examination gives a clue regarding the etiology but it never confirmatory. Earlier pleural fluid protein level of 3.0 g/dL was used as a cutoff to distinguish between transudates & exudates, Effusion was considered to be exudative if protein level in the fluid was above 3.0 g/dL.[2,3] This single criteria lead to misclassification of about 10% of effusions.[2–4] Moving a step ahead, application of Light's criteria changed the approach to pleural effusion and till date remains a very useful step in the diagnosis of pleural effusions. Additional parameters like, Lactate dehydrogenase and proteins, obtained simultaneously from the blood and serum revolutionized the management of pleural effusions for a while. With these 3 parameters about 99% of pleural effusion could be correctly classified.[4] Using Light's criteria about 98% of times we can identify exudates correctly, but about 25% of the times transudates are still mislabeled as exudates.[5] Among the exudates parapneumonic effusions (PPEs) and malignancy remain the most common causes of pleural effusion across the globe. Areas with high prevalence of tuberculosis almost a quarter of the times the effusions are attributed to tuberculosis.[6]

Pleural fluid biochemistry and adenosine deaminase (ADA) enzyme levels play a significant role in the diagnosis of tubercular effusion. Studies have shown that levels of ADA are more often higher in tubercular effusion than in any other cause for it.[7–10] Sensitivity of 92% and a more than 90% specificity of ADA level in pleural fluid for the diagnosis of tubercular effusion has given a ray of hope in ever confusing management of pleural effusions.[11] But ADA levels can also be elevated in other types of PPEs, especially complicated PPEs.[12] ADA-2 an isoenzyme of ADA, is predominantly elevated in intracellular infections like tuberculosis. While high levels of ADA-1 are seen in empyema.[13,14] Most of the laboratories measure overall ADA levels without a mention of isoenzyme type. Hence it is difficult to distinguish a tubercular pleural effusion (TPE) from other PPEs based on pleural fluid ADA levels alone. LDH/ADA ratio as an indicator for ruling out tuberculosis was analyzed in few studies with high sensitivity and specificity.[15] Addition of pleural fluid carcinoembryonic antigen to LDH/ADA ratio was also shown to be an useful index.[16]

The pleural fluid cytology has a varying sensitivity, with a maximum of only 60% and it may increase with subsequent tapping.[17,18] Closed pleural biopsy using a Cope or Abrams needle has a sensitivity up to 80% in cases of tuberculous effusion and 40%–73% in cases of Malignancies.[19] Drawback of closed pleural biopsy was false negative results. The sample obtained may not be representative of the tumor due to localized seeding of the cells.[19]

Thoracoscopy is the endoscopic visualization of the thoracic cavity. First ever thoracoscopy was done by Jacobeus as early as in 1907. Thoracoscopy can be in the form of Video assisted thoracoscopy (VATS), which is a surgeon's domain, or it can be medical thoracoscopy usually performed by the physicians. Medical thoracoscopy are of 2 types, rigid thoracoscopy or semi-rigid thoracoscopy. Semi-rigid thoracoscopy is commonly termed as pleuroscopy.

Semi-rigid thoracoscopy is a simple procedure which can be easily performed by a physician who is skilled in intercostals drain insertion.[20] Physicians are more comfortable with semi-rigid pleuroscopy as it is similar to fiberoptic bronchoscopy. Also, it is performed in the bronchoscopy suite with lower expenses. Rigid pleuroscopy is definitely better when it comes to size of the biopsy sample and adhesiolysis. But sometimes it may not be available in all the setups and may work out more expensive when operation theatres are used.[21]

The advantage of semi-rigid thoracoscopy is that. it not only allows for visualization of the pleura but also procuring the biopsies under direct visualization from the abnormal looking areas. By taking the biopsy it is useful not only to diagnose the condition but also staging can be done if turns out to be malignancy. Semi-rigid thoracoscopy is simple, safe procedure with a very high sensitivity of 93–95% in cases of malignancies.[22,23] In cases of primary pleural malignancies like mesothelioma, pleurodesis can also be done in the same setting after taking the biopsy, hence reducing the number of procedures which can cause tumor seeding in the tract of incision for biopsy.[24–27]

2. Indications of semi-rigid thoracoscopy

2.1. *Undiagnosed pleural effusions*

Sometimes etiology of pleural effusions remains undiagnosed despite repeated pleural aspirations and closed pleural biopsy.[28] Semi-rigid thoracoscopy is very useful technique in such conditions and it is known to improve the diagnostic yield up to 85% of times, particularly if we are dealing with a suspected malignancy. As the biopsy is taken under direct vision either from a polypoidal lesion or a mass or an abnormally looking pleura the yield is known to significantly increase.[29]

2.2. *Malignant pleural effusions*

Malignant pleural effusions can be primary pleural or metastatic effusions. Most common cause for metastasis is lung in males and breast in females respectively. Lymphomas, gastrointestinal malignancies and genitourinary malignancies are also known to metastasize to pleura. In an about 15% of cases the primary site may not be known at all.[30] Cytology alone has a yield of around 65% and combining with closed pleural biopsy increases the yield only by 10%, particularly if tumour seeding is around the diaphragmatic pleura or mediastinal pleura.[29] In metastatic effusions the prognosis depends on the primary site of malignancy. Shortest median survival is seen with primary lung malignancy and longest with ovarian malignancies metastasizing to pleura.[31] Hence pleuroscopic guided biopsy becomes more important in not only diagnosis of malignancy but also knowing the source of metastasis. Appearance of pleura in a malignancy may vary, sometimes it may be nodular which bleeds on touch or it may just be near normal with subtle hyperemia (Fig. 1).

In malignant mesothelioma, role of semi-rigid thoracoscopy is not still clear as the biopsy sample obtained are usually not adequate. Hence when suspicion of malignant mesothelioma is high it is better to use a rigid thoracoscopy (Fig. 2)[28] or use cryobiopsy through semi-rigid thoracoscope to obtain larger sample.[21]

2.3. *Pleural effusion due to tuberculosis*

Diagnostic yield in a pleuroscopic guided biopsy for tuberculosis is very high (98%) in endemic areas for tuberculosis. But in view of non-availability of the equipment and cost constrains, experts recommend that a closed pleural biopsy which has a diagnostic yield of 80% should suffice. But in view of increasing incidence of drug resistance in tuberculosis, it's wise to obtain a pleuroscopy guided biopsy for better culture of organism for drug sensitivity. In resource limited setting, a fiber optic bronchoscope can also be used to obtain sample with extra care for sterility of the equipment (Fig. 3).[32]

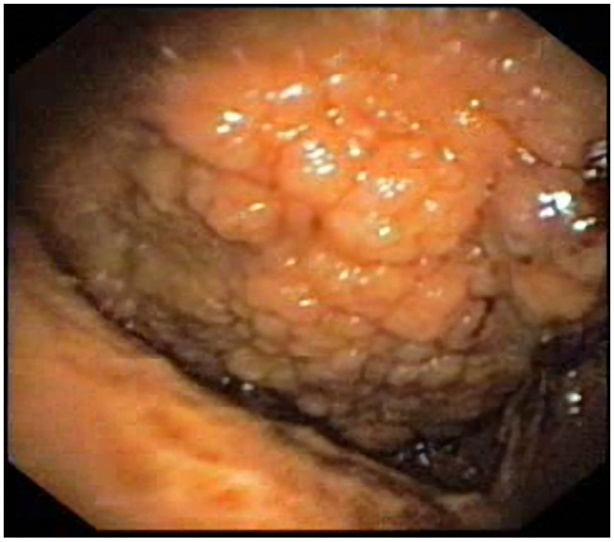

Fig. 1 – Pleuroscopic view of metastatic adeno-carcinoma.

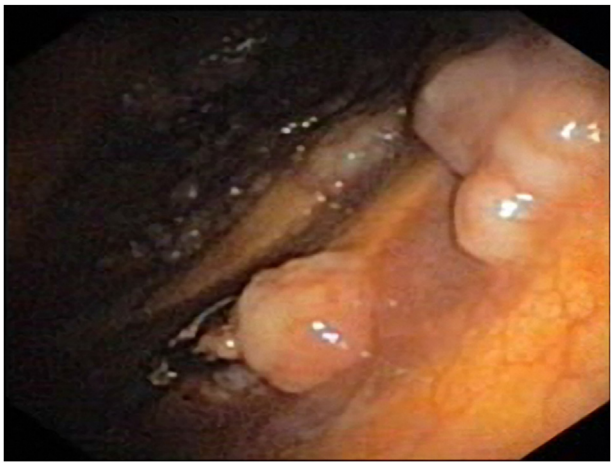

Fig. 2 – Pleuroscopic view of mesothelioma.

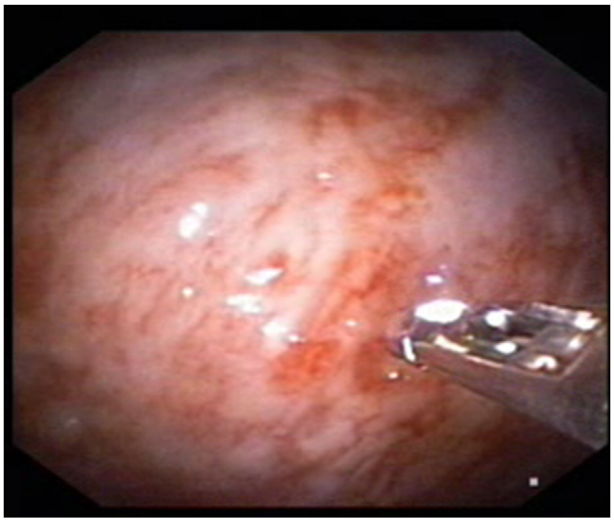

Fig. 3 – Pleuroscopic view of tuberculosis.

2.4. *Complicated parapneumonic effusions*

Semi-rigid thoracoscopy may be useful in very early stage of parapneumonic effusions. Limited adhesiolysis in cases of thin septations can be performed. Empyema ideally should be treated with rigid thoracoscope but in some cases a double port technique (Fig. 4), where a rigid forceps is introduced through a separate port can be used to break the adhesions and for a larger biopsy sample.[32]

2.5. *Recurrent pleural effusions and pneumothorax*

Apart from procuring the sample for diagnosis in cases of recurrent pleural effusion, pleurodesis using Talc can be performed in the same setting.[32] Similarly in cases of recurrent pneumothorax pleurodesis under vision can be performed, unless patient is very young and can be a potential candidate for lung transplant.

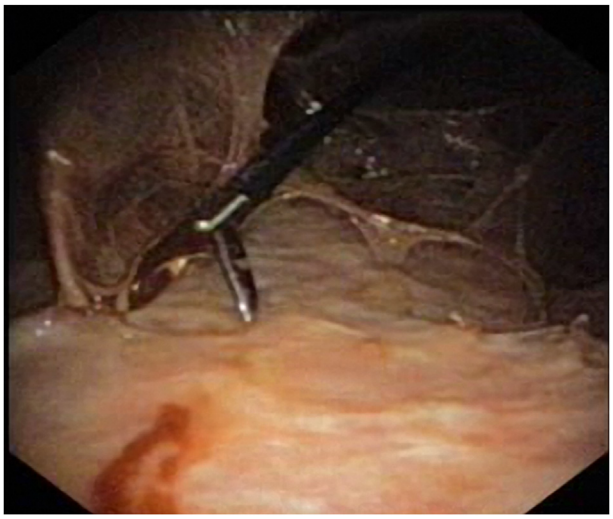

Fig. 4 – Tuberculosis, adhesiolysis with double port technique.

3. Absolute contraindications[33]

Multiple thick adhesions with no space to work in the hemithorax.

Severely dyspneic patient with hypercapnia.

Severe cough not allowing to enter the hemithorax.

Lack of informed consent in a competent patient.

4. Relative contraindications[33]

Severe obesity may make the procedure technically difficult due to limited length of canula.

Significant comorbidities like, ischemic heart disease, recent myocardial infarction (4 weeks after the initial event)

Bleeding and clotting dysfunction.

Renal failure though a relative contraindication procedure can be done if extra precautions are taken to avoid re expansion pulmonary edema.

5. Pre-procedure

A complete clinical examination and history to have pre-test probability. Routine investigations in the form of chest X ray, ultrasonography, if required a Computed tomography, complete blood count, INR, Renal function test and liver function test depending on the clinical assessment and an ECG to be done. Ideally a check list has to be maintained before any patient is subjected for the procedure. Written informed consent addressing the complications be obtained.

6. Technique

The operator and the assistant ideally should perform ultrasonography of chest on table and decide regarding the positioning (most often is lateral decubitus with affected side up) and the tentative intercostal space to be marked. Procedure is done with monitoring of vitals and ECG. Intravenous sedation (propofol, midazolam or both) can be used which is individually titrated. Initially needle aspiration is done after infiltrating lidocaine locally. Once space is confirmed, trochar is inserted with the help of a linear incision and blunt dissection. Pleural fluid drainage can be done initially either using a separate suction catheter or through the pleuroscope (Olympus Europa SE & Co. KG, Hamburg, Germany). Depending upon the amount of fluid present, around 500 ml can be aspirated initially. Either air can be actively instilled using a 50 cc syringe to create an artificial pneumothorax or just allow the space to be open to exterior for pressure equalization. Total amount of fluid to be aspirated depends on patient, in some entire fluid can be aspirated as the presence of air prevents re expansion pulmonary edema, but we have to be careful if you are going beyond 2–2.5 litres. Entire hemithorax has to be visualized including the lung. We have to see weather lung is expanding or not, if there is mass over the lung or there is visceral pleural thickening. All these observations guide us in further decisions. After visualization of parietal pleura, site for biopsy has to be decided. Biopsy can be procured using the flexible forceps, ideally 4 to 5 sample have to be procured for the adequacy of the sample. Once adequate sample is procured, and enough fluid has been drained an intercostal drain has to be kept in place through the trochar. If the ICD size is bigger in comparison to trochar diameter, than the trochar can be withdrawn and ICD has to be inserted through the same tract. Sometimes patients need negative suction for complete resorption of the introduced air.

7. Modifications in semi-rigid thoracoscopy

Limitation of the semi-rigid thoracoscopy is the smaller sample size and the more superficial sampling of the pleura. Though the smaller size of samples obtained with semi-rigid thoracoscope does not affect diagnostic yield, a larger biopsy tissue sample will always be beneficial for further subclassification using IHC and doing molecular testing if we are dealing with a malignancy. Also, it is noted that, obtaining a sample with a flexible forceps biopsy when the pleura is thickened or more fibrosed. Hence various advanced modalities are tried with semi-rigid thoracoscope to increase the sample size.

a) **Cryobiopsy** through semi-rigid pleuroscope can be used a modification to solve this issue (Fig. 5). A cryo probe (ERBE Elektromedizin GmbH, Tuebingen Germany) with size of 2.4 mm is introduced through the working channel of the semi-rigid thoracoscope (Olympus Europa SE & Co. KG, Hamburg, Germany). The probe is placed perpendicular to the surface of the parietal pleura with the tip of the probe extended well beyond the tip of the scope using the marking on the probe and with direct visualization. Freezing is done for 6–10 seconds depending on the visual assessment of pleural texture and yield at the initial pass (Fig. 6). Longer the freezing time, the larger will be the obtained sample. Once freezing is done, the semi-rigid

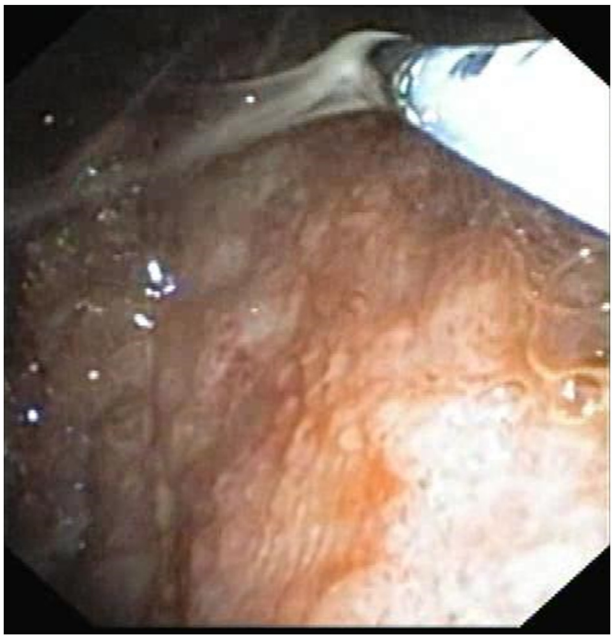

Fig. 5 – Pleuroscopic view of adeno-carcinoma, biopsy with cryoprobe.

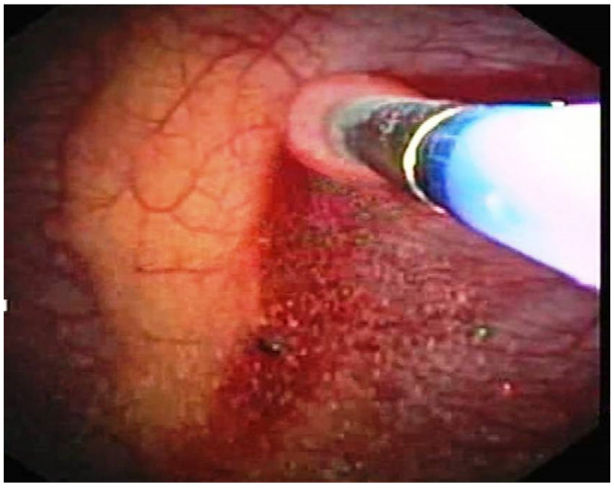

Fig. 6 – Pleuroscopic view showing the cryo probe passed through the working channel of pleuroscope and freezing an area of parietal pleura.

thoracoscope along with the probe is forcibly withdrawn *en bloc* avulsing the frozen parietal pleura. The sample attached to the tip is thawed in normal saline and detached from the cryoprobe tip. The cryoprobe is removed and immediately the semi-rigid pleuroscope is reintroduced through the port and the pleura re-visualized for bleeding. When adequate samples have been obtained, usually after 2 or 3 attempts, the semi-rigid thoracoscope is withdrawn after ensuring that no major bleeding is occurring. Literature has shown that cryo biopsy is a safer and an improved diagnostic tool for obtaining a larger biopsy sample in comparison to the conventional forceps biopsy (Fig. 7).[21]

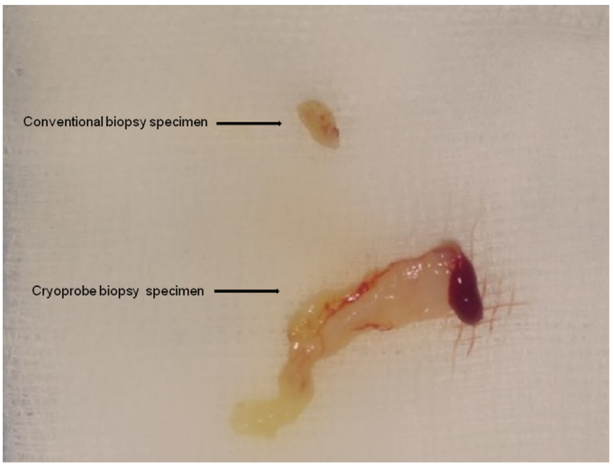

Fig. 7 – Comparison of the size of sample taken via conventional flexible biopsy forceps (smaller piece on top) and cryoprobe (larger piece).

b) **Electrocautery** guided pleural biopsy using the IT knife via semi-rigid pleuroscopy has also been tried and was shown to be superior to the standard flexible forceps biopsy.[34] Lidocaine (0.5%) mixed with saline (0.9%) and epinephrine (0.005%) was injected using an injection needle until the affected pleura was raised. After puncturing with a coagulation forceps (FD-6C-1; Olympus) a pinhole was made, through which the tip of the IT knife was inserted into the hole. The interested pleura was incised in a circular shape with full thickness by manipulating the IT knife.[34]

c) **Narrow band imaging (NBI)** enhances the endoscopic view of blood vessels. Wavelengths of light in the visible spectrum are filtered from the illumination source, with the exception of narrow bands in the blue and green spectrum (centered at 415 nm and 540 nm) which coincides with the peak absorption spectrum of oxyhemoglobin, making blood vessels more prominent when NBI mode is used.[35] Different patterns indicative of malignancy were found to be superior and statistically significant in comparison with white light.[35]

d) **Protective sheath guided pleurodesis via semi-rigid pleuroscopy:** Though semi-rigid pleuroscopy has the advantage of concurrent pleurodesis in cases of malignancy, it is done only after the pleuroscope is withdrawn. Hence it becomes a blind procedure especially when talc powderage is done. To overcome this draw back oxytetracycline solution was sprayed over the pleura under vision using the protected sheeth (Olympus) inserted via the working channel.[32] Even talc powderage was also done successfully using the same assembly in few of malignant pleural effusions (Fig. 8).

e) **Pleural infiltration of Lidocaine using TBNA needle:** During cryobiopsy, pain due to forceful avulsion of the parietal pleura sometimes becomes the limiting factor.[21] So using a TBNA needle to infiltrate the area of interest with lidocaine on the parietal pleura could reduce the pain significantly and also there was a bleb formation at the site of infiltration, which loosened the pleura and facilitated the adequate contact between the pleura and the

Fig. 8 – Protected sheath guided pleurodesis using oxytetracycline.

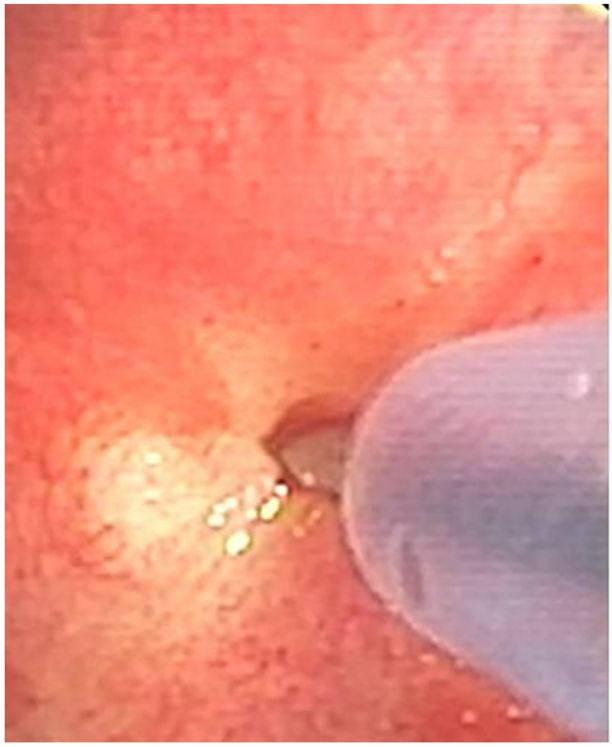

Fig. 9 – Pleural infiltration with lidocaine using TBNA needle.

probe and hence sample could be avulsed easily during cryobiopsy (Fig. 9).[21]

f) **Double port technique:** Semi-rigid thoracoscopy may be useful in limited adhesiolysis in cases of thin septations. While thicker septations ideally should be lysed with rigid forceps, hence in some cases a double port technique (Fig. 4), where a rigid forceps is introduced through a separate port can be used to break the adhesions and it also helps in procuring a larger biopsy sample.[32]

8. Complications[36–40]

Relatively a safe procedure, apart from anesthesia related and sedation related complications.

- Air leaks persisting beyond 7 days due to accidental injury to the lung or visceral pleura
- Surgical emphysema – Due to pneumothorax or insufflation of air. Typically seen in our experience when patient coughs against the clamped tube after the procedure.
- Pain & Fever – May be seen after pleurodesis due to inflammation
- Hemorrhage – Very rare from the site of incision
- Infections – Introduction of infection due to lack of sterile precautions can be seen but it's not so common
- Re-expansion pulmonary edema; Though mentioned to be rare, author has experienced in 3 patients so far in 200 patients (2 out of 3 were fatal). Hence should be careful in removing the fluid in total in clean case without any adhesions and pleural effusions with endobronchial mass. Also, it may be very useful not to drain the fluid completely and allow ICD to drain in next 24 hours to prevent this complication.

9. Summary

- Light's criteria has revolutionized the approach to pleural effusion and till date remains a very useful step in the diagnosis of pleural effusions.
- Pleural fluid biochemistry and adenosine deaminase (ADA) enzyme levels play a significant role in the diagnosis of tubercular effusion.
- Most of the laboratories measure overall ADA levels without a mention of isoenzyme type. Hence it is difficult to distinguish a tubercular pleural effusion (TPE) from other PPEs based on pleural fluid ADA levels alone.
- LDH/ADA ratio as an indicator for ruling out tuberculosis was analyzed in few studies with high sensitivity and specificity.
- The pleural fluid cytology has a varying sensitivity, with a maximum of only 60% and it may increase with subsequent tapping.
- Closed pleural biopsy using a Cope or Abrams needle has a sensitivity up to 80% in cases of tuberculous effusion and 40%–73% in cases of Malignancies.
- Semi-rigid thoracoscopy involves the visualization of thoracic cavity using a semi-rigid pleuroscope. It is usually done by a respiratory physician as a day care procedure under local anesthesia.
- It is done in undiagnosed, recurrent pleural effusions.
- Physician should be careful in selecting the cases with prior imaging. Cases with multiple thick septations and with pleural thickening should be avoided and referred for surgical approach.
- Minimum adhesiolysis can be performed by using semi-rigid thoracoscope. A double port technique may sometimes be useful for further adhesion release and for a larger sample.

- Cryo biopsy via semi-rigid thoracoscope can be used in conditions where pleura is excessively thick and a normal flexible forceps fails to procure a sample.
- Relatively a safe procedure with a very low complication rate. Re expansion pulmonary edema can be expected in excess removal of fluid.

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Conflicts of interest

All authors have none to declare.

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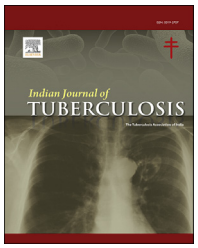

Review article

Antibiotic scintigraphy in tuberculosis: A new horizon?

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ABSTRACT

Tuberculosis (TB) continues to be a major cause of death worldwide that can be effectively treated with timely diagnosis and treatment. With the advent of nuclear imaging techniques like ^{18}Fluorine Fluoro-2-Deoxy-D-Glucose Positron Emission Tomography (^{18}F-FDG) PET/CT, the diagnosis of tuberculosis, particularly its extrapulmonary forms, has received great impetus in cases where microbiological confirmation cannot be achieved. Although detection of mycobacteria either by staining, culture or nucleic acid amplification techniques still form the gold standard of diagnosis, newer diagnostic techniques are always welcome in the field which can expedite clinical management. Use of radiolabeled antibiotics is one such evolving sphere which needs further research. Moving ahead from radiolabeled leukocytes, antibiotics are being increasingly focused upon to act as a vehicle to locate infectious lesions. Antibiotics like ciprofloxacin have been labeled with diagnostic radionuclides such as Technetium-99m (Tc-99m) and used to image many infectious diseases with encouraging results in TB. However, the nonspecific attributes of ciprofloxacin have hindered its growth to assist the diagnosis of TB. A novel approach would be to utilize ethambutol, a specific antitubercular agent, which has been found to be safe and effective in the diagnosis of TB in the available published studies. Ethambutol is known to be taken up specifically by tubercular lesions. This forms the basis of using Tc-99m labelled ethambutol for imaging TB lesions. An added advantage would be its ability to differentiate tubercular from malignant and fungal lung lesions that are the usual differentials in patients suspected of having TB. Most of the studies involving ethambutol have been done in skeletal TB and its validation in other forms of TB is still awaited. Recently the role of PET-CT has also been explored in human studies using ^{11}C Rifampicin to study the antibiotic uptake in tubercular lesions. This review summarizes the available evidence regarding diagnosis of TB by radiolabeled antibiotic imaging to emphasize the need for accelerated research in the fight against TB.

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1. Introduction

Tuberculosis (TB), caused by the bacteria *Mycobacterium tuberculosis* still remains one of the leading causes of death worldwide. Approximately 10 million people were affected by tuberculosis worldwide in 2018.[1] Global burden of TB differs among countries ranging from five to greater than 500 cases per 100,000 population per year and the global average is around 130.[1] Majority of the cases were recorded in the South-East Asia region in 2018, with India being among the top eight countries constituting 2/3rd of the total TB cases. India has also been listed as one of the countries with the highest TB burden by WHO.

TB can show multiple organ involvement (excluding hair and nails) and can present with various signs and symptoms. It is broadly classified into pulmonary and extra-pulmonary tuberculosis (EPTB). Pulmonary involvement happens to be more common of the two and is the main priority in public health sector. However, in recent years extrapulmonary involvement has increased remarkably due to the prevalence of HIV and organ transplantation.[2] EPTB constitutes 15–20% of all TB cases in HIV negative population in India, whereas the incidence ranges from 40 to 50% in HIV infected patients.[3]

TB usually spreads via respiratory route. However, only up to 30% of new TB patients have known contacts.[4] After entering the lungs, the pathogen may either be cleared by the host's immune system, may persist in a latent stage leading to reactivation later on, or may give rise to active disease depending on the host's immune status.[5] Immunosuppressive conditions like HIV, diabetes, chronic kidney disease, post-transplant patients, low socio-economic status, smoking, alcoholism, overcrowding, etc. are the major risk factors for TB.[6]

2. Methods

We searched PUBMED and GOOGLE SCHOLAR for articles published in English language from the year 2000 till now, using terms "antibiotic scintigraphy", "technetium labelled ethambutol", "technetium labelled ciprofloxacin", "ethambutol scintigraphy", "ciprofloxacin scintigraphy", "PET", "SPECT' and "Tuberculosis" or "TB". After excluding animal studies, in-vitro studies and case reports, we found 8 clinical studies in humans using radiolabeled antibiotics as a diagnostic tool in tuberculosis, which we included in our review.

3. Literature review

3.1. *Diagnosis of TB*

In order to establish diagnosis of TB, proper history, clinical examination, microbiological and histopathological investigations, along with radiological tests are necessary. Definitive diagnosis of TB can be made by culture or polymerase chain reaction detection of *Mycobacterium tuberculosis* in the sample, thereby helping to differentiate from other infectious causes, malignancy as well as other species of mycobacteria. Gold standard for diagnosis still remains culture of specimen. Ziehl Neelsen (ZN) staining and histopathology of biopsy samples also support the diagnosis of TB. However, cultures take weeks to become positive and low yields have also been reported in studies.[7] Histologic findings include caseation with granulomas, non-caseating granulomas, Langerhans giant cells and nonspecific lymphoid infiltrates. The cartridge based nucleic acid amplification test Xpert MTB/RIF approved by FDA in 2013 has boosted the diagnosis of TB. A meta-analysis of Xpert MTB/RIF compared to culture as the gold standard reported a sensitivity & specificity of 95% & 98%, respectively.[8]

3.2. *Imaging*

Imaging is an indispensable part of the diagnostic process in TB. Microbiologically negative pulmonary or EPTB is difficult to diagnose because of its similarity with other diseases like sarcoidosis and lymphomas. Computed tomography (CT) and Magnetic Resonance Imaging (MRI) are important investigations, particularly for lymph node TB (LNTB), and features like low attenuation on CT indicate central necrosis while hyperintensities on T2 weighted MR images are characteristic.[9] However, differentiating necrosis of TB from that of carcinoma remains difficult. Even after employing all the imaging modalities like CT and MR, definitive diagnosis cannot be always achieved.[10]

3.3. *Role of nuclear imaging*

The last decade has witnessed emergence of nuclear imaging techniques as the latest tool for diagnosing TB. ^{18}Fluorine Fluoro-2-deoxy-D-glucose Positron Emission Tomography (^{18}F-FDG PET) combined with CT is a non-invasive test which can detect early infection, determine disease extent, differentiate between active and inactive disease and most importantly help in evaluating treatment response before morphological changes appear on conventional imaging techniques.[11] On the other hand, it is limited by its inability to differentiate between malignant and tuberculous lesions based on Standardized Uptake Values (SUV). Studies have shown that ^{18}F-FDG PET is unable to differentiate between tubercular lymphadenitis and lymph node involvement due to malignancy because SUV of the two groups were not significantly different. This is an important observation due to the fact that the main differential of TB lymphadenitis is lymphoma.[12]

3.4. *Infection imaging beyond ^{18}F-FDG*

An ideal infection imaging agent should be specific to infection, easily administrable, should be free from side effects, should have low or negligible uptake in non-target tissues, be cost-effective and applicable for immuno-compromised patients. Various radiopharmaceuticals have been used for infection imaging namely ^{67}Gallium citrate, radiolabelled leukocytes, radiolabelled human polyclonal immunoglobulin, monoclonal antibodies, etc. These radiopharmaceuticals are specially targeted towards various components of inflammation enabling them to detect early changes during an infection

but at the same time, render them non-specific.[13] Technetium-99m (^{99m}Tc) is probably the most frequently used radionuclide owing to its decay properties, ready availability, high radiochemical purity, quick radiolabelling time and affordable cost.[14,15]

3.5. *Radiolabeled antimicrobial agents*

Radiolabeled antibiotics are the newest addition in the armamentarium for diagnosis of infectious diseases and foci of infection. The reason for their emergence is their increased specificity which in turn is attributed to the specific binding of antibiotics to the bacterial component. Using this simple principle, this binding of radiopharmaceutical with the microorganism can be picked up by gamma camera when the radionuclide used is a gamma emitter like ^{99m}Tc. Various fluoroquinolones, cephalosporins, anti-tubercular agents and other antibiotics have been developed and studied for the diagnosis of infections till date.[16]

3.6. *Radiolabeled anti-tubercular drugs*

3.6.1. *^{99m}Tc Ciprofloxacin (Infecton)*

Ciprofloxacin was the first anti-tubercular antibiotic to be radiolabeled and used in clinical studies.[17] Ciprofloxacin is a quinolone antibiotic which inhibits bacterial DNA gyrase without any effect on mammalian cells. It acts against gram positive, gram negative bacteria as well as *Mycobacterium tuberculosis* which makes it non-specific for TB. Radiolabelled ciprofloxacin is taken up by various cells and tissues but is not retained there for long and is excreted mostly by urine. However, it stays at the site of infection for prolonged periods enabling images of the infection to be taken till up to 24 hrs following injection of radiopharmaceutical.[16] It is not taken up by leukocytes, dead bacteria and if the bacterial resistance is due to cell membrane impermeability.[18] A comparative study in 1996 used Infecton for imaging bacterial infections and compared with that of radiolabelled leukocytes. They found Infecton to be better than radiolabelled leukocytes in terms of sensitivity and specificity.[19] Hall et al also confirmed high sensitivity and specificity of Infecton in bacterial imaging in their study.[18] ^{99m}Tc-Ciprofloxacin was further tested in a multicentre study of 879 patients with different types of infections which yielded a sensitivity and specificity of 85.4% and 81.7%, respectively. Sensitivity of imaging with Infecton was found to be more in case of microbiologically confirmed infections.[20] There were no adverse events in patients injected with ^{99m}Tc-Ciprofloxacin because of the minute amount of drug used for the study (approximately 1/200th of the therapeutic dose). Sensitivities and specificities varied according to the site of infection with highest sensitivity noted in orthopaedic infections, soft tissue infections, abdominal infections and TB. More than 90% specificity was observed in prosthetic joint infections, infective endocarditis and surgical infections. Serial images correlated well with treatment response which is a useful finding and particularly helpful in deciding treatment continuation. Absence of bone marrow uptake, specificity for infection, low cost and ease of preparation have been the main advantages of Infecton. In spite of initial encouraging results, subsequent studies failed to produce similar results and hence currently the ability of ^{99m}Tc-Ciprofloxacin to specifically target infection and differentiate it from inflammation is debated.[21]

There are few studies which have evaluated ^{99m}Tc Ciprofloxacin exclusively in TB patients. Lee et al assessed the efficacy of ^{99m}Tc Ciprofloxacin Single Photon Emission Computed Tomography (SPECT) for imaging active pulmonary TB and found sensitivity of 80% and specificity of 90.9%.[22] This study showed that ^{99m}Tc Ciprofloxacin SPECT could be used for differentiating between the active and inactive TB states in patients with past history of pulmonary TB. Compared to ^{111}In leukocytes, which is one of the conventional radiopharmaceuticals used for infection imaging and requires ex vivo preparation, ^{99m}Tc ciprofloxacin kit was found to be easier to prepare, less labour-intensive and quicker to use.[19]

Indian studies have assessed the role of ^{99m}Tc Ciprofloxacin in musculoskeletal tuberculosis. In these prospective studies, ciprofloxacin scintigraphy was done at baseline, 3 and 6 months after treatment to assess therapeutic response. In the study involving extraspinal osteoarticular tuberculosis, 100% patients had a positive scan while in the patients with Pott's spine, 60% positive concordance was seen. This lower positivity rate in spinal TB was attributed to the lower penetration of radiopharmaceutical into the necrotic area owing to its poor vascularity. However, the follow up scans in both the studies corroborated well with treatment response indicating that ^{99m}Tc Ciprofloxacin can well be used to determine the treatment end points in such patients but might not be reliable for monitoring disease activity.[23,24]

3.6.2. *^{99m}Tc Isoniazid (INH)*

INH is a bactericidal anti-mycobacterial agent which inhibits the synthesis of mycolic acid. Being a specific anti-TB drug, ^{99m}Tc labelled INH was used as an imaging agent for TB. Initial animal data suggested its safety as a diagnostic test and acceptable distribution of the drug similar to the unlabeled compound.[25]

A biodistribution and phase I clinical trial was conducted in 2009 by Singh et al. Among 20 patients included in the study, 13 patients had drug sensitive pulmonary and bony lesions, 2 patients had drug resistant TB and 5 patients had inactive lesions, although details regarding selection of inactive cases were not available. Scans were acquired at 1hr, 4 hr and 24 hrs, and there were no adverse effects noted. All the active lesions were picked up by imaging whereas the inactive lesions weren't. The bony lesions appeared early within 1 hr and the pulmonary lesions were visible later as the ^{99m}Tc INH accumulated gradually over 24 hrs. The investigators concluded that delayed imaging (at 24 hrs) was necessary for detecting soft tissue lesions. This was the only clinical study that we found, which showed the feasibility of ^{99m}Tc INH as diagnostic agent for tuberculosis.[26]

3.6.3. *^{99m}Tc Rifabutin*

Rifabutin is an antibiotic of the rifamycin group which has bactericidal activity against gram positive, gram negative organisms and Mycobacterium. ^{99m}Tc Rifabutin was tested in an animal model which showed high and specific uptakes in TB infected sites but till date no clinical human studies have been performed.[27]

3.6.4. ^{11}C Rifampicin

Recently ^{11}C Rifampicin has been used to study the drug distribution in TB using PET imaging. After initial animal studies, the first human study was also safely performed in a young patient with TB meningitis which showed reduced penetration of rifampicin into infected brain tissue.[28,29] Subsequently, patients with newly diagnosed rifampicin sensitive pulmonary TB were also recruited in a study to undergo PET-CT imaging after injection of microdoses of ^{11}C Rifampicin. This study was the first of its kind to show the variability in drug concentrations in infected tissues and strongly argues in favour of PET-CT imaging with radiolabeled antitubercular drugs as a non-invasive modality to predict therapeutic response and also modify the ongoing treatment.[30]

3.6.5. ^{99m}Tc Ethambutol

Considering the disadvantages of the other radiolabelled antibiotics, a specific first line anti-tubercular drug ethambutol (EMB) was chosen as a more suitable ligand to link with ^{99m}Tc for detection as well as localization of tubercular lesion. Ethambutol is a bacteriostatic agent which specifically inhibits arabinogalactan synthesis, a mycobacterial cell wall component and thus inhibits cell wall mycolic acid synthesis. Time to reach maximum concentration is about 2–3 hrs, and it is eliminated by both renal and hepatic routes.[31]

An early animal study by Verma et al showed that ^{99m}Tc Ethambutol was specifically taken up by tubercular lesions in mice and can be a potential imaging radiopharmaceutical for TB.[32] It is retained by the tubercular lesions owing to its binding property to mycolic acid but gets cleared from non-tubercular lesions thus, conferring its specificity. Being a non-invasive test is a major advantage and also provides quicker results than histopathological tests without any major adverse effects.[33]

Biodistribution studies along with clinical evaluation in patients were done by Singh et al. Patients in the study included pulmonary and bone TB. Radiolabeled ethambutol showed good radiochemical purity and stability and the pharmacokinetic properties were comparable to that of unlabeled drug. The study further noted its increased sensitivity compared to other Tc-99m radiotracers for TB due to its higher physiological concentration in lung parenchyma. This preponderance of the radiopharmaceutical to the lungs led to higher uptake by mycobacterium and thus, higher detection rate. ^{99m}Tc EMB was taken up by both pulmonary and bone lesions, with bone uptake being quantitatively higher than the lung lesions and could detect both active and resistant lesions by accumulating in rising patterns over time. The retention of ^{99m}Tc EMB in the lesions till 24 hours translated to greater specificity than ^{99m}Tc Ciprofloxacin for tubercular lesions as the latter got washed away at 4 hours of post-injection.[34]

Another retrospective cross-sectional study which was done in Indonesia where 168 suspected TB patients were subjected to ^{99m}Tc Ethambutol scintigraphy, reported a sensitivity and specificity of 93.9% and 85.7% respectively in detecting pulmonary TB. For EPTB, they reported a sensitivity of 95.5% and specificity of 77.8% while overall sensitivity was 94.9% and specificity was 83.3%. Patients included in the study were either treatment naive or within 14 days of starting treatment. After injecting 370–555 MBq ^{99m}Tc-ethambutol, whole body planar and SPECT-CT imaging was done at 1 and 4 hrs. Pathological uptake of Tc-99m Ethambutol was seen as an area of increased focal uptake in the active tubercular lesions. This study included 30.9% pulmonary TB cases and 58.4% extrapulmonary cases, with the rest being non-tubercular infections. Concordance with microbiological and histological findings were seen in 92.86% patients and discordance in 7.14%. Increased pathological uptake was seen even after 24 hrs of radiopharmaceutical administration, which increased its specificity especially in doubtful cases. Compared to the studies using Tc-99m Ciprofloxacin, they found higher sensitivity of Tc-99m Ethambutol but a slightly lower specificity which was theoretically contradictory as Ethambutol is more specific for *M. tuberculosis*.[22] A false positive result could be due to hypervascularisation that can be minimised by taking delayed 24 h images. On the other hand, false negatives could be due to necrotic lesions that result in reduced tracer uptake due to the reduced blood flow to the area of necrosis which could be correlated with anatomical imaging. It was also observed that consuming ethambutol for less than 2 weeks did not influence the results.[33]

The latest study to be published using ^{99m}Tc Ethambutol scintigraphy as a diagnostic agent, by Diah et al involved 93 suspected spinal tuberculosis patients. Planar and SPECT-CT images were taken at 1 and 3 hrs post injection of radiolabelled ethambutol. Results were compared with histopathological findings. Sensitivity and specificity in the study were 90.91% and 71.43% respectively and the accuracy was 87.5.[35] These studies have given a generally favourable result for the usage of ^{99m}Tc-EMB for TB scintigraphy.

4. Discussion

As TB continues to kill millions of people in developing countries, there have been major advances in its diagnosis. In this era of molecular imaging, infection imaging agents have been of particular interest in the field of research. ^{99m}Tc labelled radiopharmaceuticals have been the agents of choice for imaging infections.[13] Radiolabeled antibiotics have the ability to pick up the infection by specifically targeting the micro-organism. Their usage in TB diagnosis has seen a gradual shift from radiolabeled Ciprofloxacin to radiolabeled Ethambutol. ^{99m}Tc ciprofloxacin has the advantage of lower radiation exposure to the patient than conventional radiopharmaceuticals like $^{67}Gallium$ citrate.[20] On the other hand, Ciprofloxacin is a broad-spectrum antibiotic and therefore, uptake of Tc-99m ciprofloxacin is possible in many infectious diseases besides TB leading to low specificity. Non-specificity of ^{99m}Tc Ciprofloxacin towards *Mycobacterium tuberculosis* is the major hindrance in its use as an imaging agent in TB. Although radiolabelled Isoniazid has produced good results in a single study, we don't have further data to corroborate those findings.[26] Radiolabelled Rifabutin, another antimycobacterial agent, has been tested in an animal model but is yet to be tested in humans.

Table 1 – Summary of antibiotics used as radiopharmaceuticals in clinical studies in Tuberculosis.

Author	Study characteristics	Radiopharmaceutical and modality	Results	Comments
Lee et al. 2010	Prospective study involving 21 participants including patients with active and inactive PTB as well as normal patients	Tc-99m ciprofloxacin SPECT	Sensitivity: 80.0% Specificity: 90.9% PPV: 88.9% NPV: 83.3%	First study to show the feasibility of Tc-99m ciprofloxacin as an imaging agent in tuberculosis
Bhardwaj V et al. 2011	Prospective study involving 25 newly diagnosed patients of extraspinal osteo-articular tuberculosis	Tc-99m ciprofloxacin scintigraphy using gamma camera was done at treatment initiation and after 3 & 6 months of treatment	Initial scan: All patients had a positive uptake Repeat scan: Negative in 4 patients 3 months; 21 patients after 6 months of therapy	Small sample size. This study highlights the capability of Tc-99m ciprofloxacin for diagnosing as well as monitoring treatment response in skeletal tuberculosis
Agrawal et al. 2012	Prospective study involving 15 patients with newly diagnosed Pott's spine	Tc-99m ciprofloxacin scan with SPECT at 0, 3 & 6months of treatment	9 (60%) patients had initial positive bone scans. Negative scans documented in 2 patients after 3 months and in 7 patients after 6 months.	This study showed Tc-99m ciprofloxacin to be a promising agent in evaluation of Pott's spine. Small sample size precludes any definitive conclusion of this study. MRI of spine remains a pivotal tool to assess the anatomic effects and extent of disease.
Singh et al. 2009	Prospective study involving 2 healthy subjects and 20 patients of proven pulmonary and bone TB (including 5 patients with inactive TB)	Tc-99m INH scintigraphy using gamma camera with additional SPECT in 3 patients	Active lesions showed positive uptake whereas inactive lesions showed no uptake. Bony lesions were visualized earlier than soft tissue lesions.	Only human study utilizing Tc-99m INH as an effective radiolabelling agent in tuberculosis.
Singh et al. 2010	Biodistribution studies involving 2 healthy human subjects and clinical evaluation in 14 patients with pulmonary/bone TB	Tc-99m-Ethambutol scintigraphy using gamma camera	Tc-99m-Ethambutol was localized to the lung and bony lesions	First clinical study to show the safety and potential of Tc-99m-Ethambutol as an imaging agent in tuberculosis
Kartamihardja et al. 2017	Retrospective cross-sectional study with 168 patients having both pulmonary and extrapulmonary TB	Tc-99m-Ethambutol scintigraphy using gamma camera and SPECT	Sensitivity: > 90% in both PTB & EPTB Specificity: < 90%	Lower specificity contradicts the theoretical fact that ethambutol is specific anti-tubercular agent
Diah et al.2019	Retrospective study involving 93 patients with spine TB	Tc-99m-Ethambutol scintigraphy using gamma camera and SPECT	Sensitivity: 90.91% Specificity: 71.43%	Retrospective study design and low specificities are drawbacks. High sensitivity underscores the utility of Tc-99m-Ethambutol as a diagnostic radio pharmaceutical
Ordonez et al. 2020	Prospective study involving 12 patients with confirmed pulmonary TB	Dynamic ^{11}C Rifampicin PET-CT imaging	Rifampicin tissue-to-plasma AUC ratios demonstrated variable drug exposure in different lesions, with lower concentrations in cavity walls.	This study provided the first insight on the use of PET in TB imaging as a non-invasive method of monitoring drug penetration in lesions. Diagnostic capability needs to be evaluated further with different patient population and tissue involvement

Abbreviations: EPTB: Extra-pulmonary tuberculosis, INH: Isoniazid, MRI: Magnetic Resonance Imaging, NPV: Negative Predictive Value, PPV: Positive Predictive Value, PTB: Pulmonary tuberculosis, SPECT: Single photon emission computed tomography, TB: Tuberculosis.

