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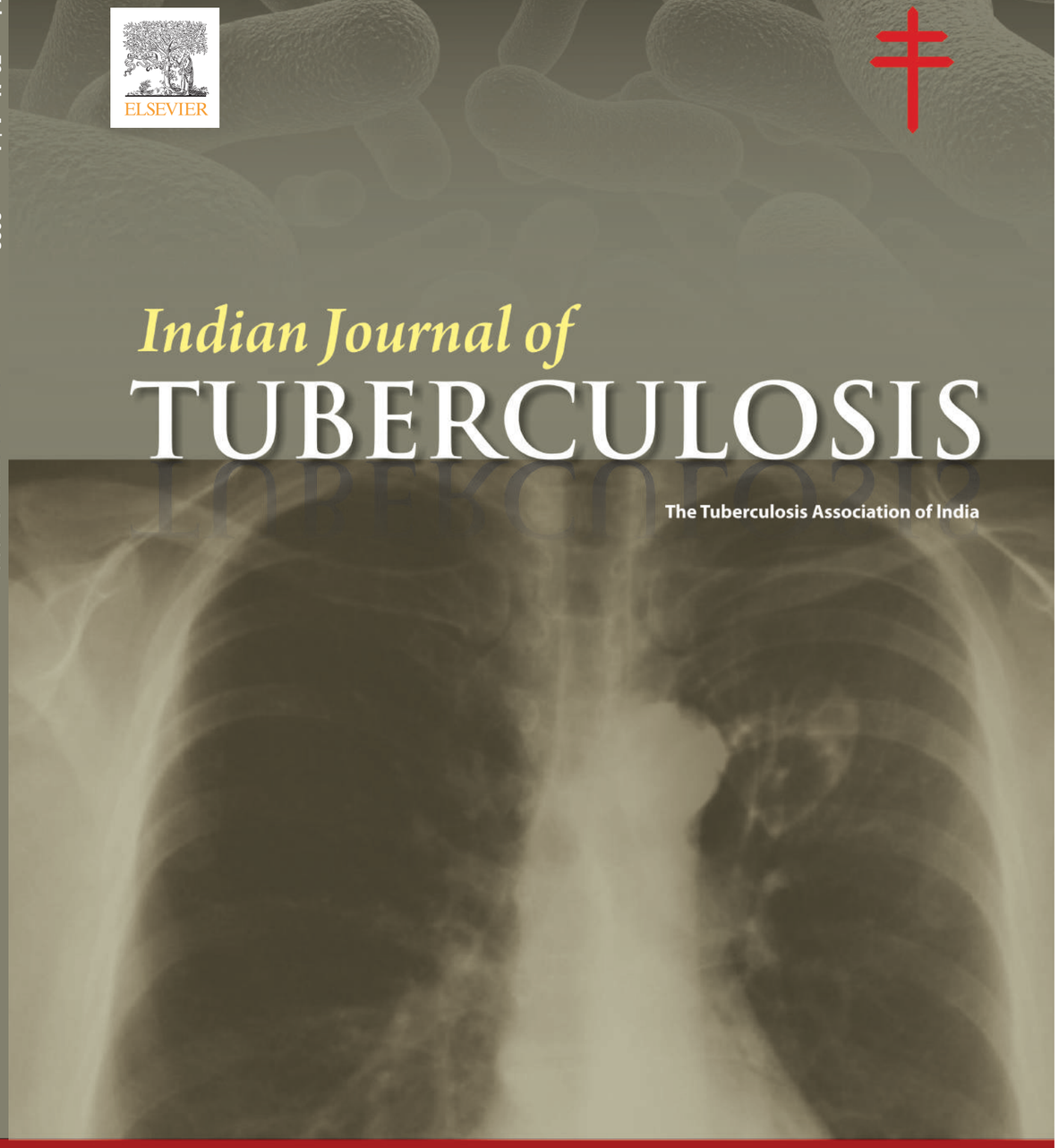

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Editorial

An odyssey from laboratory to field ? – Portable tNGS system for TB diagnosis in programmatic setting

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ABSTRACT

In spite of the elaborate diagnostic modalities available in India, there are certain shortcomings which will affect patient management. In order to address the gaps, NTEP offers scope for whole genome sequencing at few of its reference laboratories. Next generation sequencing comprising of whole genome sequencing (WGS) and targeted next generation sequencing (tNGS) are upcoming fields in TB diagnosis In a programmatic setting, tNGS offers great promise for smear positive or NAAT positive samples to be used with a Minion platform in a field setting beyond just the National reference laboratories. Once materialised, the tNGS would offer personalised patient management as well as help in public health by identification of outbreaks, transmission chain monitoring and drug resistance surveillance.