Thus, currently the focus should be on Tc-99m EMB as the agent of choice in TB scintigraphy. Published results for ^{99m}Tc EMB have been favourable in pulmonary and bone TB, but its accuracy in other forms of EPTB like LNTB is not established. It is safe to be used in humans and gives much quicker results compared to histopathological tests or GeneXpert. Further studies to prove its accuracy with larger sample sizes are needed. With stronger evidence, it could be potentially used as one of the important investigation modalities in the initial management of TB patients. This could be particularly useful in patients having deep seated or inaccessible lesions. Diah et al. had concluded in their study that suspected spinal TB patients, if found to be positive on ^{99m}Tc Ethambutol scintigraphy, can be started on ATD pending histologic results.[35]

A summary of the existing studies has been presented in Table 1. Contradictory to the theoretical aspect as mentioned above, lower specificities of the scintigraphies using radiolabelled Ethambutol can be attributed to the fact that the studies had lower number of true negative cases. Necrosis within the lesions can also affect uptake of the radiopharmaceutical and thereby influence the interpretation of results., although this is debatable. Another unexplored aspect of ^{99m}Tc Ethambutol scintigraphy is the assessment of treatment response to successful antitubercular therapy which has been addressed in the study using ciprofloxacin.[24] It can only be speculated at this point that since the lesions can be located on the basis of accumulation of antitubercular drugs, it can be a potential marker of prognostic importance which can be substantiated only by generating larger data. Compared to ^{18}F FDG PET/CT, ethambutol has a major theoretical advantage of being able to differentiate between malignant and tubercular lesions. MRI on the other hand is still indispensable in spine TB as it can clearly define the anatomic involvement and extent of disease, which may not be accurately possible with antibiotic uptake studies. Further details are required on whether imaging of tubercular lesions by planar imaging alone can be adequate or should be supplemented by SPECT-CT. SPECT-CT can provide accurate details on anatomic localization of the disease, the importance of which has been highlighted in some studies and reports.[22,36] PET-CT imaging has been utilised only for measuring anti tubercular penetration in tissues till now and needs to be further evaluated as a diagnostic modality given certain advantages over SPECT like better sensitivity, image quality and special resolution.[30,37] Nevertheless, ethambutol being a specific anti-tubercular agent, non-invasive nature of antibiotic scintigraphy and availability of quick results clearly gives it an overwhelming advantage as a diagnostic radiopharmaceutical, compared to its congeners.

5. Conclusion

In our quest for the search of the ideal radiotracer for imaging tuberculosis, ^{99m}Tc labelled ethambutol holds good promise going by the recent available data. However further large scale prospective studies are necessary particularly in other forms of EPTB before this investigation can be made widely available in clinical practice.

Conflicts of interest

The authors have none to declare.

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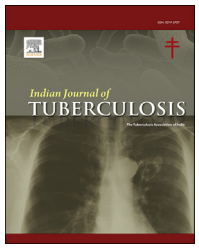

Original article

Epidemiological profile of tuberculosis in Iraq during 2011–2018

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ABSTRACT

Background: Tuberculosis (TB) is one of the most progressive infectious diseases caused by *Mycobacterium tuberculosis*. The pathogen is the first cause of mortality linked to a single pathogen worldwide, especially in poor and developing countries.

Methods: A cross-sectional descriptive study was conducted to estimate incidence rate (IR) of TB in Iraq during a period of eight years (2011–2018). TB data were extracted from the computer system of the National Specialized Center for Chest and Respiratory Diseases in Baghdad.

Results: During 2011–2018, 65,102 confirmed TB cases were reported in Iraq; 39,640 pulmonary TB (PTB) and 25,462 extra-pulmonary TB (EPTB). The average IR (case/100,000 inhabitants) of TB was 23.4 (14.2 for PTB and 9.1 for EPTB). Annual rate of TB cases showed a gradual decline over years (from 29.2 in 2011 to 18.6 in 2018). The decline in IR was more pronounced in PTB than EPTB. However PTB/EPTB ratio showed a gradual decreasing over years (from 2.04 in 2011 to 1.56 in 2018). GIS-mapping revealed that PTB and EPTB IRs show variations between the 18 governorates of Iraq. Most of the recorded PTB cases were new (average: 90.5%), followed by relapse cases (average: 7.9%). Among the reported PTB cases, percentage of males was greater than females (average: 52.1 *vs.* 47.9%), whereas an opposite trend was observed in EPTB (42.9 *vs.* 57.1%). The frequency distribution of PTB and EPTB varied between age groups, and lowest average frequency was recorded in age groups 1–4 and 5–14 year.

Conclusions: TB is still a public health threat, and although a declining trend in incidence was depicted over the years 2011–2018, the disease is still out of control in Iraq, and more investments of resource are necessitated to eliminate the disease. In this context, EPTB and PTB relapse need a recognized attention.

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by one of the most progressive bacterial pathogen in the history of human being; it is *Mycobacterium tuberculosis*. The pathogen is the first cause of mortality linked to a single pathogen worldwide, especially in poor and developing countries.[1] Pulmonary (PTB) and extrapulmonary (EPTB) are the two clinical manifestations of TB. The former affects the lungs and accounts for 80% of TB cases, while EPTB is less frequent (20% of cases) and involves organs other than the lungs.[2] Although anti-TB medicines are available for more than 50 years ago, the disease remains a major cause of mortality and *M. tuberculosis* continues to implement an enormous impact on public health worldwide.[3] In 2018, it has been estimated that around 10 million cases (132 cases/100,000 population) are suffering from TB worldwide each year. They are distributed as 5.7, 3.2 and 1.1 million among men, women and children, respectively (57, 32 and 11%, respectively). Incidence rates (IRs) at national level vary from less than 50 to more than 5000 per 1,000,000 populations per year. Most of cases occurred in the South-East Asian region (44%), followed by the African region (24%) and the Western Pacific (18%).[4] However, the IR of new TB cases declined by an average of 1.6% per year in 2018 compared to 2017. Number of TB-related deaths has also been declined by 5%.[5]

Iraq is a southwestern Asian country located in the Eastern Mediterranean region (EMR). In this region, TB accounted for 25% of the global burden of disease in 2014. A high burden of TB has been depicted in Iraq, and the country ranked seventh among EMR countries, accounting for 3% of total cases. Further, annual estimate of death cases due to TB was more than 4000. Accordingly, TB in Iraq has been considered a public health priority by the World Health Organization in 2015.[6] Employing capture–recapture modelling to estimate IR of TB in seven countries (China, Egypt, Indonesia, Iraq, the Netherlands, United Kingdom and Yemen) disclosed that TB accounted for 18% of the estimated global incidence in these countries.[4] However, it has been discussed that TB statistics in Iraq during the last two decades (years of sanction and invasion) underestimated the reported incident cases, and some discrepancy in the reported TB data has been addressed.[7] Therefore, this study sought to understand TB epidemiology in Iraq during period of eight years (2011–2018) in terms of clinical type, IR, gender, age and geospatial distribution in governorates of Iraq. Such understanding of TB epidemiology may aid Iraqi health experts in designing and implementing strategies to reduce TB burden in Iraq.

2. Methods

A cross-sectional descriptive study was conducted to estimate IR of TB in Iraq during a period of eight years (2011–2018). The study protocol was approved by the Ethics Committee at the Iraqi Ministry of Health and Environment (N11/4 on 07/03/2019). TB data were extracted from the computer system of the National Specialized Center for Chest and Respiratory Diseases (NSCCRD) in Baghdad. The center documents records of TB notification form from 18 governorates of Iraq. TB parameters were those given in the notification form of the NSCCR. Two clinical manifestations of TB was considered (PTB and EPTB), and the PTB cases were further classified into new, relapse, failure and default. PTB and EPTB were further distributed according to gender (males and females) and age groups (1–4, 5–14, 15–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years). The EPTB comprised cases with cerebral-spinal, gastro-intestinal, genito-urinary, lymph nodes, pericardium, pleura, skeleton or skin TB. IR of TB was given as case/100,000 inhabitants. The population size was based on estimates of the United Nations/Department of Economic and Social Affairs/Population Division.[8] TB data were tabulated in data sheet of the statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.), and incidence rate and frequencies were accordingly calculated. Logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence interval (CI) for EPTB in females. Chi-square test was used to assess significant differences between categorical variables. The Geographic Information System (GIS) software was used to create the geospatial distribution of PTB and EPTB IRs in Iraqi governorates.

3. Results

3.1. Incidence rate

During 2011–2018, 65,102 confirmed cases were reported in Iraq; 39,640 PTB and 25,462 EPTB. The average frequency (percentage) of PTB and EPTB during the eight years was 60.9 and 39.1%, respectively. The average IR (case/100,000 inhabitants) of TB was 23.4 (14.2 for PTB and 9.1 for EPTB). Annual rate of total TB cases showed a gradual decline as years progressed (from 29.2 in 2011 to 18.6 in 2018). The decline in IR was more pronounced in PTB (19.6–10.0) than EPTB (9.6–8.6). The annual change in TB incidence during 2012–2018 was 6.3, −2.9, −7.1, −2.2, −10.0, 8.6 and −2.3%, respectively (Table 1). However, it is worth noticing that PTB/EPTB ratio showed a gradual decreasing over the years 2011–2018 (from 2.04 in 2011 to 1.56 in 2018) (Fig. 1).

3.2. Geospatial distribution

GIS-mapping revealed that PTB IR shows variations in the 18 governorates of Iraq. Central and Eastern governorates (Diyala, Wassit, Al-Qadissiya and Thi Qar) demonstrated the highest IR (18–21 case/100,000 inhabitants), while Western governorates (Al-Anbar and Al-Najaf) were observed to have the lowest IR (9–10 case/100,000 inhabitants). Low IR was also observed in Northern and Southern governorates (11–13 case/100,000 inhabitants). Kirkuk (Northern governorate) and Al-Muthanna (Southern governorate) were exception and the IR was 16–17 case/100,000 inhabitants (Fig. 2).

GIS-mapping of EPTB contrasted the PTB geospatial distribution in Iraqi governorates. Baghdad, Karbala and Thi Qar (Central region) were among the governorates that displayed the highest IR (12–13 case/100,000 inhabitants). Northern governorates were noticed to have the lowest IR of EPTB (6 case/100,000 inhabitants), with the exception of Erbil, in which

Table 1 – Incidence rate of total, pulmonary and extra-pulmonary tuberculosis in Iraq during 2011–2018.

Year	Population size	Total TB			PTB				EPTB			
		N	IR	AC	N	%	IR	AC	N	%	IR	AC
2011	30,725,305	8977	29.2	–	6020	67.1	19.6	–	2957	32.9	9.6	–
2012	31,890,012	8782	27.5	−5.8	5521	62.9	17.3	−11.7	3261	37.1	10.2	6.3
2013	33,157,061	8634	26.0	−5.5	5360	62.1	16.2	−6.4	3274	37.9	9.9	−2.9
2014	34,411,949	8288	24.1	−7.3	5112	61.7	14.9	−8.0	3176	38.3	9.2	−7.1
2015	35,572,269	8255	23.2	−3.7	5052	61.2	14.2	−4.7	3203	38.8	9.0	−2.2
2016	36,610,632	7317	20.0	−13.8	4354	59.5	11.9	−16.2	2963	40.5	8.1	−10.0
2017	37,552,789	7707	20.5	+2.5	4396	57.0	11.7	−1.7	3311	43.0	8.8	8.6
2018	38,433,604	7142	18.6	−9.3	3825	53.6	10.0	−14.5	3317	46.4	8.6	−2.3
Total		65,102			39,640				25,462			
Average	34,794,203	8138	23.4	−6.1	4955	60.9	14.2	−9.0	3183	39.1	9.1	−1.4

TB: Tuberculosis; PTB: Pulmonary TB; EPTB: Extra-pulmonary TB; IR Incidence rate (case/100,000 inhabitants); AC: Annual change (%).

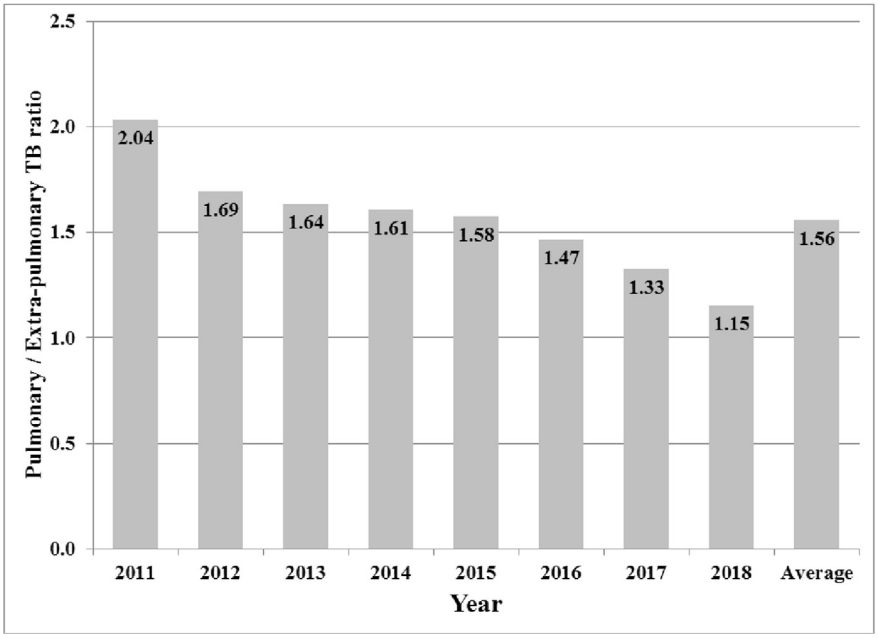

Fig. 1 – Pulmonary/extra-pulmonary tuberculosis ratio in Iraq during 2011–2018.

the incidence rate was higher (10–11 case/100,000 inhabitants) (Fig. 3).

3.3. *New, relapse, failure and default PTB*

Most of recorded PTB cases during 2011–2018 were new (average: 90.5%), followed by relapse cases (average: 7.9%). Failure and default cases were less frequent and their averages were 0.9 and 0.7%, respectively. It is noticeable that annual frequency of relapse cases showed a gradual increase as years progressed during 2011–2018 (5.9, 5.9, 5.6, 9.5, 8.9, 9.8, 9.1 and 9.6%, respectively) (Table 2).

3.4. *Gender distribution*

Among reported cases of PTB during 2011–2018, percentage of males was greater than females (average: 52.1 *vs.* 47.9%), whereas an opposite trend was observed in EPTB (42.9 *vs.* 57.1%). Thus, male:female ratio was different among PTB and EPTB (1.10:1 and 0.75:1, respectively). Logistic regression analysis revealed that females were at greater risk to develop EPTB than males over the years of study. The average OR was 1.45 (95% CI: 1.33–1.59; $p < 0.001$) (Table 3).

3.5. *Age distribution*

Frequency distribution (percentage) of PTB and EPTB varied between age groups during 2011–2018 and the differences were significant ($p < 0.001$). For PTB, the lowest average frequency was recorded in age groups 1–4 and 5–14 year (1.2 and 3.9%, respectively). Older age groups had a higher frequency of PTB; it was ranged between 14.4% in age group 55–64 year and 17.9% in age group 15–24. Age groups 25–34 and 55–64 year had a trend of reduction in frequency of cases from 2011 to 2018 (18.1–15.8% and 15.7 to 12.3%, respectively); while, an opposite trend was observed in age groups 45–54 and ≥65 year (12.6–15.2% and 14.7–18.1%, respectively). For age groups 1–4, 15–24 and 35–44 years, PTB cases tended to have approximated frequencies during 2011–2018 (Table 4).

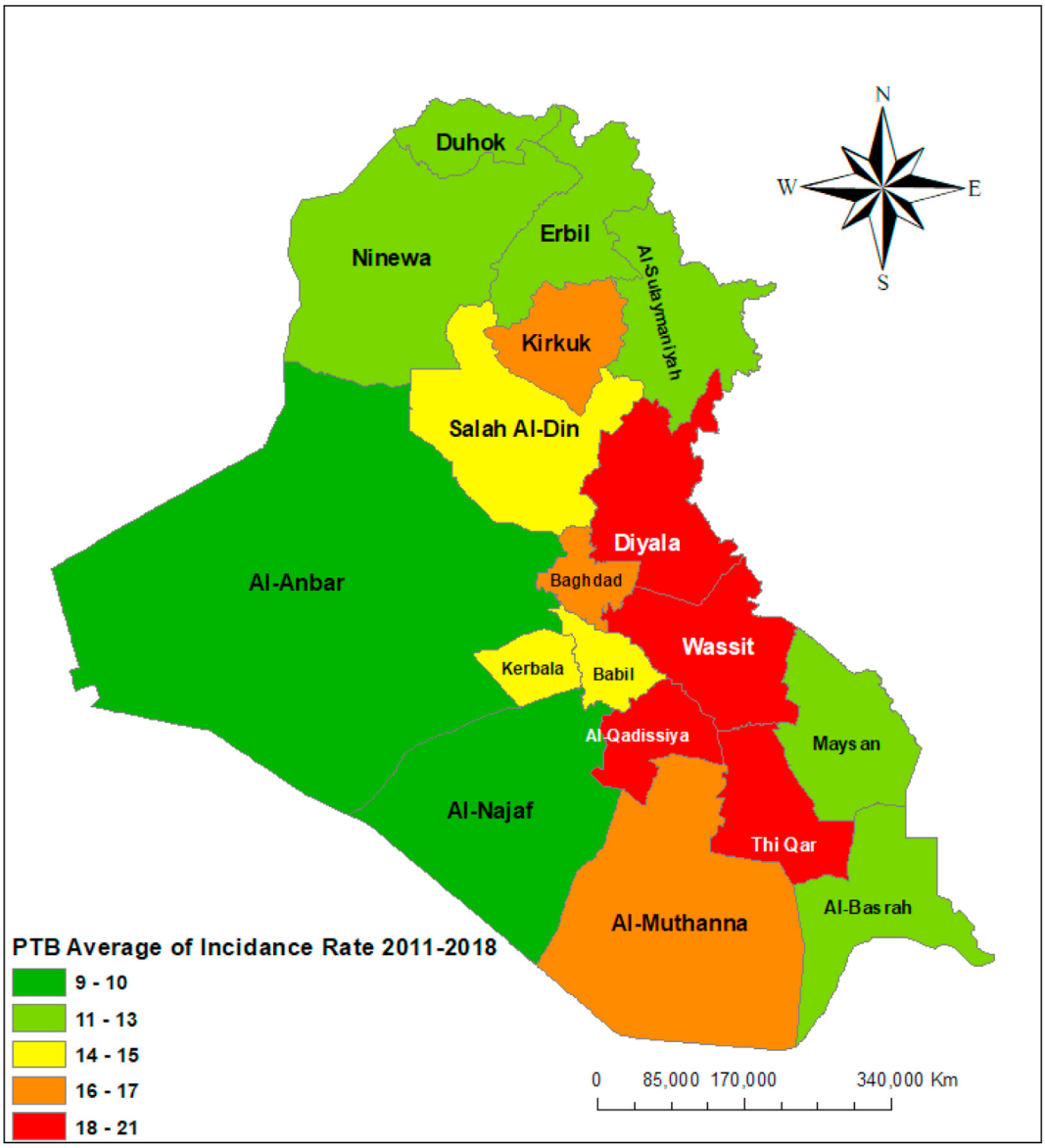

Fig. 2 – Geospatial distribution by GIS-based map of the everage estimated incidence rates for pulmonary tuberculosis (PTB) in 18 Govenonarates of Iraq during 2011–2018.

In the case of EPTB, again the age groups 1–4 and 5–14 year had the lowest frequency with an average of 4.1 and 7.7%, respectively during 2011–2018. The average frequency was higher in older age groups, and had a range of 10.3% in age group ≥60 year to 19.5% in age group 55–64 year. There was no specific trend regarding frequency distribution of EPTB in age groups during 2011–2018, with the exception of age group 15–24 year, in which the frequency tended to decrease from 21.0% in 2011 to 17.9% in 2018. The age group 45–54 is a further exception, but the frequency tended to increase from 2011 to 2018 (11.7–13.2%) (Table 5).

4. Discussion

This study demonstrated that TB shows declining trend in Iraq over the years 2011–2018, and the IR averaged at 23.4 case/100,000 inhabitants; staring from 29.2 in 2011 to 18.6 in 2018. The average annual change was −6.1%. The declined trend of IR was more pronounced compared to 2003, in which the IR was estimated at 130 case/100,000 inhabitants.[9] This decline is due to the adoption of Iraqi health authorities in 1991 for the global targets for reductions in the epidemiologic burden of TB established by the United Nations Millennium Development Goals and the WHO's End TB Strategy.[10] However, the WHO has overestimated the IR of TB in Iraq, and in 2018, it was 42 case/100,000 inhabitants,[4] while in this study, the IR was 18.6 case/100,000 inhabitants. This difference may be related to the method of estimation. The present IR was based on TB notifications, while the WHO estimation of IR was based on published capture recapture studies. In addition, a conflict was indicated between the National Tuberculosis Program (NTP-Iraq), other health authorities and the World Bank. For instance, in 2003, the NTP-Iraq reported 8258 TB

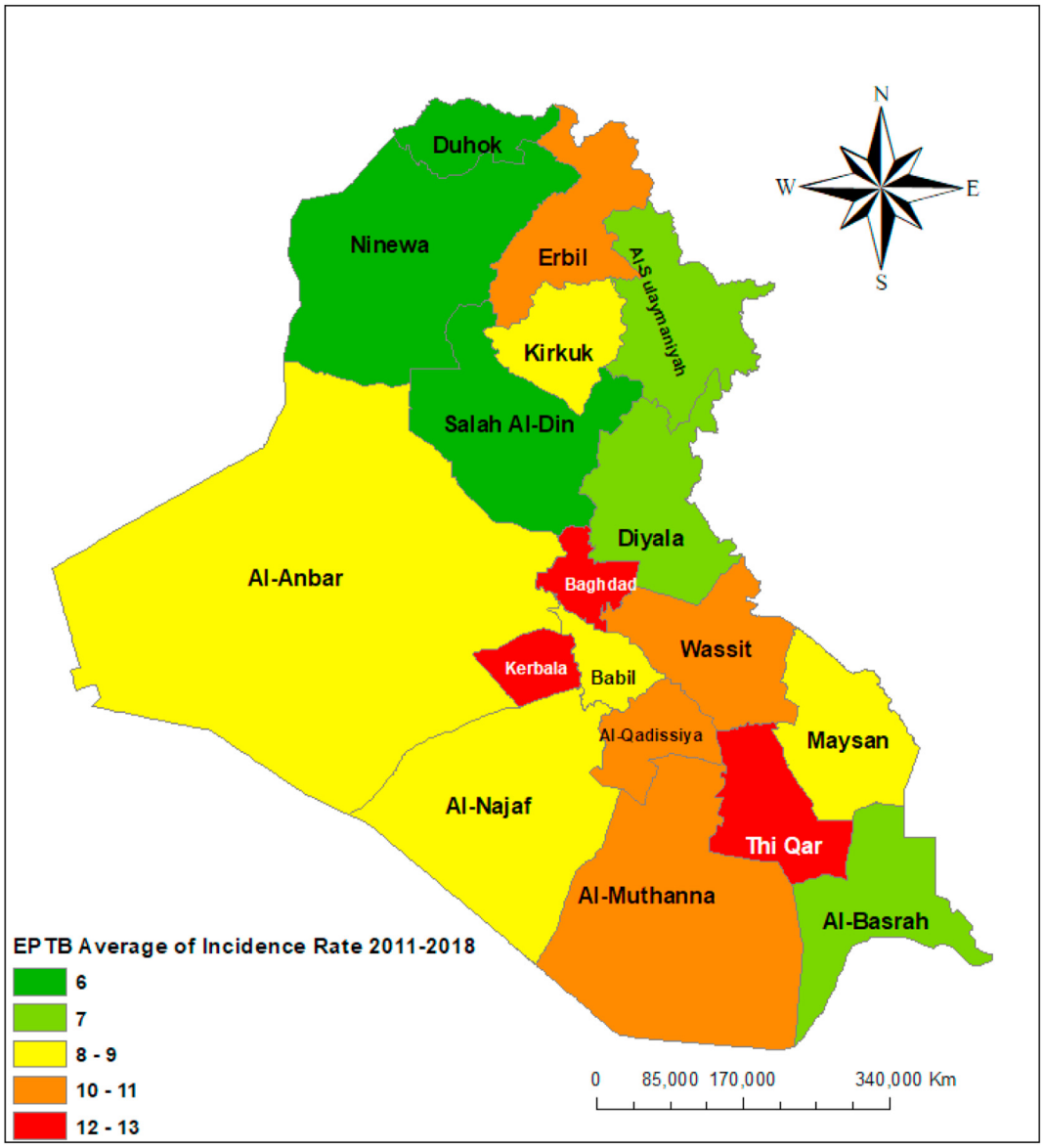

Fig. 3 – Geospatial distribution by GIS-based map of the everage estimated incidence rates for extra-pulmonary tuberculosis (EPTB) in 18 Govenonarates of Iraq during 2011–2018.

Table 2 – New, relapse, failure and default pulmonary tuberculosis cases in Iraq during 2011–2018.

Year	PTB							
	New		Relapse		Failure		Default	
	N	%	N	%	N	%	N	%
2011	5522	91.7	358	5.9	80	1.3	60	1.0
2012	5075	91.9	328	5.9	79	1.4	39	0.7
2013	4978	92.9	302	5.6	51	1.0	29	0.5
2014	4558	89.2	486	9.5	38	0.7	30	0.6
2015	4541	89.9	448	8.9	37	0.7	26	0.5
2016	3861	88.7	428	9.8	37	0.9	28	0.6
2017	3935	89.5	398	9.1	41	0.9	22	0.5
2018	3421	89.4	369	9.6	16	0.4	19	0.5
Average	4486	90.5	390	7.9	47	0.9	32	0.7

PTB: Pulmonary tuberculosis.

cases, while a higher number was reported by the Ministry of Health at the same year (11,656 cases).[7] Therefore, the IR of TB in Iraq has to be precisely re-drawn in order to orchestrate effective control programs.

A worth noticing point in this study was the change in PTB/EPTB ratio, which showed a declining trend over the years 2011–2018. In 2011, the percentage of PTB and EPTB cases were 67.1 and 32.9%, respectively, while in 2018, these figures were changed to 53.6 and 46.4%, respectively. This means that EPTB tended to have increasing proportions in Iraq over recent years. Epidemiological studies have also focused on EPTB, and found that it shows increasing rates in different countries.[11–13] Globally, 15% of the incident TB cases were notified as EPTB in 2016, and in Sub-Saharan Africa, North America, Australia and Cambodia, it exceeded 30%. The lowest frequency was estimated in the Western Pacific Region (8%), while in the EMR region, the frequency was 24%.[11] These

Table 3 – Gender distribution of pulmonary and extra-pulmonary cases in Iraq during 2011–2018.

Year	PTB					EPTB							
	Male		Female		M:F ratio	Male		Female		M:F ratio	OR	95% CI	*p*
	N	%	N	%		N	%	N	%				
2011	3225	53.6	2795	46.4	1.15:1	1354	45.8	1603	54.2	0.84:1	1.37	1.25–1.49	<0.001
2012	2915	52.8	2606	47.2	1.12:1	1361	41.7	1900	58.3	0.72:1	1.56	1.43–1.70	<0.001
2013	2806	52.4	2554	47.6	1.10:1	1462	44.7	1812	55.3	0.81:1	1.36	1.25–1.49	<0.001
2014	2634	51.5	2478	48.5	1.06:1	1333	42.0	1843	58.0	0.72:1	1.47	1.34–1.61	<0.001
2015	2638	52.2	2414	47.8	1.09:1	1375	42.9	1828	57.1	0.75:1	1.45	1.33–1.59	<0.001
2016	2209	50.7	2145	49.3	1.03:1	1263	42.6	1700	57.4	0.74:1	1.39	1.26–1.52	<0.001
2017	2272	51.7	2124	48.3	1.07:1	1389	42.0	1922	58.0	0.72:1	1.48	1.35–1.62	<0.001
2018	1971	51.5	1854	48.5	1.06:1	1371	41.3	1946	58.7	0.71:1	1.51	1.37–1.66	<0.001
Average	2584	52.1	2371	47.9	1.10:1	1364	42.9	1819	57.1	0.75:1	1.45	1.33–1.59	<0.001

PTB: Pulmonary tuberculosis; EPTB: Extra-pulmonary tuberculosis; M: F: Male:Female; OR: Odds ratio (EPTB vs. PTB; males were reference category); *p*: Pearson Chi-square test probability.

Table 4 – Age group distribution of pulmonary tuberculosis cases in Iraq during 2011–2018.

Year	Age group of pulmonary tuberculosis cases (year)															
	1–4		5–14		15–24		25–34		35–44		45–54		55–64		≥65	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
2011	96	1.6	249	4.1	1112	18.5	1091	18.1	887	14.7	756	12.6	944	15.7	885	14.7
2012	66	1.2	212	3.8	1046	18.9	947	17.1	771	14.0	738	13.4	820	14.9	921	16.7
2013	72	1.3	210	3.9	918	17.1	935	17.4	769	14.3	765	14.3	792	14.8	899	16.8
2014	45	0.9	173	3.4	915	17.9	873	17.1	735	14.4	713	13.9	807	15.8	851	16.6
2015	59	1.2	199	3.9	871	17.2	840	16.6	789	15.6	749	14.8	660	13.1	885	17.5
2016	36	0.8	191	4.4	737	16.9	711	16.3	663	15.2	650	14.9	606	13.9	760	17.5
2017	50	1.1	180	4.1	757	17.2	679	15.5	641	14.6	678	15.4	603	13.7	808	18.4
2018	46	1.2	148	3.9	723	18.9	605	15.8	559	14.6	581	15.2	469	12.3	694	18.1
Average	59	1.2	195	3.9	885	17.9	835	16.9	727	14.7	704	14.2	713	14.4	838	16.9

Chi-square test value = 150.171; Degrees of freedom = 49; $p < 0.001$.

Table 5 – Age group distribution of extra-pulmonary tuberculosis cases in Iraq during 2011–2018.

Year	Age group of extra-pulmonary tuberculosis (year)															
	1–4		5–14		15–24		25–34		35–44		45–54		55–64		≥65	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
2011	174	5.9	211	7.1	621	21.0	596	20.2	419	14.2	347	11.7	312	10.5	277	9.4
2012	108	3.3	265	8.1	661	20.3	638	19.6	508	15.6	420	12.9	337	10.3	324	9.9
2013	144	4.4	245	7.5	603	18.4	634	19.4	521	15.9	450	13.7	350	10.7	327	10.0
2014	126	4.0	233	7.3	646	20.3	616	19.4	485	15.3	399	12.6	340	10.7	331	10.4
2015	108	3.4	270	8.4	591	18.5	631	19.7	481	15.0	435	13.6	341	10.6	346	10.8
2016	128	4.3	221	7.5	528	17.8	561	18.9	475	16.0	404	13.6	320	10.8	326	11.0
2017	127	3.8	249	7.5	572	17.3	648	19.6	570	17.2	429	13.0	358	10.8	358	10.8
2018	138	4.2	270	8.1	593	17.9	632	19.1	529	15.9	478	14.4	343	10.3	334	10.1
Average	132	4.1	246	7.7	602	18.9	620	19.5	499	15.7	420	13.2	338	10.6	328	10.3

Chi-square test value = 93.500; Degrees of freedom = 49; $p < 0.001$.

findings indicate that EPTB is an important contributor to TB burden in Iraq and worldwide, and it should receive prominent attention by health authorities in their design of control strategies.

Although EPTB is a disease of low infectious potential, it represents prominent clinical challenge in terms of diagnosis and treatment, and understanding of risk factors plays a crucial role in improving overall clinical experience controlling of disease.[14] The current study shed light on two risk factors, which were gender and age. Regarding gender, PTB and EPTB followed different trends over years of study. In PTB, males outnumbered females, and the average percentage of PTB cases was 52.1 and 47.9%, respectively (male:female ratio = 1.10:1), whereas an opposite trend was noticed in EPTB (42.9 and 57.1%, respectively; male:female ratio = 0.75:1). Accordingly, it is suggested that females are more prone to

EPTB than males and the estimated OR was 1.45. Consistent observation has also been made in patients from Taiwan, and females were more likely than males to have concurrent EPTB (OR: 1.30, p = 0.013).[15] A further study reviewed 19 studies of EPTB in South Asian countries (Afghanistan, Pakistan, India, and Bangladesh) and observed a higher preponderance of EPTB in females during 2010–2016. As concluded by the authors, there is significant gap in EPTB surveillance among South Asian females. The need for greater focus on female EPTB was also emphasized.[16] This influence of gender on EPTB incidence is suggested to be related to healthcare, socioeconomic and cultural factors, as well as, sex hormones, genetic factors, and nutritional status may play roles in this gender disparity.[16,17]

PTB and EPTB tended to show elevated percentages in patients older than 14 years, and there was no obvious difference between the two clinical manifestations in this context. Most of PTB and EPTB occurred in the age range 15-≥ 65 year (95 and 88.2%, respectively). It is generally agreed that age is critical risk factor for TB, and older individuals are vulnerable to develop the disease. The 2010 Global Burden of Disease has estimated that majority of TB-related deaths depicted among individual aged 50 years and older. Further, elderly were more prone to develop EPTB.[18] This is reasoned by the fact that elderly will have diminished immunological protection from active TB due to age-related changes in functions of immune system.[19] However, young people also suffer a considerable TB burden, and in 2012, a global estimate revealed that 17% of all new TB cases were young adults.[20]

Considering PTB in term of relapse, the results documented that relapse cases showed increased percentages over the years 2011–2018 (from 5.9% in 2011 to 9.6% in 2018). One of the challenging tasks in TB control is facing relapse cases who develop active TB after successful course of treatment (at least 6 months of anti-TB drugs), and therefore, second course of treatment is necessitated. Different relapse rates have been reported among studies conducted worldwide. For instance, the rates in Vietnam and Iran were 8.6 and 8.3%, respectively,[21,22] while lower rates were reported in southern Ethiopia (4.1%).[23] Therefore, further studies have to be elaborated to identify factors that may contribute to the emergence of relapse cases in order to improve TB control.

The geospatial distribution of PTB and EPTB IR showed different profiles in Iraqi governorates. For instance, in western regions (Al-Anbar and Al-Najaf governorates), the IR of PTB was the lowest among other governorates, while the EPTB IR ranked third in the same region. Topographically, Iraq is shaped like a basin, consisting of the Great Mesopotamian alluvial plain of the Tigris and the Euphrates rivers. This plain is surrounded by mountains in the north and the east, and by desert areas in the south and west. Accordingly, four major physiographic regions are recognized (alluvial plain, desert, uplands and highlands).[24] Such diverse geography may influence the spatial distribution of TB in Iraq and reflect heterogeneity in spatial TB distribution. Employing spatial analysis and identification of TB IR may promote for targeted TB control; however, spatial analysis based on notification data alone (as in present study) could result in misleading conclusions.[25] Thus, further studies based on a population-regional-scale with emphasis on climate parameters certainly provide a better understanding of TB control.

In conclusion, TB is still a public health threat, and although a declining trend in incidence was depicted over the years 2011–2018, the disease is still out of control in Iraq, and more investments of resource are necessitated to eliminate the disease. In this context, EPTB and PTB relapse need a recognized attention.

Declaration of competing interest

The authors have none to declare.

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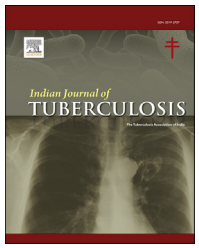

Original article

Profile of pediatric TB patients registered under Faridabad District TB centre of Haryana

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ABSTRACT

Background: Against the backdrop of Tuberculosis (TB) elimination strategy within India, all ages have assumed importance including the burden of pediatric TB. The current study was carried out to study the profile of pediatric TB patients and factors associated with treatment outcome of these patients registered in Faridabad district of Haryana, India.
Methods: This was a descriptive cross-sectional study. Record reviews of 1589 pediatric tuberculosis patients (≤14 years) registered under Revised National Tuberculosis Control Programme of Faridabad district was carried out using TB registers present at tuberculosis units. Socio-demographic data, clinical characteristics, treatment outcome and factors associated with treatment outcome were studied.
Results and conclusions: Among 1589 pediatric TB patients with records available, 62% were females, majority (68%) belonged to age group 10–14 years, 93% were new cases, and 65% had extra-pulmonary TB. Among 554 pulmonary TB cases, 41% were sputum smear-positive. Majority (97%) patients reported successful treatment outcome (cured or treatment completed). In bivariable analysis, sex, category of TB treatment, sputum result, type of TB and past history of TB treatment were significantly associated with successful treatment outcome. On multivariable analysis, patients who were female, had higher bacillary load and previously treated, had significantly lesser odds for achieving successful treatment outcomes at the end of treatment.

1. Introduction

Tuberculosis (TB) continues to be one of the most devastating and widespread infection in the world. About a quarter of the world's population is infected with *M. tuberculosis* and thus at risk of developing TB disease.[1,2] Globally, out of 10 million cases, about 1.1 million cases (11%) occur in children (<15 years of age) every year with more than 1.2 million deaths.[3] In India, proportion of children among New TB patients is around 6% with exception of few states like Delhi, Chandigarh, Madhya Pradesh, Mizoram, Nagaland, Arunachal Pradesh where it is more than 10%.[4] Infected children represent the pool from which a large proportion of future cases of adult TB will arise. In addition, childhood TB is a sentinel event, indicating on going transmission of TB within communities.

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Childhood TB is a neglected aspect of the TB epidemic. This "orphan disease" exists in the shadow of adult TB and is a significant child health problem.

Childhood tuberculosis patients under India's tuberculosis control programme are managed using diagnostic algorithms. The guidelines for the diagnosis and treatment of pediatric cases were modified through expert review and consultative process in 2012. As India is heading towards elimination of tuberculosis, more focus needs to be directed to address childhood TB component. At present, there is no special strategy to curb pediatric TB on priority basis. There is, however, limited information on the basic demographic, clinical characteristics and programme defined treatment outcomes of these patients. The present study was planned to study the profile of Pediatric TB cases and factors associated with treatment outcome of these cases registered under Faridabad district TB centre of Haryana.

2. Material & methods

2.1. Study design

This was a descriptive cross-sectional study in which we retrospectively reviewed routinely collected data under Revised National TB Control Programme in Faridabad district of Haryana.

2.2. Study period

Data collection was undertaken during the months of December 2018 to April 2019.

2.3. Study setting

Tuberculosis units functioning under District TB Centre, Faridabad, within state of Haryana. The state, with 22 districts, is the nation's eighteenth most populous state as per Census 2011 report. Faridabad district consists of 792 km^2 area with population of 17, 98, 954. District TB centre of Faridabad under Revised National Tuberculosis Programme of India covering population of 2,194,586 people. It has 9 tuberculosis units (TU) under it covering different areas of Faridabad districts (Fig. 1). The details of all TB patients registered at Tuberculosis Unit under RNCTP was maintained in TB register present at TU. Information of TB patients registered at TU is sent to District TB office every quarterly and information of each TB patient was entered in Nikshay software as soon as patient gets registered under RNTCP for treatment.

2.4. Study population

All pediatric (0–14 years) tuberculosis cases registered under RNCTP during October 2013 to December 2017 in the Faridabad district.

2.5. Methodology

In the present study, secondary analysis of pediatric tuberculosis cases registered under Faridabad district TB center was carried out through retrospective record reviews. After seeking permission from District TB officer, tuberculosis registers available at nine TUs were accessed for collecting information regarding all pediatric TB cases (0–14 years) registered during October 2013 to December 2017. Socio-demographic and treatment related data of study population was extracted from TB registers maintained at each TU. If treatment outcome of any TB case was missing, that case was excluded. This data was copied in MS excel version 16 and analyzed by STATA version 13.

2.6. Study variables

Pediatric TB case was diagnosed as per the diagnostic algorithm given under national guidelines on pediatric TB diagnosis and management 2012 till December 2017. After diagnosis, they were put on the treatment category as New or previously treated case for 6 months or 8 months respectively. The variables included into study from the TB register were:

Registration year, name of TU, age, sex, weight of patient if available, type of patient, category of treatment, history of past TB, type of sputum smear, HIV status and treatment outcome.

We defined outcome as "successful" if patient was declared as Cured or Treatment completed at the end of treatment and "Other" if patient was declared defaulter, failure, died, switched to MDR, and transferred out during or at the end of treatment. Standard definitions as per programmatic recommendations were used in this study.[5]

2.7. Ethical concerns

Ethical approval was taken from Institutional Ethics Committee, AIIMS, New Delhi. Permission from District TB office was taken for accessing of patient's data from TB registers. All information collected during the study was kept confidential. No personally identifying information was disclosed. As this was retrospective assessment of routine programme related data, hence individual patient consent was deemed unnecessary.

2.8. Data analysis plan

Data was entered into Microsoft excel and analyzed using STATA version 13. Descriptive statistics was applied to analyze type tuberculosis, site of extra-pulomonary TB, sputum result status, HIV status, treatment outcome of pediatric TB case. Variables were summarized by proportions and 95% Confidence Intervals. Bi-variable and Multi-variable logistic regression analysis was done to study factors associated with treatment outcome. Crude and adjusted odds ratios were computed. P values ≤ 0.05 were considered significant.

3. Results

In the present study, we found records of 1589 pediatric TB patients during October 2013 to December 2017. Maximum number of pediatric TB patients were registered during 2016 441 (27.8%) followed by 2014 365 (23%). Out of 1589

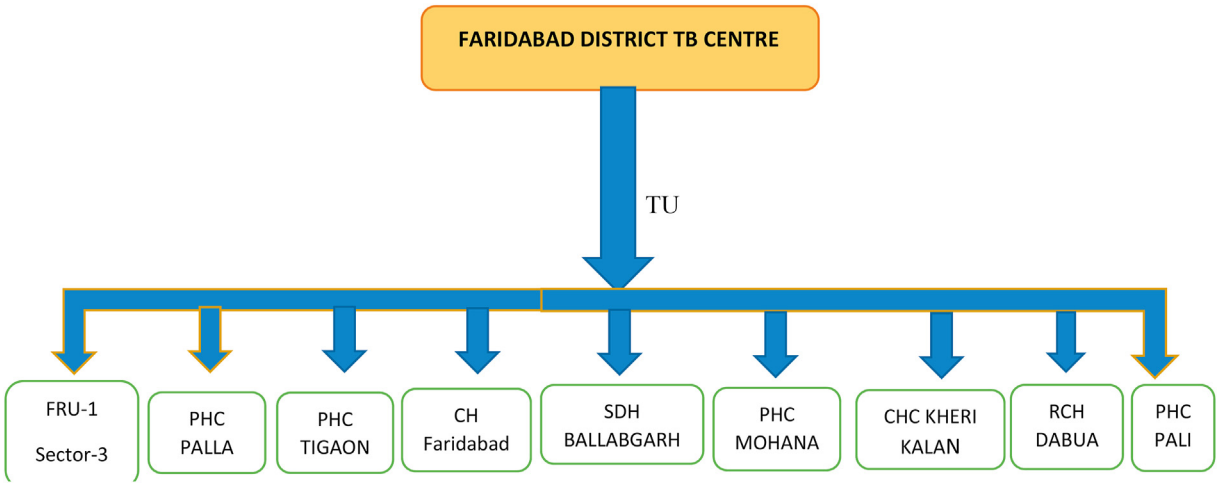

Fig. 1 – Faridabad District TB Centre & its nine Tuberculosis Units (TU). FRU-First Referral Unit; PHC- Primary Health Centre; CH-Civil Hospital; SDH- Sub District Hospital; CHC- Community Health Centre; RCH- Reproductive and Child Health Centre.

patients, nearly half (44.9%) belonged to SDH Ballabgarh TU and CH Faridabad TU.

The maximum number of pediatric TB patients were in the age group 10–14 years (67.7%), followed by 6–9 years (21.5%). The study revealed that mean age (±SD) was 10.4 years (±3.36) and range 4 months–14 years. There were more female patients (61.9%) registered during the study period and male to female ratio was 0.6:1 (Table 1).

3.1. *Clinical characteristics of pediatric TB patients*

In the present study, majority of pediatric TB patients (65.1%) had extra-pulmonary TB and this pattern was seen in all age categories (≤5 years, 6–9 years, 10–14 years). The difference was statistically significant ($p < 0.001$). Only nine percent patients had past history of TB. Majority of patients were new (92.6%) and very few belonged to relapse (1.3%) and treatment after default (0.7%). Out of 554 pulmonary TB patients, less than half (40.8%) were sputum positive TB cases. Among 1589 pediatric TB patients, HIV status was known for (84.7%) patients and no patient had HIV positivity (Table 2).

3.2. *Treatment outcome of pediatric TB patients*

Treatment outcome was studied for 1589 pediatric TB patients which was mentioned in TB register at the end of treatment. Majority (97%) patients had successful treatment outcome (cured or treatment completed). Only 18 (1.1%) defaulted the treatment and three cases were labelled as failure. Five patients were switched to MDR (Multi-drug resistance) treatment category during or end of the treatment. Four patients were transferred out to another district (Fig. 2).

3.3. *Determinants for successful treatment outcomes among pediatric TB patients*

In bivariable analysis, sex, category of TB treatment, sputum results, type of TB and past history of TB treatment were significantly associated with successful treatment outcome. In multivariable model, females were less likely to have successful treatment outcome (AOR: 0.34, 95% C.I. 0.12, 0.93). Patients having sputum result 3+ were negatively associated with successful treatment outcome (AOR: 0.19 95% C.I.:0.07, 0.52). Patients who had past history of TB treatment were less likely associated with successful treatment outcome (AOR: 0.18 95% C.I.:0.04, 0.75) (Table 3).

4. Discussion

In the present study, 1589 pediatric TB patients were studied, maximum number of patients (67.7%) belonged to age

Table 1 – Distribution of study participants by sex, type of TB and Age (N = 1589).

Age Group		<5 years	6–9years	10–14 years	Total	p-value
Variable		No (%)	No (%)	No (%)	No (%)	
Sex	Male	93 (15.4)	170 (28.1)	342 (56.5)	605 (100)	**<0.001**
	Female	79 (8.0)	172 (17.5)	733 (74.5)	984 (100)	
Type of TB	Pulmonary	32 (5.8)	67 (12.1)	455 (82.1)	554 (100)	**<0.001**
	Extra-Pulmonary	140 (13.5)	275 (26.6)	620 (59.9)	1035 (100)	

Table 2 – Distribution of study participants by clinical characteristics (N = 1589).

Sr.No	Variable		No (%)
1	Past history of TB	Yes	144 (9.1)
		No	1445 (90.9)
2	Category of treatment	I	1471 (92.6)
		II	118 (7.4)
3	Type of Patient	New	1471 (92.6)
		Relapse	21 (1.3)
		Treatment After Default	11 (0.7)
		Failure	5 (0.3)
		Others	81 (5.1)
4	Sputum smear (n = 554)	Positive	226 (40.8)
		Negative	328 (59.2)
5	HIV status	Positive	0 (0)
		Negative	1346 (84.7)
		Unknown	243 (15.3)

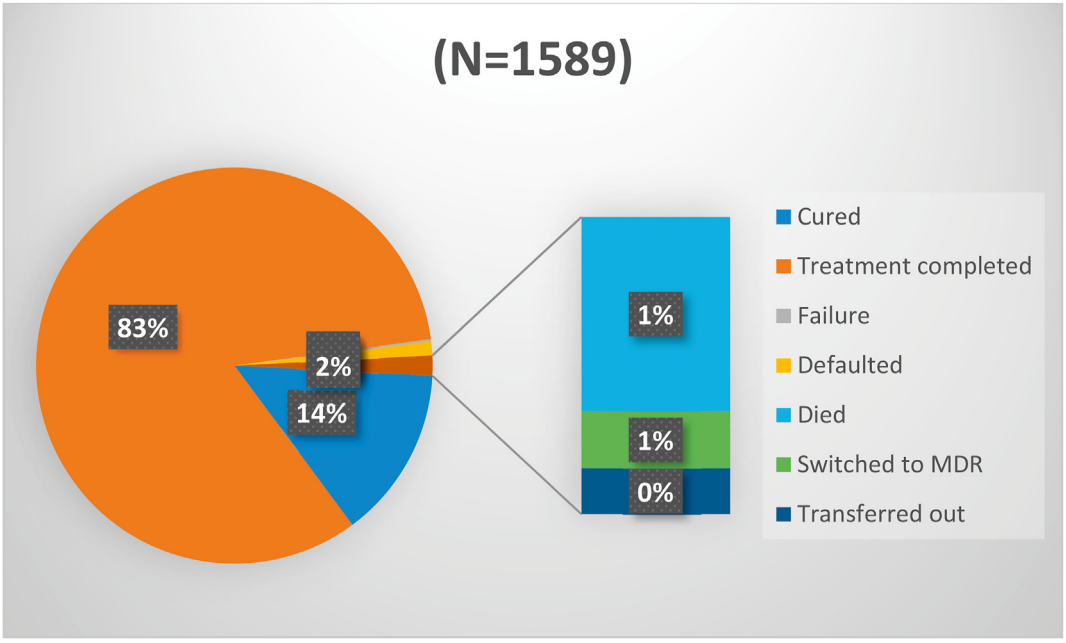

Fig. 2 – Distribution of study participants by treatment outcome.

group 10–14 years followed by age group 6–9years (21.5%). Mean age (±SD) was 10.4years (±3.36). In study done by Dhaked S et al[6] in Delhi (2015) reported maximum number of pediatric TB patients belonged in age -group 11–14 years (51.8%) and average age was 11 years which is similar to our findings. Similar finding was reported by Ruchi et al[7] in study done in Uttar Pradesh in which more than 60% cases belonged to 11–14 years age group. In study done by Gupta P et al[8] in Bareilly district, Uttar Pradesh, also reported, majority of pediatric TB patients belonged to 11–14 years (61.7%) followed by 5–9years (25.8%). Various studies done in different part of India reported similar results with commonest age-group as 11–14years. In the present study, there were higher number of female (62%) than male (38%) patients. Similar finding was reported by Satyanarayana S et al [9]and Dhaked S et al[6] in studies done in Delhi in which number of female participants were 60.6% and 63.8% respectively.

Our finding that maximum number of the pediatric TB patients (65.1%) had extra-pulmonary tuberculosis, was similar to the findings of various studies done in different part of India as they also found that more than >60% of pediatric TB patients had extra-pulmonary TB [6,9,10,11]. Pulmonary tuberculosis was commoner among females than males. In study done by Mazta SR et al[12] in Himachal Pradesh also reported more number pulmonary cases among females (71.7%) than males. In our study, only nine percent pediatric TB patients had past history of TB. Whereas, Tenali R et al[13] reported that 12.2% pediatric TB patients had past history of tuberculosis. This difference may be due to the small sample size of their study and it was done in tertiary hospital. Majority of pediatric TB patients (92.6%) belonged to category I of treatment, which was similar to findings by other studies done different parts of India (Satynarayna S et al,[9] Delhi; Jani et al,[11] Gujrat; Panigatti P et al,[10] Karnataka). In our study, out of 554 pulmonary TB patients, less than half (40.8%) had sputum positive TB. Similar finding

Table 3 – Factors associated with successful[a] treatment outcome.

Variable		No (%)	Unadjusted		Adjusted	
			OR[b](95% C.I.)	P-value	OR (95% C.I.)	P-value
Sex	Male	594 (38.5)	Ref			
	Female	948 (61.5)	0.48 (0.23, 0.98)	0.04	0.34 (0.12, 0.93)	0.036
Age in years	<5	172 (11.2)	Ref			
	6–9	336 (21.8)	2.00 (0.83, 4.78)	0.11	0.75 (0.24, 2.30)	0.62
	10–14	1034 (67.1)	1 (omitted)		1 (omitted)	
Category of treatment	I	1431 (92.8)	Ref			
	II	111 (7.2)	0.39 (0.17, 0.91)	0.03	2.55 (0.50, 12.9)	0.25
Sputum Result	Negative	315 (60.9)	Ref		Ref	
	Scanty	12 (2.3)	0.24 (0.05, 1.22)	0.08	0.23 (0.04, 1.29)	0.09
	1+	81 (15.7)	0.55 (0.20, 1.51)	0.25	0.65 (0.23, 1.81)	0.41
	2+	73 (14.1)	0.60 (0.20, 1.74)	0.35	0.67 (0.22, 2.02)	0.48
	3+	36 (7.0)	0.18 (0.07, 0.47)	<0.001	0.19 (0.07, 0.52)	0.001
History of TB treatment in Past	No	1408 (91.3)	Ref		Ref	
	Yes	134 (8.7)	0.31 (0.15, 0.65)	0.002	0.18 (0.04, 0.75)	0.019
Type of TB	Extra-Pulmonary	1025 (665)	Ref			
	Pulmonary	517 (33.5)	0.13 (0.06, 0.28)	<0.01	1 (omitted)	

[a] Successful Treatment Outcome-cured, treatment completed.
[b] OR- Odds Ratio.

was reported by Satyanaryana S et al[9] in study done in Delhi in which out of 394 pulmonary TB patients, 37.1% had sputum positive TB. In study done by Ruchi et al[7] in Varanasi also found less than half (47.3%) patients had sputum positive TB. Mazta SR et al[12] in study done in Himachal Pradesh also reported observation similar to our study. In our study, none of the pediatric TB patient had HIV infection. Whereas several studies had shown HIV prevalence between 2 and 10%. This difference may be due the low prevalence of HIV in Haryana state (General population prevalence of 0.18%) and opt-out HIV testing under RNTCP.[14]

In our study, we studied the programme defined treatment outcomes of 1589 pediatric TB patients from the available records in the tuberculosis units. We found that majority of pediatric TB patients (97%) had successful treatment outcome (cured or treatment completed). Similar treatment outcome rates among pediatric TB patients under RNTCP were reported as 94.7% by Nelliyani et al,[15] 94.6% by Panigatti P et al,[10] 96.2% by Dhaked S et al,[6] 95.7% by Bandichode ST et al,[16] 95% by Satyanarayana S et al,[9] 80% by Kabra et al.[17] This high success rate may be due to high compliance to treatment and support of parents to children during the treatment. In our study, poor outcomes were very low, out of 1589 pediatric TB patients, only three patients failed the treatment, five patients switched to MDR treatment, eighteen patients defaulted the treatment and seventeen patients died during the treatment. Our findings were supported by different studies done on pediatric TB patients in India. In study conducted by Dhaked S et al[6] in Delhi, out of 140 pediatric TB patients, only one patient died and defaulted during the treatment, three patients were failure and three were switched to Cat –4 treatment. Whereas in prospective study by Panigatti P et al[10] reported that out of 93 pediatric TB patients, four were defaulted and one patient died during the treatment. Similarly, study done by Bandichode S et al[16] in Maharashtra found, out of 93 pediatric TB patients, three patients defaulted, three died during the treatment and one patient was failure. However, studies done by Lolekha et al[18] in Thailand, Hailu et al Addis Ababa[19] and Harries AD et al[20] in Malawi reported higher death and default rate than our study. This could be due to the greater efficiency of RNTCP in India.

In this study, we categorized the treatment outcome as Successful (cured or treatment completed) and other (failure, defaulted, died, switched to MDR). We found that sex of the patient was associated with treatment outcome of the patient. It found that female sex had 66% lower odds of successful treatment outcome (AOR: 0.34, 95% C.I. 0.12, 0.93). In our study, age was not associated with successful outcome. We found that patients with sputum smear positive result (3+) and past history of TB treatment were negatively associated with successful treatment outcome. Whereas in study done by Harries AD et al[20] in Malawi found that sex was not associated with positive treatment outcome but increase in age was associated with improved outcome. They also reported that smear positive TB was associated with positive treatment outcome. This difference may be due to difference in study setting and sample size of the studies.

Our study had several strengths. This is one of the few studies that examined details about pediatric TB patients and has a large sample of subjects. This study was done using data from all nine tuberculosis units of Faridabad district; thus, it gave the insight into community-based characteristics of pediatric TB patients. It included information on all possible treatment outcomes of pediatric TB patients under RNTCP programme. This study adds the information on factors associated with successful treatment outcome among pediatric TB patients. There is paucity of data regarding factors associated with treatment outcome especially in Indian scenario. The present study had certain limitations. We missed some data due to poor maintenance of records at some tuberculosis unit which might affect our findings. Additionally, it was not possible to study the factors like weight gain during the treatment, type of extra-pulmonary TB, no. of missed doses and history of TB contact in the family due to incomplete data. Lastly, we could not evaluate adequacy of diagnosis or, adequacy of the therapy at the end of course as it

was retrospective review of programme related data. We could not corelate data from TB registers with Nikshay data as data entry was incomplete or information about most of the study variables were not available on Nikshay software of many tuberculosis units.

5. Conclusions

We found that majority of pediatric TB patients belonged to age group 10–14 years and were female. Extra-pulmonary TB was common and most of them were new cases of TB. None of the patient was HIV positive. Majority of patients had successful treatment outcomes and factors like sex, sputum smear result, past history of TB and type of TB were significantly associated with treatment outcome of patients. In view of India's envisaged targets for TB elimination that includes focus on pediatric TB patients, concerted attention will be required on female patients, those that exhibited high bacillary load within their sputum and past history of TB for enhancement of success rates within the cohort of childhood TB cases put on treatment.

Authors contribution

Bhushan Kamble: Concepts, Design› Definition of intellectual content, Literature search, Clinical studies, Data acquisition, Formal analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor. Sumit Malhotra: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Statistical analysis, Manuscript editing, Manuscript review, Guarantor

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Presentation at a meeting

Nil.

Conflicts of interest

All authors have none to declare.

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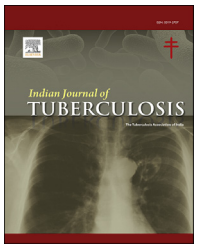

Original article

Role of GeneXpertMTB/RIF in the diagnosis of cutaneous tuberculosis

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ABSTRACT

Background: cutaneous involevemtn is an important extrapulmonary manifestation of tuberculosis. It is a paucibacillary condition and has diverse clinical presentations. Sufficient data is not available regarding role of GeneXpertMTB/RIF in cutanoues tuberculosis.
Methods: in this study, BacT/Alert3D and response to antitubercular therapy were taken as gold standard and performance of GeneXpertMTB/RIF was evaluated against it in clinically and histopathologically suspected cases of cutaneous tuberculosis.
Results: forty seven patients were included in the study of which commonest presentation was scrofuloderma (42.6%) followed by lupus vulgaris (40.4%). Granulomatous inflammation on histopathology was seen in 75.5% patients on skin biopsy. Sic patients had extracutaneous focus of tuberculosis. In 14 (29.79%), culture of skin biopsy was positive for M. tuberculosis and all showed complete response to ATT in 6 months. GeneXpertMTB/RIF detected M. tuberculsois in 4 samples.
Conclusion: GeneXpertMTB/RIF is not a reliable tool for diagnosis of cutaneous tuberculosis. Clinic-histopathological correlation along with response to ATT is needed for confirmation of diagnosis of cutaneous tuberculosis.

1. Introduction

Cutaneous tuberculosis, caused by *M. tuberculosis*, can be classified into paucibacillary and multibacillary type depending on the bacillary load in the patient. Paucibacillary type occurs in patients with good immunity to *M. tuberculosis* and includes lupus vulgaris, scrofuloderma, tuberculous chancre and tuberculosis verrucosa cutis (TBVC). Multibacillary type occurs in patients with low immunity and includes orificial tuberculosis, miliary tuberculosis and metastatic tubercular abscess.[1] The tuberculids which include lichen scrofulosorum, nodular tuberculid, nodular granulomatous phlebitis and erythema induratum of Bazin are hypersensitivity response to *M. tuberculosis*. In these conditions, *M. tuberculosis* is not identified by

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acid fast bacilli (AFB) stains, culture or polymerase chain reaction (PCR).

The diagnosis of cutaneous tuberculosis is made on clinical features in combination with mantoux test, histopathological examination (HPE) of the skin biopsy, microbiological tests like culture, Ziehl-Neelson (ZN) smear and molecular tests like PCR (polymerase chain reaction), cartridge-based nucleic acid amplification test (GeneXpertMTB/RIF), AMTDT (amplified mycobacterium tuberculosis direct test) and therapeutic trial of anti-tubercular treatment (ATT) which takes almost 5 weeks to know the result.[2]

GeneXpertMTB/RIF is a quick and reliable molecular test which detects *M. tuberculosis* in sputum and rifampicin resistance. It has proven to increase case notification in bacteriologically confirmed pulmonary tuberculosis cases by 39% and notification of rifampicin-resistant tuberculosis by five times at district level.[3] WHO also recommends use of GeneXpertMTB/RIF as initial test for detection of *M. tuberculosis* in extrapulmonary samples like cerebrospinal fluid, lymph node tissue and aspirate.[4]

Since the common presentations of cutaneous tuberculosis are paucibacillary where direct demonstration of TB bacilli is extremely difficult, culture are often negative, time consuming and variable, HPE does not confirm the diagnosis, and the value of molecular tests and electron microscopy has not been fully evaluated, in this study we proposed to evaluate the role of GeneXpertMTB/RIF in skin biopsy samples against two gold standards i.e. response to therapeutic trial of ATT and culture.

2. Materials and methods

The study was conducted in a tertiary care hospital. All untreated patients attending the Out Patient Department (OPD) with clinical diagnosis of cutaneous tuberculosis were included. Patients who had taken antitubercular drugs or aminoglycosides or quinolones for diseases other than TB in the past 6 months, patients who did not give consent and those who did not respond after 6 weeks of anti-tubercular treatment were excluded.

Digital photographs were taken. Biochemical tests (complete hemogram with ESR, liver and kidney function tests, blood sugar, urine and stool microscopy), radiological (chest radiograph and ultrasound abdomen), histopathological, microbiological and molecular investigations were done. Mantoux test using 0.1 ml of 5TU of purified protein derivative (PPD) was measured after 48–72 hours. Three skin biopsies were taken simultaneously for histopathological examination and electron microscopy, RT-PCR and BACTEC culture, and GeneXpert test (Xpert® MTB/RIF). The Xpert® MTB/RIF purifies and concentrates *Mycobacterium tuberculosis* bacilli from samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by cartridge-based nucleic acid amplification test. The process identifies Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the *Mycobacterium tuberculosis* genome. Therapeutic trial of ATT was given to all with 4 antitubercular drugs including isoniazid, rifampicin, pyrazinamide and ethambutol daily for two months followed by isoniazid and rifampicin daily for four months.

Statistical analysis was done by Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Qualitative variables were correlated using Chi–Square test/ Fisher's exact test. Inter rater kappa agreement was used to find out the strength of agreement between various methods. A p value of <0.05 was considered statistically significant.

3. Results

3.1. *Clinical features*

A total of 47 patients were included. The age of patients varied from 3 to 58 years (mean 22.43 ± 13.08 years). Majority (65.95%) were between 11 and 30 years at the time of presentation. Male to female ratio was 1.61:1. Duration of disease ranged from 1 month to 15 years. Almost 71.74% had disease for last 2 years and 28 (60.87%) patients had disease for 1 year. BCG scar was present in 18 (38.3%).

Lupus vulgaris (LV) and scrofuloderma were the commonest presentation seen in 18 (38.3%) patients each followed by lichen scrofulosorum in 6 (12.77%), tuberculosis verrucosa cutis (TBVC) in 2 (4.26%), papulonecrotic tuberculid in 1 (2.13%), LV with scrofuloderma in 1 (2.13%) and TBVC with scrofuloderma in 1 (2.13%). The most common site of involvement in LV was buttock (n = 6) followed by knee (n = 2), hand (n = 3), forearm and face in 2 each, thigh and back in 1 each. One had involvement of both buttock and knee and another had involvement of thigh and knee. In scrofuloderma, the most common site of involvement was neck and inguinal region in 6 patients each. Two other patients had involvement of both neck and inguinal region. This was followed by axilla in 3 patients and hand, back and scrotum in 1 patient each. All patients with lichen scrofulosorum had involvement of the trunk and all patients had involvement of the feet. Papulonecrotic tuberculid was seen on the legs.

Mantoux test showed induration>10 mm in 45 (95.75%) patients of which 6 (12.7%) had 10–14mm, 8 (17%) had 15–20mm, and 31 (65.96%) had >20mm. All patients were HIV-negative. Six patients (4 with lichen scrofulosorum and 2 with scrofuloderma) had extracutaneous disease in the form of hilar and mediastinal lymphadenopathy in 2 and necrotic mesenteric and retroperitoneal lymph nodes in 4.

3.2. *Histopathology*

Forty-nine biopsies were done for 47 patients. Histopathological diagnosis was consistent with clinical diagnosis of scrofuloderma in 14/20 patients (Fig. 1), lupus vulgaris in 15/19 patients, lichen scrofulosorum in all 6 patients and TBVC in 2/3 patients (Fig. 2). Non specific changes were present in 12 biopsies. Acid fast bacilli were not demonstrated in any of the biopsy. Thus histopathological examination suggested the diagnosis of cutaneous tuberculosis in 37 (75.51%) cases out of 49 clinically suspected.

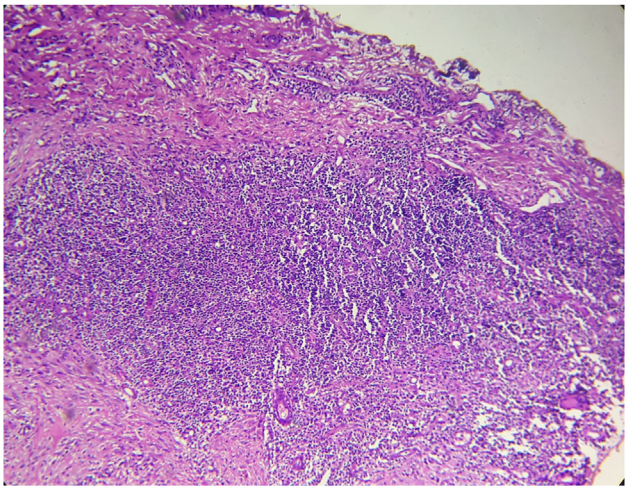

Fig. 1 – Photomicrograph of scrofuloderma showing loss of epidermis and dense inflammatory infiltrate in the upper part of dermis (H & E stain).

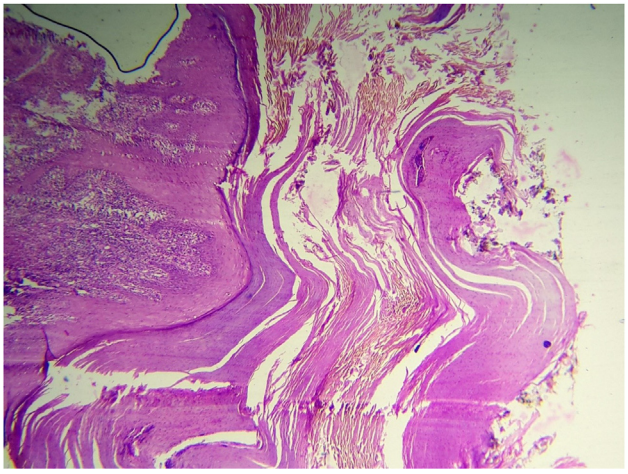

Fig. 2 – Photomicrograph of TBVC showing marked hyperkeratosis, parakeratosis, dense inflammatory infiltrate in upper dermis (H & E stain).

3.3. *Culture, real time PCR, electron microscopy*

Bact Alert 3D culture showed growth of *M. tuberculosis* in 14 (29.79%). Real time PCR was positive for *M. tuberculosis* in 4 (8.51%). Electron microscopy demonstrated *Mycobacterium* in only one tissue specimen.

3.4. *Treatment response*

Improvement was seen in all at the end of 6 weeks. All the patients completed treatment with complete resolution of the lesions at the end of 6 months (Figs. 3–6).

3.5. *GeneXpert*

GeneXpert was positive in 4 (8.7%) patients and indeterminate in 1.

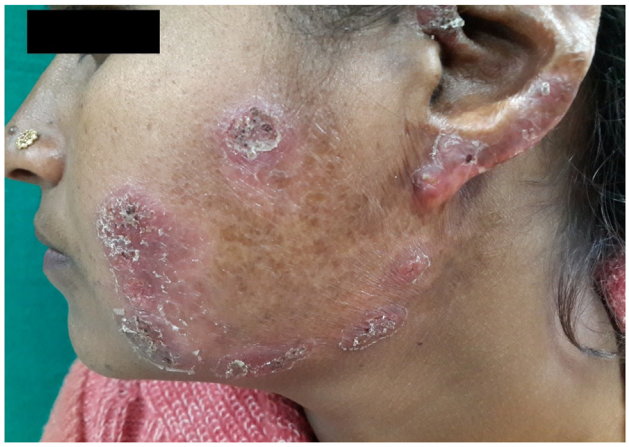

Fig. 3 – Lupus vulgaris on left side of face (before treatment).

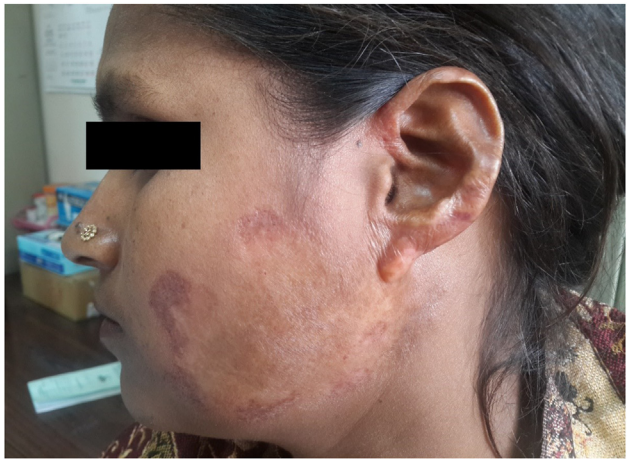

Fig. 4 – Lupus vulgaris after 6 weeks of ATT showing healing.

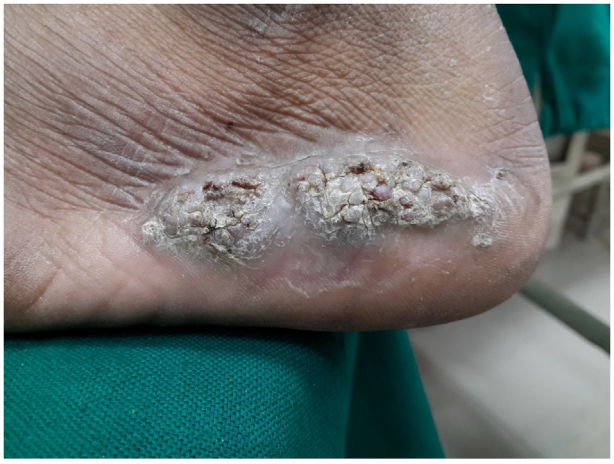

Fig. 5 – Tuberculosis verrucosa cutis on left foot (before treatment).

Keeping BactAlert 3D culture as gold standard, GeneXpert showed sensitivity of 7.14%, specificity of 90.63%, positive predictive value of 25% and negative predictive value of

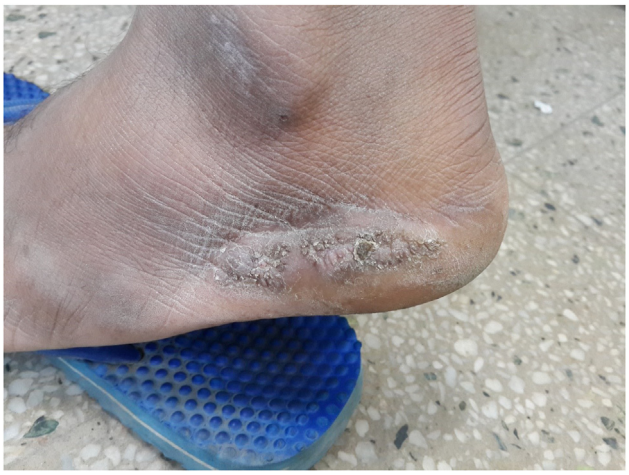

Fig. 6 – TBVC after 6 weeks of treatment showing partial healing.

69.05%.The kappa index of agreement was found to be −0.028 which is not significant (p value = 0.805).

4. Discussion

As per 2019 report, estimated incidence of tuberculosis in India was 27 lakh.[5] The percentage of cutaneous tuberculosis cases among the total dermatology outpatients varies between 0.1% and 3.5%.[6–10] In our study, the most common clinical presentation was scrofuloderma affecting 20/47 (42.6%) followed by lupus vulgaris affecting 19/47 (40.4%)patients. These results were similar to a recent study by Thakur BK et al on 42 patients in which scrofuloderma (50%) and lupus vulgaris (42.8%) were the most common presentation followed by tuberculosis verrucosa cutis (4.76%) and lichen scrofulosorum (2.38.)[11]The most common site of involvement was neck and groin for scrofuloderma, buttocks for lupus vulgaris and foot for tuberculosis verrucosa cutis which is consistent with other studies.[7–10] In India, lupus vulgaris commonly affects the lower extremities especially buttocks, probably due to accidental inoculation in children by squatting on the ground, where *M. tuberculosis* might have been deposited from the infected sputum of a family member. We also found a definite history of trauma preceding the onset of TBVC. These patients were engaged in manual work in agricultural fields, thus were more prone for frequent injuries and the resultant trauma might have provided a portal of entry to the AFB. Bravo FG et al described lichen scrofulosorum as eruption of multiple miniature follicular or parafolicullar lichenoid papules which are commonly arranged in clusters and almost always affect the trunk.[12] Similarly in our study all the cases had lesions over trunk.

Mantoux test has variable sensitivity ranging from 37.5% to 100% in the diagnosis of cutaneous tuberculosis.[11,13] Ramam et al reported sensitivity and specificity of 58.9% and 62.5% at a cut off of 10mm and concluded that mantoux test is of low accuracy in the diagnosis of doubtful cases of cutaneous tuberculosis.[14] Various reasons for false negative reaction may be cutaneous anergy because of a weakened immune system, recent TB infection (within 8–10 weeks of exposure), very old TB infection (many years), very young age (less than six months old), recent live-virus vaccination (e.g., measles and smallpox), overwhelming TB disease, concomitant viral illnesses (e.g., measles and chicken pox), incorrect method of administration, incorrect interpretation of reaction, insufficient dose and inadvertent subcutaneous injection.[15] Only two patients in our study had negative mantoux and both the patients did not have any recent or old illness or vaccination. Both were HIV negative and their chest X ray and ultrasound abdomen were normal. Thus negative mantoux result in our patients may be due to incorrect method of TST administration.

Radiological investigations were done to find out whether the disease was unifocal or multifocal and corroborated the diagnosis of cutaneous tuberculosis. In our study, an extracutaneous focus of tuberculosis was seen in 6 (12.76%) patients of which majority had lichen scrofulosorum. Ramam et al conducted radiological investigations in 107 patients which revealed extracutaneous tuberculosis in 13% patients which were pulmonary tuberculosis in 12 patients, TB spine in one and a parietal tuberculoma of brain in one.[2]

The hallmark of histology of cutaneous tuberculosis is the presence of granulomatous reaction with varying amount of caseation necrosis in the center and presence of rim of lymphocytes and monocytes around the granuloma. A similar histology may also be seen in deep fungal infections, leprosy, mycetoma and other diseases. Histopathological changes of granulomatous inflammation have shown a sensitivity ranging from 65.7% to 100% in various case series and many reports in confirming the diagnosis of cutaneous tuberculosis.[6,11,13] Histopathological examination of skin biopsies was consistent with clinical diagnosis in 37 (75.51%) biopsies out of 49. However in 12 (24.49%) biopsies, the skin biopsies showed non-specific granulomatous inflammation. It is said that around 5000-10000bacilli per ml of sample must be present to be seen by microscopy.[16] Sensitivity of AFB staining is skin biopsy samples is very low ranging from 0.0% to 13.8%. Even in other extrapulmonary samples like cerebrospinal fluid, pleural fluid, peritoneal fluid and pericardial fluid, sensitivity of AFB smear is low.[16] This explains why none of the biopsies were positive for ZN staining in our study.

BACTEC is a better method for culture than conventional Lowenstein medium. Aggarwal et al demonstrated growth in 22 (62.8%) samples in 17.3 days as compared to 9 (25.7%) samples in mean period of 31.5 days with conventional Lowenstein in 35 untreated patients of cutaneous tuberculosis.[17] Pfyffer et al showed growth in 14 (21.2%) out of 64 specimen using BACTEC culture system for skin biopsy specimens.[18] In our study BacT/Alert 3D (BACTEC) showed growth of *M. tuberculosis* in only 14/47 (29.79%) patients. PCR has showed variable results with sensitivity ranging from 25% to 100%. An Indian study reported real time PCR positive in 29.2% of skin biopsies.[19] Specificity has been reported from 73.7% to 100%.[19–22] In our study, PCR showed sensitivity and specificity of 14.3% and 93.9% respectively when compared to culture. However, keeping clinicotherapeutic correlation as gold standard, the sensitivity further dropped to 8.5%. Previous study by Malhotra et al reported higher sensitivity (83.3%) and lower specificity (78.6%). Similar drop in sensitivity from 83.3% to 29.8% was observed by them.[19] One of the reasons for low

sensitivity of PCR against clinicotherapeutic correlation as compared to culture could be the paucibacillary nature of cutaneous tuberculosis making growth in culture difficult. Another reason for low sensitivity of PCR could be due to loss of DNA in initial stage of processing during extraction. Endogenous substances which are present in blood or other body tissues may act as inhibitors to nucleic acid. Lastly inadequate sampling and technical errors may also result in low detection rates of the desired target nucleic acid material.

There is not much literature on the role of GeneXpert test exclusively on skin tissue samples of cutaneous tuberculosis patients. GeneXpertMTB/RIF is an automated test which detects presence of *M. tuberculosis* and its resistance to rifampicin in a single test. The test takes few hours to report, is quick and can be performed by minimal training. It can be conveniently used in any setting with uninterrupted power supply.[3] WHO recommends using GeneXpertMTB/RIF as initial test for diagnosis of pulmonary tuberculosis and extrapulmonary cases including tubercular meningitis and lymph node tuberculosis.[4] Few studies have been performed on extrapulmonary samples which included skin tissue biopsies also. The sensitivity and specificity ranges from 72% to 77% and specificity more than 98% respectively.[23,24] Boehme CC et al reported, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant mycobacteria and 504 of 514 (98.1%) with rifampin-sensitive mycobacteria.[23] Bankar et al evaluated GeneXpertMTB/RIF in extrapulmonary TB samples and found that 4/23 (17.39%) of biopsy samples were positive, however the authors have not mentioned from where biopsies where taken. Highest positivity rates were seen in lymphnodes (41.3% and pus (40.15%), but was lower in CSF, pleural fluid, and other fuids.[25] They observed that of all extrapulmonary samples, sensitivity of XpertMTB/RIF was highest in lymphnode and pus. In our study GeneXpert was positive in 4 (8.7%) patients out of 46 patients. It was indeterminate in 1 patient. No skin tissue sample was positive for rifampicin resistance. Usually, GeneXpertMTB/RIF performs better than culture as requires minimum of 10^2 live bacilli/ml for positive result while GeneXpertMTB/RIF requires much less and can even detect dead bacilli.

On combining RT-PCR, BacT/Alert 3D and GeneXpert, 19 (40.43%) patients were positive for cutaneous tuberculosis out of total 47. This is still low as compared to clinicotherapeutic correlation.

It has been suggested in some studies that response to anti-tubercular therapy is a reliable criterion for the diagnosis of cutaneous tuberculosis. All 47 cases in the present study responded promptly and significantly to anti-tubercular therapy. This finding is in concordance with the finding of other studies that a therapeutic trial of 6 weeks is adequate and reliable to confirm the diagnosis of cutaneous tuberculosis.[2,26]

Our study has some limitations like lack of control group, small sample size and heterogenous clinical presentations of cutaneous tuberculosis.

5. Conclusion

Our study confirms that no single investigation can make the diagnosis of cutaneous tuberculosis. Clinico-histopathological correlation along with response to ATT still remains the best option for diagnosis. GeneXperMTB/RIF does not appear to be a reliable method for detection of *M. tuberculosis* in skin samples of cutaneous tuberculosis.

Conflicts of interest

The authors have none to declare.

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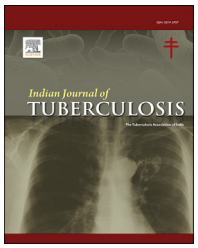

Original article

Hysteroscopic observations in 348 consecutive cases of female genital tuberculosis: A prospective study

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ABSTRACT

Study objective: To evaluate the hysteroscopic findings in female genital tuberculosis.
Design: It was a prospective study of hysteroscopic findings performed on 348 cases of female genital tuberculosis (FGTB).
Setting: It was a prospective cross-sectional study in a tertiary referral centre.
Patients: A total of 348 patients with infertility with FGTB on various tests.
Intervention: A total of 348 patients of infertility found to have FGTB on various investigations were enrolled in the study. A detailed history was taken. Clinical examination, endometrial sampling and diagnostic laparoscopy were performed was also performed in selected cases. All patients underwent hysteroscopy as part of evaluation for infertility and tuberculosis (TB) findings.
Measurements and main results: The mean age, parity, body mass index and duration of infertility was 28.2 years, 0.31,23.1kg/m^2 and 3.44 years respectively. Infertility was primary in 81.03% and secondary in 18.96% cases. Diagnosis of FGTB was made by endometrial aspirate findings of positive AFB on microscopy (4.02%), positive culture (4.88%), positive PCR (83.90%), epithelioid granuloma (14.65%), positive AFB on microscopy or culture of peritoneal cytology (1.14%) or epithelioid granuloma on peritoneal biopsy (1.72%), definitive findings of TB on laparoscopy (41.95%) or probable findings of TB on laparoscopy (58.05%). Various hysteroscopic findings observed were normal findings (28.16%), pale endometrial cavity (54.31%), features of active TB (7.47%), features of chronic TB (19.54%), features of TB sequelae like obstructed ostia (both ostia in 13.79%, one ostia 14.94%, periostial fibrosis;

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(bilateral 4.59%, unilateral 5.17%), endometrial glands atrophy (12.35%), small shrunken cavity (6.32%), distorted cavity (5.17%), various grades of intrauterine adhesions (29.88%). Hysteroscopy in FGTB was associated with increased difficulties and complications like failed procedures, difficult visualisation, false passage and uterine perforation.

Conclusion: Hysteroscopy is useful modality to detect endometrial TB but is associated with increased difficulty and complications.

1. Introduction

The global tuberculosis (TB) statistics are that there were an estimated 10.0 million new cases of tuberculosis in 2018 with Africa and Asia bearing the main burden.[1,2] Urogenital tuberculosis is the third commonest form of extrapulmonary tuberculosis (EPTB) after lymph node and pleural TB and affects between 4.7 and 10.4% of the individuals with pulmonary TB.[3,4] It is responsible for 27% cases of EPTB with genital TB seen in 9% of cases.[5,6] Urogenital tuberculosis is an important form of EPTB and usually occurs secondary to tuberculosis in other sites especially lungs and abdomen with infection being mainly hematogenous but can occur through lymphatic route or direct spread.[7–9] Female genital tuberculosis (FGTB) is an important cause of infertility especially in developing nations being responsible for 3–16% cases of infertility with incidence being much higher in tertiary referral centres (up to 18%) due to referral of difficult cases and especially with high prevalence in tubal factor infertility and in women seeking assisted conception (up to 48.5%).[10,11] Fallopian tubes are involved in almost 95–100% of cases followed by endometrium in 50–60% cases followed by ovaries (20–30%), cervix (5–15%) and vulva and vagina (1.1% cases).[6–8]

FGTB may be asymptomatic especially in early stages but later it causes menstrual dysfunction especially oligomenorrhoea, hypomenorrhoea and amenorrhoea and infertility through involvement of fallopian tubes (blockade), endometrium (Asherman's syndrome) and ovaries (decreased ovarian reserve and poor quality ova).[6–8,12,13] Diagnosis of FGTB is a dilemma due to its paucibacillary nature. Conventional methods like demonstration of acid fast bacilli (AFB)on microscopy or culture of endometrial biopsy or demonstration of epithelioid granuloma on histopathology of endometrial biopsy are gold standard for diagnosis but are positive in small percentage of cases. Polymerase chain reaction (PCR) though very sensitive has high false positivity and alone is not recommended to diagnose FGTB or to initiate treatment.[14,15] Cartridge based nucleic acid amplification test (CB-NAAT) also called gene Xpert and loop mediated isothermal amplification test (LAMP) have also been used for diagnosis of FGTB with varying accuracy.[16,17] Diagnostic laparoscopy has been invaluable in directly visualising abdomen and pulmonary TB findings and can pick up more cases than gold standard tests and can show definitive findings like tubercles, caseous nodules and beaded tubes or probable findings like hydrosalpinx, pyosalpinx, encysted ascites, pelvic, abdominal and perihepatic adhesionsandtubo-ovarian masses.[18–20] Diagnostic hysteroscopy is also useful for diagnosis of FGTB.[21] To increase the detection rate of FGTB, Composite Reference Standard has been used for diagnosis of FGTB like other EPTB in which combination of tests like AFB on microscopy or culture of endometrial biopsy, histopathology of epithelioid granuloma, result of Gene Xpert and definitive and probable findings of FGTB on laparoscopy are taken into consideration.

The present study discusses findings of 348 consecutive cases of hysteroscopy performed in a tertiary referral centre on infertility patients diagnosed to have FGTB on composite reference standard.

2. Material and methods

It was a prospective study of hysteroscopy conducted over a period of 9 years (July 2010 to July 2019) on 348 consecutive cases of FGTB in females with infertility from a tertiary referral hospital diagnosed on Composite Reference Standard. It was part of our ongoing reference study on FGTB for which ethical approval was taken from the Institute Ethical Committee (IEC). Participants were enrolled for this study from the infertility screening OPD during this period. Informed written consent was taken from the women with infertility. Detailed history including the past and family history of TB, any comorbidity, symptoms including general symptoms, menstrual symptoms and infertility were taken in all the cases. Meticulous general physical examination was performed in all the cases to detect any other sites of TB like lymphadenopathy, pallor, heart, chest examination, abdominal followed by gynaecological examination including speculum and bimanual pelvic examination. All infertile women were subjected to baseline investigations which included complete blood count (CBC), blood sugar, Mantoux test and endometrial sampling in premenstrual phase (between cycle day 21–23). The endometrial sample was sent for AFB on microscopy, culture, gene Xpert (done only for last 167 cases done in last 4 years as the test was not available before), PCR and histopathology examination for epithelioid granuloma. All patients suspected to have FGTB clinically or on PCR or radiological methods were subjected to undergo diagnostic laparoscopy and hysteroscopy. During laparoscopy peritoneal biopsy was taken from suspicious areas for AFB and histopathology and findings of FGTB were noted.

Though a total of 374 patients were found to have FGTB on investigations during this time but hysteroscopy could be done in only 348 patients who were enrolled in this study. Laparoscopic findings have been reported in other paper.

Composite Reference Standard was taken in this study in which various tests were combined to increase the detection rate of FGTB; including positive AFB on microscopy or in culture of endometrial or peritoneal biopsy, epithelioid granuloma on histopathology of endometrial or peritoneal biopsy and positive findings of FGTB (presence of tubercles, caseous nodules and beaded tubes) on laparoscopy or probable findings of FGTB on laparoscopy which included straw coloured fluid, fluid filled cavities, encysted ascites, shaggy areas, peritubal, pelvic, abdominal and perihepatic adhesions, hyperemic or convoluted tubes, hydrosalpinx, pyosalpinx and tubercular masses with at least 3 positive findings of probable TB. Women with only one or two findings but normal pelvic anatomy were not taken as probable FGTB. We appreciate that various probable findings of FGTB like straw coloured fluids and pelvic adhesions, hydrosalpinx, hyperemic convoluted tubes and other findings can also occur in other causes of pelvic inflammatory disease (PID) like chlamydia, mycoplasma and gonorrhoea. Unfortunately, due to financial and logistics constraints, we could not do testing for these infections, but as all these women had positive PCR for mycobacterium tuberculosis and had more than 3 findings of probable TB, we took them as a case of probable FGTB. Some of these cases may have been due to other causes of PID. Those women who had positive PCR but normal laparoscopy or only 1–2 abnormal probable finding were not taken as FGTB in the present study. We could not subject sample to newer molecular tests due to financial and logistics constraints. PCR was not taken as part of composite reference standard but was indication of doing laparoscopy. All patients of FGTB also underwent diagnostic hysteroscopy using 2.7 mm telescope with 4.5 mm outer diameter double flasksheath (Karl Storz Tuttlington, Germany). Whole of endometrial cavity was carefully examined for evidence of any lesion of tuberculosis including active TB (tubercles, caseous nodules), chronic endometritis (hyperemic endometrium, stromal edema, micropolyps), tubercular sequelae like ostial obstruction, any periostial fibrosis, colour of cavity, endometrial glands atrophy, distorted or shrunken cavity and intrauterine adhesions including their grading as per European Society for Gynaecological Endoscopy (ESGE) classification of intra uterine adhesion (IUAs). All hysteroscopies and laparoscopies were done or supervised by the first author (JBS) of the study. This scoring system was adopted from the classification developed for the former European Society for Hysteroscopy, by Wamsteker and De Block.[21] Any difficulties encountered or perioperative and postoperative complications were carefully noted. All patients were given a single dose of antibiotics and were discharged on the same evening. Hysteroscopy was not used to diagnose FGTB. The diagnosis FGTB was made on Composite reference standard (positive AFB on microscopy or culture or positive histopathology or definite and probable findings of FGTB. We only documented findings of hysteroscopy in these FGTB cases as an addition to the knowledge on this important aspect for the benefit of readers. The diagnosis of FGTB was made by composite reference standard which is well accepted for diagnosis of extrapulmonary TB including FGTB which is a paucibacillary disease. To avoid subjectivity and overdiagnosis, all cases were done or supervised by single author and all laparoscopies and hysteroscopies were done in post menstrual phase and only definite or at least 3 probable findings were taken for diagnosis of FGTB.

All patients diagnosed to have FGTB on composite reference standard were treated with full course of six months of anti tubercular therapy using Directly Observed Treatment Short Corse (DOTS) strategy of National Tuberculosis Elimination Programme (NTEP) of India using daily doses of four drugs namely Isoniazid (H), Rifampicin (R), Pyrizinamide (Z), Ethambutol (E); HRZE for two months in intensive phase followed by three drugs (Isoniazid, Rifampicin, Ethambutol (HRE) for 4 months in continuation phase). All patients were followed up regularly for improvement in their symptoms and to monitor for any adverse effects of drugs. Liver function tests were done when indicated. In this study we did not collect data on pregnancy and live birth rate as we followed them during antitubercular treatment only in this study.

2.1. *Statistical analysis*

The obtained data were analysed using standard descriptive methods (minimum, maximum, mean, standard deviation, ± standard error of the mean (SEM), median values) and analytical statistical methods (Student t-test, Chi-square test, Fisher test, Mann–Whitney test, Kruskal–Wallis, Friedman test). All data are presented as mean ± SEM. Single-factor and multi-factor analysis of variance was used for repeated measurements, while the Wilcoxon test method of regressive analysis was applied fir categorical variables. A p value of less than 0.5 was taken as significant.

3. Results

It was a prospective study carried out over a period of 9 years (July 2010 to July 2019) on 348 consecutive cases diagnosed to have FGTB on composite reference standard on whom hysteroscopy was done or supervised by the first author of the study (JBS). The baseline characteristics of the patients are shown in Table 1. The age ranged from 19to 44 years with mean being 28.2 ± 4.4 years. A total of 282 (81.03%) women were nulliparous, 47 (13.50%) women were para 1, 18 (5.17%) women were para 2 while one woman (0.28%) was para 4. Body mass index (BMI) ranged from 15.9 to 34.8kg/m^2 with mean being 23.1 ± 2.71 kg/m^2. Past history of TB was observed in 133 (38.21%) with pulmonary TB being in 84 (24.13%) and extra pulmonary TB being in 49 (14.08%) cases. The duration of infertility ranged from 1 to 12 years with mean being 3.44 ± 1.45 years; while the infertility was primary in 282 (81.03%) cases and secondary in 66 (18.96%) cases. Most patients belonged to lower (65.22%) or middle (32.47%) socioeconomic status. History of BCG vaccination was obtained in 264 (75.86%) cases.

The clinical features and baseline investigations are shown in Table 2. Raised temperature was seen in 61 (17.52%), loss of appetite in 118 (33.90%), loss of weight in 112 (32.18%), malaise in 105 (30.17%), night sweats in 72 (20.68%) cases. Normal menstruation was seen in 187 (53.73%) cases. Menstrual abnormalities in the form of heavy menstruation was seen in 6 (1.72%) cases, hypomenorrhoea in 92 (26.43%), oligomenorrhoea in 99 (28.44%), secondary amenorrhoea in 23 (6.60%) and

Table 1 – Characteristics of patients (N = 348).

S.NO.	Characteristics	Number	Percentage (%)
1	**Age (years)**		
	Range	19–44	
	Mean ± Standard deviation	28.2 ± 4.4	
2	**Parity**		
	• Nulliparous	282	81.03
	• Para 1	47	13.50
	• Para 2	18	5.17
	• Para 3 and above	1	0.28
3	**Body Mass Index (BMI)**		
	Range	15.9–34.8	
	Mean ± Standard deviation	23.1 ± 2.71	
4	**Past history of TB**	133	38.21
	• Pulmonary	84	24.13
	• Extra pulmonary	49	14.08
5	**Duration of infertility**	1–12	
	Mean ± Standard deviation	3.44 ± 1.45	
6	**Type of infertility**		
	• Primary	282	81.03
	• Secondary	66	18.96
7	**Socioeconomic status**		
	• Lower	227	65.22
	• Middle	113	32.47
	• Upper	8	2.29
8	**History of BCG injection**	264	75.86

dysmenorrhoea in 24 (6.89%) cases. Abdominal pain and chronic pelvic pain were seen in 30 (8.62%) and 39 (11.20%) cases respectively, while lymphadenopathy and abdominal mass were seen in 10 (2.87%) and 28 (8.05%) cases respectively. On speculum examination abnormal vaginal discharge was seen in 136 (39.08%) cases, cervical growth in 3 (0.86%) cases while on bimannual examination adenexal mass was seen in 59 (16.95%) cases; being unilateral in 40 (11.49%) and bilateral in 19 (5.45%) cases. On baseline investigations shown in Table 2, anemia (haemoglobin <11 g/dl) was seen in 59 (16.95%) cases, total leucocyte count (TLC) ranged from 3910 to 12,162/mm^3 with mean being 5439 ± 2198/cubic mm and random blood sugar ranged from 80 to 198 mg/dl with mean being 111.48 ± 14 mg/dl. Abnormal Mantoux test (>10mm) was seen in 128 (36.78%) cases, borderline Mantoux (5–10 mm) was seen in 154 (44.25%) cases while a negative Mantoux (<5mm) was seen in 60 (18.96%) cases. Low Mantoux test may be due to BCG vaccination in only 264 (25.86%) cases and our dependence on diagnosis on Composite reference standard. ESR ranged from 13 to 68with mean being 31.48 ± 11.98 mm/hr. Chest X ray findings were normal in 313 (89.94%) and old healed lesion of TB were found in 21 (6.03%) cases and mediastinal lymphadenopathy in 14 (4.02%) cases.