End-TB strategy envisaged a reduction in TB deaths by 35% in 2020 though only a quarter to expect 9.2% drop in TB deaths was achieved between 2015 and 2020. COVID pandemic resulted in reduced TB notifications in India, a country with high burden TB accounting for a 41% drop, the highest among overall global drop in year 2020. WHO released a list of 30 high burden countries worldwide for TB, HIV-TB and MDR/RR-TB cases, where India is listed in all three categories that warrants need for improved diagnosis and clinical management.[1] TB diagnosis algorithm in India followed under the National TB Elimination program offers upfront Nucleic acid amplification testing (NAAT) for confirmation of presumptive TB, with molecular diagnosis slowly replacing the conventional microscopy. Following TB detection, the samples are tested by line probe assay (GenoType MTBDRplus and GenoType MTBDRsl) to detect any resistance to first- and second-line drugs. Upon detection of resistance, drug susceptibility testing for pyrazinamide (Z), moxifloxacin (MFX), bedaquiline (BDQ), delamanid (DLM), clofazimine (CFZ) and linezolid (LZD) is performed. The turnaround time (TAT) for NAAT is 2 hours

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and LPA is 3 days while the TAT for DST is variable and ranges from 4 to 8 weeks (including primary culture).[2]

In spite of the elaborate diagnostic modalities available in India, there are certain shortcomings which will affect patient management. Few key issues include delay in sample transportation, significant delay in obtaining DST results which affect patients who are resistant to drugs like Z, BDQ, DLM, CFZ and LZD, lack of trained professionals to perform the test and External Quality Assurance for DST of newer drugs, certification for newer drug DST across NTEP laboratories leading to lack of DST results, and lastly discordance between molecular and phenotypic tests which needs to be addressed sooner at least for fluoroquinolones.

In order to address the gaps, NTEP offers scope for whole genome sequencing at few of its reference laboratories. Next generation sequencing comprising of whole genome sequencing (WGS) and targeted next generation sequencing (tNGS) are upcoming fields in TB diagnosis.[2,3]

Although WGS from clinical isolates have been reported earlier, it is complicated, time consuming and less cost effective compared to tNGS. WGS is preferred from cultures over direct sputum owing to the requirement of high-quality DNA.[3] Illumina platform is a well-established protocol for *Mycobacterium tuberculosis* WGS while Oxford Nanopore technology offers cost effective, simple and portable solution. Upon evaluation in comparison to Illumina Miseq platform, Minion nanopore based technology showed good genotypic and phenotypic resistance analysis similar to Miseq (phenotypic resistance agreement between the two was 98.1% for first line drugs, fluoroquinolones and second line injectables). Minion and Miseq had 96% and 96.2% concordance with phenotypic DST for drug resistance prediction. Both had similar TAT of 2–4 days and considered suitable in clinical setting for use in public health.[4] The accuracy of mutation prediction is much superior in Miseq than Minion during WGS though more evaluation studies are required to comprehend the same.[5] No studies have yet evaluated the mutation predictions of both platforms for the newer and repurposed drugs like BDQ, DLM, CFZ and LZD. Besides, mutations pertaining to newer drugs need to be better defined in terms of their significance to drug resistance.[6] Although Nanopore technology has eased the utility of WGS, it is not yet ideal for routine use of TB diagnosis. Besides, requirement of culture positive samples is still a drawback for use of WGS in rapid diagnosis either with MiSeq or Nanopore.

Recently, targeted NGS gained impetus for *M. tuberculosis* drug resistance. WGS has some major limitations like capital cost, requirement of sophisticated infrastructure, expertise and culture positivity which are overcome by targeted NGS for TB diagnosis. Previous studies had developed amplicon sequencing of specific drug resistance genes (Next Gen-RDST assay) which could help in targeted NGS for prediction of drug resistance with 97% and concordance with phenotypic DST using a MiSeq platform. Further evaluation with latest

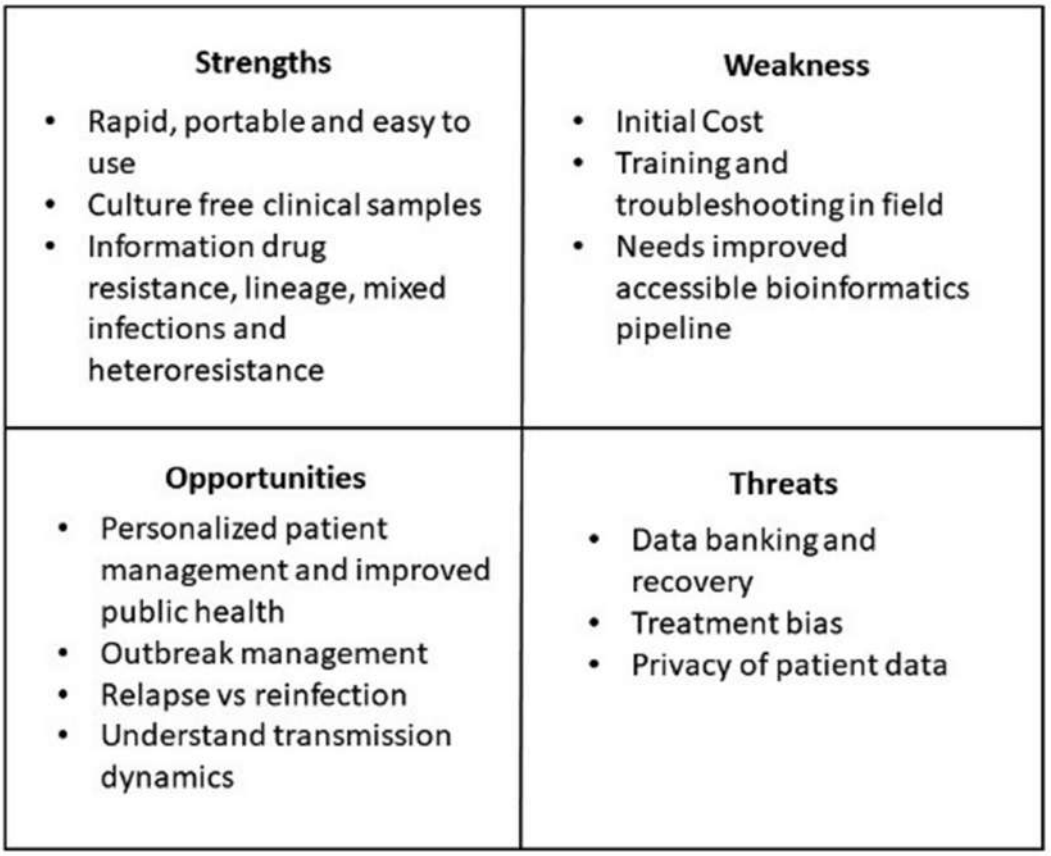

Fig. 1 – SWOT analysis for evaluation and strategic planning of use of portable tNGS for TB diagnosis in programmatic setting.

iSeq platform in comparison to the Miseq yielded 99.6% agreement with the Miseq SNP predictions.[7] Deeplex Myc-TB, a tNGS kit developed by Genoscreen has gained lot of attention worldwide for its use with direct smear or NAAT positive uncultured sputum sample and screening for 18 drug resistance genes of *M. tuberculosis* including some of the newer and repurposed drug. When assessed on a MiSeq platform, the overall sensitivity and specificity of the kit for all drug resistance genes were 84% and 100% as compared to phenotypic DST.[8] The same when evaluated in a MiniSeq and NextSeq 500 platform gave 96.7% concordance with phenotypic DST for first line drugs, 67% for FQs and 100% for second line injectables. Smear positive samples (all positive grades of 1+, 2+ and 3+) yielded complete resistance predictions.[9] Another study compared the effectiveness of tNGS in MiniSeq and Nanopore Minion system where the read quality, mapping data and variant calls were similar with composite reference coverage of 99% and full concordance on genotypic analysis and resistance prediction. Though the raw error rates were bit higher with Minion, the study does establish the use of Deeplex Myc-TB kit with a Minion platform.[10] A single tube multiplex PCR based on Deeplex Myc-TB and performed in house using both platforms for 9 drug resistance and 2 phylogeny determining regions showed strong concordance between MiSeq and Minion thus offering great promise for customized tNGS.[11]

Overall, tNGS has advantages over currently used LPA albeit with comparable TAT of 3 days by providing information on drug resistance of all TB drugs (including Z, BDQ, DLM, LZD, CFZ which are not currently covered by LPA) as well as strain lineage. Compared to WGS, tNGS in a Minion platform offers advantage of ease of operation, portability, lower instrument cost, reuse of flow cell, reduction in TAT to 3 days due to use of direct sputum compared to isolates in WGS, simpler bioinformatics platform and data storage that offers great promise in detection of drug resistance upon implementation of tNGS to field setting. Apart from drug resistance prediction, tNGS could provide information on heteroresistance, mixed infections and lineage. In addition to individualised patient treatment, tNGS could help public health professionals by providing information on cluster outbreaks, transmission dynamics, relapse or reinfection in addition to drug resistance surveillance. Further, evaluation of direct use of DNA extracted for TrueNAT with tNGS could ease the operation and transportation offering scope to continue the present diagnostic algorithm with minimal changes. Implementation of tNGS in field setting is still challenging (Fig. 1) in terms of initial investment, data management and security, training requirements and skill development posing tremendous preparation in the programme.