Microbiological and other investigations for diagnosis of FGTB are shown in Table 3. Endometrial biopsy (aspirate) showed positive (AFB) on microscopy in 14 (4.02%) cases, positive culture in 17 (4.88%), positive gene Xpert (done in only last 167 cases) was seen in 31 (18.56%) cases, positive PCR in 292 (83.90%) cases, positive epithelioid granuloma on histopathology in 51 (14.65%) cases. Biopsy from peritoneal lesion or caseous nodules during laparoscopy showed positive AFB on microscopy or culture in 4 (1.14%) cases while epithelioid granuloma on histopathology of biopsy from peritoneal lesion or caseous nodules was seen in 6 (1.72%) cases and in biopsy from cervical growth was seen in 3 (0.86%) cases. Definitive findings of FGTB on laparoscopy (caseous nodules, beaded tubes and tubercles) were seen in 146 (41.95%) cases while probable findings of FGTB on laparoscopy were seen in 202 (58.05%) cases. Diagnosis of FGTB was made on Composite Reference Standard in which positive AFB on microscopy or culture of endometrial or peritoneal biopsy or positive epithelioid granuloma on endometrial or peritoneal biopsy and definitive or probable findings of FGTB on laparoscopy were taken into consideration.

Various hysteroscopic findings in the study are shown in Table 4. In 18 (5.17%) cases hysteroscopy could not be done. Normal hysteroscopic findings in the form of pink, smooth endometrium with gland openings and no tubercles, no adhesions with normal ostia were seen in 98 (28.16%) cases. Pale endometrial cavity (Fig. 1) was seen in 189 (54.31%) cases. Features of active TB were seen in 26 (7.47%) cases with tubercles in 18 (5.17%), shaggy areas (white deposits) (Fig. 2) in 6 (1.72%) and caseous nodules (Fig. 3) in 2 (0.57%) cases. Features of chronic tuberculous endometritis were observed in 68 (19.54%) cases with hyperemic and congested endometrium in 44 (12.64%) cases, stromal edema in 18 (5.17%) cases, micropolyp formation in 6 (1.72%) cases. Features of TB sequelae seen were obliteration of both ostia in 48 (13.79%), obliteration of one ostium in 52 (14.94%) cases, bilateral periostial fibrosis in 16 (4.59%) cases, unilateral periosteal fibrosis in 18 (5.17) cases andpinhole one ostium in 6 (1.72%) cases, endometrial glands atrophy in 43 (12.35%), shrunken uterine cavity (Figs. 4 and 5) in 22 (6.32%), distorted uterine cavity in 18 (5.17%) cases. Intrauterine adhesions (Figs. 4–6) were seen in 104 (29.88%) cases with grade 1adhesions in 54 (15.57%), grade 2 including transverse band (Fig. 4) in 28 (8.04%), grade 3 (Fig. 5) in 16

Table 2 – Clinical features and baseline investigations in patients of FGTB.

S.NO.	Symptoms	Number	Percentage (%)
1	Raised temperature	61	17.52
2	Loss of appetite	118	33.90
3	Loss of weight	112	32.18
4	Malaise	105	30.17
5	Night sweats	72	20.68
6	Menstrual symptoms		
	• Normal menstruation	187	53.73
	• Heavy bleeding	6	1.72
	• Hypomenorrhoea	92	26.43
	• Oligomenorrhoea	99	28.44
	• Secondary Amenorrhoea	23	6.60
	• Dysmenorrhoea	24	6.89
7	Abdominal pain	30	8.62
8	Chronic pelvic pain	39	11.20
9	Lymphadenopathy	10	2.87
10	Abdominal mass	28	8.04
S.NO.	Signs	Number	Percentage (%)
1	**Speculum examination**		
	• Normal	209	60.05
	• Abnormal vaginal discharge	136	39.08
	• Cervical growth	3	0.86
2	**Adenexal/pelvic mass**	59	16.95
	• Unilateral	40	11.49
	• Bilateral	19	5.45
Baseline investigations			
S.NO.	Investigation	Number	Percentage (%)
1	**Anemia** (Hb < 11g/dl)	59	16.95
2	**Total leucocyte count (Cubic mm)**		
	Range	3910–12162	
	Mean ± Standard deviation	5439 ± 2198	
3	**Random blood sugar (mg/dl)**		
	Range	80–198	
	Mean ± Standard deviation	111.48 ± 14	
4	**Mantoux test**		
	• Negative (<5 mm)	66	18.96
	• Borderline (5–10mm)	154	44.25
	• Positive (>10mm)	128	36.78
5	**Erythrocyte sedimentation rate (mm/hr)**		
	Range	13–68	
	Mean ± Standard deviation	31.48 ± 11.98	
6	**Chest X- Ray**		
	• Normal	313	89.94
	• Old healed lesion of TB	21	6.03
	• Mediastinal lymphadenopathy	14	4.02

*Some patients had more than one symptom or sign.

Table 3 – Laboratory investigations for FGTB in the study (N = 348).

S.NO.	Test	Number	Percentage (%)
1	**Endometrial biopsy or sampling**		
	• Positive AFB on microscopy	14	4.02
	• Positive culture	17	4.88
	• Positive PCR	292	83.90
	• Positive epithelioid granuloma on histopathology	51	14.65
2	**Peritoneal or caseous nodule biopsy on laparoscopy**		
	• Positive AFB on microscopy	4	1.14
	• Positive epithelioid granuloma on histopathology	6	1.72
3	**Epithelioid granuloma on cervical biopsy**	3	0.86
4	**Definitive findings of FGTB on laparoscopy** (caseous nodules, beaded tubes, tubercles)	146	41.95
5	**Probable findings of FGTB on laparoscopy** (hyperemic tubes, hydrosalpinx, pyosalpinx, peritubal adhesions, pelvic and perihepatic adhesions, tubo-ovarian masses)	202	58.05

Table 4 – Hysteroscopic observations in patients of FGTB (N = 348).

S.NO	Finding	Number	Percentage (%)
1	**Inability to perform the procedure** (failed or abandoned procedure)	18	5.17
2	**Normal finding** (pink smooth cavity, endometrial glands opening seen, no tubercles, no adhesions, both ostia patent)	98	28.16
3	**Pale endometrium and cavity**	189	54.31
4	**Features of active tuberculosis**	26	7.47
	• Tubercles	18	5.17
	• Shaggy areas	6	1.72
	• Caseation	2	0.57
5	**Features of chronic tubercular endometritis**	68	19.54
	• Hyperemic and congested endometrium	44	12.64
	• Stromal edema	18	5.17
	• Micropolyp formation	6	1.72
6	**Features of tubercular sequalae:**		
	• Obliteration of both ostia (both ostia not seen)	48	13.79
	• Obliteration of one ostium (one ostia not seen)	52	14.94
	• Bilateral periostial fibrosis	16	4.59
	• Unilateral periostial fibrosis	18	5.17
	• Pinhole ostium	16	1.72
	• Endometrial glands atrophy	43	12.35
	• Shrunken uterine cavity	22	6.32
	• Distorted uterine cavity	18	5.17
	• Intrauterine adhesions: overall	104	29.88
	a) Grade 1	54	15.57
	b) Grade 2	28	8.04
	c) Grade 3	16	4.59
	d) Grade 4	6	1.72

Note: Many patients had more than one finding.

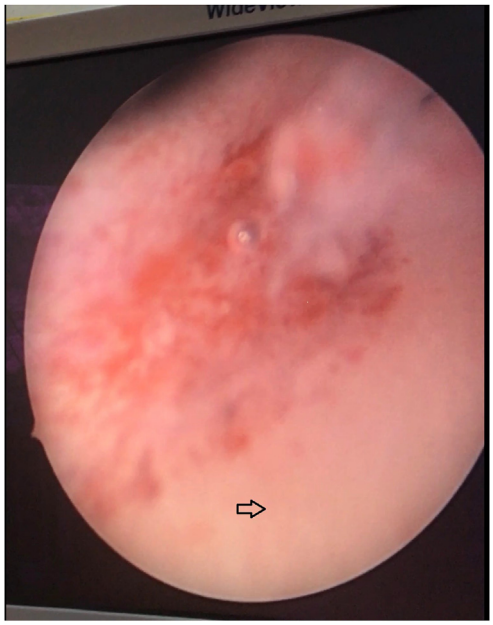

Fig. 1 – Hysteroscopy showing partially pale cavity (arrow) and endometrial atropy in lower part of uterus in a case of FGTB.

(4.59%) and grade 4 (Fig. 6) in 6 (1.72%) cases with many patients having more than one finding.

Performing hysteroscopy in FGTB is more difficult and is associated with more complications as shown in Table 5. There was inability to dilate cervix in 18 (5.17%) cases, difficulty in distension of endometrial cavity in 19 (5.45%) cases, inability to visualise the cavity due to thick adhesions in endocervix in 4 (1.14%) cases, difficulty in visualisation in 32 (9.19%), excessive bleeding (controlled with cautery or inflated foley's catheter for 12 hours) in 22 (6.32%) cases, false passage in 4 (1.14%) cases. Uterine perforation in 8 (2.28%) cases (treated at the same time by laparoscopic application of oxidised cellulose), flare up of TB in 12 (3.44%) cases, increased hospital stay (>24 hours) in18 (5.17%) cases, postoperative

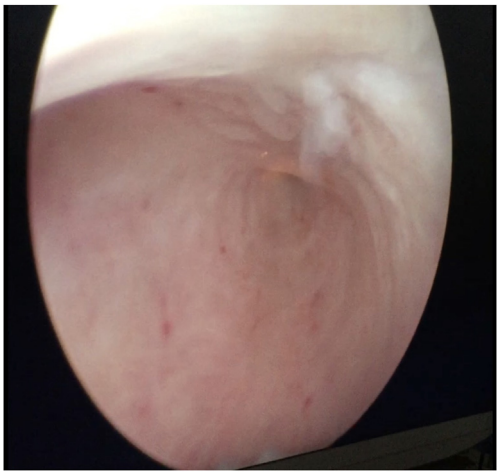

Fig. 2 – Hysteroscopy showing small uterus with white deposits and cavity (shaggy areas)near left ostium in a case of FGTB.

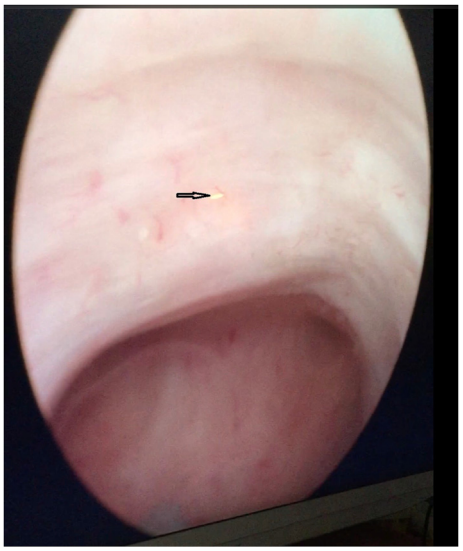

Fig. 3 – Hysteroscopy showing pale endometrium cavity, caseous nodule (arrow) and small uterine cavity in a case of FGTB.

infection in 16 (4.59%)cases, postoperative excessive pain (necessitating injection diclofenac) in 14 (4.02%) cases and need of repeat admission in 2 (0.57%) cases. None of the patients required laparotomy.

4. Discussion

Female genital tuberculosis is an important cause of infertility in developing countries being responsible for about 10% cases

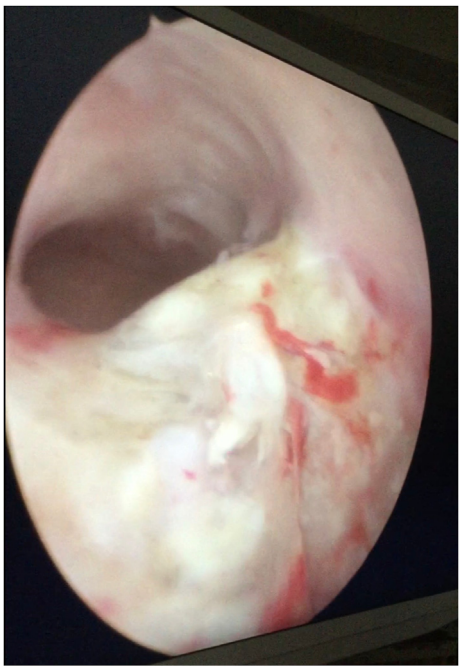

Fig. 4 – Hysteroscopy showing small and distorted uterine cavity with large transverse white transverse band (adhesion) in a case of FGTB.

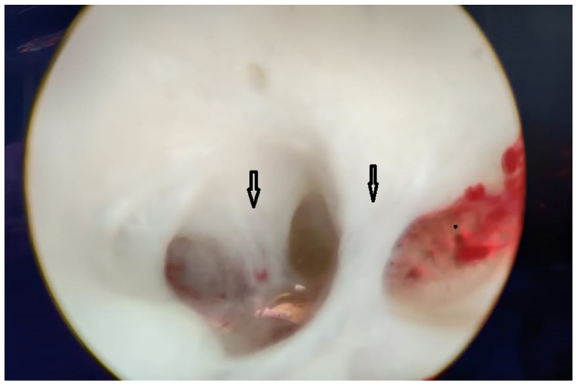

Fig. 5 – Hysteroscopy showing small shrunken and distorted cavity with pale endometrium and grade III adhesion (arrow) in a case of FGTB.

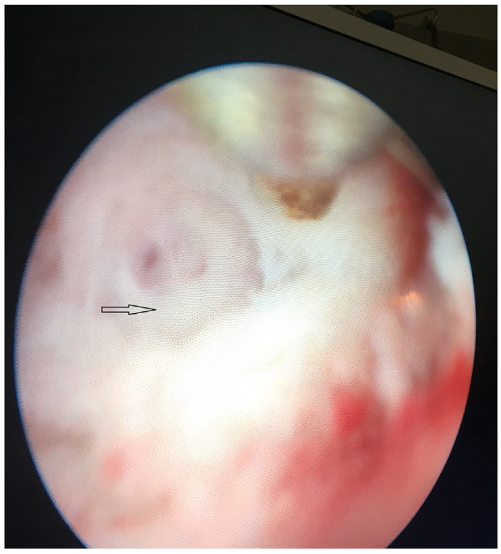

Fig. 6 – Hysteroscopy showing grade IV adhesions (arrow), periosteal fibrosis and adhesions in a case of FGTB.

of infertility.[6–8,10] It causes infertility through involvement of fallopian tubes (95–100% cases), uterine endometrium (endometrial atrophy and intra uterine adhesions) and through defective ovarian function.[6–8,12,13] Being a paucibacillary disease, diagnosis of FGTB is difficult as conventional gold standard methods like detection of AFB on microscopy or culture of endometrial biopsy or histopathological demonstration of epithelioid granuloma on endometrial, cervical, peritoneal biopsy are positive in small percentage of cases with risk of missing the diagnosis.[6–8] Gene Xpert though useful also has lower sensitivity.[16] Diagnostic laparoscopy is a useful modality in detection of asymptomatic abdominopelvic TB by direct visualisation of TB lesions and in prognostication for infertility.[18,19,22] Radiological imaging methods like ultrasound, computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomography (PET) are more useful for tuberculoustubo-ovarian masses.[23,24] In the present study positive Mantoux test was seen in 36.78% cases, borderline in 44.25%, negative Mantoux in 18.96% cases which may be due to only 75.86% BCG vaccination and our dependence on CRS for diagnosis of FGTB. In the present study, the hysteroscopy was performed to observe and document hysteroscopic findings in cases of FGTB. Hysteroscopy was not used to make diagnosis of FGTB. The diagnosis of FGTB was made by Composite reference standard by combining various reliable methods of diagnosis for higher pick up of disease.

For improving diagnosis of FGTB, Composite Reference Standard has been recommended in line with other EPTB cases.[25] It can detect higher number of cases by combining various methods like detection of acid fast bacilli on microscopy or culture of endometrial biopsy or peritoneal biopsy, detection of gene Xpert on endometrial or peritoneal biopsy along with definite (caseous nodules, tubercles, beaded tubes) or probable (pelvic, abdominal and peritoneal adhesions, shaggy areas, calcified tubes, hydrosalpinx, pyosalpinx, hyperemic tubes, tubo-ovarian masses) findings of FGTB.[25]

In the present study conducted over a period of 9 years, a total of 374 cases of FGTB were detected in infertility patients on Composite Reference Standard. However, hysteroscopy could be done in only 348 patients where various findings of

Table 5 – Difficulties encountered and complications observed in hysteroscopy in FGTB patients (N = 348).

S.NO	Difficulties or complications	Number	Percentage (%)
1	Inability to dilate cervix	18	5.17
2	Inability to put hysteroscope due to fibrosed cervix	18	5.17
3	Difficulty in distension of endometrial cavity	19	5.45
4	Inability to see cavity due to thick adhesions at endocervix	4	1.14
5	Difficulty in visualisation	32	9.19
6	Excessive bleeding (controlled with cautery or inflated foley's catheter for 12 hours)	22	6.32
7	False passage	4	1.14
8	Uterine perforation (managed by laparoscopic application of oxidised cellulose)	8	2.29
9	Flare up of tuberculosis	12	3.44
10	Increased hospital stay	18	5.17
11	Postoperative infection	16	4.59
12	Postoperative excessive pain	14	4.02
13	Readmission required	2	0.57

FGTB were demonstrated. Hysteroscopy was normal in 28.16% cases of FGTB diagnosed on Composite Reference Standard. Various abnormal findings of FGTB were pale endometrial cavity in 54.31%, features of active TB (tubercles, shaggy areas, caseation) in 7.47% cases, features of tuberculosis sequelae like obliteration of bilateral ostia (13.79%) or unilateral ostium (14.94%), bilateral periostial fibrosis (4.59%), unilateral peritostial fibrosis (5.17%), pinhole ostium (1.72%), endometrial gland atrophy (12.35%), shrunken uterine cavity (6.32%), distorted uterine cavity (5.17%) and intrauterine adhesions (29.88%) with grade 1 adhesions (15.57%), grade 2 adhesions (8.04%), grade 3 adhesions (4.59%) and grade 4 adhesions (1.72%) cases. Normal findings in 28.16% cases is probably due to non involvement of uterus in these cases as uterus is involved in 50–70% of cases as compared to 95–100% incidence of fallopian tube involvement.[6–8] Various authors have described hysteroscopic findings in FGTB. Mohakul et al[26] observed ostial and periostial fibrosis, intrauterine fibrosis and irregular cavity surface in cases of latent endometrial tuberculosis in infertility.

Fowler et al[27] also observed Asherman's syndrome and infertility due to pelvic tuberculosis. Prado et al[28] observed formation of cotton like white deposits in a case of endometrial TB confirmed by demonstration of epithelioid granuloma in histopathology of biopsy from the endometrial lesion.

Hysteroscopy is an important diagnostic tool for detection of endometrial TB and can test tubal patency also in combination with laparoscopy.[21] The most common finding of FGTB on hysteroscopy are thin endometrial thickness with dirty appearance, irregular and pale endometrium with whitish deposits on the endometrial surface, presence of varying grades of intrauterine adhesions and a small and poorly expandable uterine cavity.[29,30] Kumar et al[29] described starry sky appearance of endometrial TB by application of methylene blue dye in which case any tubercular deposit appears like a white star due to non absorption of dye against the blue background of endometrium which absorbs the dye. They also described cobwebs and intra cavitary adhesions along with white deposits in the endometrial TB cases.[29,30] Malhotra et al[22] also observed chronic endometritis, obstructed ostia, atrophic glands, intrauterine adhesions and distorted cavity on hysteroscopy in their study on FGTB. In the present study we observed increased difficulties and complications while performing hysteroscopy possibly due to cervical fibrosis (making dilatation of cervix difficult with increased chances of failure, false passage) and distorted and shrunken endometrial cavity causing increased risk of uterine perforation as was observed by our previous study also.[31] Hence hysteroscopy should be performed only by experienced hysteroscopic surgeons. Hysteroscopic adhesiolysis should only be performed after completion of six months course of anti tubercular therapy. Benefit of hysteroscopic adhesiolysis in terms of fertility outcome are mainly in lesser grades of adhesions (Asherman's grade 1–3). In advance stages of grade 4 or 5 adhesions adhesiolysis is not only difficult but also hazardous and may not achieve pregnancy even after assisted conception due to endometrial atrophy.[6,7]

5. Conclusion

Hysteroscopy performed in combination with laparoscopy is a useful modality in diagnosis of FGTB in females with infertility by direct visualisation of lesions of FGTB and for prognostication of infertility. Unfortunately, in this study we did not keep a record of conception of patients as we followed them only during antitubercular therapy when they were not trying for conception which is a limitation of the study. In the present study, we could not do testing for chlamydia, mycoplasma and gonorrhoea which is a limitation in the study as some of the cases may have been due to these infections. We included only women positive for PCR for *Mycobacterium tuberculosis* and had at least 3 of the probable findings to minimise the bias.

Unfortunately, due to financial and logistics constraints, we could not subject the endometrial and peritoneal biopsy samples to newer and molecular methods like loop mediated isothermal amplification and other methods which is a limitation of the study.[14,17] However, we used Composite reference standard which is an established method of diagnosis of extrapulmonary TB including FGTB and used only definite findings of FGTB or at least 3 probable findings of FGTB. All cases of laparoscopy and hysteroscopy were done in postmenopausal phase by single author is the strength of the study.

Conflicts of interest

The authors have none to declare.

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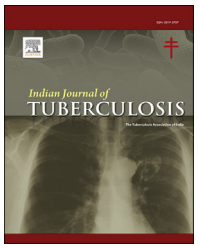

Original article

Computed tomographic findings in female genital tuberculosis tubo-ovarian masses

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ABSTRACT

Female genital tuberculosis (FGTB) is a common cause of infertility in developing countries. It can manifest as menstrual disturbances, infertility and pelvic masses.

Objective: To evaluate the role of computed tomography in diagnosis of female genital tuberculosis with tubo-ovarian (adnexal) masses.

Methods: It was a prospective study over a four year period (July 2015 to August 2019) in a tertiary referral centre over 33 patients presenting with tuberculosis and tubo ovarian masses only. 75 total cases of FGTB diagnosed on composite reference standard (evaluation of AFB bacilli in microscopy or culture or endometrial biopsy, gene expert, epitheloid granulomas on endometrial biopsy or definitive or possible findings of FGTB on laparoscopy). Detailed history taken, clinical examination, baseline investigations and endometrial biopsy were done in all cases. Computed tomography was performed in women presenting with infertility, tubo ovarian masses on clinical examination and laboratory investigations. A total of 33 cases were evaluated.

Results: Mean age, body mass index, parity and history of TB contact were 27.5 ± 4.2 year, 22.7 ± 3.6 kg/m^2, 0.27 ± 0.13 and 44.4% respectively. Infertility was primary in 72.72% and secondary in 27.23%. Case wise mean duration being 5.8 years, menstrual dysfunction was seen in 45.45% cases. Abdominal discomfort with pain and lump were seen in all 33 (100%) cases. Abdominal lumps were felt in 4 (12.12%) cases while adnexal mass was seen in all 33 (100%) cases being unilateral in 18 (54.54%) and bilateral in 15 (45.45%). Mean ESR was 33.4mm in first hour while mean leucocyte count was 6128 ± 2854 per cubic mm. Infectious mantoux test (>10mm) was seen in 14 (42.82%) cases while abnormal X ray chest was seen in 9 (27.27%) cases.

Diagnosis of FGTB was made by positive AFB n microscopy or culture of endometrial biopsy in 5 (15.15%) cases, positive gene expert in 6 (18.18%) cases, positive polymerase chain reaction in 32 (96.96%) cases, epitheloid granulomas on histopathology of endometrial biopsy in 7 (21.21%) cases, definitive findings of tuberculosis in 15 (45.45%) cases and a possible findings of tuberculosis inn 18 (54.54%) cases. Various CT findings were pelvic mass (100%), unilateral pelvic mass in 18 (54.54%), bilateral pelvic mass in 15 (45.45%), cystic mass (24.2%), solid mass (21.2%), mixed mass (54.54%), mass showing multilocular

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caseous necrotic enhancements (12.12%), ascites (42.4%), thickening and enhancement of peritoneum in 14 (42.42%), nodules in 24.2%, smooth in 18.8%, pelvic adhesion in 6 (18.18%), lymphadenopathy in 8 (24.3%) with calcifications (9.09%) and central necrosis (52.5%). Other CT findings were thickening and enhancement of bowel wall (12.12%), hepatic TB (3.03%), splenic TB (3.03%), omental thickening (9.09%) and omental calcification (3.03%) cases.

Conclusion: Computed tomography appears to be a useful diagnostic modality in diagnosis of tuberculosis tubo ovarian masses and may help avoid unnecessary surgery.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease with worldwide distribution and major health implications.[1,2] The disease is more prevalent in developing countries and its increased incidence globally is attributed to human immunodeficiency virus (HIV) infection, more liberal immigration, drug addiction, low economic status, occupational exposure and lack of BCG vaccination.[3]

Female genital tuberculosis (FGTB) is an important variety of extrapulmonary TB with devastating effect on female fertility by causing irreversible damage to the fallopian tubes through tubal damage, endometrium through endometrial atrophy and intrauterine adhesions (Asherman's syndrome) and through decreasing ovarian reserve and quality of ova.[4–7] The prevalence of FGTB amongst infertile women ranges from 1 percent in USA to up to 18 percent in Africa and India.[8,9] Abdominopelvic TB also affects peritoneum, bowel, and abdominal and pelvic lymph nodes and can masquerade as ovarian cancer with ascites and even raised CA125 levels and makes it difficult to differentiate from ovarian cancer.[10,11] It causes involvement of omentum by producing omental thickening and ascites and liver and gall bladder through perihepatic adhesions and hanging gall bladder.[12,13] It also produces various abdominal and pelvic adhesions like ascending colon adhesions, sigmoid colon adhesive band and Sharma's parachute sign in which ascending colon is adherent to the anterior abdominal wall.[13–15] Diagnosis of FGTB is made by demonstration of acid fast bacilli on microscopy and culture of endometrial biopsy or positive gene Xpert on endometrial sampling or demonstration of epitheloid granulomas on histopathology of endometrial biposy.[4,5,8,16] Polymerase chain reaction alone is not taken for diagnosis due to high false positivity.[4,17] Diagnostic laparoscopy is useful in diagnosis of abdominopelvic TB including peritoneal, bowel, gall bladder, liver and omental disease.[18] It can detect definite and probable findings of FGTB. Imaging methods like ultrasound, CT scan, MRI and PET CT are more useful in the diagnosis of tubo ovarian masses and abdominopelvic tuberculosis.[19,20]

We present our study on 33 women out of 175 women with tuberculous tubo ovarian masses to evaluate the role of computed tomography in FGTB with adnexal mass.

2. Material and methods

It was a prospective study on 175 infertile women diagnosed to have female genital tuberculosis (FGTB) on composite reference standard from a tertiary referral centre over 4 year period from July 2016 to August 2019. Out of 175 women, a total of 33 women (18.85%) had adnexal masses on clinical examination. All patients were diagnosed to have FGTB and adnexal masses on clinical examination and composite reference standard which included positive AFB on microscopy or culture of endometrial biopsy or positive gene Xpert on endometrial sampling or demonstration of epitheloid granulomas on histopathology of endometrial biopsy and definite (tubercles, caseous nodules, beaded tubes) or probable (tubo ovarian masses, convulated tubes, hyperaemic tubes, hydrosalpinx, pyosalpinx, pelvic, peritubal, abdominal or perihepatic adhesions or encysted ascites.

Computed tomography was performed on these masses using a 128- MDCT scanner (SIEMENS SOMATOM definition flash dual energy). The CT parameters for 128 detection rows, a beam collimation of 128 × 0.6mm, a pitch of 1.295mm slice thickness and 5mm reconstruction intervals. Unenhanced and contrast enhanced CT scans were performed during a single breathhold with patients in supine position. In selected patients, intravenous contrast material (90ml) was given and CT images were obtained using arterial, venous and delayed phases at 30, 60 and 180 seconds after contrast injection. The CT features were recorded on computer and were reviewed by consultant radiologist (SH) in all the cases for the presence or absence and characterisation of pelvic masses including its nature whether solid, cystic or mixed, its contour whether ovoid, round, tubular or lobulated, its margins whether well -defined or ill defined, its enhancement pattern like caseous necrosis, homogenous or heterogeneous and presence or absence of calcification.

The CT features were also evaluated for any ascites, its nature (high density or low density), thickening and enhancement of peritoneal wall at different areas in pelvis and abdomen, thickening and enhancement and caking of omentum, any adhesions in the pelvis and abdomen. Any lymphadenopathy in pelvis and abdomen was noted including the lymph nodes involved, any calcifications or central necrosis. Any thickening and enhancement of bowel wall was also noted on CT scan. Any associated lesion in other abdominal organs were also noted carefully.

All patients were started with anti-tubercular therapy for 6 months with daily Rifampicin, Isoniazid, Pyrazinamide, Ethambutol (RHZE) for 2 months (intensive phase) followed by 4 months of RHE daily. Patients were followed for any adverse effects of drugs and for amelioration of symptoms of TB. Informed consent was taken from all patients. The study was part of our ongoing large project on FGTB for which ethical clearance was given by the Institute Ethical Committee. CT scan not done in all cases of FGTB due to financial constraints.

2.1. *Statistical analysis*

Data Analysis was carried out using STATA software v 12.0. Continous variables were tested for normality assumption

Table 1 – Characteristics, Clinical Features and Basic Investigations of the patient.

Sr no	Characteristics	No	Percentage
1.	Age (years)		
	Range	24–36	
	Mean ± SD	27.5 ± 4.2	
2.	Body Mass Index (kg/m^2)		
	Range	17.5–31.8	
	Mean ± SD	22.7 ± 3.6	
3.	Parity		
	Range	0–3	
	Mean	0.27 ± 0.13	
4.	History of TB contact	14	42.42
5.	History of BCG vaccintion	25	75.75
6.	Type of infertility		
	Primary	24	72.72
	Secondary	9	27.27
7.	Duration of Infertility (years)		
	Range	2–13	
	Mean ± SD	5.85 ± 2.65	
8.	Menstrual symptoms		
a)	Normal menstruation	14	42.42
b)	Menstrual Dysfunction	19	57.57
i)	Abnormal uterine bleeding	2	6.06
ii)	Hypomenorrhea	8	24.24
iii)	Oligomenorrhea	7	21.21
iv)	Amenorrhea	2	6.06
v)	Dysmenorrhea	9	27.27
9.	Anorexia	12	36.36
10.	Weight loss	14	42.42
11.	Pyrexia	11	33.33
12.	Dyspareunia	9	27.27
13.	Vaginal discharge	11	33.33
14.	Abdominal or Pelvic pain	33	100
15.	Abdominal or Pelvic lump	33	100
Examination Findings			
1.	Pallor	11	33.33
2.	Lymphadenopathy	9	27.27
3.	Abdominal lump	4	12.12
4.	Speculum examination		
a)	Abnormal discharge	23	69.69
b)	Adnexal mass	33	100
i)	Unilateral	18	54.54
ii)	Bilateral	15	45.45
Baseline investigations			
1.	Anemia	11	33.33
2.	ESR (Erthrocyte sedimentation Rate) in mm first hour (Mean ± SD)	33.4 ± 11.48	
3.	Leucocyte count (mean ± SD)	6128 ± 2854	
4.	Infectious mantoux test (>10mm)	14	42.42
5.	Abnormal chest Xray	9	27.27
6.	CA 125 levels (IU/ml)		
	Range	15–500	
	Mean ± SD	75.4 ± 38.5	

Total FGTB Patients = 175.
Tubo ovarian masses = 33 (N = 33).
Percentage of TB tubo ovarian masses in FGTB = 18.85%.

using KOLMOGOROV-SMIRNV test. Descriptive statistics such as Mean, Standard deviation, range values were carried for normally distributed datas. Comparison of two groups means were tested using Student's 't' independent test. Categorical data were presented as frequency and percentage values. Comparison of categorical values were tested using Chi-Square/Fischer's exact test.

3. Results

It was a prospective study on 33 out of 175 FGTB patients with tubo ovarian masses with incidence of adnexal masses in FGTB being 18.85%. The characteristics of the patients are given in Table 1. The age ranged from 24 to 36 years with mean age being 27.5 ± 4.2 years. The body mass index ranged from 17.5 to 31.8 with mean being 22.7 ± 3.6 kg/m^2 while the parity ranged from 0 to 3 with the mean being 0.27 ± 0.13. There was history of TB contact in 14 (42.42%) cases while history of BCG vaccination was seen in 25 (75.75%). All 33 (100%) patients were infertile with 24 (72.72%) having primary infertility while 9 (27.27%) patients having secondary infertility. Duration of infertility ranged from 2 to 13 years with mean being 5.85 ± 2.65 years.

Various menstrual symptoms were normal menstruation in 14 (42.42%) cases, abnormal menstruation in 19 (54.54%) cases with abnormal uterine bleeding in 2 (6.06%) cases, hypomenorrhea in 8 (24.24%), oligomenorrhea in 7 (21.21%) cases, amenorrhea in 2 (6.06%) and dysmenorrhea in 9 (27.27%) cases. Various other symptoms were anorexia (12, 36.36%), weight loss (14, 42.42%), pyrexia (11,33.33%), abdominal or pelvic pain (33, 100%) and abdominal or pelvic lumps in all (33, 100%) cases.

In examination findings, pallor was seen in 11 (33.33%) cases, lymphadenopathy in 9 (27.27%) cases, abdominal lumps in 4 (12.12%), abnormal discharge on speculum examination in 23 (69.69%) cases, adnexal masses in 33 (100%) cases with unilateral mass in 18 (54.54%) and bilateral in rest 15 (45.45%) cases. On routine baseline investigations, anemia (Hb < 11gm/dl) was seen in 11 (33.33%) cases. Mean erythrocyte sedimentation rate was 33.4 ± 11.48 mm in first hour while mean leucocyte count was 6128 ± 2854 per cubic mm. Infectious Mantoux test (>10mm) was seen in 14 (42.42%) cases while abnormal X-ray chest with old healed TB lesions or hilar lymphadenopathy was seen in 9 (27.27%) cases. CA 125 levels ranged from 15 to 500 IU/ml with mean being 75.4 ± 38.5 IU/ml which was higher than normal.

The method of diagnosis of FGTB in the 33 cases of FGTB are shown in Table 2. Acid fast bacilli (AFB) on microscopy or culture of endometrial biopsy or aspirate was seen in 5 (15.15%) cases while positive gene Xpert was seen in 6 (18.18%) cases and positive polymerase chain reaction was in 32 (96.96%) cases. Epitheloid granulomas on histopathology of endometrial biopsy was seen in 7 (25.21%) cases. All patients had diagnosis of FGTB made by laparoscopy and to rule out ovarian cancer. Definite findings of tuberculosis on laparoscopy were seen in 15 (45.45%) cases while probable findings were seen in rest 18 (54.54%) cases.

The computed tomography findings in 33 cases of tuberculous tubo ovarian masses are shown in Table 3. Thus pelvic masses were seen in all 33 (100%) cases with unilateral in 18 (54.54%) cases and bilateral in rest 15 (45.45%). The mass was cystic in 8 (24.24%) cases, solid in 7 (21.21%)cases and mixed in 18 (54.45%) cases and it showed multilocular caseous necrotic enhancement in 4 (12.12%) cases. Ascites was seen in 14 (42.42%) cases being high density (>18HU) in 12 (36.36%) cases and low density in 2 (6.06%) cases. On CT scan, thickening and enhancement of peritoneum was seen in 14 (42.42%) cases with nodular thickening in 8 (24.24%) and smooth thickening in 6 (18.18%) cases. Pelvic adhesions on CT scan were seen in 6 (18.18%) cases. Lymphadenopathy was seen in 10 (33.33%) cases being para aortic in 2 (6.06%), mesenteric in 3 (9.09%) and pelvic in 5 (15.15%) cases. Calcifications in lymph nodes were seen in 3 (9.09%) cases and central necrosis in lymph nodes was seen in 4 (12.12%) cases. Other additional findings on CT scan were thickening and enhancement of bowel wall in 4 (12.12%) cases, associated hepatic tuberculosis in 1 (3.03%) cases, splenic tuberculosis in 1 (3.03%) case, omental thickening in 3 (9.09%) cases and omental caking in 1 (3.03%) case.

Fig. 1A and B Axial CECT shows disseminated abdominopelvic TB with bilateral adnexal multilocular cystic lesions (* in A and B). Necrotic bilateral obturator lymph nodes are also seen (arrows in A).

Fig. 1C Axial CECT at the level of aortic bifurcation reveals mesenteric lymphadenopathy (arrowhead), omental nodularity (*) and wall thickening of caecum and terminal ileum (white arrows). And Fig. 1D. Axial lung window shows volume loss and bronchiectasis in left upper lobe (*) with multiple air space nodules and centrilobular nodules (arrow) in right upper lobe.

Fig. 2 shows abdominal tuberculosis with Fig. 2A showing Axial CECT reveals bilateral adnexal multilocular cystic lesions. The right adnexal lesion is tubular and predominantly

Table 2 – Diagnosis of FGTB in tubo ovarian masses (N = 33).

Sr no	Test	No	Percentage
1.	AFB on microscopy or culture of endometrial biopsy	5	15.15
2.	Positive gene Xpert	6	18.18
3.	Positive polymerase chain reaction	32	96.96
4.	Epitheloid granulomas on histolopathology of endometrial biopsy	7	21.21
5.	Definite findings of tuberculosis on laparoscopy	15	45.45
6.	Probable findings of tuberculosis on laparoscopy	18	54.54

*Some patients had more than one finding.

Table 3 – CT scan findings in tuberculosis tubo ovarian masses (N = 33).

Sr no	CT scan findings	no	percentage
1.	Pelvic mass	33	100
i)	Unilateral adnexal masses	18	54.54
ii)	Bilateral adnexal masses	15	45.45
iii)	Cystic mass	8	24.24
iv)	Solid mass	7	21.21
v)	Mixed mass	18	54.54
vi)	Mass showing multilocular caseous necrotic enhancement	4	12.12
2.	Presence of ascites	14	42.42
i)	High density ascites (18HU)	12	36.36
ii)	Low density ascites	2	6.06
3.	Thickening and enhancement of peritoneum	14	42.42
i)	Nodular thickening	8	24.24
ii)	Smooth thickening	6	18.18
4.	Pelvic adhesions	6	18.18
5.	Lymphadenopathy	10	33.33
i)	Para aortic	2	6.06
ii)	Mesenteric	3	9.09
iii)	Pelvic	5	15.15
iv)	Calcification in lymph nodes	3	9.09
v)	Central necrosis in lymph nodes	4	12.12
6.	Thickening and enhancement of bowel wall	4	12.12
7.	Hepatic Tuberculosis	1	3.03
8.	Omental thickening	3	9.09
9.	Omental caking	1	3.03
10.	Splenic TB	1	3.03

*Some patients had more than one findings.

cystic with thick enhancing walls and septations (arrow). The left adnexal lesion is solid cystic in morphology (*). Fig. 2B reveals Concentric wall thickening of the terminal ileum (arrow). Fig. 2C; Coronal CECT reveals ileocecal junction thickening (arrow) and bilateral adnexal complex cystic lesions (*) and Fig. 2D Sagittal CECT reveals wall thickening of terminal ileum (*) and necrotic mesenteric lymph nodes (arrow).

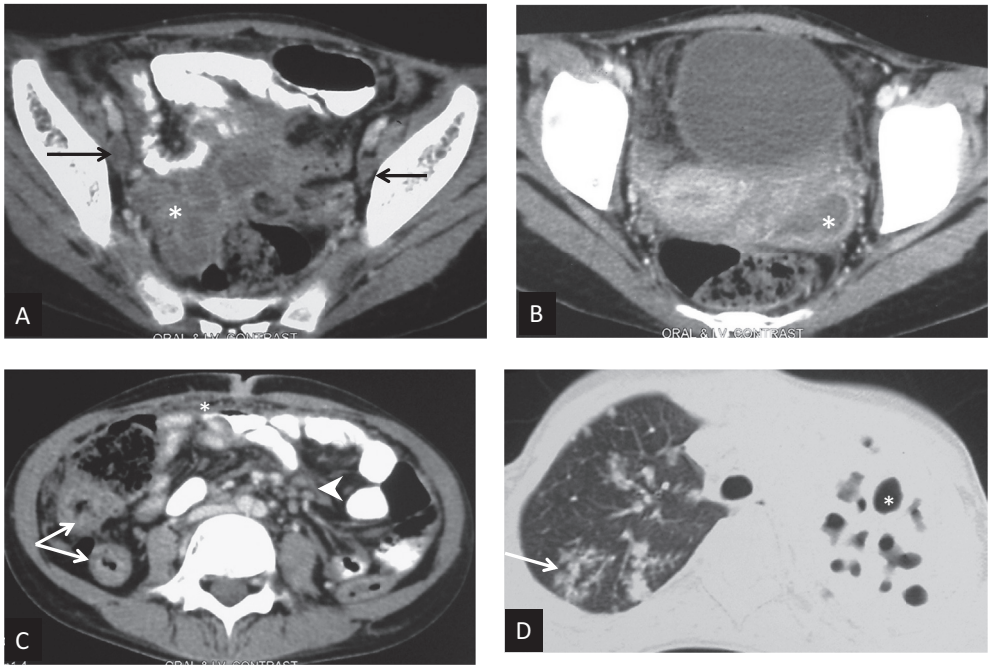

Fig. 1 – A and B. Axial CECT reveals bilateral adnexal multilocular cystic lesions (* in A and B). Necrotic bilateral obturator lymph nodes are also seen (arrows in A). Fig. 1C. Axial CECT at the level of aortic bifurcation reveals mesenteric lymphadenopathy (arrowhead), omental nodularity (*) and wall thickening of caecum and terminal ileum (white arrows). Fig. 1D. Axial lung window shows volume loss and bronchiectasis in left upper lobe (*) with multiple air space nodules and centrilobular nodules (arrow) in right upper lobe.

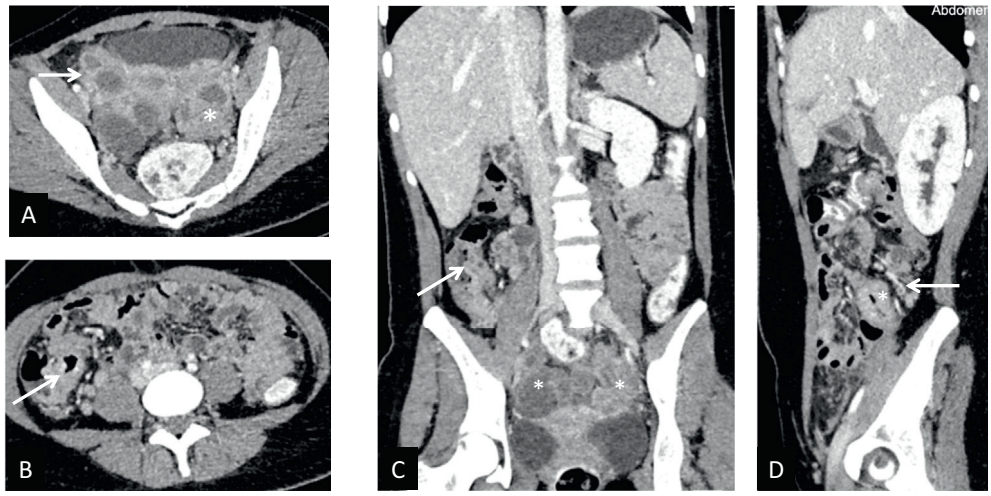

Fig. 2 – A Axial CECT reveals bilateral adnexal multilocular cystic lesions. The right adnexal lesion is tubular and predominantly cystic with thick enhancing walls and septations (arrow). The left adnexal lesion is solid cystic in morphology (*). Fig. 2 B reveals Concentric wall thickening of the terminal ileum (arrow). Fig. 2C Coronal CECT reveals ileocecal junction thickening (arrow) and bilateral adnexal complex cystic lesions (*). Fig. 2D Sagittal CECT reveals wall thickening of terminal ileum (*) and necrotic mesenteric lymph nodes (arrow).

All patients were treated with 6 month course of antitubercular drugs under Directly Observed Treatment short course (DOTS) free of cost using 4 drugs (rifampicin, isoniazid, pyrazinamide and ethambutol).

4. Discussion

Tuberculosis continues to be a major public health problem globally with more burden in developing countries like India.[1,2] Abdominopelvic and female genital TB are types of extrapulmonary TB.[4,5] FGTB is an important cause of infertility in India being responsible for about 10% cases of infertility.[4,8] It can also cause menstrual dysfunction, abdominal and pelvic pain and abdominopelvic lumps and ascites and may mimic an ovarian cancer often necessitating needless laparotomies.[10,11] It also causes various types of thick and vascular pelvic and abdominal adhesions like peritubal adhesions, peihepatic adhesions (Fits-Hughs-Curtis Syndrome), ascending colon adhesions, sigmoid colon adhesive band and Sharma's parachute sign (adhesion of ascending colon to anterior abdominal wall resembling an open parachute).[12–15]

Being paucibacillary in nature, the diagnosis of FGTB is difficult.[4] The gold standard method of diagnosis like demonstration of AFB on microscopy or culture of endometrial and peritoneal biopsy, positive gene Xpert on endometrial biopsy, positive epitheloid granuloma on histopathology are positive in small percentage of cases and diagnosis may be missed.[4,5,8,21] PCR though highly sensitive has low specificity and alone is not used to diagnose FGTB.[4,17] Diagnostic laparoscopy is a useful modality to detect abdomino pelvic TB and can visualise even subtle changes and can help in early diagnosis so that timely treatment can be started to prevent permanent damage to genital structures.[18] It can detect definite (tubercles, caseous nodules and beaded tubes) and probable (pelvic, peritubal, abdominal and perihepatic adhesions, encysted ascites, shaggy areas{white deposits} convuluted and hyperaemic tubes, hydrosalpinx, pyosalpinx and tubo ovarian masses). However, laparoscopy is an invasive procedure performed under general anaesthesia and needs expertise and maybe associated with difficulties and increased complications.[22] Radiological methods like ultrasound, computerised tomography, magnetic resonance imaging and positron emission tomography have been used in diagnosis of tuberculous tubo ovarian masses and to differentiate abdominopelvic TB from ovarian cancer with varying results.[19,20] They have the advantage of being non invasive and reasonable accuracy in diagnosis.[21] However, MRI and PET are more expensive and are not routinely available in smaller centres. Ultrasound though economical has lesser accuracy than CT and MRI.[24] Computed tomography is the imaging modality of choice for pelvic TB.[23] In the present study performed on FGTB with adnexal masses, we observed various CT findings like unilateral (54.54%) or bilateral pelvic masses (45.45%), cystic (24.24%), solid (21.21%) or mixed (54.54%) mass, mass with multilocular caseous necrotic enhancement (12.12%), ascites (42.42%), thickening and enhancement of peritoneum (42.42%), pelvic adhesions (18.18%), lymphadenopathy (33.33%), calcified lymph nodes (9.09%), necrotic lymph nodes (12.12%). We also observed other associated findings of CT scan in abdomen and pelvis like thickening and enhancement of bowel wall (12.12%), omental thickening (9.09%), omental caking (3.03%), associated hepatic TB (3.03%) and associated splenic TB (3.03%). Our results are similar to Sah et al[23] who observed unilateral or bilateral pelvic mass, cystic, solid or mixed mass, high density ascites, thickening and enhancement of peritoneum and omentum and lymphadenopathy with calcification and necrosis. They opined that integrated with clinical history and laborotary tests, pelvic TB should be considered in young female patients with elevated CA 125 levels and CT findings of adnexal masses with multilocular caseous necrotic

enhancement, high density ascites and thickened and enhanced peritoneum. Such patients can be started antitubercular therapy on time and unnecessary laparotomy can be avoided.[24] da Rscha et al[25] also reported usefulness of computed tomography and MRI for diagnosis of abdominal and pelvic TB. Other authors have also reported usefulness of CT scan in diagnosing abdominal TB and its utility in differentiating abdominal TB from ovarian and peritoneal cancer.[26–29] Various authers have observed utility of CT scan in detecting abdominal and peritoneal TB and tuberculous ascites which can be wet type with multiple fine septae with diffuse, smooth and regular peritoneal thickening and fibrinous type of peritonitis and with diffuse peritoneal thickening associated with bowel loops conglomerates.[25–29] The present study confirms the utility of computed tomography in diagnosis of TB tubo ovarian masses. However, the limitation of study is that CT scan could only be done in 33 out of 175 (18.85%) cases of FGTB with tubo ovarian masses due to financial and logistic constraints. Performing CT scan on all 175 women with FGTB could have been interesting. We may have missed findings in these cases by not performing CT scan on them.