In conclusion.

1. In a programmatic setting, tNGS offers great promise for smear positive or NAAT positive samples to be used with a Minion platform in a field setting beyond just the National reference laboratories.
2. Development of a nationwide drug resistance user friendly pipeline for use in NTEP could help predict drug resistance better in a high burden country like India.
3. Once materialised, the tNGS would offer personalised patient management as well as help in public health by identification of outbreaks, transmission chain monitoring and drug resistance surveillance.
4. tNGS can be used in different platforms and any discrepancies from field could be resolved with Illumina platforms in National Reference Laboratories.

Conflicts of interest

The authors have none to declare.

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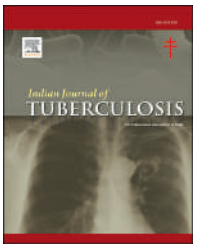

Viewpoint

Tuberculosis elimination: Looking beyond chemotherapy

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ABSTRACT

National TB elimination programs mainly focus on TB elimination through the microbiological approach of early diagnosis and treatment and thereby curtailing the transmission of the disease. But looking back, it is observed that despite this approach and various advances in research made in this front, lives are still lost due to TB. Various voices in the past have attempted to showcase the importance of socioeconomic and psychological factors that contribute to the disease causation. This oration was to highlight that we need to look at social determinants of disease causation in TB and to create a roadmap addressing these determinants for eliminating TB in the future. The various attempts being made in NTEP program to address these social issues are also highlighted.

My narration is on **Tuberculosis control: Looking beyond Chemotherapy**.

Looking back, I remember my post graduate days in JIPMER, when we used to counsel TB patients for three days on the disease causation and transmission, drugs and side effects and importance of good nutrition and treatment adherence before starting them on ATT. This system developed by **Prof. Arora sir,** long before the advent of DOTS, led to high success rate and low rates of lost to follow up. Being a doctor is not just about writing a prescription for a diseased person but involves looking beyond chemotherapy at the social, economic, and psychological factors that led to the disease in the first place and addressing these issues for a holistic cure.

1. End TB strategy and gains made so far

WHO adapted the End TB strategy in 2014 with a bold vision of *"A world free of Tuberculosis, zero deaths, disease and suffering due to TB"* with the goal of ending the world TB epidemic. The main targets are 95% reduction in TB deaths in 2035, 90% reduction in TB incidence rates compared to 2015 and zero families having catastrophic costs due to TB by 2035.[1]

There has been a paradigm shift from TB control to TB elimination.

TB control is a strategy aimed at cutting down the transmission of disease by early diagnosis and treatment, in order to break the chain of transmission. TB elimination strategy widens the control strategy by identifying and treating latent TB cases from where future cases are generated thereby preventing the emergence of new cases.[2]

TB elimination, defined as less than one TB case per million population, is a scenario where the prevalence is so low that it ceases to be a public health problem.[2]

According to WHO,[1] the current rate of decline of TB is 1.5% per year. Optimising use of current and new tools, pursuing univeral health care and social protection measures can lead to about 5% decline in prevalence per year. To

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achieve the targets of TB elimination by 2035, newer tools including vaccines, newer prophylaxis and treatment regimens and point of care testing will be required to achieve the decline of 17% needed to meet the targets.

The National Strategic plan (2017–25) for ending TB has framed appropriate strategies under four pillars based on the foundations of "universal coverage" and "social protection".

"Prevent – Detect – Treat – Build"

Preventive strategies for treatment of Latent TB infection have been introduced in NTEP. Focus on earlier diagnosis using molecular diagnostics like Xpert Mtb/XDR, TRUNAT, next generation sequencing are being introduced. DRTB treatment guidelines have been updated and focus is on all oral shorter DRTB regimen wherever feasible.[3]

The TB pipeline report 2021,[4] gives insights into new avenues of research being undertaken to achieve the global goal of TB elimination. Various preventive strategies including trials on newer vaccines and treatment of LTBI, newer diagnostic tools for earlier TB diagnosis and universal drug susceptibility testing are in vogue. Newer diagnostics tools for LTBI like TBST, Newer molecular diagnostics like Xpert MTb/XDR, focus on incipient and subclinical TB using computer aided diagnostics, POCUS, POC testing are being investigated. Various clinical trials to shorten treatment; make it all-oral; optimize drug doses, combinations, and duration; minimize toxicities; and expand treatment indications are being undertaken.

2. Fatality as a disease outcome indicator

Robert Koch in his publication in 1882, after announcing the discovery of TB bacilli to the world, observed that "*If the importance of a disease is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera, and the like. Statistics have shown that 1/7 of all humans die of tuberculosis …*"

In our institution, Government hospital of Thoracic Medicine (GHTM), Tambaram sanatorium we had analyzed the cause of TB mortality in 2016 and 2020.[5] These deaths were among approximately 5000 TB cases admitted in the institution in those years. Most of the deaths (>80%) in both these years were in males in the economically productive age group of 20–60 years. More than 70% of these deaths were in sputum positive Pulmonary TB cases with 60% of these cases being newly diagnosed with no prior exposure to Anti TB treatment. The commonest comorbidities observed were Diabetes mellitus and HIV in 30% of patients. In more than 60% of cases it was observed that death was due to respiratory failure and within a week of diagnosis. 90% of deaths were in Drug sensitive TB cases, 7% in MDR TB cases, 2% in INH mono resistant TB cases and 1% in XDR TB.

These observations lead us towards the following inferences.

- Most deaths were in males in productive age group reflecting the socioeconomic burden of the disease
- Most deaths were in PTB and in sputum positive reflecting transmission before the time of diagnosis
- Most common comorbidities DM and HIV indicating need for better management of these comorbidities
- Most deaths were in newly diagnosed cases, within a week of admission and respiratory failure indicating the delayed diagnosis and loss of pulmonary reserve by the time the diagnosis was made
- Most deaths in DSTB indicating a need to refocus on these cases

More than 90% of TB deaths were observed to be in DSTB than in DRTB which highlights the importance on earlier diagnosis and appropriate treatment of these cases to bring down the mortality and prevent the emergence of drug resistant TB.

Many a young productive life of writers like Keats, George Orwell, Thoreau, Kafka, Chekov, Scientists like Braille, Laennec, Schrodinger, various statesmen and other personalities have had their life cut short by Tuberculosis. The loss to mankind is immeasurable for they had the potential to steer the course of mankind.

Reflecting on the mortality data, makes us believe that despite so many advances in various fronts on diagnosis and treatment, there are factors other than chemotherapy that must be addressed if we have to eliminate TB. Maybe it's time to step back and see the whole picture.

As the American author Jonathan Kellerman said, "Life *is a prism and what you see depends on how you turn the glass*". Tuberculosis can be viewed as a disease caused by bacteria, or it can be seen as a social disease, the causation of which lies in the socioeconomic and psychological make up of an individual. If TB must be eliminated, it is vital that both these views are accepted, and the disease seen beyond microbiological factors and chemotherapy.

3. Social determinants of tuberculosis

"*The past speaks to us in a thousand voices, warning and comforting, animating and stirring to action*," said Felix Adler.

In the past, various voices have spoken about the *"Germ Theory of Disease"* versus the *"Seed and Soil Theory"* of disease. While the germ theory envisages disease as a result of various microbes, in TB this concept has led us towards using the medical technique of early diagnosis and treatment for control of the disease. The seed soil theory emphasises that for the seed to flourish the soil must be conducive. For us it means that the vulnerability of patients for TB is the soil on which the seed, the TB bacilli, multiplies and grows. Understanding this is vital while looking at TB elimination from a public health point of view.

One such voice was that of Dr. David Chowry Muthu who founded the Thambaram TB sanatorium in 1928. In his book "Pulmonary Tuberculosis, its etiology and treatment' published first in 1921, he said "Cause of tuberculosis is not the tubercle bacillus but that the state of the patient is brought about by various social and economic conditions, by want of fresh air, natural surroundings, sufficient food, by the overwork and worry produced chiefly by the industrial system …."[6]

Another voice was that of Jean Dubos whose *book "The white plague. Tuberculosis, man and the society"* was first published in 1952. Dubos debated that the discovery of TB bacilli led us away from the concept of inherent susceptibility. Success of chemotherapy was a therapeutic shortcut to disease control and perceived a danger in scientific and social logic that aims to treat the disease rather than modify the underlying cause of vulnerability. Dubos stressed in his book that it was imperative to investigate the human and environmental factors that determine resistance to infection and suggested two ways to attack the progression of the disease, decreasing risk of infection and boosting resistance.[7]

Dubos effectively argued that throughout the 20th century, even before the introduction of effective chemotherapy, TB incidence declined steadily in most industrialized countries although it did increase temporarily during the two world wars. The reasons for this decline in TB incidence were period of economic growth, social reform, poverty reduction and improved living conditions as well as important advances in medicine and public health.[7]

Speaking of war, it leads to not only direct casualty but lot of indirect casualty and disruption of health and health delivery services. Wars and armed conflicts are powerful public health enemies that destroy basic medical infrastructure, hinder health agendas, hamper immunization programs, and cause significant shortages in healthcare workers and medicines.[8] The recent political events in Ukraine are a cause for concern as Ukraine is a country with a high prevalence of DRTB and disruption of health care services together with large scale migration will have catastrophic consequences on the global TB elimination programs. Covid pandemic has taught us that in the era of globalization and international travel, we can't afford to ignore events happening seemingly far away from us.