Conflicts of interest

All authors have none to declare.

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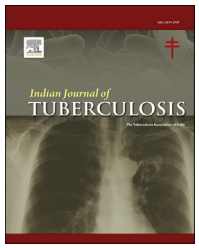

Original article

Risk factors for non-adherence among people with HIV-associated TB in Karnataka, India: A case–control study

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ABSTRACT

Setting: Five select districts of Karnataka, India, providing anti-tubercular and antiretroviral therapy (ATT and ART) to people with Human Immunodeficiency Virus (HIV) - associated Tuberculosis (TB) through a single window care approach at the ART centres (seven ART centres and 16 link ART centres).

Objectives: To determine the factors associated with non-adherence to concurrent therapy.

Design: We conducted a case–control study involving primary and secondary data collection. Starting January 2019, we consecutively enrolled people on at least three months of ATT until we enrolled 125 cases (non-adherent to concurrent therapy) and 375 controls (adherent to concurrent therapy). Adherence was defined as taking >95% ART doses and >90% ATT doses, every month over the last three months. We performed multivariable logistic regression to identify factors associated with non-adherence.

Results: The mean age of the cases and control was similar: 39.8 (standard deviation: 8.8) years. The risk factors for non-adherence were status non-disclosure (aOR = 2.06), zidovudine-based ART (aOR = 4.87), >3 side effects (aOR = 6.45), not receiving counselling before ATT initiation (aOR = 5.25) and non-receipt of co-trimoxazole prophylaxis (aOR = 9.90).

Conclusion: Major determinants for non-adherence were clinical and treatment related factors.

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1. Introduction

Globally, Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) are the top killers among infectious diseases.[1] People living with HIV (PLHIV) are up to 19 (15–22) times more likely to fall ill with TB.[2] In 2018, the estimated annual incidence of HIV-associated TB was 862 000 and 251 000 people died due to HIV-associated TB, a third of AIDS related deaths. The treatment success rate among people with HIV-associated TB (2017 cohort) is around 75%.[3,4]

Understanding HIV-associated TB is of great importance because of the severity of clinical presentation, rapid progression and challenges in treatment adherence and treatment success. Despite the availability of free treatment, the adherence to treatment of both the diseases has always been a challenge. In addition to the inherent nature of both the diseases, the patient has to now consume a handful of drugs, which pose problems like overlapping toxicity, drug–drug interactions and pill burden which may ultimately lead to treatment failure.[4] Factors associated with non-adherence to concurrent treatment among HIV-associated TB has not been studied extensively. In a study from South Africa (2008–10), extra-pulmonary TB and non-disclosure of HIV status were the risk factors for non-adherence.[5] Another study conducted around the same time in South Africa suggested poverty, having co-morbid health condition, being a high risk for alcohol misuse and a partner who is HIV positive were the risk factors.[6]

India has the highest TB burden in the world and accounts for 27% of the global TB burden.[2] In 2018, India accounted for 10 million cases of HIV, and the estimated annual incidence of HIV-associated TB was 92 000 with 9700 deaths. TB treatment success rate among the 2017 HIV-associated TB cohort was 71%.[4,7]

Traditionally, national TB and HIV programmes have functioned separately. This has changed with the World Health Organization (WHO) recommendations on total integration and one stop service for people with HIV-associated TB.[8] In this context, by the year 2008 a new window of care and daily treatment regimen using fixed-dose combination was adopted throughout the country by India's National Tuberculosis Elimination Program (NTEP).[9] People with HIV-associated TB now receive both antiretroviral therapy (ART) and anti-tubercular therapy (ATT) in a single-window at the ART centre. Patients are expected to collect the drugs once a month and self-administer the treatment.[9,10] To ensure adherence to treatment various initiatives like mobile phone-based technology with real-time monitoring of daily intake of treatment (99 DOTS)[11] were added into the programme. However, these newer initiatives had poor acceptability resulting in failure to manage adherence.[12] Understanding the factors associated with poor adherence in this setting will help the programme in designing specific interventions and target patients that are at risk of non-adherence.

Thus, we conducted a study to determine the factors associated with non-adherence to concurrent ATT and ART among the people with HIV-associated TB being managed in single window treatment centres in south India.

2. Methods

2.1. Study design

We conducted a case control study involving primary and secondary data collection.

2.2. Settings

2.2.1. Study sites

We carried out the study in the southern state of Karnataka, India, with a population of 67.7 million.[13] In 2018, the state had, 0.1 million TB patients with about 10% (3.4% nationally)[4] of them co-infected with HIV. The care and support services for HIV-associated TB patients are provided through 64 ART centres as per national guidelines.[7]

We included ART centres (seven ART centres and 16 link ART centres) across five select districts of Karnataka (Bengaluru Urban, Bengaluru rural, Ramanagara, Mandya and Mysuru). The study districts have an approximate population of 0.14 million.[13]

2.2.2. Management of HIV-associated TB

ART centres are actively involved in management of people with HIV-associated TB and are monitored by staff nurses, dedicated counsellors and also TB-HIV supervisors. The details of management are maintained in-house through separate records and people with HIV-associated TB are provided with white and green cards containing information regarding TB and HIV management, respectively.[7] The cards contain socio-demographic details, diagnostic and treatment details, adherence to treatment based on pill count and follow up dates.

Current guidelines recommend ART initiation for all new HIV diagnoses irrespective of clinical stage or CD4 count. Timing of ART in relation to start of TB treatment differs for people with HIV-associated TB. When ATT is initiated first then, ART is started as soon as TB treatment is tolerated (after 2 weeks and before 2 months).[9]

2.3. Study population

The study participants included people with HIV-associated TB registered for care and completing three months of ATT in the study districts. Using flies method with correction factor, we arrived at the sample size (125 cases and 375 controls) assuming the least extreme odds ratio to be detected as 1.83 (proportion of controls with exposure being 45%, proportion of cases with exposure being 60%), case control ratio of 1:3, 80% power and 5% alpha error (OpenEpi Software version 3).[14]

Starting from January 2019, we included consecutive patients with HIV-associated TB who completed three months of ATT (date of completing three months of ATT on or after 1 January 2019) as cases (non-adherence to concurrent ATT and ART) and controls (adherence to concurrent ATT and ART) until we attained the target sample size. We attained the desired sample size for controls by May 2019, while for cases it was attained by June 2019. Adherence was defined as taking >95% ART doses and >90% ATT doses, every month over the

last three months. We classified the rest as cases. The cut offs used were as per prevailing national guidelines[9,15] and we assessed them using the entries in records. We excluded children (<18 years), drug resistant TB, people on second line ART and bed ridden patients.

2.4. Data variables, sources of data and data collection

We extracted variables like co-morbidities, treatment duration, treatment regimen, side effects and past history of disease through record review. Before starting the face-to-face interview, we confirmed the correct classification of cases and controls using 28-day recall method (we did not find a single instance of misclassification). We collected socio-demographic details,[16] smoking and alcohol use in past 6 months for more than once a week, details of counselling sessions, HIV disclosure status, present nutritional status and reasons for non-adherence during the face-to-face interview. We interviewed most of the patients at the ART centres during their scheduled visit to collect medicines. Where an interview at the centre was not possible, we contracted and interviewed the patients at their homes. The participation rate was 100% for cases and controls. We have summarized the operational definition of various exposure variables in Table 1.

2.5. Analysis and statistics

We single-entered data into Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and performed the analysis using SPSS version 20.0. We performed logistic regression to assess the association between various exposure variables and non-adherence using unadjusted and adjusted odds ratio (95% confidence intervals). We included age, sex and variables found to be significantly associated ($p < 0.05$ using chi square test) on unadjusted analysis in the multivariable regression model.

2.6. Ethics

Bangalore Medical College and Research Institute Ethics Committee, Bengaluru, Karnataka approved the study. Trained researchers collected the data after prior approval from the Karnataka state AIDS prevention society. We obtained written informed consent from the study participants and the process was approved by ethics committee.

3. Results

We have summarised and compared the baseline characteristics of cases (n = 125) and controls (n = 375) in Tables 2–4. The mean age of the cases and control was similar: 39.8 (standard deviation: 8.8) years. The frequency (proportion) of males in cases was 82 (65.6%) while it was 233 (62.1%) in controls. Factors associated with non-adherence on unadjusted analysis were: type of family, wage loss, past history of TB, family history of TB, presence of co-morbidities, alcohol use and duration of HIV. Programmatic characteristics like type of treatment supporter, counselling before initiation of treatment, type of HIV regimen, side effects to treatment, disclosure of status to spouse/

Table 1 – Operational definitions of variables included in the study.

Study variables	Operational definitions
Socioeconomic status (SES)	Modified BG Prasad's classification was used for determining SES of the participant both in urban and rural areas. It is based on per capita monthly income divided into five classes based on consumer price index.
Type of family	Nuclear family is defined as family with married couple and their children living together while children were dependents. Joint family is group of married couples and their children with their other siblings and relatives living together in the same household.
Wage loss	Loss of wages by the participants incurred in a month during their visits to treatment centers for follow up or due to admission to hospital because of the disease.
Type of TB	New case was defined as a case which never had been treated for TB. Previously diagnosed is one in which patients were diagnosed and treated in the past and either were failure, relapse, defaulters or loss to follow up.
Type of diagnosis	Bacteriological specimen positive by smear microscopy or culture was a bacteriologically confirmed case. Clinically diagnosed case was one which was diagnosed based on symptoms, X-ray abnormalities or suggestive histology.
Co-morbidities	Presence of Diabetes, Hypertension, Thyroid disorders, Malignancy, Congenital heart diseases and other diseases along with TB and HIV.
Smoking	Smoking tobacco for the past 6 months.
Alcohol use	Consuming alcohol for the past 6 months at least 3 days in a week.
Body mass index (BMI)	BMI was calculated using the formula weight in kilograms divided by meters square. Divided into underweight (<18.5), Normal (18.5–23.4), Overweight (23.5–27.4), Obese (≥27.5).
Place of diagnosis	Designated microscopy centers and reference laboratories (by CBNAAT) are established by the government exclusively for TB diagnosis. Those participants who were diagnosed in private labs and reported to ART center for treatment were also included.
HIV regimen	Among the two Nucleoside reverse transcriptase inhibitors in the treatment – Zidovudine or Abacavir or Tenofovir were chosen for classification.
Side effects to treatment	Nausea, vomiting, gastritis, fatigability, hepatitis, hypersensitivity reactions, cutaneous reactions and rarely psychosis, seizures, joint pains etc were defined as side effects to treatment.
Status disclosure	Disclosure of HIV and TB to their spouse, significant partner or to one of their family member on whom the participant was being dependent.

SES – socioeconomic status, TB – tuberculosis, HIV – human immunodeficiency virus, CBNAAT – cartridge-based nucleic acid amplification test, ART – antiretroviral therapy.

Table 2 – Socio-demographic characteristics of participants associated with non-adherence[g] to ATT[a] and ART[b] in five districts of Karnataka, India (2019).

Socio-demographic details		Non-Adherent		Adherent		OR[c]	(95% CI[d])	p value
		n	(%)	n	(%)			
Total		125	(100)	375	(100)			
Age in years	18–27	10	(8.0)	24	(6.4)	0.71	(0.31–1.60)	0.265
	28–37	51	(40.8)	127	(33.9)	0.74	(0.46–1.18)	
	38–47	45	(36.0)	151	(40.3)	ref		
	48–57	12	(9.6)	59	(15.7)	1.46	(0.72–2.96)	
	≥58	7	(5.6)	14	(3.7)	0.59	(0.22–1.56)	
Sex	Male	82	(65.6)	233	(62.1)	ref		0.58
	Female	43	(34.4)	140	(37.3)	1.14	(0.74–1.75)	
	Transgender	0	(0.0)	2	(0.5)	–		
Religion	Hindu	119	(95.2)	355	(94.7)	ref		0.97
	Muslim	4	(3.2)	13	(3.5)	1.08	(0.34–3.40)	
	Others	2	(1.6)	7	(1.9)	1.23	(0.25–6.04)	
SES[e]	Lower class	18	(14.4)	70	(18.7)	1.73	(0.88–3.41)	0.27
	Lower middle	37	(29.6)	138	(36.8)	1.66	(0.94–2.93)	
	Middle class	35	(28.0)	87	(23.2)	1.10	(0.61–1.99)	
	Upper middle	29	(23.2)	65	(17.3)	ref		
	Upper class	6	(4.8)	15	(4.0)	1.11	(0.39–3.16)	
Family type	Nuclear family	116	(92.8)	364	(97.1)	ref		0.03[f]
	Joint family	9	(7.2)	11	(2.9)	2.56	(1.03–6.34)	
Wage loss	<250	29	(23.2)	130	(34.7)	ref		0.04[f]
	250–499	41	(32.8)	91	(24.3)	0.49	(0.28–0.85)	
	500–749	31	(24.8)	93	(24.8)	0.66	(0.37–1.18)	
	750–999	19	(15.2)	56	(14.9)	0.65	(0.34–1.26)	
	≥1000	5	(4.0)	5	(1.3)	0.22	(0.06–0.82)	

[a] Anti-tubercular therapy
[b] Anti-retroviral therapy
[c] Odd's ratio
[d] Confidence Interval.
[e] SES – Socio-economic status according to modified BG Prasad's classification.
[f] Statistically significant.
[g] Criteria for non-adherence used was <95% for ART and <90% for ATT (using entry in treatment cards). People fulfilling the criteria every month for last three months were classified as controls (adherent patients). Others were classified as cases (non-adherent).

significant people, co-trimoxazole prophylaxis were also associated with non-adherence.

On adjusted analysis, the risk factors for non-adherence were status non-disclosure (aOR = 2.06, 95% CI: 1.19–3.58), Zidovudine-based ART (aOR = 4.87, 95% CI: 2.69–8.83), presence of >3 side effects (aOR = 6.45, 95% CI: 3.03–13.74), non-receipt of co-trimoxazole prophylaxis (aOR = 9.90, 95% CI: 2.92–33.55) and not receiving counselling before ATT initiation (aOR = 5.25, 95% CI: 3.01–9.17) (Table 5).

4. Discussion

To our knowledge this is the first study in India to assess the factors associated with treatment non-adherence among people on concurrent ATT and ART. The major determinants for non-adherence were clinical and treatment related factors. The risk factors for non-adherence were Zidovudine based ART, and side effects to drugs. The protective factors for non-adherence were status disclosure to significant people, co-trimoxazole prophylaxis and counselling of patients before the initiation of ATT.

Non-disclosure of HIV status has mostly been linked to non-adherence, similar to studies conducted in South Africa (2008–10)[5] and in Ethiopia (2010).[17] Mazinyo et al[5] in their study showed non-adherence was 1.96 times more associated with non-disclosure of status when compared to adherent individuals. K A Rowe et al,[18] in their study mentioned disclosure of HIV status among the family and social circle builds up support and has a positively influenced adherence. The patients may not disclose their status to avoid social stigma. Status disclosure has the potential to draw support, both economically and emotionally from the family. This could result in shared responsibility of family members towards the diseased.

Side effects have been a significant factor in treatment adherence. In studies conducted in South Africa (2008–10) and Uganda (2009), side effect have resulted in non-adherence to the extent that they were lost to follow up.[5,19] Elbireer et al[19] in their study showed that side effects to drugs was 5.5 (OR) times more associated with non-adherence to treatment. Chang et al,[20] (Hong Kong 2000) also states side effects were 13.3 (aOR) times more associated with non-adherence.

Also there are studies which suggests side effects to drugs were not merely associated with non-adherence. Amuha GM et al,[21] (Uganda, 2008) in their study have expressed that there was no significant association between side effects and drug non-adherence. However, side effects to treatment leads to

Table 3 – Clinical characteristics of participants (≥18 years) associated with non-adherence[f] to ATT[a] and ART[b] in five districts of Karnataka, India (2019).

Clinical details		Non-Adherent		Adherent		OR[c]	(95% CI[d])	p value
		n	(%)	n	(%)			
Total		125	(100)	375	(100)			
Type of TB	Pulmonary	77	(61.6)	209	(55.7)	0.78	(0.51–1.18)	0.142
	Extra-pulmonary	48	(38.4)	166	(44.3)	ref		
Type of diagnosis	BC	63	(50.4)	184	(49.1)	ref		0.437
	CC	62	(49.6)	191	(50.9)	0.94	(0.63–1.42)	
Past history	New case	95	(76.0)	315	(84.0)	ref		0.031[e]
	Previously diagnosed	30	(24.0)	60	(16.0)	1.65	(1.01–2.71)	
Family history of TB	Yes	22	(17.6)	7	(1.9)	0.08	(0.03–0.21)	<0.001[e]
	No	103	(82.4)	368	(98.1)	ref		
Co-morbidities	Yes	18	(14.4)	11	(2.9)	0.18	(0.08–0.39)	<0.001[e]
	No	107	(85.6)	364	(97.1)	ref		
Smoking	Yes	15	(12.0)	38	(10.1)	1.20	(0.64–2.28)	0.331
	No	110	(88.0)	337	(89.9)	ref		
Alcohol use	Yes	22	(17.6)	46	(12.3)	0.65	(0.37–1.13)	0.061
	No	103	(82.4)	329	(87.7)	ref		
BMI(Kg/m^2)	Underweight	72	(57.6)	176	(46.9)	ref		0.152
	Normal	47	(37.6)	175	(46.7)	0.27	(0.18–0.40)	
	Overweight	6	(4.8)	20	(5.3)	0.89	(0.34–2.35)	
	Obese	0	(0)	4	(1.1)	–	–	
Duration of HIV (years)	<3	48	(38.4)	183	(48.8)	1.52	(1.01–2.31)	0.041[e]
	>3	77	(61.6)	192	(51.2)	ref		

[a] Anti-tubercular therapy
[b] Anti-retroviral therapy
[c] Odd's ratio
[d] Confidence Interval.
[e] Statistically significant.
[f] Criteria for non-adherence used was <95% for ART and <90% for ATT.

increased pill burden and hospitalisation. This creates a sense of ill-being and people will be scared to continue the treatment on a regular basis.

Zidovudine-based therapy is associated with greater risk of drug toxicity[22] and this may explain its association with poor adherence. On the other hand co-trimoxazole prophylaxis and ART are known to have a synergistic effect and known to reduce morbidity and mortality among people with HIV-associated TB.[23] This explains why those who received co-trimoxazole prophylaxis demonstrated better adherence to treatment than those who did not receive prophylaxis.

Counselling sessions before the initiation of ATT, have played a significant role in adherence to treatment. Similar findings have been demonstrated in studies conducted in Ethiopia (2010)[17] and Uganda (2009).[19] According to study findings of Gebremariam et al[17] also suggest that adherence counselling before initiation of treatment facilitates adherence. O' Donnell M R et al,[24] in their article opined that medication adherence and care for TB-HIV could be improved by team-based patient-centered care which empowers patients with counselling and support. Increased health care provider–patient interaction will create a sense of warmth and care among the participants.

4.1. *Strengths and limitations*

The adopted criterion for adherence was stringent and was according to the guidelines by the individual programmes. However, most of the other studies have employed less stringent criteria (<80% for ATT and <90% for ART - Mazinyo EW et al, 2008–10)[5]. The misclassification bias was ruled out during face-to-face interviews.

Some of the variables like side effects and status disclosure are always subject to individual and observer variations. For assessing adherence and co-morbidities, we used only history and past treatment records. We did not perform laboratory tests to confirm adherence. As blinding was not possible, we cannot rule out interviewer bias. Data entry errors cannot be ruled out as double entry and validation was not done.

4.2. *Implications*

Most of the barriers can be tackled by counselling, especially providing counselling before the start of ATT. Counselling can address issues of alcohol misuse, status non-disclosure and management of side-effects. Strengthening the services of counselling by providing a dedicated counsellor to manage people with HIV-associated TB may be considered. Programme should consider effective strategies to ensure increased coverage of co-trimoxazole prophylaxis and use of non-Zidovudine-based ART regimen. The recommended ART regimen for HIV-associated TB (similar to HIV without TB) in adolescents and adults is Tenofovir (TDF 300 mg) plus Lamivudine (3TC 300 mg) + Efavirenz (EFV 600 mg) in a single pill once a day.[25]

Though the study provides a good insight into factors associated with non-adherence to concurrent ATT and ART,

Table 4 – Treatment and programmatic characteristics of participants (≥18 years) associated with non-adherence[f] to ATT[a] and ART[b] in five districts of Karnataka, India (2019).

Treatment details		Non-Adherent		Adherent		OR[c]	(95% CI[d])	p value
		n	(%)	n	(%)			
Total		125	(100)	375	(100)			
Place of diagnosis	DMC	25	(20.0)	90	(24.0)	1.35	(0.81–2.25)	0.366
	Private labs	20	(16.0)	72	(19.2)	1.35	(0.77–2.36)	
	IRL[g]	80	(64.0)	213	(56.8)	ref		
Type of treatment supporter	Health care worker	96	(76.8)	212	(56.5)	ref		<0.001[e]
	Family/friend	28	(22.4)	160	(42.7)	0.38	(0.24–0.61)	
	Private/NGO	1	(0.8)	3	(0.8)	0.73	(0.07–7.16)	
Counselling before initiation of TB treatment	Yes	53	(42.4)	301	(80.3)	ref		<0.001[e]
	No	72	(57.6)	74	(19.7)	5.52	(3.57–8.55)	
HIV regimen	Tenofovir based	69	(55.2)	368	(98.1)	ref		<0.001[e]
	Zidovudine based	53	(42.4)	49	(13.1)	5.01	(3.13–7.98)	
	Others	3	(2.4)	7	(1.9)	1.98	(0.50–7.85)	
Side effects of treatment	Nil	41	(32.8)	220	(58.7)	ref		<0.001[e]
	<3	53	(42.4)	121	(32.3)	4.89	(2.71–8.82)	
	>3	31	(24.8)	34	(9.1)	2.08	(1.16–3.73)	
Status disclosure	No	60	(48.0)	109	(29.1)	2.25	(1.48–3.41)	<0.001[e]
	Yes	65	(52.0)	266	(70.9)	ref		
Co-trimoxazole prophylaxis	Yes	97	(77.6)	367	(97.9)	ref		<0.001[e]
	No	28	(22.4)	8	(2.1)	13.24	(5.85–29.9)	

[a] Anti-tubercular therapy
[b] Anti-retroviral therapy
[c] Odd's ratio
[d] Confidence Interval.
[e] Statistically significant.
[f] Criteria for non-adherence used was <95% for ART and <90% for ATT.
[g] Intermediate reference laboratory (IRL) with CBNAAT or any other CBNAAT centre.

Table 5 – Multivariable logistic regression model for factors[g] associated with non-adherence[f] to ATT[a] and ART[b] in five districts of Karnataka, India (2019).

Variable		Non-Adherent		Adherent		aOR[c]	(95% CI[d])	p value
		n	(%)	n	(%)			
Total		125	(100)	375	(100)			
Counselling before initiation of TB treatment	Yes	53	(42.4)	301	(80.3)	ref		<0.001[e]
	No	72	(57.6)	74	(19.7)	5.25	(3.01–9.17)	
HIV regimen	Tenofovir based	69	(55.2)	368	(98.1)	ref		0.013[e]
	Zidovudine based	53	(42.4)	49	(13.1)	4.87	(2.69–8.83)	
	Others	3	(2.4)	7	(1.9)	3.71	(0.60–22.7)	
Side effects of treatment	Nil	41	(32.8)	220	(58.7)	ref		
	<3	53	(42.4)	121	(32.3)	2.26	(1.25–4.08)	0.006[e]
	>3	31	(24.8)	34	(9.1)	6.45	(3.03–13.7)	0.001[e]
Status disclosure	No	60	(48.0)	109	(29.1)	2.06	(1.19–3.58)	0.012[e]
	Yes	65	(52.0)	266	(70.9)	ref		
Co-trimoxazole prophylaxis	Yes	97	(77.6)	367	(97.9)	ref		<0.001[e]
	No	28	(22.4)	8	(2.1)	9.90	(2.92–33.5)	

[a] Anti-tubercular therapy
[b] Anti-retroviral therapy
[c] Adjusted Odd's ratio
[d] Confidence Interval.
[e] Statistically significant.
[f] Criteria for non-adherence used was <95% for ART and <90% for ATT.
[g] Age, sex and variables found to be significantly associated on univariate logistic regression were included in the multivariate analysis, only significantly associated variables have been presented.

qualitative systematic enquiry is required to explore the 'why' and 'how' which will further guide the implications of the findings of this study.

5. Conclusion

We aimed to assess the factors associated with non-adherence to concurrent ATT and ART provided through a single window care approach at ART centres in south India. Non-adherence was associated with zidovudine-based ART, occurrence of side-effects, status non-disclosure, non-receipt of counselling before ATT and non-receipt of co-trimoxazole prophylaxis. This study identifies the key role of programmatic interventions to increase co-trimoxazole prophylaxis coverage, use of non-zidovudine-based ART and ensuring counselling before ATT to address the barriers like alcohol use, status non-disclosure and management of side-effects.

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Disclosure

The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the official position or policy of the NTEP or the State TB Office, Karnataka or the NTEP State Task Force Operational Research Committee, Karnataka or The Union, France.

Authors' contributions

TSR, SGK, BNS, NSS conceived and designed the study; TSR, SGK, RR, HJDM, BV collected and entered the data; HJDM, BV, HDS analysed, interpreted and visualized the data; HJDM, BV, HDS prepared the first draft; all authors critically reviewed the manuscript for important intellectual content and approved the final draft for submission.

Data availability statement

The data is available on request from the corresponding author SG Kishore (dr.kishoregowda@gmail.com).

Conflicts of interest

The authors have none to declare.

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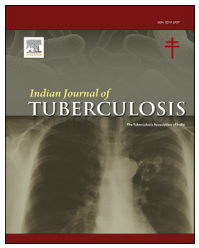

Original article

Tuberculosis notification: An inquiry among private practitioners in Pimpri-Chinchwad municipal corporation area of Maharashtra, India

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ABSTRACT

Background: The Government of India implemented mandatory TB notification policy since 2012. After that India's TB notifications from the private sector steadily increased; however, less is known about private practitioners' (PP's) experiences with TB notification. The present study aims to fulfil this gap.

Methods: We conducted a cross-sectional study during November 2019 to March 2020 in Pimpri-Chinchwad Municipal Corporation (PCMC) area of Maharashtra State. We used a mixed methods approach which involved a survey of 200 PPs and in-depth interviews (IDIs) with 7 PPs and 8 National TB Elimination Program (NTEP) staff. The data were presented in the form of frequencies and percentages and thematic analysis was performed on the qualitative data.

Results: The study revealed that most PPs (194 of 200; 97%) were aware of TB notification and 75% reported that they notify TB cases to the NTEP. Of those who notify, majority (129 of 145; 89%) reported that they use paper-based notification being the convenient method due to in-person visit and help by the NTEP staff. Only a third of PPs were aware of electronic notification methods. The main reasons behind low utilization of web based and mobile application were unfamiliarity and technical issues such as poor network connectivity. A third of PPs were aware about monetary incentives for notification and only 17% reported actual receipt of incentive at some point.

Conclusions: Our study identifies several areas where the NTEP can undertake interventions to strengthen the implementation of mandatory TB notification policy. Low awareness about electronic notification methods and preference for paper-based notification in this Study area suggest that more efforts are necessary for successful transitioning from paper-based to electronic notification system.

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1. Introduction

Tuberculosis (TB) is a lead infectious killer and globally there were estimated 10 million cases of TB and 1.4 million deaths in 2019.[1] India carries a fourth of global burden of TB cases. Several studies report that the private sector in India manages substantial proportion of TB cases either partly or completely and thus it has a key role in TB care.[2–5] It is noteworthy that despite being expensive, private practitioners (PPs) often remain the first point of help seeking for the reasons such as their availability in the community, trust and confidentiality that offers protection against social stigma associated with conditions such as TB.[3,5]

Nevertheless, there were several concerns about the private sector. First, until 2012, a vast majority of cases from the private sector were not being reported to the Revised National Tuberculosis Control Program (RNTCP) since TB was not a notifiable disease in India. Second, in the absence of notification/reporting of cases from the private sector, there was uncertainty about the actual burden of TB in the country. Third, outcomes of TB cases treated in the private sector were unknown.[4] Lastly, previous studies had reported poor diagnostic and prescribing practices involving deviation from the standard guidelines by PPs.[5–8]. Given the fact that PPs manage a significant proportion of TB cases, there is a clear need to identify ways to involve them in TB control activities.

To address some of the above described concerns, the Government of India brought mandatory TB notification policy in May 2012.[9] Fig. 1 based on the data in Table 1 indicates an increasing trend in TB notifications from the private sector with an apparent increase in total notifications.

The mandatory TB notification policy entails that all health care providers in all sectors shall notify TB cases which they treat, to the RNTCP. To facilitate the notification, the RNTCP launched a web-enabled application called 'NIKSHAY'. Under NIKSHAY, there are different modalities viz. paper, web and mobile application. The RNTCP report 2014 revealed that there were no TB cases notified from the private sector in New Delhi State in 2013.[11] However, a study by Yeole et al in Pimpri-Chinchwad Municipal Corporation (PCMC) area indicated that the private sector contribution to total TB notification was 20%. However, there were several barriers in the notification process since the implementation of mandatory TB notification policy was in a primitive stage.[17] Since 2018, Joint Effort for Elimination of TB (JEET) program was initiated by the RNTCP (which is presently referred as National TB Elimination Program or NTEP) to involve private sector in TB control activities. Tuberculosis notifications sharply increased with the private sector contribution from 2.7% (38,596 of 1,410,880) in 2013 to 28.2% (678,895 of 2,404,815) in 2019.[10–16] Nevertheless, little is known about PPs' awareness and actual experiences with TB notification process.

To address this gap, we undertook a study with following objectives: (1) To assess PP's awareness about the TB notification and modalities used for that; (2) To document PPs' experiences with TB notification process and barriers that they encounter.

2. Methods

2.1. Study area, design and the population

We undertook the study in PCMC area of Maharashtra during November 2019 to March 2020. The area is an industrial belt adjacent Pune city. The PCMC area has population of 1,727,692.[18] The study had a cross-sectional design and it used mixed methods approach. The quantitative component involved a face-to-face survey of 200 PPs and the qualitative component involved in-depth interviews with selected PPs and the NTEP staff.

2.2. Ethics approval

The study was approved by the Institutional Ethics Committee of Dr. D.Y.Patil Vidyapeeth, Pune (Ref. DYPV/EC/381/2019).

2.3. Sample size and study procedures

Given a small study grant spanned over 5 months, we decided to include a purposive sample of 200 PPs (which is approximately 24% of 831 PPs in PCMC area[17]) who were assessing and/or diagnosing and/or treating TB cases. We demarcated the study area into two parts and it was decided that two study staff would personally visit practicing PPs in that area during their clinic hours. Since PPs were busy during the evening time, the study staff visited majority of the PPs during the morning hours as per PPs' preference. PPs were interviewed using a semi-structured interview schedule after obtaining a written consent. An inquiry was made into following areas; educational qualification, years of practice, degree, awareness about TB diagnosis and notification, and barriers to notification etc. Besides structured interviews, we conducted 15 in-depth interviews (7 with PPs and 8 with NTEP staff). The NTEP staff included 4 TB health visitors (TBHV), 1 Medical officer and 3 JEET workers. The interviews with NTEP/JEET staff were conducted mainly to understand their experiences with PPs.

2.4. Statistical analysis

The data from semi-structured interviews were entered and processed using MS Excel 2013 and were presented in the form of frequencies, percentages, median and interquartile ranges (IQR). The data from in-depth interviews were processed for thematic analysis using MAXQDA (Version 18) program.

3. Results

3.1. Sample characteristics

Overall 223 PPs were approached of which 23 were not included in the study for the reason that they refused to participate in the study either due to no time or were unwilling.

Thus the study was conducted among 200 PPs. The median age for participants was 39 years (IQR 34–45). Seventy percent

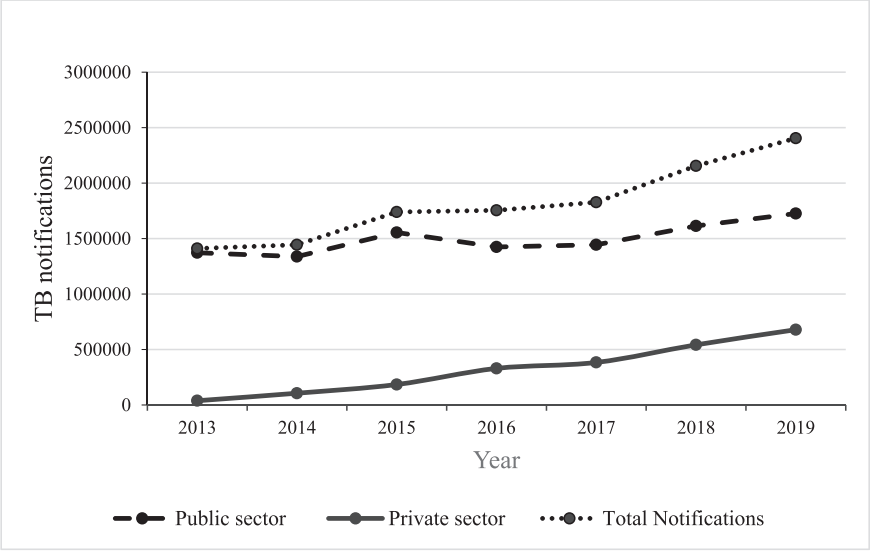

Fig. 1 – Tuberculosis case notifications in India.

were males. Forty-seven percent belonged to allopathy and remaining belonged to the alternative systems of medicine (Ayurveda, Unani, and Homeopathy). With regards to place of practice, 65% PPs reported private clinic, 27% reported private clinic as well as hospital, and 8% mentioned only hospitals (Table 2). The mean of years of practice was 11.7 (Standard Deviation ± 10.2).

3.2. *TB notification*

Table 3 presents findings related to TB notification. Most (194 of 200; 97%) PPs reported that they were aware of TB notification. Major sources of information on notification were the Government Resolution, NTEP staff and Continuing Medical Education (CME) sessions/conferences. Majority (165 of 194; 85%) of PPs were of aware of paper-based notification, whereas only a third were aware of NIKSHAY mobile app and web-based platform.

Three fourths of PPs (145 of 194; 75%) reported that they notify TB cases to the NTEP. Of these, 89% (129 of 145) PPs mentioned that they use paper based notification for notifying cases and 85% reported it as the convenient mode for the reasons such as ease of filling records, less time consuming, convenient to carry patients' follow up and ready format provided by NTEP/JEET staff etc. Reasons behind low utilization of web or mobile application were unfamiliarity and technical issues such as poor network connectivity. Further 88% (127 of 145) PPs reported that the NTEP/JEET staff regularly visit them to collect TB notification data. Among those who notify, majority (134 of 145; 92%) of PPs mentioned that they notify ≤2 cases per month, 6% mentioned 3–10 cases and only 2% mentioned more than 10 cases per month. Regarding awareness about notification related monetary incentives, only 37% (72 of 194) PPs provided affirmative response. However, only 25 of 145 (17%) PPs who mentioned that they notify TB cases, confirmed that they actually received monetary incentives at some point.

Table 1 – Tuberculosis case notifications in India.

Year	Public sector	Private sector n (%)	Total notifications (TB India Report)
2013	1,372,284	38,596 (2.7)	1,410,880
2014	1,337,528	106,414 (7.4)	1,443,942
2015	1,555,633	184,802 (10.6)	1,740,435
2016	1,424,769	330,186 (18.8)	1,754,955
2017	1,444,175	383,784 (21.0)	1,827,959
2018	1,613,504	542,390 (25.2)	2,155,894
2019	1,725,920	678,895 (28.2)	2,404,815

Data Sources: TB India Reports 2013-19[10–15]; For the Year 2015, WHO's Global TB Report 2016 was referred since we were unable to locate the figure in TB India Report 2016.[16]

3.2.1. *Barriers in notification process and suggested remedial measures*

The thematic analysis of data from in-depth interviews elaborated barriers in notification along with possible remedial measures for overcoming those barriers. Some private practitioners reported that the NIKSHAY mobile app is not user friendly and web based NIKSHAY platform consumes lot of time so they are unable to register the patient in the first attempt. One PP mentioned that there remains a communication gap among application developer, NTEP personnel and the end users (PPs). Sometimes there is lack of communication from the JEET staff, in which case, PPs get in touch with TB health visitors (TBHVs) due to their prior rapport and provide notification details to them. Some PPs do not have time to do notification because of busy clinic hours. So those PPs either prefer to call TBHVs or notify through JEET staff. Some PPs forget to notify cases and they need frequent reminders. PPs further expressed need for having clarity on periodic notification so that when the NTEP/JEET staff is planning to visit, they should inform and request PPs to keep records ready for submission. Some PPs also expressed a need for conducting refresher trainings on TB notification procedures. Lastly, a couple of PPs mentioned that things have become easier with

Table 2 – Sample characteristics.

(n = 200)	n (%)
Gender	
Male	141 (70.5)
Female	59 (29.5)
Age group (years)	
<30	21 (10.5)
31–40	93 (46.5)
41–50	63 (31.5)
>50	23 (11.5)
Branch	
Allopathy	94 (47.0)
Ayurveda	53 (26.5)
Homeopathy	49 (24.5)
Unani	2 (2.0)
Place of Practice	
Private clinic	130 (65.0)
Hospital	16 (8.0)
Private clinic and Hospital	54 (27.0)

the JEET staff appointment as they simply provide their data and the JEET staff helps to enter that data in NIKSHAY.

A couple of PPs explained another problem with notification. When they try to notify some cases, they find that those cases are already notified to the system by the laboratory personnel (who generally gets the microbiological confirmation before the clinician) though they are referred by the PP. For such patients referred by a PP to a private lab, the lab gets incentive and not the PP. If a patient is notified by the lab, it is impossible for the PP to give the outcome of that patient on NIKSHAY platform.

4. Discussion

Nearly after eight years of implementation of mandatory TB notification policy in India, our study among PPs in urban Indian setting reflects its current ground reality. It provides the first-hand information on PPs' awareness and experiences with TB case notification and methods used for the same. While most PPs included in the study were aware of TB notification in general, we found that majority were aware of paper–based notification and only a third were aware of electronic notification systems. The study by Uplekar et al reports that with the technical advances, some of the high TB incidence countries including India are now transitioning from paper-based notification to electronic case notification.[19] However, the present study shows that in practice, PPs still have a high preference for the conventional paper-based notification being the convenient mode. Visits by JEET/NTEP staff to collect notification data was the motivating factor for paper-based notification, whereas lack of knowledge and technical glitches were factors behind less preference for electronic notification methods.

PPs reported difficulties in registration with the notification system and its functionality. Similar observations were previously reported by Satpati et al.[20] Other studies reported barriers such as lack of knowledge about TB notification, TB related stigma and breach of confidentiality, and lack of trust and coordination with the government health system.[17,21] While confirming the existence of these barriers, our study further elaborates several other barriers such as unfamiliarity with the electronic notification systems, technical problems, communication gap between them and the NTEP/JEET staff etc. The Mandatory TB notification Gazette for PPs states that PPs will receive nominal incentive of Rs.1000/- per TB case. Of this Rs.500/- will be given on notification (once it is verified) and Rs.500/- on treatment completion.[22] Since June 2019, PPs were supposed to get incentives from the NTEP for notifying TB cases. However, the present study revealed that majority of PPs were not aware about such incentives and only few PPs confirmed actual receipt of incentives at some point. Lastly, our study also reveals the systemic lacunae in capturing the outcome of patients treated in the private sector.

To bridge the above described gaps in TB notification, the JEET Project was initiated. It spans across 45 cities with intensive engagement activities and an additional 361 cities have a light touch provider engagement model.[23] However, the JEET staff elaborated practical difficulties which they routinely encounter while dealing with PPs. These include PPs' reluctance to provide information on notified cases, busy clinics etc. The JEET staff further explained how PPs' long standing relationship with the NTEP staff (such as TB health visitors etc.) sometimes raise challenges for them and raise questions of trust as they took over NTEP staff's role. Our findings underscore a need for the NTEP to educate PPs regarding the notification procedures and make provision for appropriate incentives. The JEET staff can work as a link in the process of incentivization and to help provide feedback to PPs. Uplekar et al reported that the enforcement of mandatory TB notification policy remains weak in many high incidence countries including India and highlight a need to build in mechanisms for that.[19] The present study thus re-emphasizes the need for creating mechanisms and support systems for notification to make it realistic. With regards to target groups for notification data, both NTEP and JEET staff reported that chest physicians are the primary targets that are followed by general physicians and providers with BAMS (Bachelor's Degree in Ayurveda) qualification who prescribe anti-TB treatment. However, in our opinion, this strategy may lead to missing out few cases who are being managed by other specialities such as orthopaedics, gynaecology etc.

Our study has some limitations. First, the duration of the study was short, hence we could cover only around 24% of registered PPs in the study area. Though we made an attempt to include PPs who evaluate and treat TB cases at some point, the representation may be lacking. Second, PPs did not share information on actual notifications, so we had to rely on their verbal reporting of approximate monthly notifications. Lastly, many PPs were busy with their clinics and were reluctant to appear for an in-depth interview. Hence we could conduct only a handful of interviews that elaborated actual barriers and/challenges in notification.

In conclusion, though there is an overall increase seen in notifications since the launch of mandatory TB notification policy, our study reveals that adequate initiative toward TB notification is lacking from PPs' side and there remains a communication gap between the NTEP/JEET staff and PPs. Low awareness about electronic notification methods in this region suggests that aggressive efforts are still necessary for

Table 3 – TB notification.

	n (%)
Awareness about TB Notification (n = 200)	194 (97.0)
[a]Source of Information on notification (n = 194)	
Government Resolution	98 (50.5)
Informed by RNTCP staff	105 (54.0)
Media publicity	27 (14.0)
Informed by another private practitioner	15 (8.0)
CME sessions/conferences	59 (30.0)
[a]*Awareness about modes of notification(n = 194)*	
Web	68 (35.0)
Paper	165 (85.0)
Mobile app	62 (32.0)
Notification of TB cases (n = 194)	
Yes	145 (75.0)
No	49 (25.0)
Mode of Notification used (n = 145)	
Web	6 (4.1)
Paper	129 (88.9)
Mobile app	5 (3.4)
Web and mobile app	2 (1.3)
Paper and mobile app	1 (1.0)
Paper and web	2 (1.3)
Convenient mode for TB case notification (n = 145)	
Web	12 (8.0)
Paper	123 (85.0)
Mobile app	10 (7.0)
NTEP staff visit to collect TB notification data (n = 145)	
Yes	127 (88.0)
No	18 (12.0)
Approximate number of cases notified per month (n = 145)	
≤2	134 (92.0)
3 to 10	9 (6.0)
>10	2 (2.0)
Incentives	
Awareness about notification related monetary incentive (n = 194)	72 (37.1)
Receipt of monetary incentive at any time (n = 145)	25 (17.0)

[a] Multiple responses.

successful transitioning from paper-based to electronic TB notification. The possible solutions as brought out by this study are: notification related hands-on-training to PPs, making the NIKSHAY platform user-friendly for PPs by reducing number of fields to be filled up to only a few (for e.g. Patient and PP's name, phone number, microbiological confirmation, type of TB: pulmonary or extra pulmonary etc.) and remaining to be filled by the JEET staff, finding solution for appropriate incentives and their prompt disbursement. Simple non-monetary incentives such as appreciation letters/certificates can also help to motivate PPs in getting involved with TB notification.

Author contributions

Tushar Sahasrabudhe, Study conceptualisation, tool development, data analysis and manuscript writing. **Madhusudan Barthwal,** Study conceptualisation, tool development, comments on the manuscript. **Trupti Sawant,** Study conceptualisation, tool development, data entry and analysis, manuscript writing. **Sunil Ambike,** Tool development, data collection and input in writing of the manuscript. **Jayshri Jagtap,** Data collection and analysis and input in writing of the manuscript. **Shashank Hande,** Conceptualisation, tool development, data analysis and comments on the manuscript. **Sachin Atre,** Study conceptualisation, tool development, data analysis, manuscript writing. All authors approved the final draft of the manuscript.

Conflicts of interest

The authors have none to declare.

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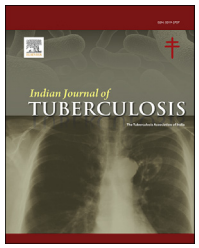

Original article

Era of TB elimination: Growing need to understand diversities of *Mycobacterium tuberculosis* lineages!

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ABSTRACT

Introduction: The *mycobacterium tuberculosis complex* (MTBC) has highly clonal population structure which made the organism spread globally mirroring human migration out of Africa and resulted in the formation of seven lineages. We conducted this study to determine the proportion of spoligotype lineages and drug susceptibility profile of *Mycobacterium tuberculosis* isolates among smear positive TB patients attending a tertiary care hospital in Mysore, Karnataka, India.

Methods: It is a descriptive study conducted at JSS Hospital a tertiary care centre at Mysore, India during 2018–19. The sputum smear positive samples were subjected to solid culture and drug susceptibility testing and spoligotyping for identification of lineages.

Results: Of the 100 samples which were culture positive, 94 isolates were clustered into five spoligotype international types with SIT-126 (EAI-5) being the largest cluster of 46 (46%) isolates, followed by SIT-62 (H1) with 24 (24%), SIT -26 (CAS 1-DELHI) with 20 (20%), SIT-53 (T1) with 03 (3%) and SIT-482 (BOV-1) with 01 (1%). Among the remaining six isolates, two had unique Cameroon spoligotypes and four were orphans

Conclusion: The study finding reveals that a diverse pattern of genotypes is circulating in the region of which EAI-5, Harleem (H1) and CAS-DELHI pattern forms the majority (88%). It is evident that there is a wide range of MTB genetic lineages in circulation and further research is needed to understand the diversity across the country.