Incidentally, Selman Walksman, the discoverer of Streptomycin was born in Ukraine which makes me reflects how many noble laurates and people who have the potential to alter the history of humankind die as a result of unnecessary armed conflicts. Men must be in peace to realize their full potential.

WHO had set up a **commission on social determinants of health (CSDH)** in 2005 for improving health by addressing the "causes of the causes of ill health". The **key determinants** for this were.

- The inequitable distribution of TB among various nations
- Clustering of TB cases among disadvantaged groups such as the poor, the hungry and ethnic minorities
- Growing awareness of social determinants of health in other areas esp. HIV/AIDS.[9]

Broadly social determinants can be classified as downstream (providing clinical care), midstream (addressing individual's social needs) and upstream (improve community conditions) determinants.[10]

The downstream and midstream determinants in TB are addressed in various steps being taken in the program including active case finding, airborne infection control, air pollution reduction, tobacco cessation, better management of comorbidities like HIV, DM, Malnutrition, and various lung diseases. This serves to curtail the transmission of the disease and addresses vulnerability issues like in HIV and DM.

The National TB elimination program has recognized these social determinants and various strategies have been put in place by the Central TB division. Steps taken include the nutritional support program "Nikshay Poshan yojana", Gender responsive approach to TB, integrating Maternal and child health services with the program to reduce maternal and infant mortality due to TB, TB free workplace campaign and TB forums to reach the unreached and support TB patients through the treatment and recovery phase.

The best solution regarding the upstream determinants is those envisaged by Dr. David Chowry Muthu in 1921.[6]

- Stop all wars and war expenditure, reduce armaments, and promote peace
- Reduce taxation, free imports, and exports, cheapen the necessities of life
- Encourage industry, promote agriculture and small holdings, so that people can live on fresh foods, carry out land reform and land development
- Help to multiply garden cities and garden suburbs, clear slums and build houses

4. *In conclusion*

- First, the sine qua non of TB care and control remains high quality diagnostics and treatment
- Second, NTPs could strengthen collaboration with other public health program to contribute to the prevention, treatment and management of HIV, malnutrition, smoking-related conditions, diabetes, and alcohol abuse.
- Third, and most challenging, interventions outside the health sector will have to be strengthened

As Thiruvalluvar, the Tamil poet who lived in the first century BC said

நோய்நாடி நோய்முதல் நாடி அதுதணிக்கும்

வாய்நாடி வாய்ப்பச் செயல்.

nōynāṭi nōymutal nāṭi atutaṇikkum

vāynāṭi vāyppac ceyal.

Diagnose the illness, trace its cause,

Seek the proper remedy and apply it with skill.

I wish to express my heartfelt gratitude to Prof. Arora sir and TBAI for this award, Dr. Jaikishan sir and the organising team of NATCON for inviting me here and Prof. Sridhar sir for mentoring and moulding me.

I dedicate this oration, to our "Temple of Learning", Government Hospital of Thoracic medicine, Tambaram sanatorium and to all those ever associated with the institution.

Thank you for this opportunity.

Transcript of the Dr. S.N.Tripathi Memorial Oration delivered during 76th NATCON on 13.4.2022 by Prof. Vinod Kumar Viswanathan

Conflicts of interest

The author has none to declare.

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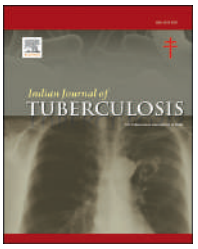

Review article

The contribution of private health facilities to the urban tuberculosis program of Afghanistan

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ABSTRACT

Setting: Although the prevalence of tuberculosis (TB) is generally higher in urban areas than in rural areas, coordination between the private and public sectors for TB control is weak.
Objective: To share experience from an urban DOTS program in five cities of Afghanistan.
Design: An urban DOTS project was designed in 2009 in Kabul, Afghanistan, and later expanded to Kandahar, Jalalabad, Herat, Mazari-i-Sharif, and Paul-i-Khomri cities.
Results: In total, 57 public health facilities and 49 private facilities provided DOTS services in the five cities from 2015 to 2018. A total of 28,542 (10.6%) adults (aged ≥15) screened were diagnosed with TB (all forms). The private sector contributed 5,618 (19.7%) of those. Positivity rates among presumptive TB cases in public facilities were 18.9%, 12.5%, 14.4%, and 4.8% in 2015, 2016, 2017, and 2018, respectively. In private facilities, positivity rates were 25.8%, 39.5%, and 27.4% in 2016, 2017, and 2018, respectively.
Conclusion: The private sector's contribution to case detection was very high and the TB positivity rate among people screened in the private sector was high, which could be due to more selective screening rather than all health facility visitors done by public health facilities.

1. Introduction

With rapid urbanization of the world, we expect population health to improve overall because people will have better access to health services and infrastructure. However, other factors, including overcrowding, proliferation of slums, and lack of access to health services because of cost or cultural factors, aggravate TB transmission in cities.[1] In one European study, the rate of TB in cities was two times higher than the national notification rates.[2] In many countries, the urban TB burden is much higher than the rural one; for example, in Bangladesh the prevalence of TB in urban areas was 334 per 100,000 population, while it was 274 per 100,000 in rural

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areas.[3] In the same national survey of TB in Bangladesh, the TB death rate was higher in urban areas, at 8.5% versus 4% in rural areas.[3] In a Kenyan national prevalence survey, the urban TB prevalence rate was 760 per 100,000 population, while rural TB prevalence was 453 per 100,000.[4] In Pakistan, the reverse is true: prevalence of TB in rural areas was 471 per 100,000 population, while it was 309 per 100,000 in urban areas.[5] A study comparing multidrug-resistant TB isolates in rural India and Mumbai city found that 51% of isolates from Mumbai residents were multidrug-resistant, while the proportion was only 2% in patients who lived in a nearby rural region. The authors posit that Mumbai has become a hot spot for MDR-TB because private-sector practitioners, a major source of health care in the city, are not regulated and do not follow the nationally approved regimens for treatment of TB.[6] In a South African study, cases of extremely drug-resistant TB(X-DR TB) were clustered in poor neighborhoods, characterized by lower educational attainment (12% vs. 9%), higher unemployment (29% vs. 20%), and a lower proportion of homes with flush toilets (36.4% vs. 68.9%).[7]

In Afghanistan the incidence of TB was estimated at 189 per 100,000 in 2017, a level that has remained the same for the past 17 years, while the case notification rate increased from 103.7 per 100,000 in 2008 to 131.3 per 100,000 in 2017.[8] The case notification rate varies by province, from as low as 46 per 100,000 in Panjshir Province to 300.2 per 100,000 population in Kunar Province (WHO database, 2017), but no disaggregated data for urban and rural areas exist for Afghanistan. In Kabul, a TB project performance report reported that case notification increased from 59 per 100,000 in 2009 to 125 per 100,000 population in 2015.[9]

We analyzed the data collected in the routine health information system for five cities, excluding Kabul, to show the increase in case notification and treatment outcomes.

2. Study population, design, and methods

2.1. Settings

In 2017 we published an analysis of six years of experience in Kabul with urban DOTS,[9] a successful approach that was expanded to five other cities of Afghanistan with a combined population of 1.9 million: Kandahar, Jalalabad, Herat, Mazari-i-Sharif, and Paul-i-Khomri.[10] In 2015, 35 (42%) of 83 public health facilities were providing DOTS services, and 18 (18.3%) of 98 private health facilities were DOTS centers (National TB Program [NTP], surveillance database, 2015).

2.2. The DOTS expansion approaches

We also published information about the approaches we used for DOTS expansion in 2017,[9] so this article briefly summarizes them. Two types of health care providers serve urban areas: the public sector managed and funded by the government, and the private for-profit sector. TB diagnosis and treatment services are free even in the private sector, except for the consultation fee for the first visit of a client to a private health facility. The Ministry of Public Health recognized some of the private health facilities as DOTS centers, based on criteria such as existence of a laboratory and outpatient department (OPD), and advised clients to use those facilities, as well as public-sector health facilities, to obtain TB prevention and care services.

A baseline assessments of both the public and private health facilities at the beginning of the expansion was done with the objective of assessing human resources, and diagnostics capacities, as well facilities space adequacy to give TB services. Based on the findings, training as given to health workers on the national comprehensive TB care and prevention guideline, and standard operating procedures, reagents for diagnostics, drugs, and other supplies supplied. Provincial or NTP program officers supervised and mentored both the private and public DOTS centers quarterly.

2.3. Data collection and analysis

The data we used were collected in Excel using the routine NTP recording and reporting forms and reported quarterly to the provincial offices and the NTP. Although TB services were provided in urban areas, clients came from both rural and urban areas. In our analysis we included patients aged 15 years and above. Data were entered in Excel and analyzed manually. Proportions, rates, and ratios were used to analyze the secondary data.

2.4. Ethical statement

We used only routine program data for this analysis, and we solicited approval from the NTP to publish the data. The research was implemented in close collaboration with the NTP, which reviewed and approved the manuscript for publication.