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1. Introduction

Being evolved from a single ancestor, *mycobacterium tuberculosis complex* (MTBC) has highly clonal population structure which made the organism spread globally mirroring human migration out of Africa and resulted in the formation of seven lineages.[1–7] Lineage 1, the Indo-Oceanic strain is predominantly seen in East Africa, Southeast Asia and Southern India. Lineage 2, the East Asian strain seen in East Asia, Russia and South Africa. Lineage 3 (East African-India) isolates are usually from East Africa, northern India, and Pakistan. Isolates from Lineage 4 (Euro-American) are found in the Americans, Europe, North Africa, and the Middle East. Lineage 5 isolates are found in Ghana, Benin, Nigeria and Cameroon, and are referred to as *Mycobacterium africanum* subtype 1 (Clade1), while isolates from Lineage 6 are found in Senegal, Guinea-Bissau and The Gambia, and are referred to as *M. africanum* sutbtype 1 (Clade 2).[8] Lineage 7 was described from Ethiopia in 2013.[9] Molecular typing of *Mycobacterium tuberculosis* can be an essential tool in investigating the spread of such lineages, which may differ from one geographical location to another.[8] Application of molecular typing methods such as Restriction Fragment Length Polymorphism (RFLP), Spoligotyping and Mycobacterial interspersed repetitive units-Variable number of tandem repeats (MIRU-VNTRs) can be useful in determination of predominant lineages circulating in a geographical region and their transmission within the population. Over a decade, the most widely used molecular typing method was Spoligotyping because of its own advantages over RFLP and MIRU-VNTR. Spoligotyping is a fingerprinting tool which is based on Polymerase Chain Reaction (PCR) that can be used to analyse the polymorphism in the spacer sequences, which are present in the direct repeat (DR) region of MTBC strains. This molecular epidemiology is very much useful in differentiating between the strains, assessing their diversity, and measuring the proportion of the most circulating strains in this particular area. There are no studies regarding the circulating lineages in Mysore city in the South Indian state of Karnataka, which is one of important tourist attractions, both domestic and international, including Tibetan settlements in the nearby areas. Hence, this study was aimed to determine the proportion of spoligotype lineages and drug susceptibility profile of *Mycobacterium tuberculosis* isolates among smear positive TB patients attending a tertiary care hospital in Mysore, Karnataka, India (Fig. 1).

2. Methods

2.1. Settings

It is a descriptive study conducted at Department of Microbiology, JSS Medical college Hospital, Mysore, a city in Karnataka State of South India with a population of approximately 1,210,000. The JSS hospital is a tertiary care teaching hospital with 1800 beds and provides out-patient services to roughly 500 to1000 patients per day and it caters to a vast geographical area of Mysuru and its neighbouring districts. The Department of Microbiology has an established and functional designated microscopy centre (DMC) under National Tuberculosis Elimination Programme (NTEP) and is equipped with Binocular Light Emitting Diode (LED) fluorescent microscope. The DMC receives approximately 1200–1500 sputum samples per year for the diagnosis of TB. Apart from conducting, fluorescence smear microscopy using Auramine O the centre also conducts *M. TB culture* on Lowenstein Jensen's (LJ) media and its identification using MPT 64Ag.

2.2. Study population

We included all the consecutive sputum samples from presumptive TB cases which were received at DMC during June 2018 to May 2019.

2.3. Sample processing

The sputum smear examination for Acid Fast Bacilli (AFB) was done using Auramine phenol technique and examined under LED Fluorescent microscope. The results of smear examination were graded according to NTEP guidelines (Table 1). All the sputum smear positive specimens were processed for digestion and decontamination through standard sodium hydroxide-N-acetyl-L-cystein (NaOH-NALC) method and subjected to solid culture. The LJ Media which was prepared in-house using standard precautions were used for culture. The centrifuge deposits of the samples were inoculated on two Lowenstein-Jensen slants (L-J slants) and incubated at 37 °C till growth appeared on the slants or upto two months, whichever is earlier. After culture growth, the organisms were confirmed as MTBC using MPT 64Ag ICT Test.

After confirmation and identification of organisms, the drug sensitivity testing (DST) was performed at Intermediate Reference Laboratory (IRL), Bengaluru, using economic version of proportion method. As per the standard guidelines of NTEP, Isoniazid (H) with potency of 1gm to 1gm substance was used to obtain a final concentration of 0.2ug/ml; Rifampicin (R) with final concentration of 40ug/ml was used; Streptomycin (S) of 4ug/ml; and Ethambutol (E) of 2ug/ml as final concentration was used. For control, Para Nitro benzoic Acid (PNBA) was used, 75mg of PNB was dissolved in 2.5 ml of Di-methyl formaldehyde. All the antitubercular drugs were obtained from Sigma Pvt. ltd and were standardized before the test. Briefly, 2–3 colonies of M. tb grown on LJ-media was scrapped and emulsified in autoclaved distilled water and was matched with 0.5 McFarland solution. Master mix of 200ul was transferred to sterile MaCartney bottle containing one ml of autoclaved distilled water (10^{-2}). The same was repeated to obtain the final concentration of 10^{-4}. A loopful suspension from 10^{-2} and 10^{-4} was taken and inoculated into 2 sets of drug-containing media and the growth was recorded simultaneously. The media was observed for the growth once weekly, till the end of 42 days.

2.4. DNA extraction

An appropriate number of bacterial cells were transferred into a microcentrifuge tube containing 400 μl Tris-EDTA (TE) buffer. They were incubated for 20 min at 80 °C in a water bath to kill the bacteria and 50 μl of 10 mg/ml lysozyme was added

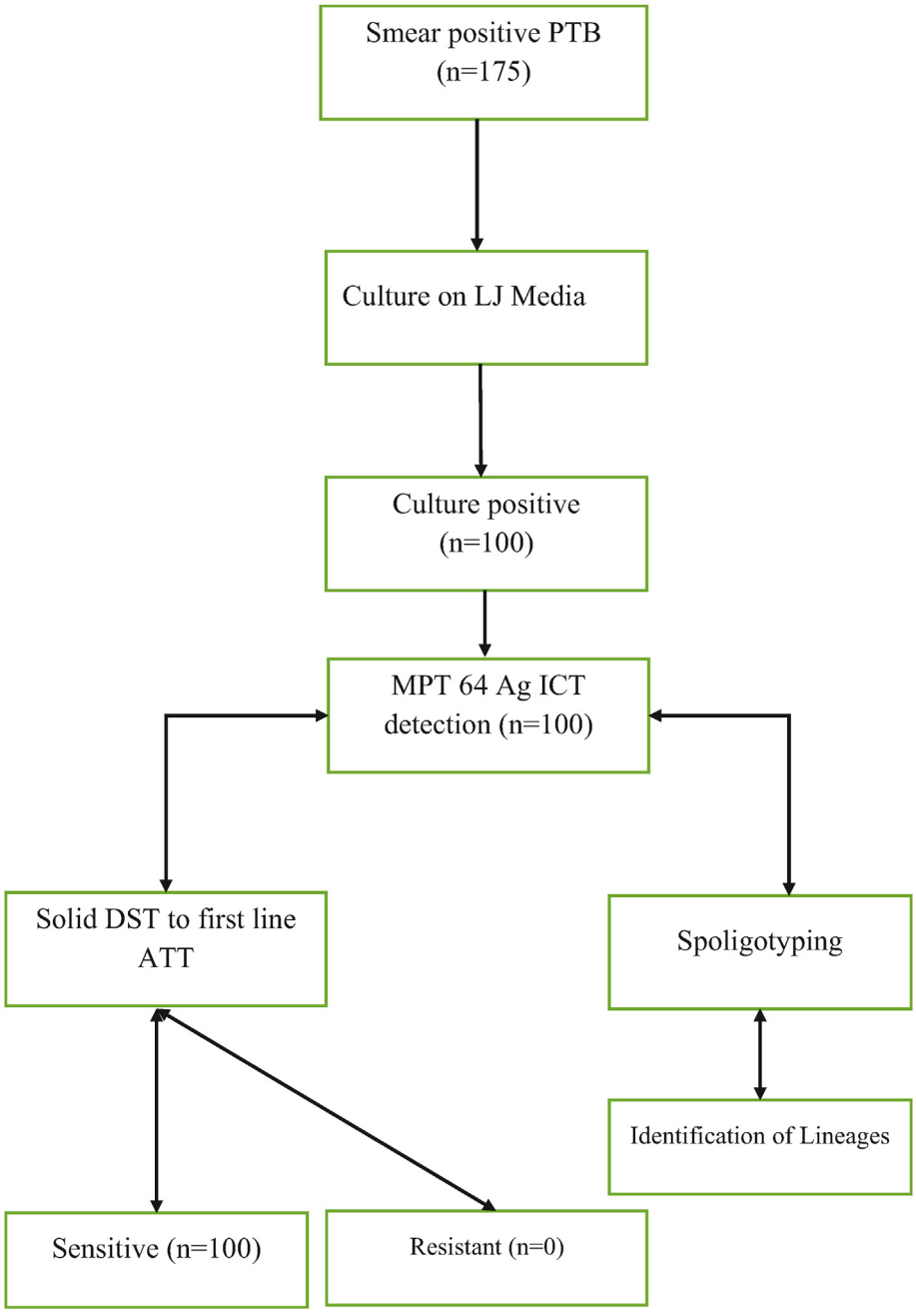

Fig. 1 – Drug susceptibility testing and Spoligotyping of MTB isolates in smear positive PTB patients attending JSS Hospital, Mysore. (2018–19).

Table 1 – Florescent smear microscopy grading of the sputum samples according to NTEP guidelines.

Examination (400X1 length = 40 Fields = 200 HPF	Result	Number of fields to be examined
Zero AFB/1 length	Negative	40
1-19 AFB/1 length	Scanty	40
20-199 AFB/1length	1+	40
5-50 AFB/1 field an average	2+	20
>50 AFB/1 field length	3+	08

AFB- Acid fast bacilli.

and vortexed then incubated for at least 1 hour at 37 °C. Then 75 µl of 10% Sodium dodecyl sulphate (SDS)/proteinaseK mix (5 µl Proteinase K (10 mg/ml) + 70 µl 10% SDS) was added, vortexed shortly and incubated for 10 min at 65 °C. One hundred µl of 5 M Sodium chloride (NaCl) was added followed by 100 µl -N-cetyl-N, N, N,-trimethyl ammonium bromide/ NaCl.

(CTAB/NaCl) mix (prewarmed at 65 °C) and vortexed until the liquid content became white and was incubated for 10 min at 65 °C. Approximately 750 µl chloroform/isoamyl alcohol mix (24:1) was added and vortexed for 10 seconds then

centrifuged for 10 min at 12,000 g at room temperature. The aqueous supernatant was transferred in a new tube and 0.6 volume (450 μl) isopropanol added, mixed carefully and incubated for 30 min at −20 °C. This was then centrifuged for 15 minutes at 12,000 g. Most of supernatant was removed and 500 μl of cold 70% ethanol was added and spinned for 5 min at 12,000 g. The supernatant was discarded and the tube spinned for 5 minutes at 12,000 g. The last μl of the supernatant was discarded cautiously and the pellet dried for about 10 min at room temperature. 80μl TE buffer was added and the DNA dissolved for 10 minutes at 60 °C. Quality and concentration of the DNA was checked using Nanodrop.

2.5. *Spoligotyping*

The Spoligotyping was carried out by amplifying the whole DR region using the commercially available kit (Mapmygenome, Hyderabad) according to a standardized method using the designated primers pairs of DRa and DRb6. BIO-RAD PCR was used for DNA amplification. The amplified PCR products were hybridized with nitrocellulose membrane having covalently linked 43 spacer oligonucleotides following the manufacturer's instructions. The hybridized fragments were identified using enhanced chemiluminescence system (GE Healthcare, UK). The spoligotypes were initially reported as 43 digits binary representation of 43 spacers, one (1) was scored for positive hybridization and zero (0) for negative hybridization. The binary codes were converted into octal codes. The octal codes were analysed using the SITVIT WEB (*http://www.pasteurguadeloupe.fr:8081/SITVIT_ONLINE*/) database to locate the families of spoligotypes.[10] A cluster was defined as two or more isolates with identical spoligotype patterns. However, if the spoligotype pattern in the SITVIT WEB database corresponded to only one isolate, then the pattern was called 'unique'. The spoligotypes that did not match any pattern in the SITVIT WEB database were defined as 'orphan'.

2.6. *Sample size and sampling technique*

A total of 175 smear positive sputum samples were included in the study. The total smear positive sputum samples during the study period was included, as conventional solid culture on LJ-Media would take time, convenient sampling technique was adopted, and the first 100 isolates were randomly chosen for the study.

2.7. *Data collection and procedures*

AFB smear microscopy data was collected from the DMC lab register, DST results was obtained from the IRL and spoligotyping results from mapmygenome laboratories, Hyderabad, India.

2.8. *Data variables*

The data variables required for the study was recorded from DMC lab registers and IRL DST register.

2.8.1. *Data entry and analysis*

The data was entered in Microsoft Excel and statistical analysis was done using OpenEpi (Open Source Epidemiologic Statistics for Public Health) software. Odds ratio with 95% Confidence Intervals were calculated and '*p*'value < 0.05 was considered statistically significant.

2.8.2. *Ethics approval*

Institutional Ethics committee approval was obtained from JSS Medical college & Hospital, JSS Academy of Higher Education and Research (JSSAHER), Mysore, before initiation of the study (Ref No: JSSMC/IEC/0102/13NCT/2018-19). As this study involves the samples received at DMC, waiver for the informed consent was requested and the same was obtained.

3. Results

A total of 175 smear positive PTB sputum samples were included during the study period and were cultured on LJ Media. The first 100 samples that yielded the growth of MTB were processed for DST and spoligotyping. The mean age of the study population was 42.82 years (±18.98 years).

All 100 (100%) isolates of MTB were found to be pan-sensitive to first line anti-tubercular drugs (Isoniazid, Rifampicin, Streptomycin and Ethambutol) by solid DST. Of them, 94 isolates were clustered into five spoligotype international types with SIT-126 (EAI-5) being the largest cluster of 46 (46%) isolates, followed by SIT-62 (H1) with 24 (24%), SIT -26 (CAS 1-DELHI) with 20 (20%), SIT-53 (T1) with 03 (3%) and SIT-482 (BOV-1) with 01 (1%). Among remaining 6 isolates, 2 had unique Cameroon spoligotypes and 4 were orphans (Table 2). EAI-5 was the most common clade (46%) is our study. Of the 2 HIV seropositive patients, one each belonged to SIT-126 (EAI-5) and SIT- 26 (CAS-DELHI). Among the 7 patients with diabetes mellitus, 4 had EAI-5 clade, 2 had CAS-DELHI and had H1 clade.

4. Discussion

To our knowledge, this is the first study conducted in the region to determine the lineages of MTB bacilli using spoligotyping technique. The study finding reveals that a diverse pattern of genotypes is circulating in the region of which EAI-5, Harleem (H1) and CAS-DELHI pattern forms the majority (88%).

Spoligotyping is one of the genotyping methods that helps in surveillance of the disease and to define the origin and spread of pathogen in the community for effective control and prevention of MTB infection. This study would give a valuable insight into the genetic diversity of circulating MTB strains in and around Mysore. There are no studies conducted in South India and our study reveals that there are diverse pattern of genotypes circulating in this region.

The majority of the identified lineage in our study were East African-Indian [EAI-5] (46%) followed by Harleem (H1) (26%), and CAS-DELHI(20%) respectively. The East African-Indian (EAI) lineage is more prevalent in Southeast Asia,

Table 2 – Spoligotyping lineages of MTB in new PTB patients attending JSS Hospital, Mysore, (2018–19).

Spoligotype Lineages[a]	Number (%) of isolates (n = 100)
Eai-5	46 (46)
H1	24 (24)
Cas-Delhi	20 (20)
Orphans	04 (4)
T1	03 (3)
Cameroon	02 (2)
Bov-1	01 (1)

[a] Eai-5- east african indian; H1- harleem; Cas-Delhi- central asian lineage; Bov-1- bovine lineage.

particularly in the Philippines, Myanmar, Malaysia, Vietnam, Thailand, India, and East Africa and yet is relatively rare in the Americas. Regarding the Indian subcontinent, EAI lineage strains are mostly found in the southern parts of India, whereas CAS lineage strains are predominantly present in the north India. The present study reveal that EAI-5/SIT-126 (46%) to be the predominant lineage which is in concordance with previously published data. According to the Global TB Report 2019, the estimated proportion of TB cases with MDR/RR-TB in India among new PTB cases in 2018 is 2.8% (2.3–3.5). Beijing genotype, a member of EAI lineage which originated from China is known to be associated with drug resistance and prevalent worldwide. No drug resistance has been reported in our study, this may due to the small sample size. We did not find any Beijing genotypes, however all other Non-beijing types were found to be pan-sensitive to first line antitubercular drugs.

A study from costal Karnataka reported that prevalence of Harleem (H1) was very low, but in our study it is the second largest cluster (24%) identified from the patients in and around Mysore, which suggest there could be difference in type of strains circulating in different parts within the state itself.

Literatures state that CAS1-Delhi lineage was supposed to be originated from India and later disseminated to other regions such as Saudi Arabia, Kenya, South Africa, Malaysia, Myanmar, Australia, the USA, and parts of Europe through frequent migration. CAS-DELHI in our study is the third most common lineage (20%).

Beijing strains have been reported to be in association with HIV seropositive patients but few other studies also reported association of HIV seropositive with other MTB lineages. A study from Malawi reported that HIV seropositive patients are more associated to have infection with Lineage 1. Our study reported the similar findings. Of 2 HIV seropositive, each one of the isolate has association with EAI-5 (Lineage 1) and CAS-DELHI (Lineage 3).

Interestingly we found two unique strains (Cameroon genotype) in our study which were not reported in South India earlier to the best of our knowledge. Four clinical isolates were identified as Orphans and did not match with any of the spoligotypes that are available in the SITVIT WEB database. These results suggest there may be few newer strains circulating in this region, which need further exploration.

No significant association was found between age, sex, sputum smear grading and the most common MTB lineages (EAI-5). This may be due to the limited sample size. Studies with larger sample size would have given an insight into the association of the lineages.

The study has following programmatic implications. First, the study provides base line data on the genotypes of MTB circulating in this region and would be helpful in differentiating between the strains, assessing their diversity, and measuring the proportion of the most circulating strains in a particular geographical area with high burden of TB. The programme should consider mapping and identifying of MTB genes periodically from all the districts in the country by building the capacity of all intermediate reference laboratories (IRLs). Currently, the country does not have any programmatic database of molecular epidemiology; India, being the highest TB burden country globally and with a set ambitious target of achieving elimination of TB by 2025 needs to adapt newer and innovative techniques for genetic surveillance. Second, there is a dire need for the programme to build its research capacity in molecular epidemiology by collaborating with nationally and globally acclaimed research institutes. Over the last few years, there has been a sea change in the field of TB diagnostics and drug regimens, and it is important to understand these impacts on MTB lineages. To conclude, there is a wide range of MTB genetic lineages in circulation and further research is needed to understand the diversity across the country.

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Disclaimer

The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the official position or policy of the NTEP or the State TB Office, Karnataka or the NTEP State Task Force Operational Research Committee, Karnataka or The Union, France.

Conflicts of interest

The authors have none to declare

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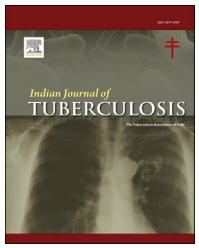

Original article

P2X7 polymorphism (rs3751143) and its reliability as a diagnostic biomarker for tuberculosis: A systematic review and meta-analysis

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ABSTRACT

Tuberculosis (TB) is one of the most important infectious diseases and is accounted for as the second most common cause of death due to infectious agents after HIV. It is estimated that a quarter of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*), and 5–10% of whom will be infected with active TB. Introducing a biomarker to predict TB can help control the disease and reduces the burden of mortality from this infectious disease. P2X7/P2X7R is one of the most important axes of the innate immune system, which its activity increases the clearance of the residual bacteria in macrophages. Numerous studies have shown the association between rs3751143 polymorphism and susceptibility to TB. The present study aimed to evaluate the diagnostic value of this polymorphism in predicting TB. In the current quantitative analysis, we studied the data from twenty relevant case–control studies, consisting of 10,544 volunteers. We found that, although rs3751143 polymorphism causes susceptibility to TB, but based on statistical analysis, it cannot be considered as a reliable biomarker for the diagnosis of TB.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). This disease has remained one of the threats to human health.[1,2] According to the World Health Organization (WHO) reports, in 2019, near the 10 million people were infected with TB worldwide, and approximately 1.5 million died from the disease.[2] Also, the lack of an effective vaccine for adults, the HIV pandemic, and the emergence of drug-resistant TB strains, all have exacerbated the condition and made it impossible to eradicate TB.[3–5] About a quarter of the world's population is infected with *Mtb*, and these people are considered as latent TB infection cases. However, about 5–10% of these cases develop to active-TB.[6] Numerous factors such as comorbidity with other infectious agents particularly HIV, genetic characteristics of *Mtb* strains,

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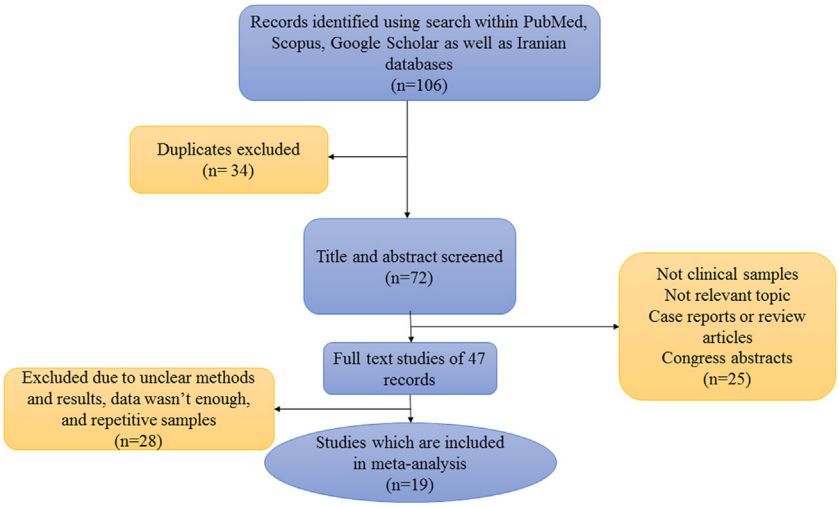

Fig. 1 – Flowchart of study selection.

host genetics and polymorphisms of genes responsible for the immune system, epigenetic events, and environmental conditions can have a significant impact on the development of the latent tuberculosis infection (LTBI) to the active TB.[7–9] Continuous monitoring of the molecular events and host genetic factors during the TB immunopathogenesis can lead to the development of therapeutic options and the introduction of biomarkers to predict the development of LTBI into the active disease.[10,11] The Purinergic receptor P2X ligand-gated ion channel 7 (P2X7) is encoded by the P2X7 gene located on chromosome 12q24.31.[12] P2X7R is a P2X7 receptor that is expressed on a wide range of immune cells, especially macrophages.[13] P2X7R is activated by the extracellular adenosine triphosphate (eATP) and then acts as an ATP gated ion channel so that its activity leads to influx of Ca^{2+}, Na^{+} and efflux of K^{+} out of the cell. Studies show that the ATP/P2X7R pathway triggers the several immune system mechanisms such as the formation of inflammasome and pro-inflammatory cytokines, induction of apoptosis, formation of reactive oxygen species (ROS), stimulation of phospholipase D, and facilitation of phagosome-lysosome fusion.[14] According to the literature review, P2X7R has an important role in the pathogenesis of infectious pathogens and its polymorphism can have a significant role in the final outcomes of the infectious agents.[13,14] Recently, much attention has been paid to the role of P2X7R in the intracellular killing of *Mtb*, and it has been suggested that polymorphisms in its canonical regions cause its loss of activity and consequently susceptibility to infection by *Mtb*. The role of polymorphisms in P2X7, especially rs3751143, was well investigated in susceptibility to TB in the previous studies.[15,16] The aim of our study was to evaluate the diagnostic value of rs3751143 polymorphism in predicting the active TB.

2. Methods

2.1. *Publication search strategy*

A computer-assisted search was applied for collecting the candidate studies regarding the role of polymorphism rs3751143 in the active TB infection. The search was performed using the keywords based on MeSH including "*M. tuberculosis*", "polymorphism", "P2X7", "rs3751143", and "mutation" up to July 2020, and the potentially relevant case–control studies were retrieved from PubMed, Scopus, Embase, Cochrane library and Google scholar databases.

2.2. *Study selection criteria*

The inclusion criteria consisted of 1) case–control articles, 2) English language articles, 3) articles which investigated the effect of rs3751143 polymorphism in *Mtb* infection, and 4) articles which contained data for the case and control groups. However, the articles published in non-English languages, review articles, case reports, letter to the editor and congress abstracts, studies that had ambiguous results or used repetitive samples, as well as the studies that used non-standard methods to diagnose *Mtb* infection and rs3751143 polymorphism were considered as exclusion criteria and were excluded from the study. The search strategy and the selection criteria process are presented in Fig. 1.

2.3. *Data extraction*

The required information including the first author, publication year, country, genotyping method, number of cases and controls, type of TB infection, distribution of rs3751143 polymorphisms, and Hardy–Weinberg equilibrium (HWE) are listed in Table 1.

2.4. *Statistical analysis methods*

Statistical analysis was performed using Meta-DiSc, version 1.4 software (Ramon y Cajal Hospital, Madrid, Spain). For this purpose, the pooled sensitivity and specificity with 95% confidence intervals (CIs) were measured using a random effect model, based on the Mantel-Haenszel method. Moreover, the pooled DOR was calculated using the DerSimonian-Laird method. Finally, a summary receiver operating characteristic (sROC) curve plot was generated. Heterogeneity between

Table 1 – Characteristics of included studies.

First Author	Year	Country	Type of TB	Genotyping Method	Case/Control	Case			Control			HWE *p*-Value
						AA	AC	CC	AA	AC	CC	
Amiri	2018	Iran	PTB	PCR-RFLP	100/100	76	21	3	40	58	2	0.001
Ben-Selma	2011	Tunisia	PTB/EPTB	PCR-RFLP	223/150	149	57	17	104	40	6	0.395
Chaudhary	2018	India	PTB/EPTB	ARMS-PCR	245/247	105	115	25	141	95	11	0.315
De	2017	India	PTB	PCR-RFLP	56/60	26	18	12	36	21	3	0.978
Fernando	2007	Southeast Asia	PTB/EPTB	TaqMan	185/167	89	83	13	169	89	11	0.845
Li	2002	Gambia	PTB	PCR-RFLP	325/297	261	58	6	256	37	4	0.057
Mokrousov	2008	Russia	PTB	PCR-RFLP	188/126	120	59	9	96	27	3	0.511
Nino-Moreno	2007	México	PTB	PCR-RFLP	94/110	53	33	8	70	38	2	0.215
Ozdemir	2014	Turkey	PTB/EPTB	PCR-RFLP	160/160	91	52	17	76	63	21	0.176
Sambasivan	2010	India	PTB	PCR-RFLP	156/100	89	55	12	71	21	8	0.002
Shamsi	2016	Iran	PTB	PCR-RFLP	100/100	33	66	1	83	16	1	0.817
Sharma	2010	India	PTB/EPTB	ARMS-PCR	281/177	110	88	6	48	3	300	0.515
Singla	2012	India	PTB/EPTB	PCR-RFLP	357/392	207	134	16	258	123	11	0.420
Souza de Lima	2016	Brazil	PTB	TaqMan	288/287	170	95	23	141	184	89	0.450
Taype	2010	Peru	PTB/EPTB	PCR-RFLP	619/513	434	167	18	347	149	17	0.838
Tekin	2010	Turkey	EPTB	PCR-RFLP	74/192	39	28	7	141	46	5	0.595
Velayati	2013	Iran	PTB	PCR-RFLP	79/50	42	35	2	37	12	1	0.981
Wu	2015	China	PTB	PCR-RFLP	103/87	33	49	21	51	27	9	0.075
Xiao	2009	China	PTB/EPTB	PCR-RFLP	96/384	51	37	8	221	119	44	0.001
Zheng	2017	China	PTB	TaqMan	1595/1521	972	551	72	900	544	77	0.655

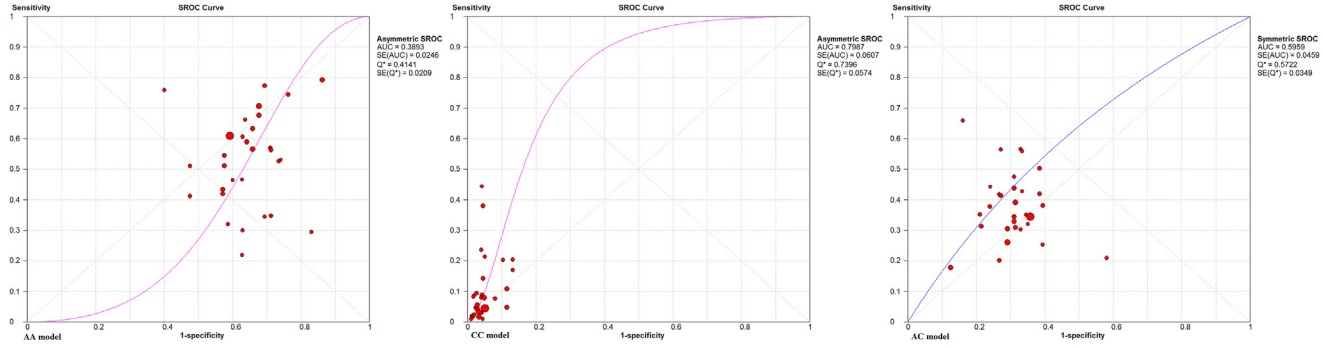

Fig. 2 – The SROC plots for each of the genetic models of rs3751143 polymorphism.

studies was determined in the present study, using the inconsistency index (I square).[17]

3. Results

Based on the initial search, 106 studies were obtained. After evaluating the title, abstract, and compliance with inclusion criteria, twenty studies were included in the present meta-analysis.[18–36] All studies were conducted during 2002–2018. In the present quantitative analysis, the data from 5324 TB patients (both pulmonary and extra-pulmonary TB) and 5220 controls were evaluated. The rs3751143 polymorphism was investigated in these studies by PCR-RFLP, ARMS-PCR, and TaqMan methods. The most important characteristics of the studies were summarized in Table 1.

Based on statistical analysis, the calculated pooled sensitivity for AA, CC, and AC genetic models was estimated 59.4% (58–60.7 with 95% CIs), 6% (5.3–6.7 with 95% CIs), and 34.3% (33–35.6 with 95% CIs) respectively. Also, the pooled specificity for AA, CC and AC models was measured 36.3% (35.2–37.4 with 95% CIs), 95% (94.5–95.5 with 95% CIs) and 68.6% (67.6–69.7 with 95% CIs) respectively. Furthermore, the pooled DOR for AA, CC and AC models was 0.637 (0.512–0.793 with 95% CIs), 1.908 (1.374–2.650 with 95% CIs) and 1.347 (1.110–1.634 with 95% CIs) respectively. Based on the results of the SROC Curve plot, the diagnostic accuracy of the homozygous codominant (CC) genetic model was higher compared to the homozygous/heterozygous codominant (AA and AC) models (Fig. 2).

Based on the available evidence, the rs3751143 polymorphism has a significant effect on susceptibility to TB; however, considering the value of the areas under the curve (AUC), it can be concluded that the rs3751143 polymorphism cannot be a reliable biomarker for predicting TB. In addition, in recently published articles, it seems that there is a relationship between rs3751143 polymorphism and susceptibility to TB in the Asian population, we show a significant association between rs3751143 polymorphism and risk of fallen to TB in the Asian population (OR: 1.48; 1.09–2.00 with 95% confidence intervals). But given the present meta-analysis, the rs3751143 polymorphism was not a reliable diagnostic biomarker for TB in the Asian population. The pooled sensitivity of AA, AC, and CC alleles was measured as 56.4%

(54.7–58.1 with 95% CIs), 37.3% (35.7–39 with 95% CIs), and 61% (53–69 with 95% CIs) respectively. Moreover, the specificity of AA, AC, and CC alleles in Asian was 43.8% (42.2–45.4 with 95% CIs), 69.3% (67.8–70.8 with 95% CIs), and 86.7% (85.6–87.8 with 95% CIs) respectively. The pooled Diagnostic-OR for AA, AC, and CC genetic models of the rs3751143 polymorphism in Asian population were 0.78 (0.48–1.26 with 95% CIs), 1.70 (1.09–2.63 with 95% CIs), and 0.92 (0.36–2.36 with 95% CIs) respectively. In final, based on the value of the areas under the curve (AUC), it was also revealed that the rs3751143 polymorphism was not reliable biomarkers in the Asian population which were calculated for different variant models AA, AC, CC as 0.509, 0.359, and 0.136 respectively.

4. Discussion

P2XR7 can play a significant role in the innate immune responses against intracellular pathogens such as *Chlamydia*, *Toxoplasma*, and *Toxoplasma*, and particular *Mtb*. Overall, P2XR7 activates several intracellular pathways including NLRP3 inflammasome, NF-kB, NFAT, GSK3β, and VEGF.[37] Fairbairn et al (2001) showed that the treatment of *Mtb* infected macrophages with ATP can lead to the increased apoptosis and intracellular killing of bacteria.[38] P2X7R is also involved in the pathogenesis of the Chlamydia genus, which is a mandatory intracellular parasite.[39] Coutinho-Silva et al (2001) in their study showed that ATP stimulation leads to the killing of 70–90% of *Chlamydia psittaci*.[40] Various pieces of evidence have been released about the role of the host genetic factors and sensitivity to the infectious factors.[41] P2X7 is one of the responsible genes in the immune system, which regulates the innate immune system responses [13–14]. According to the literature, pathogenic bacteria use a variety of strategies to block the activity of the P2X7R. For instance, *Porphyromonas gingivalis* that secretes anti-apoptotic enzyme nucleoside diphosphate kinase (NDK) causes the cleavage and hydrolysis of ATP, or *Mtb*, which secretes NDK converts GTP to active GDP.[42,43] Recent studies have indicated the role of polymorphism in the P2X7 gene and the sensitivity to infectious pathogens, particularly *Mtb*.[16,44] Numerous meta-analysis studies have suggested the role of P2X7 polymorphism in susceptibility to TB. Most of these studies suggest that the Glu498Ala mutant plays a pivotal role in the susceptibility of human beings to TB infection.[16,44–46] Chi et al (2018) stated that rs3751143 polymorphism can be considered as a biomarker for TB in the Caucasian population.[16] According to the literature review, biomarkers can screen the high-risk individuals for exposing TB and help for reducing the TB burden and mortality rate. IL-33 and adenosine deaminase is considered among the biomarkers in TB pleural effusion. However, the diagnostic accuracy of these markers for pulmonary TB is limited.[47,48] In the present study, we attempted to evaluate for the first time the diagnostic power of rs3751143 polymorphism as a biomarker for TB. Based on the results of statistical analysis, we found that the sensitivity and specificity of each of the homozygous codominant and heterozygous codominant genetic models were low. Therefore, the AUC values for each of the genetic models were not sufficient to make this polymorphism a reliable biomarker for the diagnosis of TB. The present study had several limitations: 1) we included only articles in English, 2) different qualification and underlying condition of individuals can be considered as a potential source of bias, 3) sample size was low, and 4) heterogeneity in some cases above (higher than 25%) which causes unreliability of the results.

5. Conclusion

Although rs3751143 polymorphism plays an important role in the susceptibility of individuals to TB, at the same time, according to the results of the present study, this polymorphism cannot be considered as a biomarker for predicting TB.

Conflicts of interest

The authors have none to declare.

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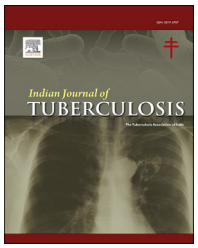

Original article

Th2 immune response by the iron-regulated protein HupB of *Mycobacterium tuberculosis*

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ABSTRACT

Background: HupB is an iron-regulated protein essential for the growth of *Mycobacterium tuberculosis* inside macrophages. To investigate if HupB induced a dominant Th2 type immune response, we studied the effect of rHupB on PBMCs from TB patients and by infecting mouse macrophages with wild type and *hupB* KO mutants.

Methods: PBMCs from pulmonary TB (n = 60), extra pulmonary TB (n = 23) and healthy controls (n = 30) were stimulated with purified HupB and the cytokines secreted were assayed. The sera were screened for anti-HupB antibodies by ELISA. Mouse macrophages cell line (RAW 264.7) was infected with wild type, *hupB* KO and *hupB*-complemented strains of *M. tuberculosis* grown in high and low iron medium and the expression of cytokines was assayed by qRT-PCR.

Results: Murine macrophages infected with the *hupB* KO strain produced low levels of the pro-inflammatory cytokines IFN-γ, TNF-α, IL-1, and IL-18 and high levels of IL-10. HupB induced IL-6 and IL-10 production in PBMCs of TB patients and down-regulated IFN-γ and TNF-α production. The influence of HupB was remarkable in the EPTB group.

Conclusion: HupB shifted the immune response to the Th2 type. Low IFN-γ and elevated IL-10 in EPTB patients is noteworthy.

1. Introduction

Tuberculosis (TB) is a major killer among infectious diseases, with 10 million people infected worldwide.[1] *Mycobacterium tuberculosis*, the causative organism resides predominantly in the lungs causing pulmonary tuberculosis (PTB) but can disseminate to other parts of the body, causing extra pulmonary tuberculosis (EPTB). In 2017, 14% of the 6.4 million new cases of TB were EPTB patients[1] where the mortality is about 14%.[2]

The pathogen, upon entry into the lungs of healthy human host is phagocytosed and killed by the alveolar macrophages. In the ensuing host-pathogen responses, bacilli that escape the host innate immune response reside as dormant bacilli in

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granuloma, whose formation is considered to be beneficial to both the pathogen and the host.[3] Most infected individuals mount an efficient cell-mediated immune response by producing a timely and coordinated expression of pro-inflammatory Th1 cytokines, including IFN-γ and TNF-α. While these cytokines primarily kill the bacilli, they also damage the host cells. Among them, tissue damage by TNF-α is well known and this is attributed to the cytokines eliciting an exaggerated inflammatory response and necrosis.[4] The mammalian host produces anti-inflammatory Th2 cytokines such as IL-10 and TGF-β to balance the ratio of pro-inflammatory *vs* anti-inflammatory cytokines. Clinical TB is often associated with a mixed Th1/Th2 response with a shift towards a dominant Th2 type.[5] The disturbance of the relative levels of Th1/Th2 cytokines not only determines the severity of the disease but also the dissemination of the pathogen to sites other than the lungs, causing EPTB.[6–8] The pathogen, in the lungs and in other sites can reside as dormant bacilli or be activated to produce disease manifestations in these compartments.[9]

The pathogen is known to disturb the Th1/Th2 balance by expressing antigens that produce a dominant Th2 response that facilitates the establishment of the pathogen and progression of the disease. Heparin-binding hemagglutinin antigen (HBHA;[10,11]) and a secreted protein CFP32[12] of *M. tuberculosis* are two such antigens, whose expression is associated with high levels of anti-inflammatory cytokines and a strong humoral immune response. Our focus here is on the iron-regulated protein HupB (Rv2986c). Experimental infection of the macrophage RAW cell line with a *hupB* KO strain of *M. tuberculosis* established the essentiality of HupB in the growth and survival of the pathogen inside macrophages.[13] This feature and the presence of high levels of anti-HupB antibodies in TB patients led us to suggest that HupB must confer an advantage to the pathogen in disease development. Our hypothesis is that it induces a Th2 type immune response and in this study, we present the cytokines produced by the PBMCs of TB patients stimulated with purified HupB. Additionally, we present the cytokines expressed by the RAW cell line infected with WT and *hupB* KO strains of *M. tuberculosis* respectively.

2. Methods

2.1. Bacterial strains

Three strains of *M. tuberculosis* were used in the study: WT, wild type (ATCC 27294), *hupB* KO (*M.tbΔhupB*) and the *hupB*-complemented (*M.tbΔhupB*/pMS101) strains respectively [the latter two strains were generated from a previous study (13)]. Cloning and expression of rHupB was performed using *E. coli* DH5α and *E. coli* BL21 (DE3) as hosts, respectively.

2.2. Growth of the mycobacterial strains

All the three strains were grown in Middlebrook 7H9 liquid medium, supplemented with 10% ADC (Difco, MD, USA) and 0.2% glycerol,[14] with hygromycin (250 μg/mL) and kanamycin (25 μg/mL) added to KO and the complemented strains respectively. Since HupB is induced upon iron limitation, the strains were grown in Proskauer and Beck medium supplemented with 0.02 and 8 μg Fe/mL (low and high iron respectively) as reported elsewhere.[14]

2.3. Experimental infection of RAW 264.7 cell line and analysis of cytokine expression

This was done as described earlier.[14] Briefly, each of the three strains of *M. tuberculosis* grown in low and high iron media were prepared by dispersing the bacteria using glass beads, diluting to McFarland 1 and finally adjusting to an $OD_{600\ nm} = 0.15$ for all the cultures. They were added to identical lots of the adherent RAW 264.7 cells, with infection done at MOI of 10:1 (bacteria: macrophage). After removing adherent organisms after 24 hours, the macrophages were lysed, the phagocytosed mycobacteria removed by centrifugation and the lysate was used for isolating the host total RNA.

1 μg of total RNA was converted to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen, CA, USA) as per manufacturer's instructions. This cDNA was used for measuring the transcript levels of cytokines, using the mouse-specific primers, listed in Table 1 (Primer Bank at https://pga.mgh.harvard.edu/primerbank/); β-actin was used as the

Table 1 – List of primers for quantification of cytokines.

Gene	Primer sequence
IFN-γ	For: 5′-ACAGCAAGGCGAAAAAGGATG-3′ Rev: 5′-TGGTGGACCACTCGGATGA-3′
TNF-α	For: 5′-CCCTCACACTCAGATCATCTTCT-3′ Rev: 5′-GCTACGACGTGGGCTACAG-3′
IL-1β	For: 5′-GCAACTGTTCCTGAACTCAACT-3′ Rev: 5′-ATCTTTTGGGGTCCGTCAACT-3′
IL-4	For: 5′-GGTCTCAACCCCCAGCTAGT-3′ Rev: 5′-GCCGATGATCTCTCTCAAGTGAT-3′
IL-6	For: 5′-CCAAGAGGTGAGTGCTTCCC-3′ Rev: 5′-CTGTTGTTCAGACTCTCTCCCT-3′
IL-10	For: 5′-GCTCTTACTGACTGGCATGAG-3′ Rev: 5′-CGCAGCTCTAGGAGCATGTG-3′
IL-12p40	For: 5′-TGGTTTGCCATCGTTTTGCTG-3′ Rev: 5′-ACAGGTGAGGTTCACTGTTTCT-3′
IL-18	For: 5′-GACTCTTGCGTCAACTTCAAGG-3′ Rev: 5′-CAGGCTGTCTTTTGTCAACGA-3′
β-actin	For: 5′-GGCTGTATTCCCCTCCATCG-3′ Rev: 5′-CCAGTTGGTAACAATGCCATGT-3′

internal control for normalization. qRT-PCR was performed in a reaction mixture containing 4 μL of cDNA, 5 pmol of each primer and 5 μL of 2X SYBR Green (Applied Biosystems, Warrington, UK) in a total volume of 10 μL. Amplification was done in the Eppendorf Mastercycler RealPlex 2 (Eppendorf, Hamburg, Germany) using the thermocycling program consisting of 95 °C for 5 min, 40 cycles of 95 °C for 15 sec and 60 °C for 1 min..

2.4. Cloning, expression and purification of rHupB

The 645 bp full length *hupB* gene was amplified using established PCR program[15] with the following modifications: the primers were (forward: 5′-CCC GGA TCC GAT GAA CAA AGC AGA GCT CA-3′) and (reverse: 5′-CCC AAG CTT TTT GCG ACC CCG CCG AG-3′) and the vector was pET-22b (+) (Novagen, Wisconsin, USA). This was done to obtain soluble rHupB. The recombinant *E. coli* BL21 (DE3) was induced with 0.1 mM IPTG (Hi Media Laboratories, Mumbai, India) for 15 h at 18 °C. The harvested cells were sonicated and the soluble rHupB in the supernatant was purified on Ni-Sepharose column (GE Healthcare, India) as detailed. The column was equilibrated with buffer A (20 mM phosphate buffer, pH 7.4 containing 20 mM imidazole and 0.5 M NaCl), loaded with the sample, washed to remove unbound proteins using buffer A supplemented with 50 and 100 mM imidazole respectively. The bound rHupB was eluted with buffer B (20 mM phosphate buffer, pH 7.4 containing 200 mM imidazole), concentrated and passed through Sephadex G-25 column (GE Healthcare, Buckinghamshire, UK) to remove imidazole. The purity of rHupB was checked by immunoreactivity with commercial anti-His monoclonal antibody (1:1000 dilution; Santa Cruz Biotechnology, Texas, USA) and with rabbit anti-HupB antibodies (1:2500). The rHupB was passed through agarose beads bound with polymyxin B (Sigma–Aldrich, MO, USA) to remove endotoxin based on manufacturer instructions and sterilized by filtration through 0.2 μm filter (Millipore Corporation, MA, USA).

2.5. Clinical studies with TB patients

2.5.1. Study subjects

Table 2 gives the demographic details of the subjects in this study. Patients were grouped as PTB based on radiological, clinical, and smear (three consecutive AFB-positive sputum samples) examinations. Clinical examination was predominantly used for diagnosis of EPTB. Exclusion criteria included cases with HIV, chronic diabetes and other conditions with immunosuppression. The control group included 30 healthy controls who did not present any symptoms of the disease.

10 mL of heparinized blood (for isolation of PBMC) and 2 mL blood for serum were collected from all the study subjects, with informed written consent obtained from them. The study was approved by the Institutional Ethical Committees of AP Chest & General Hospital and University of Hyderabad.

2.5.2. Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated by density gradient separation using Histopaque (Sigma–Aldrich, MO, USA), washed and resuspended in RPMI 1640 (Sigma-Adrich, MO, USA) containing 5% FBS (fetal bovine serum), 100 U/mL Penicillin, 100 mg/mL Streptomycin, and 25 μg/mL Amphotericin B (all of which were purchased from GIBCO, NY, USA). Viability of the cell suspension was determined by Trypan Blue dye exclusion test and used immediately for the immunoproliferation studies.

2.5.3. Stimulation of PBMCs with rHupB

2×10^5 viable PBMCs in a total volume of 200 μL were added to 96-well flat-bottomed tissue culture plates (Corning, NY, USA). Two such identical sets, performed in triplicate were stimulated with the reference antigen PPD (3 μg; Span Diagnostics Ltd., India) and rHupB (2.5 μg) respectively; additional controls included mitogen-stimulated PBMCs (1 μg of Concanavalin A; Sigma–Aldrich, MO, USA) and the negative control (PBMCs without any added mitogen or antigen). The plates were incubated at 37 °C in an atmosphere of 5% CO_2 for 72 h. 150 μL of the culture supernatant from each well was removed and stored at - 80 °C for assay of cytokines. The cells were subjected to MTT assay as follows to determine the cell proliferation: 10 μL of MTT [3 (4,5 dimethyl thiazol-2-yl) 2,5 diphenyl tetrazolium bromide; stock 5 mg/mL; Hi–Media, India) was added to each well, incubated for 4 h at 37 °C in an atmosphere of 5% CO_2, centrifuged the plates at 2000 rpm for 10 min, removed the supernatant, solubilized the purple formazan crystals with 100 μL DMSO (Qualigens, India) and measured the absorbance at 570 nm, with 630 nm as reference.

Table 2 – Demographic data of the study groups.

S. No.	Group	Sample size (n)	Age (years)	Sex		AFB positivity	
				Male	Female	(+)	(–)
1.	Pulmonary TB (PTB)[a]	60	38.2 ± 13.71	51	9	49	11
2.	Extrapulmonary TB (EPTB)[b]	23	28.48 ± 12.94	15	8	2*	
3.	Healthy controls (HC)	30	26.43 ± 3.53	25	5	ND	

[a] This group included 33 newly diagnosed cases, 42% of whom did not start anti-tubercular therapy (ATT) at the start of this study, while the others initiated therapy during a span of one month before recruitment for this study. 24 cases were defaulters of previously initiated ATT who presented with symptoms of the disease and 3 were cases of miliary TB, who were included in this group (as indicated in the WHO guidelines).

[b] EPTB cases comprised of 20 pleural effusion cases, 2 lymphadenitis cases and 1 ophthalmic Koch. * AFB testing of biopsy material was done for two lymphadenitis cases; the remaining 21 samples were not done; ND= Not determined.

Proliferation was expressed as stimulation index (SI) which was calculated as follows:

$$SI = \frac{\text{Test OD570}_{nm}\text{(PBMCs stimulated with antigen/mitogen)}}{\text{Control OD570}_{nm}\text{(PBMCs minus antigen/mitogen)}}$$

SI value ≥ 3 was considered positive.

2.5.4. *Bio-plex kit assay of cytokines*

The culture supernatants from the stimulated PBMCs were analysed for IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, IFN-γ and TNF-α using Bio-Plex Pro Human Cytokine Group I Panel 8-Plex (Bio-Rad, CA, USA) as specified by the manufacturer. The standards, blanks, and 1X coupled beads in the assay buffer were first prepared. 50 μL of the bead suspension was added after pre-wetting and washing the filter plate twice. 50 μL of standards/test samples were added to the respective wells, plate was sealed and incubated in dark at RT with shaking at 300 rpm for 30 min. After washing, 25 μL of the suitably diluted detection antibody was added, plate was sealed and incubated with shaking at 300 rpm for 30 min. After washing, 50 μL of streptavidin-PE was added, plate was sealed and incubated with shaking at 300 rpm for 10 min. The plate was washed, the beads re-suspended in 125 μL of assay buffer, mixed by shaking at 1100 rpm for 30 sec and immediately read on the Bio-Plex Analyzer using Bio-Plex Manager software (Version 5.0). The standard curve was plotted using a five-parameter regression formula.

2.6. *Screening for anti-HupB antibodies in the serum of TB patients*

ELISA was performed as reported earlier.[16] Briefly, 250 ng of rHupB was used as antigen and 100 μL of diluted (1:200) patients' serum was used as primary antibody. Goat anti-human IgG peroxidase conjugate (1:10,000 dilution; Sigma–Aldrich, MO, USA) was added as the secondary antibody and the colour was developed using freshly prepared tetramethyl benzidine TMB (1 mg in 10 mL of 0.05 M phosphate-citrate buffer, pH 5.0 and 3 μL of 30% H_2O_2) as substrate and the absorbance read at 450 nm in an ELISA reader (Model 680XR; Bio-Rad, CA, USA). Controls included antigen and antibody blanks and a known positive serum sample with high titre of anti-HupB antibody, established from our previous study.[16]

2.7. *Statistical analysis*

Data was analyzed using GraphPad Prism software version 5.01 (GraphPad Software, Inc., San Diego, CA) and Sigma Plot software (Version 10.0; Systat Software Inc., CA, USA). The non-parametric Mann–Whitney U test was used to compare the differences between the groups. The threshold for significance was set at $P < 0.05$ (two-tailed). Receiver-operating-characteristic curves (ROC) were calculated and expressed as areas under the curve (AUC), with an asymptotic 95% confidence interval (CI) using the MedCalc Statistical Software (Version 15.2.2; MedCalc Software, Ostend, Belgium).

3. Results

3.1. *Cytokines produced by infected macrophages in the experimental infection study*

Association of iron levels, HupB expression and synthesis of siderophores[14] makes it difficult to dissociate the role of iron and HupB on cytokine production. Since iron levels regulate HupB expression (13), the mycobacterial strains were grown in high and low iron medium. The fold change in the cytokines induced by iron-deficient vs iron-replete organisms is shown in Fig. 1A for each of the three strains of *M. tuberculosis*. While it can be argued that these effects could also be due to HupB, the constitutive expression of HupB in the complemented strain can be considered to negate the effect of HupB and reflect the iron status of the infecting strain. It can be inferred from Fig. 1A that the iron status of the infecting strain had little or no influence on IFN-γ, TNF-α, IL-1, and IL-18 but the iron-deficient WT which expressed HupB and HupB complemented mutant strains markedly triggered the release of IL-6 and IL-10 (~12 fold) and a moderate rise in IL-4 and IL-12 implying a definite role for HupB in inducing IL-6,IL-10, IL-4 and Il-12.

Since HupB is expressed by the WT *M. tuberculosis* only under iron limitation and we were interested to study the influence of HupB on cytokine expression, bacteria grown under iron limitation were included. Fig. 1B represents the relative expression of the cytokines induced by the KO strain *vs* the HupB-positive WT/*hupB*-complemented strains respectively; H37Rv LI/HI was included as the control. The KO strain induced expression of several cytokines, with maximal up-regulation of expression of IL-1β, IL-12 and IL-18 (mention fold increase here), and moderate increase in IFN-γ and TNF-α while down regulating the transcription of IL-6, IL-10 and IL-4.

3.2. *HupB-mediated humoral and cell-mediated immune response in TB patients*

3.2.1. *High levels of circulating anti-HupB antibodies in TB patients*

Antibodies against HupB were significantly high ($P < 0.0001$) in both the PTB and EPTB patient groups (Fig. 2). We reported earlier high titres of anti-HupB antibodies in EPTB patients,[14] while in this study the titres were almost identical in both PTB and EPTB patients. We think it is likely to be due to the inclusion of the defaulters and the miliary cases in the PTB group in this study.

3.2.2. *Immuno proliferation and cytokine production by PBMCs of TB patients stimulated by rHupB*

Stimulation Index (SI) values indicated better proliferation of the HupB-stimulated PBMCs of the two patient groups compared to the endemic normal group (Fig. 3); the sensitivity and specificity was higher than that achieved with PPD (Table 3).

Fig. 4 shows the cytokines released by the rHupB-stimulated PBMCs from the three study groups. IFN-γ was

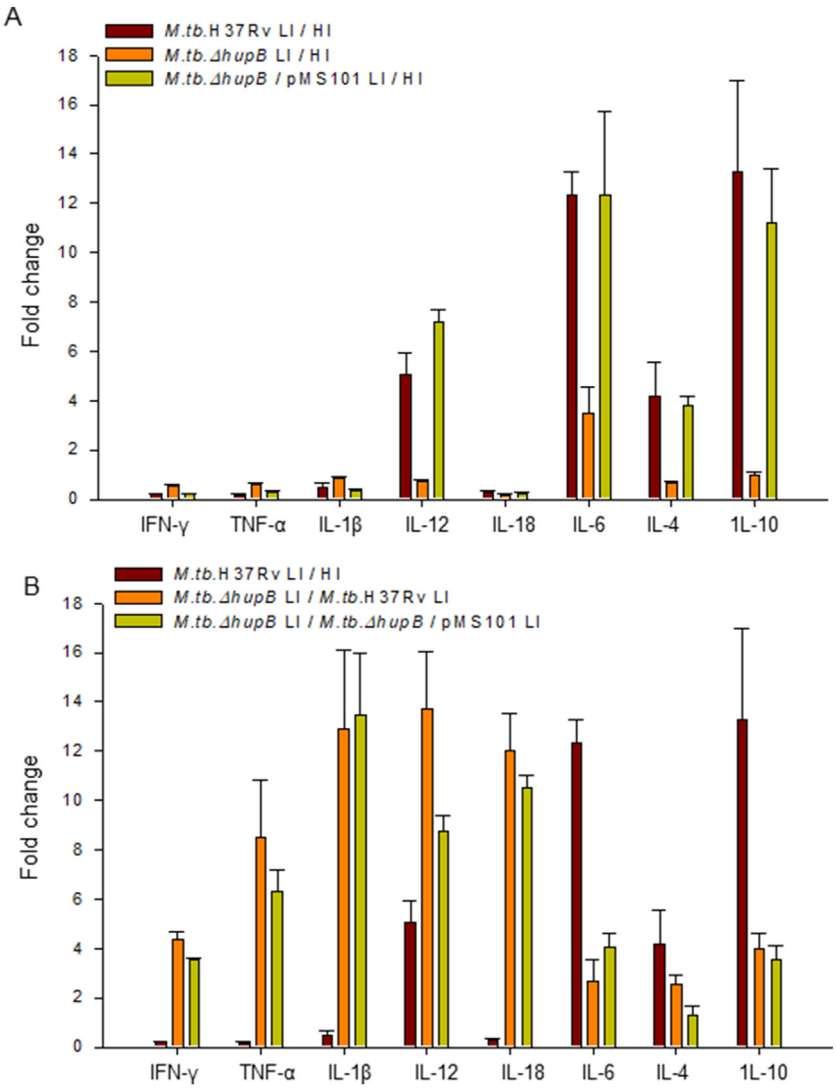

Fig. 1 – qRT-PCR evaluation of the cytokine transcripts produced by macrophages infected with the mycobacterial strains. Identical sets of the monolayer cultures of the mouse macrophage cell line were incubated for 24 h with iron-deficient and iron replete WT, KO and *hupB*-complemented strains of *M. tuberculosis* respectively. From the total RNA extracted from the macrophages, qRT-PCR was performed with primers specific for IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-10, IL-12, and IL-18 respectively; β-actin was included as the internal control for normalization. In Panel A, the fold change in cytokine expression, calculated by the $2^{-\Delta\Delta CT}$ method represents the ratio of the cytokine induced by iron-deficient vs iron replete organisms of the respective strain. Considering only the cytokines induced by iron-deficient organisms, Panel B represents the fold change of cytokine expression of the KO strain relative to the WT/hupB-complemented strains (*M. tbΔhupB*/*M. tb* H37Rv and *M. tbΔhupB*/pMS101/M.tb H37Rv); the fold change of the WT (*M.tb* H37Rv LI/HI) was included as a control. The error bars represent standard deviation calculated from two identical experiments performed in duplicates.

low in the PTB [median was 122.5 (interquartile range was 110.5–135) pg/mL]; and EPTB groups [median was 151 (113.0–184) pg/mL] when compared to the endemic healthy controls [median 228.5 (190.3–276.3) pg/mL]. TNF-α was low in PTB patients [median value of 458.0 (278–811) pg/mL] compared to healthy controls [median value of 749.5 (590.3–896.5) pg/mL) and was considerably high in EPTB patients [median value of 1239 (593.5–2015) pg/mL].

The anti-inflammatory cytokine IL-10 was high in EPTB patients (median 3768 (2530–4893) pg/mL) compared to PTB (median 2295 (1641–3044) pg/mL) and healthy controls (median 2760 (2460–2848) pg/mL IL-6 was elevated in PTB and EPTB groups, with median values of 20,642 (19,733–21,463) pg/mL and 21,251 (20,354–22,123) pg/mL while the healthy controls produced IL-6 with a median value of 17,456 (16,807–17872) pg/mL. IL-8, IL-4 and IL-2 showed no remarkable differences.

4. Discussion

The first report on HupB (reported as Histone like proteins) by Prabhakar and group[17] was followed by several other reports (13) which established it as a multi-functional mycobacterial

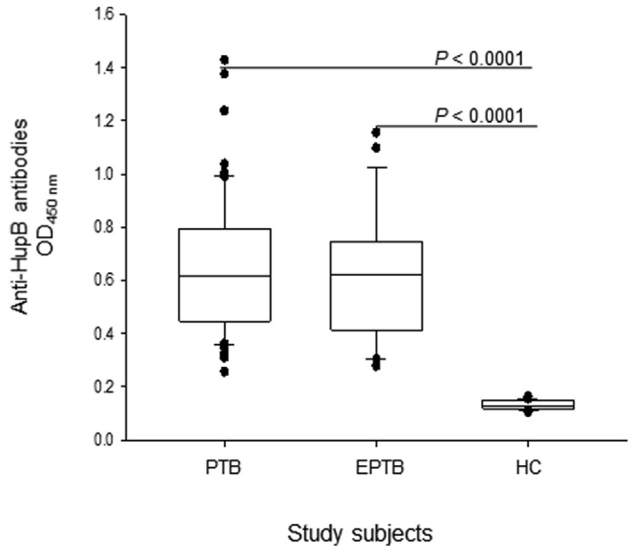

Fig. 2 – Screening of serum samples for anti-HupB antibodies by ELISA. Serum samples of pulmonary TB (PTB), extrapulmonary TB (EPTB), and healthy controls (HC) were tested in triplicate for anti-HupB antibodies. In the Tukey box plots, the box boundaries display the median and inter-quartile ranges and the whiskers display the maximum and minimum values excluding the outliers that are shown as points. The threshold for significance was set at P < 0.05.

protein. Our lab identified its association with iron acquisition and demonstrated its role as a transcriptional activator of the *mbt* operon in *M. tuberculosis*. The HupB-deficient KO strain produced negligible mycobactin and carboxy-mycobactin.

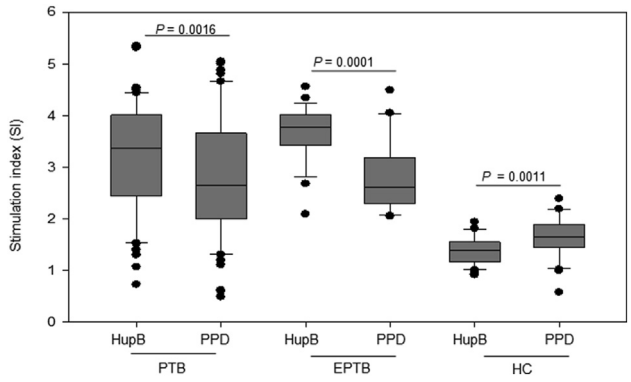

Fig. 3 – Proliferation of PBMCs upon addition of HupB. The figure shows the Tukey box plots representing the stimulation indices of PBMCs from pulmonary TB (PTB), extra pulmonary TB (EPTB), and healthy controls (HC) treated with rHupB and PPD respectively. The box boundaries display the median and inter-quartile ranges; whiskers display the maximum and minimum values excluding the outliers (shown as points). The threshold for significance was set at P < 0.05. The experiment also included the mitogen Concanavalin A. The mean SI values, not included in the figure are 3.068 ± 1.00, 3.465 ± 0.57 and 3.248 ± 0.49 respectively with PBMCs from PTB, EPTB and HC subjects.

The inability of the mutant to acquire iron, as seen with the *ideR* mutant strain resulted in low survival inside macrophages.[13] This study includes two components: first, an experimental infection of mouse macrophages with the WT and mutant strains and the second, a clinical component to study the response of PBMCs of TB patients to HupB.

Mouse macrophages were infected with HupB-positive WT and HupB-negative mutant strains of *M. tuberculosis* that made possible the identification of cytokines influenced by HupB. The reversal of the findings upon complementation of the mutant strain with *hupB* confirmed the role of HupB as identical responses were seen with WT and the *hupB*-complemented strains. From the elevated cytokine levels induced by the KO strain lacking HupB, it can be inferred that HupB down-regulated the pro-inflammatory cytokines IFN-γ, TNF-α, IL-1, and IL-18. Both iron and HupB caused the up-regulation of IL-6 and IL-10 by the macrophages.

The two anti-inflammatory cytokines IL-6 and IL-10 were elevated in the supernatants of the HupB-stimulated PBMCs of EPTB patients and the pro-inflammatory cytokines, IFN-γ and TNF-α were low in both PTB and EPTB patients. It is well established that high levels of circulating anti-HupB antibodies are seen in TB patients, and more so with dissemination of the disease in the EPTB cases.[15,16] We hypothesized that HupB, like HBHA (heparin binding hemagglutinin; discussed below) facilitates the establishment of the pathogen at sites other than the lungs and additionally tilts towards a Th2 type immune response, thus promoting pathogen survival inside the human host. This was addressed in this study by investigating the role of HupB on the immune response of the PBMCs from three study groups, two with disease (PTB and EPTB groups) and the third, a control group of healthy endemic individuals.

Iron status and the immune response of the mammalian host are inter-linked.[5] With the iron-withholding of the human host and the adaptation of bacterial pathogens well understood, it should not be not surprising that the tubercle bacillus has adapted to these conditions *in vivo*.[13] Cytokines influence iron within the macrophages where the pathogen resides. For example, IFN-γ lowered iron levels within the macrophages by down-regulating the transferrin receptors on its cell surface.[18] In another experimental study, it was shown that IL-4 activation of macrophages infected with *M. tuberculosis* caused upregulation of the transferrin receptor, thereby facilitating the uptake of iron by the macrophages. When IL-10 was administered as a therapy in Crohn's disease, there was an increase in the levels of serum ferritin in these patients.[19] From our observations in the laboratory and clinical settings, HupB down-regulated the expression of the pro-inflammatory cytokines IFN-γ and TNF-α and triggered the production of the anti-inflammatory cytokines IL-6 and IL-10. It is thus evident that HupB confers advantage to the pathogen. The Th2 shift also explains the antibody response that does not offer any protection to the host, by virtue of the intracellular localization of the pathogen.

The usefulness of HupB as an antigen over PPD deserves mention here. With high sensitivity and specificity seen with HupB, the proliferation of the PBMCs of the endemic normals showed baseline values compared to PPD. The study group is from an endemic region, vaccinated with BCG and exposed to

Table 3 – T cell proliferation of PBMCs of PTB and EPTB patients to HupB.

Antigen	Stimulation index mean ± SD			Area under the ROC curve						
	PTB	EPTB	HC	AUC	SE	P value	95% CI		% Sensitivity	% Specificity
HupB	3.194 ± 1.08	3.653 ± 0.55	1.378 ± 0.26	0.892	0.0358	<0.0001	0.766	0.97	81.71	96.77
PPD	2.830 ± 1.20	2.792 ± 0.67	1.643 ± 0.38	0.781	0.0468	<0.0001	0.693	0.853	70.89	85.29

PTB: pulmonary TB; EPTB: extrapulmonary TB; HC: healthy controls; AUC: area under the curve; SE: standard error; CI: confidence interval. The SI obtained with the mitogen Concanavalin A was 3.068 ± 1.00, 3.465 ± 0.57 and 3.248 ± 0.49 respectively when added to PBMCs from PTB, EPTB and HC subjects.

environmental mycobacteria. The healthy controls therefore responded to the heterogenic mixture of antigens present in PPD. On the contrary, HupB is expressed only by live organisms exposed to the iron-limiting conditions in the human host and therefore the PBMCs of the infected patients are sensitized to HupB.

IFN-γ and TNF-α are two clinically relevant pro-inflammatory cytokines in tuberculosis. They effectively eliminate *M. tuberculosis in vivo* by triggering the production of reactive oxygen and nitrogen species,[20,21] thus playing important roles in determining the progression of the disease. As these two cytokines target the elimination of the pathogen, their expression by the PBMCs needs to be compared with their levels in the circulation and at the site of infection to derive any meaningful conclusion. The mycobacterial proteins produced to lower these two cytokines will promote pathogen establishment. In this study, the role of HupB on the PBMCs was confirmative of such an antigen. Studies must be performed on larger groups determining the levels of these cytokines in circulation and in the lungs and extra-pulmonary sites must be determined. This will help to establish the influence of HupB on the pathogenesis of EPTB. IFN-γ is produced primarily by CD4 and CD8 T lymphocytes, with its production enhanced by other cytokines such as IL-12 and IL-18. It is possible that HupB lowers IFN-γ production through IL-12 and IL-18 in TB patients. This is substantiated by the higher production of IFN-γ, IL-12, and IL-18 by the mouse macrophages infected with the HupB-negative KO mutant strain. IFN-γ level is also controlled by the relative amounts of the anti-inflammatory IL-10. The latter was up-regulated by iron and HupB in the experimental macrophage infection study and in the HupB-stimulated PBMCs from patients, specifically EPTB. There are several reports on IL-10 down-regulating the production of IFN-γ,[22,23] possibly by inhibiting IL-12, the T_H1-polarizing cytokine.[8,24] IL-10 and TGF-β, another immuno-suppressive molecule must play important roles in the establishment of the disease, with high levels of these cytokines reported in macrophage and neutrophil-rich bronchoalveolar lavage (BAL) fluid of patients.[25] It will be meaningful to see how HupB influences the expression of these cytokines *in-vivo* at the site of infection.

IL-4 and IL-6 production by the rHupB-stimulated PBMCs corroborated other reports on their role in disease development. IL-4 was reported to be unaffected in TB patients.[26] Suppression of IFN-γ coupled with steady-state of IL-4 production contributes to the persistence of the pathogen and thus the progression of tuberculosis in susceptible individuals.[27] IL-6 known to interfere with the functioning of IFN-γ[28] was elevated in all the TB groups in this study. This chemokine contributes to pathogen containment by attracting both acute and chronic inflammatory cells of active infection.[29]

Little importance was given to the role of humoral immunity in disease development since the cell-mediated immune response, specifically of the Th1 type was thought to contribute to the containment and killing of *M. tuberculosis* within the macrophages. This, however, is changing with reports of mycobacterial antigens shifting the immune response to a dominant Th2 type associated with the production of antibodies. Since the humoral response to mycobacterial antigens, such as antigen 85A, 85B, ESAT-6, 30 kDa protein are well documented,[23,30] we wanted to understand how HupB influenced the immune response, particularly in EPTB patients with disseminated disease. HupB shares similar structure and immune response with the *M. tuberculosis*-specific heparin-binding haemagglutinin (HBHA), an antigen known to be involved in dissemination and establishment of *M. tuberculosis* in sites other than the lungs.[11,31–33] Both of them are iron-regulated proteins, present on the cell surface and possess unique lysine and arginine-rich C-terminal region, whose methylation by post-translational modification is mediated by a common methyl transferase.[31] The methylation of HBHA has been demonstrated to facilitate the interaction with the epithelial cells and contribute towards eliciting an effective immune response.[11] Further studies are needed to understand how methylation of specific lysine/arginine residues by the bacterial methylases affects its functioning. This is needed in light of our recent study, which showed for the first time how methylation of HupB by the host histone methyl transferase SUV39H confers protection to host.[34]

It is well documented that the type and numbers of the immune cells stimulated with mycobacterial antigens causes variation in the profile of the circulating cytokines, at the site of infection or on PBMCs isolated from the blood of TB patients.[35] Hence, it may not be appropriate to extrapolate the above observed effects of HupB on the PBMCs of TB patients to the events occurring at the site of infection. It is well known that IFN-γ levels are high at the site of infection but low when PBMCs are stimulated with mycobacterial antigens.[35] Another noteworthy aspect is that the PBMCs, a heterogenous mixture of different types of immune cells can produce different levels of cytokines based on the percentage of individual cell types; for example, the levels of IL-10, can vary based on the percentage of monocytes present in the PBMC preparation.

In conclusion, HupB expressed by *M. tuberculosis* promotes Th2 type immune response by increasing the ratio of the anti-

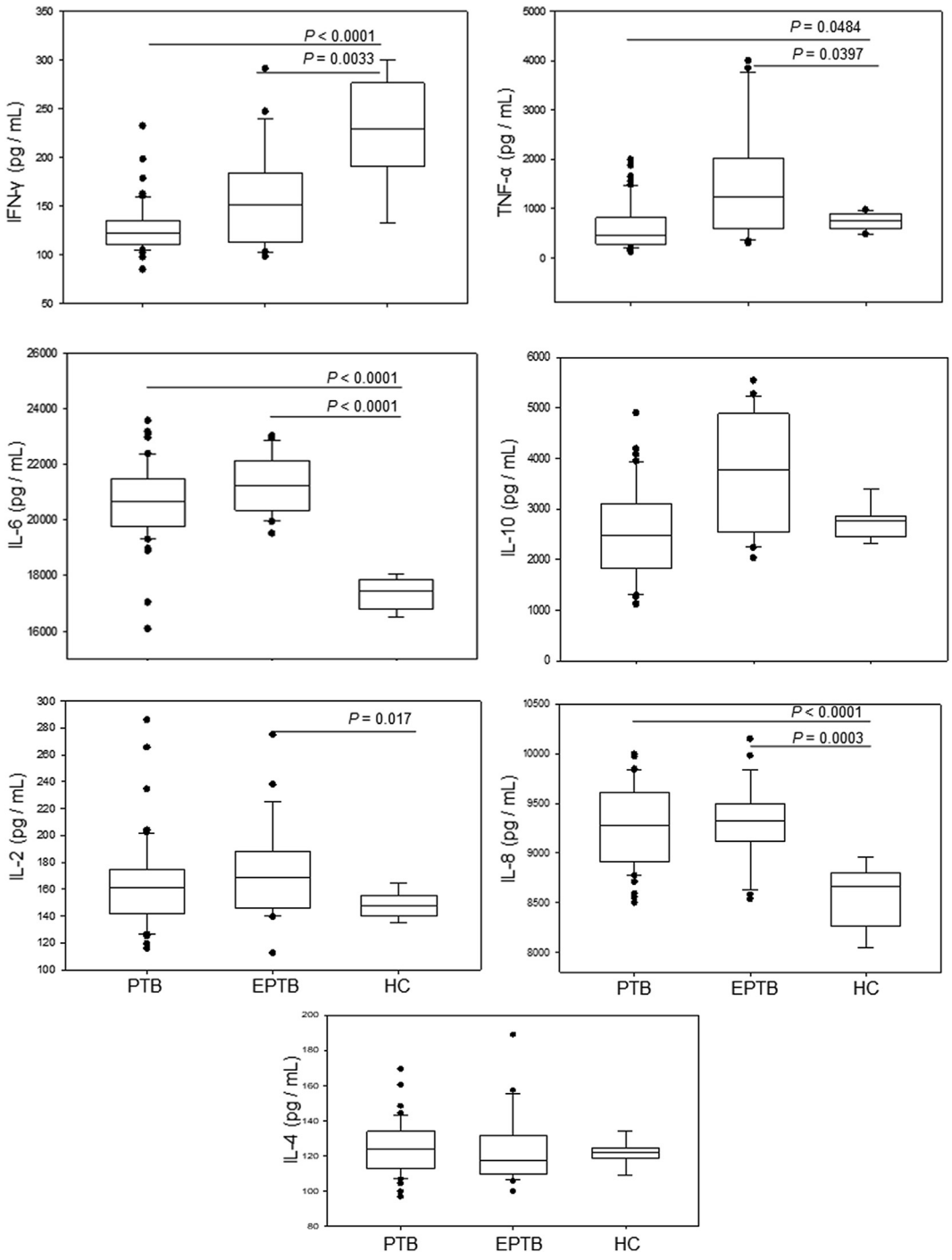

Fig. 4 – Expression of cytokines by PBMCs stimulated with rHupB. The culture supernatants of the PBMCs stimulated with rHupB were assayed for cytokines as described in Methods. The Tukey box plots represent the levels of IFN-γ, TNF-α, IL-6, IL-10, IL-2, IL-4 and IL-8 expressed by pulmonary TB (PTB), extra pulmonary TB (EPTB) and healthy controls (HC). The box boundaries display the median and inter-quartile ranges and the whiskers display the maximum and minimum values excluding the outliers (shown as points). The threshold for significance was set at P < 0.05.

inflammatory cytokine IL-10 to the pro-inflammatory IFN-γ in TB patients. This effect is more pronounced in EPTB patients as it appears highly likely that HupB, like HBHA promotes pathogen establishment in epithelial cells, promoting dissemination to sites other than lungs. HupB has been explored as a candidate target for tuberculosis control[36] and our findings here support its candidature.

Author contributions

MS and VS conceptualized and designed the study. MC and VJ collected patient data and samples. MC carried out data entry, experiments and data analysis. KS helped in selection, recruiting and direct interaction with patients for blood

collection. MC and MS prepared and finalized the manuscript, with critical suggestion inputs from VS.

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Ethical approval

The study was approved by the Institutional Ethical Committees of AP Chest & General Hospital and University of Hyderabad. Informed written consent was obtained from all study participants.

Conflicts of interest

The authors have none to declare.

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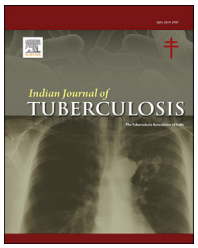

Short communication

Adherence to Isoniazid Preventive Therapy among children living with tuberculosis patients in Delhi, India: An exploratory prospective study

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ABSTRACT

INH Preventive Therapy (IPT) substantially reduces the risk of incidence of TB disease in pediatric household contacts of TB patients. The National TB Elimination Program (NTEP) of India prescribes a daily regimen of Isoniazid to all under-6 pediatric contacts for 6 months duration.

We conducted, this exploratory prospective study (June to Nov' 2020) to assess adherence to IPT and reasons for nonadherence among child contacts of microbiologically confirmed, drug sensitive, non-PLHIV Tuberculosis patients in Delhi, India. The study outcomes included the initiation, adherence and completion of IPT. The caregivers of the child TB contacts were interviewed face to face by the field investigator. The data were entered on EpiData 3.1 and analysed with IBM SPSS 25.

The INH adherence was assessed in a total of 86 household child TB contacts. IPT had been initiated in 62 (72.1%) child TB contacts of which 61 (98.4%) received INH within 1 month of starting of ATT-DOTS therapy in the index TB patient of the household. Furthermore, the failure to initiate IPT was reported by 24 (27.9%) child TB contacts. Within the cohort of child TB contacts who were not initiated with IPT, the ATT-DOTS duration in the index-TB patient was ≥5 months in 18 (75%) cases, 1–2 months in 3 (12.5%) cases, and <1 month in also 3 (12.5%) cases. Reasons for non-initiation (n = 24) were reported as refusal by the family in 12 (50%) cases mostly due to concern over side-effects of the drug, while non-provision of the drug by the DOTS provider was also observed in 12 (50%) cases.

The mean (SD) INH adherence in the INH initiated cohort was 5.6 (2.0) (n = 62). Reasons for INH non-adherence were attributed to forgetfulness (n = 23, 37.1%), carelessness (n = 24, 38.7%), and intermittent stopping of the medication (n = 17, 27.4%) on the child falling sick, perceived drug side effects, and running out of drug stocks.

INH non-adherence defined as at-least two missed INH doses in the previous 7 days was observed in 47 (54.7%) participants (n = 86). On bivariate analysis, none of the household

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sociodemographic characteristics showed any statistically significant association with the rate of INH non-adherence in the child TB contacts.

The findings of the present study indicate the need to periodically assess adherence and persistence to IPT in the child TB contacts as high intermittent missed dosing rates can undermine the effectiveness of IPT in preventing incident disease.

1. Introduction

Tuberculosis (TB) is an airborne, infectious bacterial disease, which is leading cause of under-5 mortality in endemic regions with most cases occurring from lack of treatment.[1] According to the World Health Organization (WHO), 1.2 million children contracted TB worldwide during 2019.[2] More than 80% of pediatric tuberculosis deaths globally occur in the under-5 age-group of which 70% are concentrated in the WHO South East Asian and the African regions.[1] In India, the country with the highest global burden of TB, child TB is estimated to constitute 10% of the total adult incidence, albeit, with significant underreporting of cases.[3] Moreover, the high burden of latent tuberculosis infection, correlates with high incidence of TB disease among household child contacts of TB patients usually within 1 year of primary infection with Mycobacterium tuberculosis.[4,5]

Nevertheless, it is well-established that Tuberculosis Preventive Therapy (TPT) including an effective regimen containing Isoniazid (INH) can reduce the risk of TB disease by more than half in children aged <15 years.[6] Currently, the National Tuberculosis Elimination Program (NTEP) in India prescribes a daily regimen of Isoniazid to all under-6 pediatric contacts of microbiologically confirmed drug sensitive TB patients for a duration of 6 months, which is consistent with the WHO guidelines.[7]

A standardized taxonomy of INH nonadherence can be classified as: (i). late initiation or non-initiation, (ii). discontinuation or lack of completion, and (iii). suboptimal adherence due to intermittent missed dosing.[8] Although, inadequate implementation of IPT in terms of delayed initiation and lack of completion have been reported from Southern and Central India, the prevalence of intermittent missed INH dosing were not assessed in prior studies.[9,10] However, the phenomenon of high prevalence of nonadherence to long-term therapies' especially when the perceived risk of disease is low, renders it crucial to identify the extent of non-adherence to IPT and the associated health-system and patient related barriers in achieving optimal INH adherence. Furthermore, significant periods of drug holiday due to lowering of adherence could potentially undermine the effectiveness of INH chemoprophylaxis in protecting against TB disease and contribute towards drug resistance. Consequently understanding the patterns of INH nonadherence is crucial towards understanding the need for evaluating and incorporating appropriate IPT adherence support within the NTEP.

1.1. Objective

To assess adherence to Isoniazid Preventive Therapy and reasons for nonadherence among child contacts of Tuberculosis patients in Delhi, India.

2. Methods

2.1. General setting

This study was conducted in the Central district of Delhi. As per the Indian TB control guidelines, all household contacts of a TB case initiated on anti-tubercular treatment (ATT) require to be screened for active TB, and in its absence, the child contacts aged below 6 years, are provided the Isoniazid Preventive Therapy (IPT) for six months.

Study design, setting, and participants: We conducted, this exploratory prospective study from June to Nov' 2020. Details of index microbiologically confirmed adult TB patients currently on ATT after exclusion of those with MDR-TB/XDR-TB/extra-pulmonary-TB/PLHIV were extracted from their TB treatment cards, who were then contacted by trained field investigators. The index TB patients having children aged below 6 years in their households were asked for consent to visit and to interview the primary caregiver of the asymptomatic children to assess the initiation and persistence to the recommended IPT.

Operational definitions:

(i). Child TB contact: A child below 6 years of age without any TB disease symptoms living in the same household as the index-TB patient on ATT-DOTS.
(ii). INH non-adherence: A child TB contact who received less than six doses of the prescribed daily INH chemoprophylaxis in the previous 7 days from the day of interview, which signified <80% adherence.

2.2. Study procedure

The caregiver of the child TB contacts were interviewed face to face by the field investigator with the data collected using a pretested interview schedule that was used to collect information on adherence to INH chemoprophylaxis. Completion of INH chemoprophylaxis was ascertained through telephonic follow-up.

2.3. Statistical analysis

EpiData version 3.1 was used for data entry (single-entered) and validation (EpiData Association, Odense, Denmark). The data were analysed with IBM SPSS Statistics for Windows (Version 25.0. Armonk, NY: IBM Corp). Summary statistics of the key variables were reported. The significance of association between INH non-adherence of the child TB contact (dependent variable) and sociodemographic characteristics of the household (independent variables) was assessed using the chi-square test. A p-value < 0.05 was considered as statistically significant.

2.4. Ethical considerations

Written and informed consent was obtained from the caregiver of the child that were interviewed. The study was approved by the Institutional Ethics Committee.

3. Results

The INH adherence was assessed in a total of 86 household child TB contacts of which 37 were from single child households, 17 from two-child households, and 15 from three-child households. The median (IQR) age of the child TB contacts was 3.5 (2, 5) years including 50 (58.1%) male and 36 (41.9%) female children.

In the study setting, INH chemoprophylaxis (IPT) was usually initiated by the DOTS-provider after symptom screening of the child contacts for TB disease at the DOTS-clinic or health facility, and the first dose of INH was administered to the child under supervision. INH refills of 1 month duration were dispensed which was recorded for every month in the TB treatment cards of the index TB case until 6 months.

IPT had been initiated in 62 (72.1%) child TB contacts of which 61 (98.4%) received INH within 1 month of starting of ATT-DOTS therapy in the index TB patient of the household. Furthermore, the failure to initiate IPT was observed in 24 (27.9%) child TB contacts. Within the cohort of child TB contacts who were not initiated with IPT, the ATT-DOTS duration in the index-TB patient was ≥5 months in 18 (75%) cases, 1–2 months in 3 (12.5%) cases, and <1 month in also 3 (12.5%) cases. Reasons for non-initiation (n = 24) were reported as refusal by the family in 12 (50%) cases mostly due to concern over side-effects of the drug, while non-provision of the drug by the DOTS provider was also observed in 12 (50%) cases.

Among child-TB contacts successfully initiated on IPT (n = 62), lack of adequate drug stocks within the previous 30 days was reported in 13 (20.9%) cases by the caregivers because of the inability to obtain timely INH refill from the DOTS clinic. Within this cohort (n = 62), a total of 36 (58.1%) child TB contacts were reported as receiving daily INH chemoprophylaxis in the previous 7 days, while at-least one missed dose was reported in 26 (41.9%) children and ≥2 missed doses in 23 (37.1%) children. The mean (SD) INH adherence in the INH initiated cohort was 5.6 (2.0) (n = 62). Reasons for INH non-adherence were attributed to forgetfulness (n = 23, 37.1%), carelessness (n = 24, 38.7%), and intermittent stopping of the medication (n = 17, 27.4%) on the child falling sick, perceived drug side effects, and running out of drug stocks.

INH non-adherence as per operational definition was observed in 47 (54.7%) participants (n = 86). On bivariate analysis, none of the household sociodemographic characteristics showed any statistically significant association with the rate of INH non-adherence in the child TB contacts (Table 1).

The completion of the 6-months Isoniazid Preventive Therapy was reported in 42 (87.5%, n = 48) child TB contacts that were initiated on exclusion of the 14 (22.6%) contacts lost to telephonic follow-up.

4. Discussion

The present study is one of the first from Northern India to report on the status of INH preventive therapy (IPT) among household child contacts of non-PLHIV TB patients. IPT was not initiated in nearly one in four eligible child contacts because of parental or caregiver objection due to the perceived concern over drug adverse effects or the failure of programmatic initiation. Even in the child contacts initiated on IPT, suboptimal adherence due to intermittent missed dosing was observed in a majority of the cases.

Previous studies from Southern India have reported unsatisfactory implementation of IPT in child TB contacts.[9] Fear of the drug side effects and reduced risk perception of caregivers have been previously reported to reduce the acceptability of IPT.[10] In this study, the respondents attributed the lack of initiation of IPT to both factors, although we were unable to verify lack of INH availability leading to non-initiation, which is a study limitation.

Another important programmatic implication of our study findings is the need to periodically assess adherence and persistence to IPT in the child TB contacts as the potentially high intermittent missed dosing rates can undermine the

Table 1 – Distribution of factors associated with INH non-adherence in household child contacts of TB patients in Delhi (N = 86).

Characteristic	Total (N = 86)	INH non-adherence (n = 47)	p-value
Age of child			
≤3	43 (50)	24 (55.8)	1.000
≥4	43 (50)	23 (53.5)	
Gender of child			
Male	50 (58.1)	29 (58)	0.515
Female	36 (41.9)	18 (50)	
Education of index TB case			
Illiterate	30 (34.9)	13 (43.3)	0.173
Literate	56 (65.1)	34 (60.7)	
Median per-capita income			
≤Low	61 (70.9)	32 (52.5)	0.635
>High	25 (29.1)	15 (60)	
Family history TB			
Yes	43 (50)	24 (55.8)	1.000
No	43 (50)	23 (53.5)	
Household type			
Single-child	37 (43)	23 (62.2)	0.276
Multi-child	49 (57)	24 (49.0)	

effectiveness of IPT in preventing incident disease. Training of DOTS providers for correctly estimating INH adherence and the sensitization of caregivers and index TB patients towards achieving optimal INH adherence for prevention of TB disease warrants prioritization in India's NTEP.

There are certain study limitations. First, the sample size was small and the study was conducted in a single district which limits the external validity of the findings. Second, the study lacked qualitative inquiry for identifying perspectives of all stakeholders on challenges in INH implementation in Indian settings.

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Conflicts of interest

The authors have none to declare.

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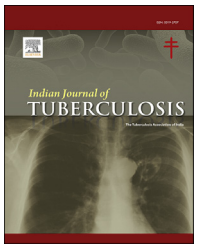

Short communication

Pediatric TB detection in the era of COVID-19

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ABSTRACT

The effect of COVID-19 and measures in response to it on human lives, including healthcare, was enormous. The necessary healthcare services including communicable diseases, such as Tuberculosis (TB) were badly affected. Here an attempt has been made to trace the number of notified Pediatric TB cases during and after COVID-19 lockdown and unlock period, and then compared with the same period of previous year. The epidemic data on notified pediatric TB cases for 2019 and 2020 were extracted from the Health Management Information System (HMIS) database. The absolute numbers of monthly pediatric TB notifications from January to September for the year 2020 were compared to 2019, and the percentage decrease was estimated. The HMIS data shows that there is a significant decrease in pediatric TB notifications during COVID-19 epidemic in India. Especially, when the lockdown and related restrictions in response to COVID-19 was imposed, notifications were significantly decreased compared to the same period during the previous year. Even, the reduction numbers of pediatric TB notifications during post-lockdown are still more worrying. Though, little improvements were observed suddenly after lockdown was removed, but then-after again consisted decrease was reported; and these numbers again substantially lower than the numbers of previous year. Adequate measures to diagnose, control, and prevent TB focusing young children, should be implemented simultaneously with response to COVID-19 pandemic. Further, effective steps should be taken to remove the fear arising due COVID-19 pandemic among masses, so that the healthcare seeking may be improved.

1. Introduction

The first case of COVID-19 (CoronaVirus Disease 2019) – an infectious respiratory disease – was identified in Wuhan, China in December, 2019 and severely out broke across the globe[1] including India (the first case of COVID-19 in India was identified on January 30, 2020 in Kerala[2]). In India, like other countries, the effect of COVID-19 and measures in response to it on human lives was enormous, including healthcare.[3,4] The necessary healthcare services including communicable diseases, such as Tuberculosis (TB) were badly affected.[5]

TB, a biggest infectious killer, is a major public health crisis across the world. India contributes more than one-fourth of new TB cases and around one-third of TB deaths globally.[6] Though, roughly a million children (aged <15 years) TB cases estimated globally each year (11% of global TB cases), but the risk of death is much higher among children (14% of global TB

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deaths).[6] Early detection and quick treatment initiation as well as prevention of transmission from TB positive adult family members, since household source is most commonly implicated for young children,[7–9] are crucial.

These important elements of cascade of care are affected because the entire focus of healthcare was diverted to COVID-19. Moreover, the impact of COVID-19 on overall TB notification is well documented,[10] however, nothing is known about the specific impact of COVID-19 epidemic on detection of Pediatric TB cases in India, especially, during lockdown and unlock period. Here, an attempt has been made to analyze the real-time monthly service delivery data of 2020 and compared to that of 2019.

2. Data source and methods

The monthly notified childhood disease-TB cases and Child immunization-BCG for 2019 and 2020 were extracted from the Health Management Information System (HMIS), a web-based administrative database under the Ministry of Health and Family Welfare (MoHFW). The services on which the HMIS provides data range from Maternal and Child services, immunization and family planning to the treatment of disease. The absolute numbers from the January to September for the year 2020 were compared to that of 2019 figures. The percentage decrease during April to September in 2020 was estimated by comparing the previous year of 2019. The HMIS data is available in public domain at https://nrhm-mis.nic.in/SitePages/Home.aspx.

3. Results

Pediatric TB data illustrate that drastic drop was observed during April (−36%) in notified pediatric TB cases after COVID-19 forced lockdown was imposed. Though, little improvements were observed during May (−12% decrease) and June (−14%) months, but then-after consisted and drastic decrease was observed in next months. In August 2020, case notifications were down by more than half (−53%) compared with the same month in 2019 (Fig. 1). Similarly, the number of cases of pediatric TB registered in April 2020 fell to just half the February levels. Moreover, in August 2020 these numbers fell to more than 55% compared to the February levels (Fig. 1). During the overall lockdown period, a total 2953 Pediatric TB cases were reported compared to 3888 cases during the same period of 2019, a reduction of 24%. However, a more worrying reduction of 36% was observed during the post-lockdown period (June to September, 2020); a total of 6251 Pediatric TB cases were reported compared to 9821 cases in 2019, 3570 less cases in absolute numbers (Table 1).

In addition to Pediatric TB detection, the HMIS data shows serious disruption in providing BCG vaccine - which provides protection against childhood TB. At the national level a decline of 15 and 37% were observed during March and April 2020 compared to the same period of 2019. There-after also on an average around 15% decrease was observed in every month. In April 2020, over 6 lakh fewer BCGs were provided to the children than in April 2019. In subsequent months, there was some evidence of an improvement, but these numbers are also significantly fewer than the previous year of 2019 (Fig. 2). When compared to the January 2020 figure, the number of children receiving BCG vaccine was over 2.6 lakh fewer in March 2020. The decline in April was even sharper – the number of children received the BCG in April 2020 fell to just half to the January 2020 levels (Fig. 2). In particular, a total of 213,983 (−4%; 5,732,168 vs. 5,946,151), 820,680 (−23%; 2,683,552 vs. 3,504,232) and 1,058,784 (−13%; 7,114,513 vs. 8,173,297) fewer BCG immunizations were done during overall period of pre-lockdown, lockdown and post-lockdown (June–September) compared to 2019, respectively (Table 1). Overall, at the national level around 2.1 Crore children were vaccinated for BCG during January–December 2020 compared to more than 2.4 Crore in 2019, more than three million fewer children.

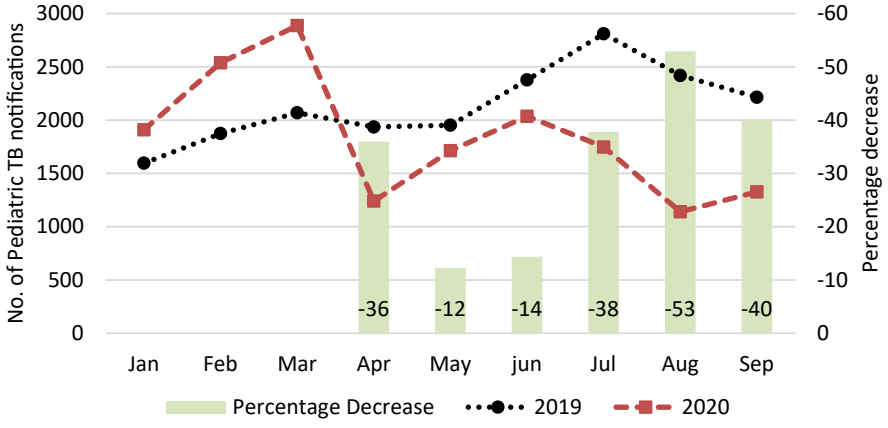

Fig. 1 – Trends in registered Pediatric TB notifications for January to September 2020 in comparison to 2019 and percentage decrease during COVID-19 epidemic, India, HMIS, 2019–20. Note: Nationwide COVID-19 forced lockdown was imposed during March 25, 2020 to May 31, 2020; COVID-19, coronavirus disease 2019; HMIS, Health Management Information System; TB, tuberculosis. Source: Author's calculations based on pediatric TB notifications data extracted from the HMIS database.

Table 1 – Number of Pediatric TB cases and BCG immunization data reported before, during and after implementation of COVID-19 forced lockdown in 2020 compared with the same period during the previous year of 2019, India, HMIS, 2019–20.

Period	No. of cases, 2019 (*n*)	No. of cases, 2020 (*n*)	Absolute difference (*n*)	Percentage Decrease (%)
Pediatric TB Cases				
Pre-lockdown	5539	7334	(1795)	32%
Lockdown	3888	2953	935	–24%
Post-lockdown	9821	6251	3570	–36%
BCG immunization				
Pre-lockdown	5,946,151	5,732,168	213,983	–4%
Lockdown	3,504,232	2,683,552	820,680	–23%
Post-lockdown	8,173,297	7,114,513	1,058,784	–13%

Note: () figure in parenthesis is excess number of Pediatric TB notifications during 2020 compared to 2019; nationwide COVID-19 forced lockdown was imposed during March 25, 2020 to May 31, 2020. Hence, pre-lockdown period was considered from January to March, lockdown form April to May and post-lockdown from June to September; COVID-19, coronavirus disease; HMIS, Health Management Information System; TB, tuberculosis.
Source: Author's calculations based on pediatric TB notifications and BCG immunization data extracted from the HMIS database.

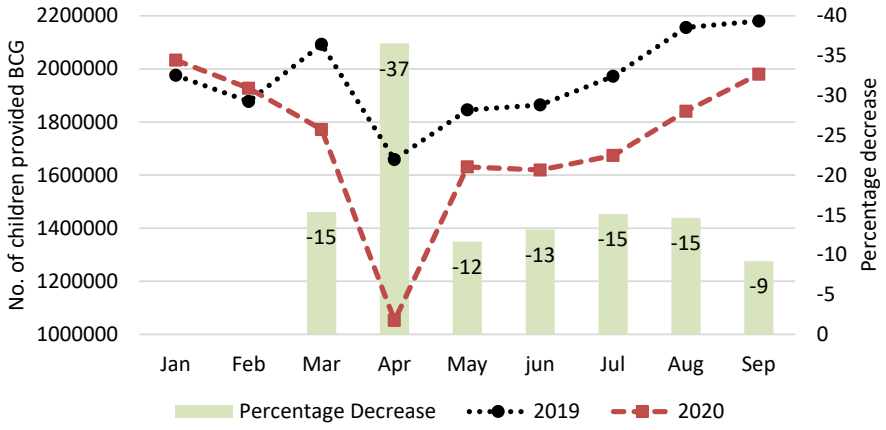

Fig. 2 – Trends in routine BCG immunization data for January to September 2020 in comparison to 2019 and percentage decrease during COVID-19 epidemic, India, HMIS, 2019–20. Note: Nationwide COVID-19 forced lockdown was imposed during March 25, 2020 to May 31, 2020; COVID-19, coronavirus disease 2019; HMIS, Health Management Information System; TB, tuberculosis. Source: Author's calculations based on BCG immunization data extracted from the India's HMIS database.

4. Discussion

While, almost similar symptoms are being seen both in COVID-19 and TB,[11–14] and as COVID-19 does not appear generally to have similar affect among children compared to adults, hence, it is assumed that children will cope better and do not need similar attention from healthcare services for COVID-19.[15] Additionally, during the era of COVID-19, where the entire focus of healthcare is diverted to tackle COVID-19 epidemic and other healthcare services are neglected,[16] the disruptions in access to the prevention, monitoring and timely treatment of TB is expected, particularly among young children. Moreover, everyone is discouraged from using healthcare services, unless severely unwell, due to fear of getting infected and labeled as COVID-19 positive.[17] Hence, families are reluctant to bring unwell children to the healthcare facilities for investigation.

Usually, pediatric TB is relatively neglected and the all emphasis has been on adult disease.[18] Apart from this, TB rarely presents as an acute, severe illness in children but progresses silently. If not regularly reviewed for timely diagnosis and early initiation of treatment then TB can be more fatal among young children compared to adults. However, the data shows severe disruptions in the detection of pediatric TB during COVID-19 epidemic. Though, Stop TB Partnership advocates that a 10% decrease in TB case notification may be attributed to maintaining physical distancing in high TB burden countries,[19] but it may not be true in case of children. Because, as most TB in young children is acquired in their own household, hence, social distancing measures that keep a family together for a long period of time are likely to result in more exposure of children to infectious TB cases.

5. Conclusion

In order to protect young children from the risk of getting TB infection, it is being suggested that the COVID-19 screening

should gather information about TB in the household. However, to effectively tackle the TB during COVID-19, the health ministry has issued guidance for bi-directional TB-COVID screening, TB screening for ILI and SARI cases,[20] this should be strictly implemented for COVID-19 screening. Lastly, it must be noted that TB is the oldest and more killer than the other communicable disease; and hamper in measures to diagnose, control, and prevent TB during this pandemic may dampen the government of India's aim to eradicate TB by 2025, which also consequently lead to great loss in achieving global committed targets to end TB in general. Hence, the situation warrants continuity of essential TB interventions through the national TB program should be implemented simultaneously with response to COVID-19 pandemic. Further, effective steps should be taken to remove the fear arising due COVID-19 pandemic among masses, so that the healthcare seeking may be improved.

Disclosures and declaration

Availability of data and materials

The data used for the study is obtained from the India's Health Management Information System (HMIS) web-portal under union health ministry which is available in public domain. No separate ethics statement and consent for publication was required for this study.

Informed consent

Not applicable.

Author's contributions

Sole author.

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Conflicts of interest

The author has none to declare.

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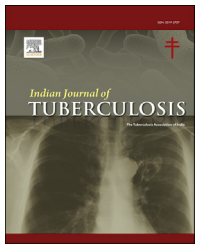

Case report

Sjogren's syndrome—An interesting case

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ABSTRACT

Objective: To present a case of Sjogren syndrome with pulmonary manifestations in an adult female and discuss its assessment and management.
Design: Case Report.
Setting: Tertiary care hospital.
Patient: One.
Results: A 50 yrs female admitted with complaints of dryness of eyes with decreased salivation causing difficulty in swallowing since last 3 years, with persistent dry cough since 10–15 days and progressive dyspnea since 4–5 days. Anti-nuclear antibody (ANA) profile revealed **Anti- Ro/SS-A and Anti- La/SS-B Positive.** Also, sub-lingual excisional biopsy was done which was consistent with findings of Sjogren's syndrome. Patient showed significant improvement after starting oral glucocorticoids, systemic anti inflammatory agents (Tab. HCQS), artificial tear drops, oral iron supplements and other supportive treatment.
Conclusion: Sjögren syndrome (SS) is a chronic inflammatory disorder characterized by diminished lacrimal and salivary gland function and associated with lymphocytic infiltration of exocrine glands, and can affect extraglandular organ systems including the skin, lung, heart, kidney, neural, and hematopoietic systems. We present a case of Sjogren syndrome in an adult female presenting with xerostomia and dyspnea and was diagnosed upon detection of anti-Ro and anti-La antibodies and confirmed by histopathological examination of lip biopsy. Patient was started on oral steroids and other supportive treatment, General condition improved significantly and is doing very well on regular follow-up.

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1. Introduction

Sjögren's syndrome is the second most common autoimmune disease after rheumatoid arthritis. Sjögren's syndrome mostly affects middle aged women, with a peak at 56 years of age; men are affected less frequently and later in life, mostly after the age of 65 years. Interstitial lung disease (ILD) is the most common pulmonary abnormality in primary Sjögren syndrome (pSS).

Sub lingual excisional biopsy is the gold standard for diagnosis of sjogren's syndrome. A lip/salivary gland biopsy takes a tissue sample that can reveal lymphocytes clustered around salivary glands, and damage to these glands due to inflammation.

2. Case history

Asha Rani, 50 yrs female admitted in Jaipur golden hospital with complaints of dryness of eyes with decreased salivation causing difficulty in swallowing since last 3 years, with persistent dry cough since 10–15 days and progressive dyspnea since 4–5 days. There was no history of bilateral jaw swelling, palpable purpura, dysuria, nocturia, flank pain, seizures, any neurological deficit, joints pain, swelling, malar/discoid rash, photosensitivity, raynaud phenomenon and any drug intake. On general examination, General condition stable, Vitals-all WNL, SpO2- 95% (O2@2 L/min), pallor present, no icterus, cyanosis, clubbing, pedal edema, lymphadenopathy. Eye examination-movement of tears on schirmer test: 8 mm right eye & 9 mm left eye. Respiratory examination-bilateral air entry present with right basal fine crepts, CVS examination - S1 S2 present, no murmurs. Abdominal examination - soft, non tender, Bowel sounds (BS) present, CNS-conscious, oriented, no focal neurological deficit. Routine investigations showed low Hb(8.3 g/dl), slight leucocytosis (11,600 cells/cubic mm), slightly deranged Kidney function test (KFT)(BUN-31 mg/dl, S. Creatinine- 1.68 mg/dl), rest investigations normal, iron studies done were suggestive of iron deficiency anemia. Pulmonary function test showed moderate restriction with a low diffusing capacity (48%). Chest skiagram showed slight right lower zone haziness with no other significant findings (Fig. 1). High resolution computed tomography (HRCT) chest showed intralobular reticulation with right lower lobe predilection (Nonspecific Interstitial pneumonia pattern) (Fig. 2). Bronchoscopy was done, Bronchoalveolar lavage (BAL) taken from Right middle lobe (RML) and Right lower lobe (RLL) which came negative for Gram stain, Pyogenic culture, Fungal smear, Acid fast bacilli (AFB) smear, GenXpert -MTB not detected. Cryo lungbiopsy was done from right lower lobe and sent for histopathological examination, which showed moderate lympho plasma cells infiltrate in sub-epithelial connective tissue stroma around submucous glands with focal destruction. Connective tissue- Interstitial lung disease (CT-ILD) Workup was done which showed RA Factor-<10 IU/ml, Montoux test – negative, LE Cell phenomenon – negative, Serum Angiotensin converting enzyme (ACE) level – 29 U/L (normal), ANA profile revealed **Anti- Ro/SS-A and Anti-La/SS-B Positive** which are positive markers for Sjogren's Syndrome, Sub-lingual biopsy was done which was consistent with findings of Sjogren's syndrome (Fig. 3). Patient showed significant improvement after starting oral glucocorticoids (Chest skiagram post 2 months of treatment showed complete clearance of haziness), systemic anti inflammatory agents (Tab. HCQS), artificial tear drops, oral iron supplements and other supportive treatment and was discharged on same treatment.

3. Discussion

The aetiopathogenesis of Sjögren's syndrome combines environmental factors such as viruses or solvents, genetic predisposition and hormonal deregulation leads to an initial

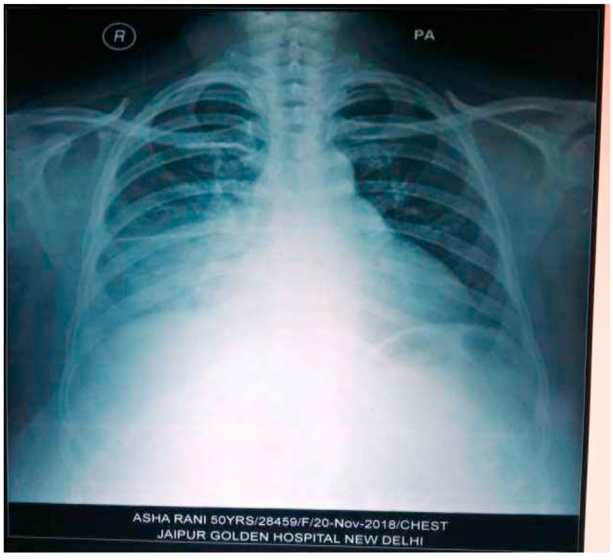

Fig. 1 – Chest skiagram showing right lower zone haziness.

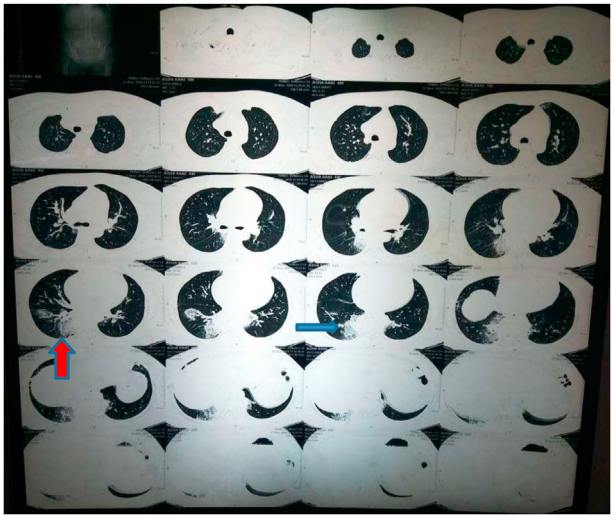

Fig. 2 – HRCT chest showing intralobular reticulation with right lower lobe predominance.

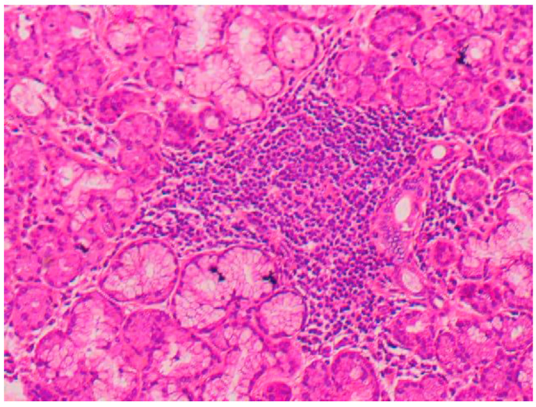

Fig. 3 – HPE picture showing lymphoplasmacytic infiltrate in sub epithelial connective tissue stroma around sub mucous glands with focal destruction.

glandular inflammation called autoimmune epithelitis and a deregulated immune response. B-cell demethylation, activation and proliferation are abnormal, and activated B-cells promote plasma cell secretion of anti-Ro (SSA) and anti-La (SSB) autoantibodies directed to the small cytoplasmic RNP-bound peptides.[1–3]

This immune response, combined with local inflammation, gland infiltration and pathogenic anti-Ro52 autoantibodies, could cause the bronchopulmonary damage observed in Sjögren's syndrome patients.[4,5]

The symptoms and signs of Sjogren syndrome- Interstitial lung disease (SS-ILD) depend on the type and severity of lung parenchymal and lower airway involvement. The clinical features include dyspnea, Cough without sputum, chest pain, fever, clubbing which is rare. Occasionally, patients with SS-ILD will report Raynaud phenomenon, arthralgias, fever, or rash.[6,7] Our patient presented with dryness of eyes, dryness of mouth and progressive dyspnea.

Bibasilar crackles are noted on physical examination in approximately 60 percent of patients with SS-ILD. Cutaneous vasculitis occurs in approximately 10 percent of patients with SS and can present with urticarial lesions, purpura, or punctate erosions.[6,7] Thoracic manifestations of Sjögren's syndrome include bronchiolitis, bronchiectasis, pulmonary infections, Interstitial lung diseases, Pulmonary amyloidosis, Pulmonary lymphoma, Pulmonary embolism and pulmonary hypertension.[6,7] In our case described she presented with features of diffuse parenchymal lung disease.

The diagnostic approach follows the general approach to interstitial lung disease with CT-ILD workup and cryo lung biopsy.

Chest radiograph typically shows a fine reticular or nodular pattern with basilar prominence, but may be normal or less commonly show cysts or pleural abnormalities.[8–10] In patients with pSS-associated ILD, HRCT abnormalities include

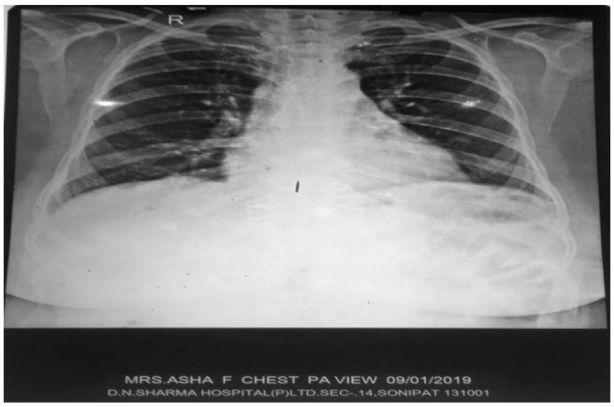

Fig. 4 – Chest skiagram post 2 months of treatment.

ground-glass attenuation, subpleural small nodules, non-septal linear opacities, interlobular septal thickening, bronchiectasis, and cysts.[11,12]

When ILD is suspected in a patient with SS, original diagnosis of SS is usually confirmed by reviewing or obtaining serologic studies such as the antinuclear antibody, anti-Ro/SSA and anti-La/SSB antibodies, rheumatoid factor, erythrocyte sedimentation rate [ESR], total globulins, and immunoglobulin quantitation.[11,12] Anti-Ro/SSA and anti-La/SSB antibodies were positive in the case described.

Bronchoalveolar lavage (BAL) cell count patterns in Sjogren syndrome (SS) are nonspecific, so the main role of BAL is to exclude other causes of diffuse parenchymal lung disease, such as eosinophilic pneumonia, infection, lymphangitic tumor, and hemorrhage.[10,11]Transbronchial or cryobiopsy, video-assisted thoracoscopic, or surgical lung biopsy may be performed for confirmation of diagnosis.

For symptomatic patients with SS-associated Non specific Interstitial pneumonia (NSIP) who have worsening symptoms, Prednisone is usually started at a dose of 1 mg/kg ideal body weight per day. The response to therapy is assessed after 4–6 weeks with evaluation of symptoms and Pulmonary function test (PFTs).[13]

4. Conclusion

Sjögren syndrome (SS) is a chronic inflammatory disorder characterized by diminished lacrimal and salivary gland function and associated with lymphocytic infiltration of exocrine glands, especially the lacrimal and salivary glands. SS can also affect extraglandular organ systems including the skin, lung, heart, kidney, neural, and hematopoietic systems. In our case, patient presented with dryness of eyes, decreased salivation, persistent dry cough and progressive dyspnea, and was diagnosed upon detection of anti-Ro and anti-La antibodies and confirmed by histopathological examination of lip biopsy. Patient was started on oral steroids and other supportive treatment, and has responded well to the treatment, recent chest skiagram clear (Fig. 4), General condition improved significantly and is doing very well on regular follow-up.

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Case report

Atypical presentations of cutaneous tuberculosis: Series of 10 cases☆

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ABSTRACT

Cutaneous tuberculosis classically presents as Lupus vulgaris, scrofuloderma, tuberculosis verrucose cutis and tubercular abscess. Hypersensitivity reaction to the bacilli leads to Lichen scrofulosorum and papulonecrotic tuberculids. At the same time, it can have myriad of clinical presentations, many of which are still undescribed. It is important to regularly update ourselves with these unusual manifestations so as to ensure early treatment and reduction of overall morbidity. In this case series tuberculosis manifesting as rapidly progressing diffuse facial granulomas, sporotrichoid tuberculosis, tuberculosis mimicking squamous cell carcinoma, scrofuloderma as tubercular ulcer, lupus vulgaris with nasal septal perforation, lupus vulgaris resembling furuncle, psoriasis, dermatitis and BT Hansen are described in immunocompetent individuals. These cases highlight the importance of recognition of atypical forms of cutaneous tuberculosis to minimize scarring and dissemination of bacilli.

1. Introduction

Tuberculosis (TB), caused by mycobacterium tuberculi is a growing health concern, especially in developing countries. Cutaneous tuberculosis (CTB), a form of extra pulmonary TB can present with diverse clinical morphology depending upon host's immunity and mode of infection. Although, CTB occurs in only a small proportion (<1%–2%) of all cases of TB,[1] for a country with high incidence of TB, the cases of CTB correspondingly are very high. Most of the cases of CTB are easy to diagnose clinically, but some cases, especially with atypical morphology can pose diagnostic dilemma, thus causing future ramifications like disfigurement and spread of the infection. Despite having an extensive TB program and control measures, adequate knowledge about skin TB and its timely diagnosis is still lacking. We have described CTB as atypical if it presented at unusual site or with an uncommon morphology.

☆ 9 cases out of 10 was presented as an e-poster by the corresponding author in e-Dermacon 2021(5-7 February).

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2. Report

This case series describes 10 patients with atypical and unusual morphological forms. Table 1 summarizes the demographic and morphological profile of these patients. All our patients were immunocompetent and had no underlying chronic illness. None of them had family history of tuberculosis and BCG scar was present in eight out of ten cases. Chest radiographs were performed in all the patients and did not reveal any signs of coexisting pulmonary tuberculosis. Face was the most common site involved and most common morphological type was Lupus vulgaris. The duration varied from 2 months to maximum of 20 years. The histopathology from these lesions showed granulomatous inflammation in the dermis with negative acid fast bacilli staining Fig. 1(c), caseation necrosis could be seen in a few. Standard category 1 Anti tubercular therapy was given to the patients. The healing was complete with residual scarring in all the 9 cases, the septal perforation in case no 7 persisted for which he was referred to ENT for further management. None of these patients received any topical or oral corticosteroids. All the patients had a positive Mantoux test and CB-NAAT was done in 3 cases (Table 1), two from skin tissue and one from the lymph node aspirate (case 9). Only the one from FNAC sample was positive. Culture report could be traced for 5 patients and were all negative (Table 1). In all other cases, the presence of induration in the lesions, subtle evidence of scarring along with a positive tuberculin test and response to ATT helped in confirming the diagnosis of cutaneous tuberculosis.

Written Informed consent was obtained from participating adult subjects or from parents or legal guardians for minors or incapacitated adults.

3. Discussion

Tuberculosis is a chronic disease caused by *Mycobacterium tuberculosis*. It poses a major public health problem especially in the developing countries. Cutaneous TB (CT)accounts for a small fraction of extrapulmonary tuberculosis varying from approximately 1–1.5% in various parts of the world[2] and approximately 25% cases of CT have co-existing systemic focus of tuberculosis.[3,4]

CT can be acquired by inoculation, in an unsensitized or sensitized individual. The source of infection could be either exogenous or endogenous. Endogenous infection could be due to reactivation of the dormant organisms or hematogenous or lymphatic spread from an active internal focus. When a tubercular bacteria spreads to human skin, a series of events resulting from host and pathogen related factors determine the disease progression and presentation. Tuberculosis is a TH1 pathway mediated disease where INFϒ, TNFα along with various interleukins, cytokines, macrophages leads to its pathogenesis. Recently interleukins IL-17 and IL-23 stimulated Th17 cells pathway has been studied and found to be important in granuloma formation.[5] The same tubercular bacilli can produce different clinical forms even in same individual.

Broadly, the lesions of CT can be classified into true CT and the so-called tuberculids. True CT consists of Primary cutaneous tuberculosis (Tubercular chancre and primary military tuberculosis) and secondary cutaneous tuberculosis (Lupus vulgaris, scrofuloderma, tuberculous gumma, tuberculosis verrucosa cutis, miliary tuberculosis, orificial tuberculosis) depending upon the mode of acquisition of bacilli. The tuberculids are regarded to be hypersensitive manifestations to the tuberculous infection. The most common presentations of tuberculids are lichen scrofulosorum, papulonecrotic tuberculid and erythema induratum of Bazin.[6]

Diagnosis can be suspected clinically in most common forms of tuberculosis. Clinical diagnosis of early lesions of TB and atypical forms is often difficult and may need to be supplemented by other tests which include the tuberculin test, direct smear examination and histopathology. Culture and CB-NAAT are not only useful when histopathology is not conclusive but also provides information about drug resistance. A therapeutic trial with ATT for 5–6 weeks can be given in doubtful cases with high index of suspicion with non-corroborative histological or bacterial tests.[6,7]

Most cases of CT are given a Multidrug treatment with a total of four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) daily for first 2 months comprising the intensive phase followed by isoniazid, rifampicin and Ethambutol daily for further 4 months as continuation phase.

Cutaneous tuberculosis presenting with atypical features are not so uncommon and are frequently misdiagnosed in clinical practice which may lead to delayed diagnosis with a chronic and disfiguring sequalae. Extremities, Trunk, neck are common sites of involvement in India with gradual progression of lesions. Six out of 10 patients in the above series had facial involvement, which is a less common site and has a significant morbidity associated with it. One of our patients (case 1) presented with diffuse and rapidly progressive facial lesions. Despite an extensive literature search, we were not able to come across any report of such a florid presentation and rapid progression of cutaneous tuberculosis on the face and hence we intend to describe rapidly progressive facial tuberculosis as a unique entity. Two other cases who presented with facial lesions had involvement of oral mucosa and nasal mucosa leading to loss of teeth and nasal septal perforation respectively. Although Lupus vulgaris causing septal perforation has been reported,[4,8] its rare to see such presentation. Similarly the linear pustular lesion of the eyelid could have been missed easily for small furuncle if the slight infiltrated edge was missed.

Sometimes the disease may follow its classical course but may be misdiagnosed because of atypical site and pattern, as in our cases with palmar, planter lesion and lesion on the check of the child with subtle evidence of scarring. They resembled psoriasis and dermatitis. Sporotrichoid cutaneous tuberculosis is rare and accounts to around 3% of all lesions of cutaneous TB.[9] It is more commonly seen in children owing to efficient lymphatic drainage and high physical activity. Similarly, the female with long standing erythematous plaque on face resembled tuberculoid leprosy. Normal sensory -motor examination, no nerve thickening and strongly positive Mantoux test were clue to the diagnosis.

Many other atypical variants of CTB have been described in literature like, tuberculosis ulcerosa cutis, tubercular cellulitis, lupus vulgaris as turkey ear, annular lesions, port wine

Table 1 – Demographic and clinical profile of cutaneous tuberculosis patients.

Case No	Age (years)	Sex	Duration	Site of involvement	Type of skin TB	Morphological variant	Scarring at presentation	Additional information
1. Fig. 1	17	M	2 months	Face (entire face including eyelids, ear lobes)	Lupus vulgaris	Rapidly progressing facial granuloma	No	• CB-NAAT negative, • Culture negative • Healed with extensive facial scarring
2. Fig. 2	58	F	20 years	Face (centro-facial area)	Lupus vulgaris	Mimicking BT Hansen	No	• Culture negative
3. Fig. 3	40	F	6 months	Face	Scrofuloderma	Mimicking squammous cell carcinoma	Yes	• Associated with gingival involvement.
4. Fig. 4	15	M	2 years	Sole	Lupus vulgaris	Mimicking planter psoriasis	No	
5. Fig. 5(b)	30	F	1 year	Palm	Lupus vulgaris	Mimicking palmer psoriasis	Yes	• Culture negative
6. Fig. 5(a)	35	M	8 months	Palm	Lupus vulgaris	Resembling sporotrichosis	No	• Presentation as discrete linear lesions
7. Fig. 6	10	F	2 years	Face	Lupus vulgaris	Resembling Leishmaniasis	No	• Associated with nasal septal perforation • CB-NAAT negative • culture negative
8. Fig. 7	2	M	2 year	Face	Lupus vulgaris	Mimicking Discoid eczema	Yes	• Healed with hypo-pigmented scar
9. Fig. 8	48	F	6 years	Neck	Scrofuloderma	Ulcerative variant	No	• With underlying lymph node enlargement • CB-NAAT positive: from FNAC lymph node
10. Fig. 9	39	F	3 months	Face	Lupus vulgaris	Resembling furuncle	No	• Involvement of eyelid

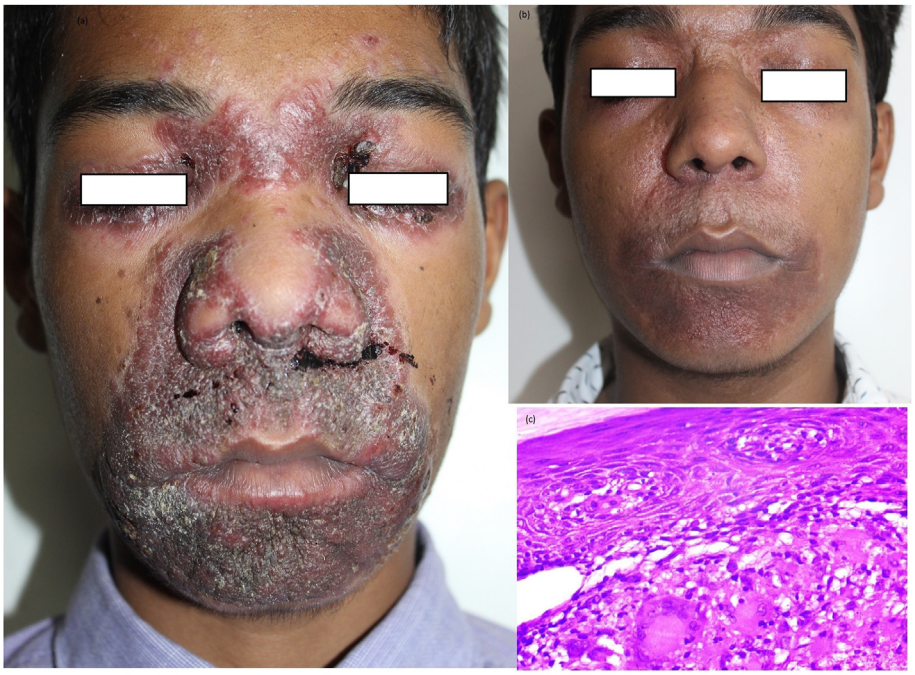

Fig. 1 – (a) Erythematous papules and plaques symmetrically distributed on face, (b) resolution of lesions with residual scarring, (c) Histopathology (H&E 400X) showing granulomatous inflammation in the dermis.

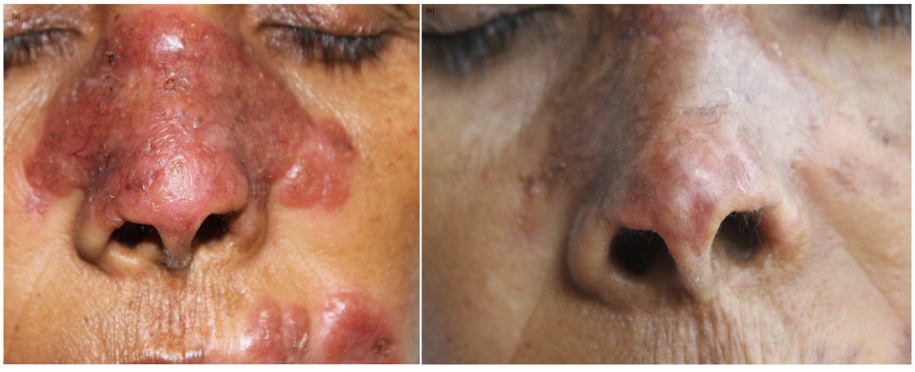

Fig. 2 – (a) Erythematous plaques on nose and upper lip area with overlying telangiectasia (b) healed lesion with residual scarring.

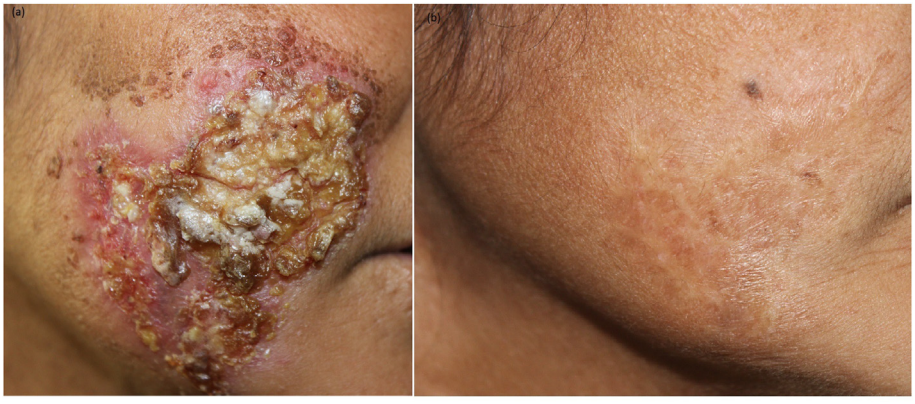

Fig. 3 – (a) Erythematous crusted plaque on check (b) healed lesion with scarring.

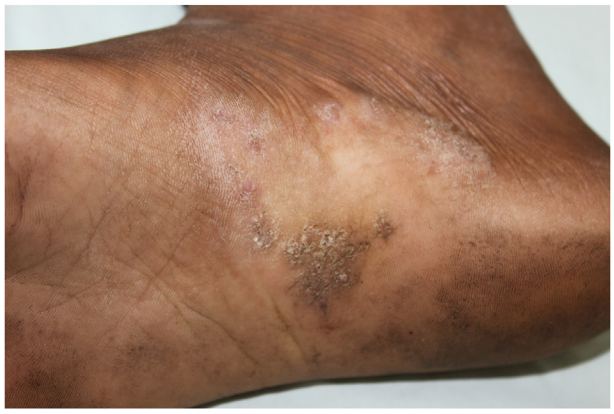

Fig. 4 – Scaly plaque with mild erythema on inner edge of sole associated with scaling and fissuring.

stain, mycetoma like lesions etc.[10–13] Lupus vulgaris is classically described as infiltrated plaque with scarring at regressing edge. Out of 8 cases of lupus vulgaris scarring was present only in 2 cases. Carefully observing for Infiltration and satellite lesions specially in nonresponsive dermatosis may help in clinching the diagnosis of cutaneous tuberculosis. Finding apple jelly sign with dermatoscopy may be of additional help. Ramesh et al in their review have mentioned that

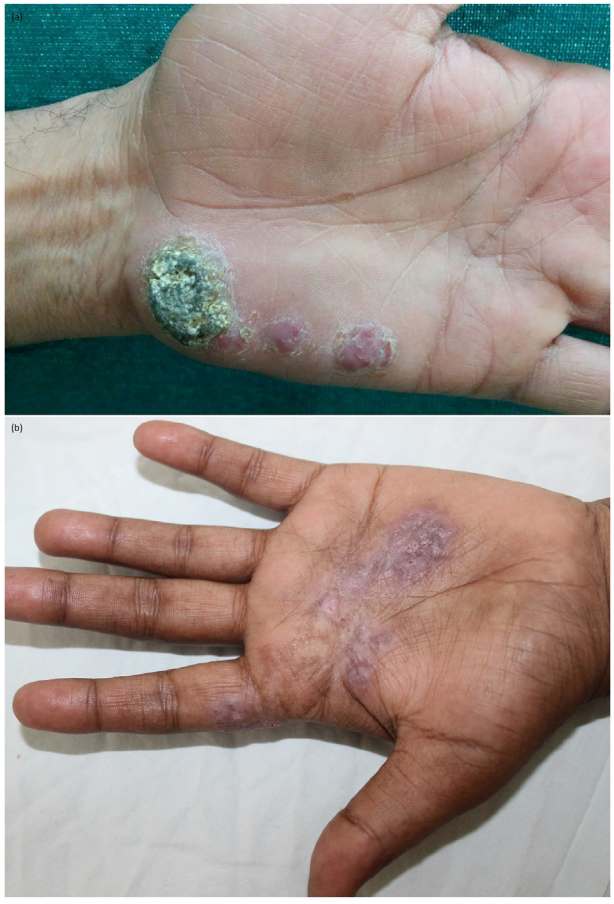

Fig. 5 – (a) Linearly arranged erythematous discrete plaques with crusting. (b) Erythematous infiltrated plaque with subtle scarring and mild scaling.

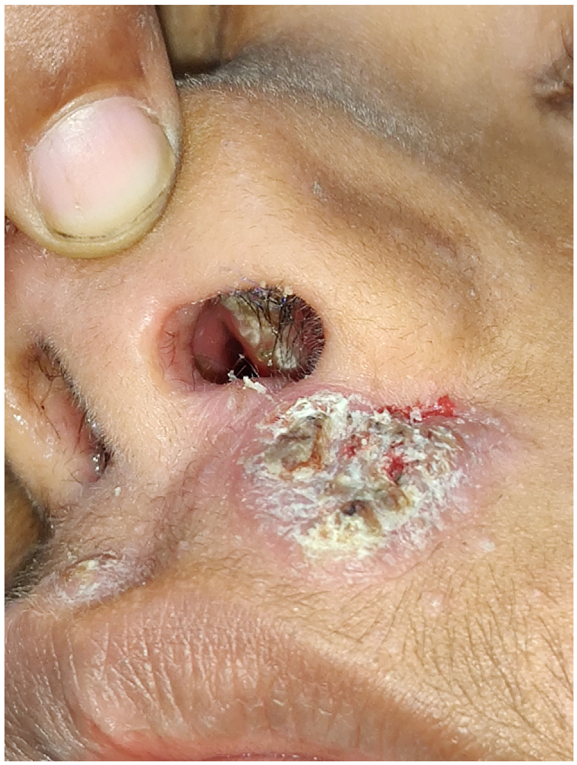

Fig. 6 – Erythematous plaque covered with crust associated nasal septal perforation.

a positive intradermal tuberculin test carries significance in cutaneous tuberculosis and tuberculin reactions of more than 20 mm is often helpful in detecting CTB.[6] Histopathology, PCR (polymerase chain reaction), CB-NAAT and culture may further confirm the diagnosis. Histopathology from the lesions was supportive of the diagnosis in all our cases, as was the response to standard antitubercular therapy. Cutaneous tuberculosis is usually a paucibacillary disorder and isolating tubercular bacilli is always difficult from skin specimen, as was evident from the biopsy reports in above series. The stain

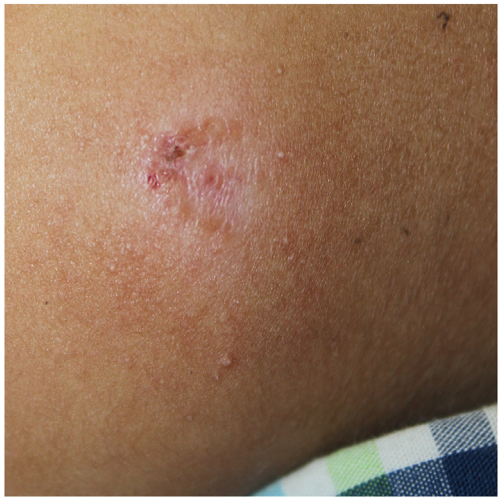

Fig. 7 – Ill-defined plaque with erythema and areas of hypopigmentation and mild scarring, present on check.

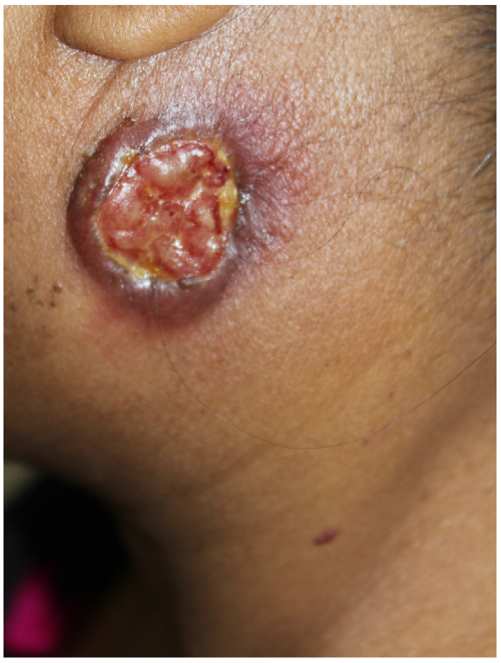

Fig. 8 – Single large ulcer with rolled edge present on neck, covered with red granulation tissue and skin puckering at the margins.

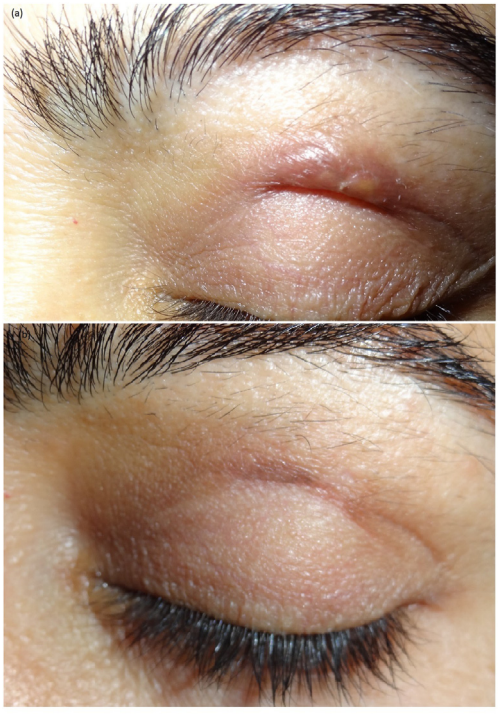

Fig. 9 – (a)Single small pustular plaque on eyelid (b) Clearing of lesion with ATT, leaving behind thin linear scar.

for AFB was negative and culture reports and CB-NAAT (from skin tissue) test where available were also negative.

It would be imperative to note that while most of the clinical presentations in our series are known to occur in immunocompromised individuals with underlying systemic conditions, all of our patients were in good health with no prior history of tuberculosis in self or family. With such bizarre presentations, and low sensitivity of tissue smears and culture in paucibacillary lesions and restricted availability of PCR, cutaneous tuberculosis threatens towards persistence and causing morbidity especially in developing nations. To conclude, atypical manifestations of cutaneous tuberculosis along with the emergence of multidrug resistant tuberculosis lately, a broader approach to diagnosis with high index of suspicion and judicious use of newer diagnostic technique like CB-NAAT (cartridge based nucleic acid amplification test), the age old tuberculin test and the therapeutic trial of ATT should be utilized for early diagnosis and treatment. The significance of understanding disease epidemiology and of educating society and physicians about the nature of infection and its management, to decrease associated morbidity cannot be over emphasized.

Conflict of interest

The authors have none to declare.

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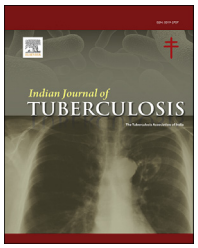

Letter to editor

Nocardial pneumonia as an opportunistic infection in Covid treated patient

Keywords:
Covid 19
Nocardial pneumonia
SARS CoV2

Dear Editor,

We are still evolving with respect to the new virus of SARS CoV2. As the time passes we are coming across new information pertaining to Covid -19. There are various post Covid complications listed in published literatures.[1]

Here we are presenting a case of pulmonary nocardiosis as an opportunistic infection in a covid treated case.

A 47-year-old female came to our hospital with a history of Covid infection in the preceding month. Patient was discharged previously on steroid as a continuation of Covid therapy.

The patient presented with complains of continuous moderate to high grade pyrexia for 15 days, troublesome irritant cough, breathing discomfort, generalized weakness for 10 days in the department of emergency. The patient was admitted for further work up.

On admission patient was febrile, conscious and well oriented. Examination revealed HR 84/min, BP 140/70, SpO 2 95%, Respiratory 16 rate/minutes, Temperature 99.6 °F.RT-PCR testing for detection of Covid 19 was negative at the time of admission.

Sputum for Grams stain, culture sensitivity, acid fast bacilli (AFB) stain, gene xpert for TB, blood culture was sent to lab for evaluation.

Blood cultures grew *Klebsiella pneumoniae* on day 2. Patient was given polymixin, doxycycline as per sensitivity report. Patient improved by day 6 but again developed cough and fever.

Sputum smear examination revealed Gram positive branching, thin filamentous structures resembling *Nocardia. sp* and modified AFB smear showed acid fast branching filamentous structure as seen in Fig. 1a and b.

Chest X ray and CT scan was done during course of stay. CT scan of thorax was suggestive of mass like consolidation involving apico-posterior segment of left upper lobe as shown in Fig. 1c and d. Repeated sputum smear examination revealed Gram positive branching thin filamentous structures resembling *Nocardia* sp. so the patient was started on cotrimoxazole.

Patient was followed up and repeat X rays and CT scan after 3 months was suggestive of complete resolution of mass like consolidation and residual post infective fibrosis as shown in Fig. 1e and f. Patient improved completely without any sequelae.

Nocardia infection generally occurs in immunocompromised host and can present as lung infection. Most cases have an underlying illness including renal transplantation, hemato-oncological disease, human immunodeficiency virus infection, and long-term steroid therapy. Diagnosis of pulmonary nocardiosis depends on the isolation or demonstration of the organism from respiratory secretions such as sputum or tissue specimens.[2]

Treatment guideline have included steroid as therapy for Covid -19. Corticosteroids, though not approved by IDSA, were widely prescribed to prevent the development of ARDS in patients with COVID-19 pneumonia.[3]

Corticosteroid has a disadvantage of masking signs of current infection. It can exacerbate the latent infections, including those caused by Amoeba, Candida, Cryptococcus, Mycobacterium, Nocardia, Pneumocystis, Toxoplasma, and fungal infection.[4] There is currently dearth of data regarding complication with steroid therapy in Covid patients.

This is first case report where patient after treatment of Covid 19 presented with symptoms of pneumonia. Extensive

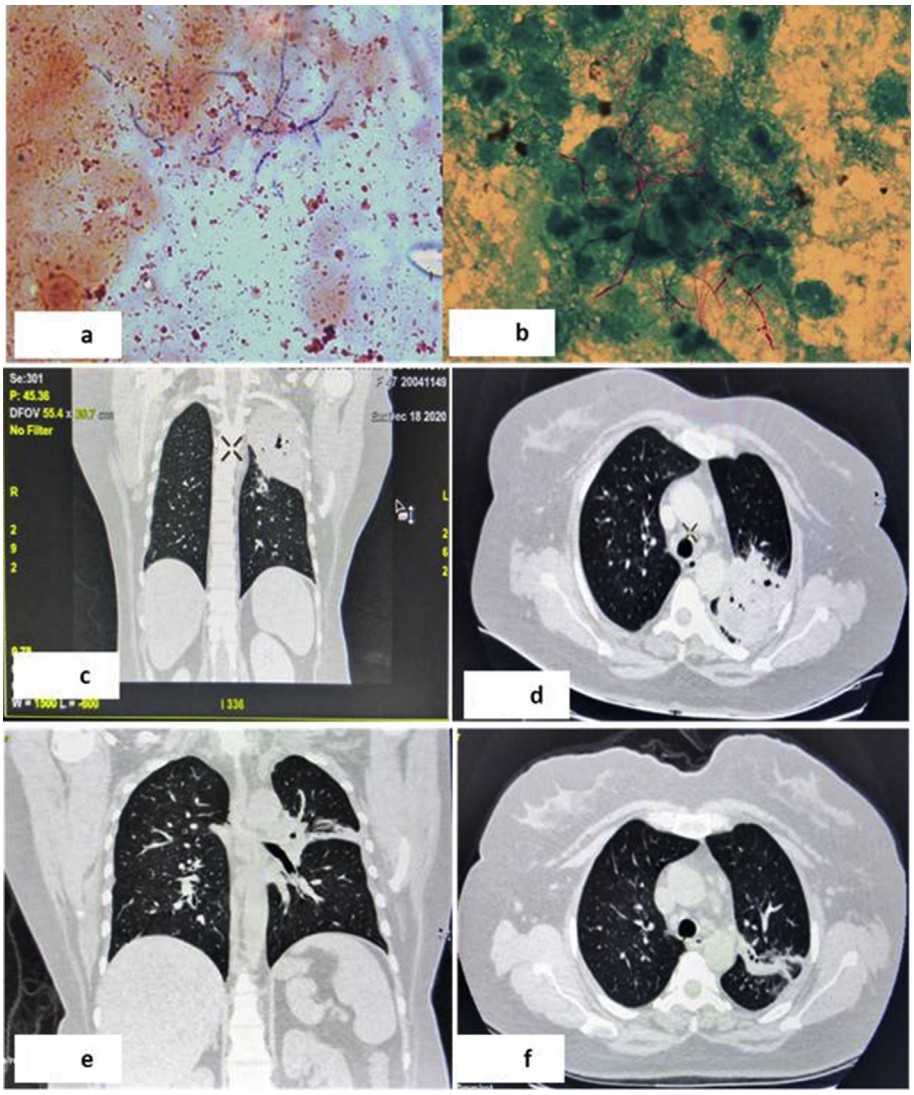

Fig. 1 – a–b:Gram positive and modified ZN smear showing acid fast branching filamentous structure, Fig. 1 c–d CT: mass.

work up confirmed nocardial infection. This case shows the importance of considering Nocardial infection as a differential diagnosis in post covid patients presenting with pneumonia. Those patients wherein steroid was added during covid treatment can later present themselves with pulmonary disease or sometimes non resolving pneumonia. In these cases all the opportunistic pathogens must be ruled out before starting any definitive therapy.

Availability of data material

All data presented in paper.

Consent to publish

Yes.

Conflicts of interest

The authors have none to declare.

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