3. Results

In total 181 health facilities had DOTS capacity as per the nationals standard, and we were able to engage 106 (58.5%) of them as DOTS centers. The number of DOTS centers in the private sector grew from 18 in 2015 to 57 in 2018. Of the total of 3,581,079 OPD visitors over the age of 15 years old, the majority of patients (2,960,718 [(89.6%]), visited public health facilities. The number of people seeking care in public health facilities increased from 502,112 in 2015 to 862,191 in 2018. The same trend of increase in patient load, from 65,547 in 2015 to 107,868 in 2018, was also observed in private health facilities. The striking difference between the public and private health facilities was that the private health facilities did not screen all OPD visitors except those whose major complaints coincided with sign and symptoms of TB. Of all screened OPD visitors (unfortunately the number screened was not recorded), 268,677 were reported to be presumptive TB cases and 28,542 (10.5%) were diagnosed with TB (all forms). The positivity rates for the public health facilities were 18.2%, 12.2%, 13.9%, and 4.5% in 2015, 2016, 2017, and 2018, respectively. In the private sector, the positivity rates were higher, at 25.1%, 38.1%, and 26.2% in 2016, 2017, and 2018 respectively (Table 1).

Out of 28,542 TB cases (all forms) diagnosed in the public and private health facilities in the four years, 10,156 (35.5%) were bacteriologically confirmed. There was a large difference

Table 1 – Screening for TB and TB cases diagnosed in public and private health facilities, 2014–2019.

Year	2015		2016		2017		2018		Total
Indicator	Public	Private	Public	Private	Public	Private	Public	Private	
Total number of Health facilities (HFs) with DOTS capacity	67	71	70	83	83	90	83	98	181
Number (%) of HFs that were DOTS centers	35	18	38	37	42	45	49	57	106
Number of patients ≥15 years who visited OPDs	502,112	65,547	792,000	73,036	804,415	94,765	862,191	107,868	3,301,934
Total number of presumptive TB cases identified	27,444	NA	41,278	4,511	39,921	5,134	142,761	7,628	268,677
Total number of all forms of TB patients diagnosed	5,191 (18.9)	329	5,196 (12.5)	1,166 (25.8)	5,757 (14.4)	2,030 (39.5)	6,780 (4.7)	2,093 (27.4)	28,542 (10.6)
Number (%) bacteriologically confirmed TB patients	1,918 (36.9)	50 (15.2)	2,037 (39.2)	310 (26.6)	2,222 (38.6)	401 (19.7)	2,714 (40.0)	504 (24.1)	10,156 (35.5)
Treatment success rate	81	NA	82	83	85	84	85	85	NA

in the proportion of bacteriologically confirmed TB cases between the public and private health facilities. In the public sector, 8,891 (38.1%) cases were bacteriologically confirmed TB, while the proportion was 1,265 (22.5%) in the private health facilities. Clinically diagnosed TB (11,822 [41.4%]) and extrapulmonary TB (6,564 [23.0%]) constituted the majority of the TB diagnoses in both public and private DOTS centers.

Treatment success rates also improved, from 81% in 2015 to 86% in 2018. The treatment success rate was not different between the public and private health facilities: it was 81% in 2015 and increased to 85% in 2018 in the public sector, and it rose from 82% in 2016 to 85% in 2018 in the private health facilities.

4. Discussion

Based on the experience gained in Kabul, the NTP and Challenge TB together expanded the urban DOTS program to five more cities, which resulted in increasing case notification of all forms of TB from 5,520 in 2015 to 7,988 in 2018. Many of the changes came from private-sector engagement. In 2015 only 329 cases were notified by the private sector. This number reached 2,093 in 2018—an almost 536% increase, while the increase in public health facilities was only 30%. The increases can be explained by the expansion of TB DOTS to the private sector and the capacity-building efforts in the public sector. The TB positivity rate out of all presumptive TB cases has decreased from 18.9% in 2015 to 4.8% ($p < 0.05$) in 2018 in the public health facilities. In the private sector, the all forms of TB positivity rate was 25.8%, 39.5%, and 27.4% in 2016, 2017, and 2018 respectively. Because all visitors in the OPD are screened for TB in the public health facilities, the positivity rate is low in the public sector.

In an evaluation of passive and active case-finding approaches in health facilities of Afghanistan, the rate of TB (all forms) diagnosed was 0.3% in the first year and 0.5% in the second year,[11] which is a little lower than our findings in the public sector. The NTP should decide if blanket TB screening in OPDs is effective in Afghanistan, from a time and cost perspective, as compared to targeted screening of contacts and people from high-risk groups.

We saw also a difference in the proportions of extrapulmonary TB and bacteriologically confirmed TB diagnoses between the public and private centers. Of all forms of TB diagnosed, 8,891 (38.8%) were bacteriologically confirmed in the public centers, while only 5,618 (22.5%) were bacteriologically confirmed in the private sector ($P < 0.05$). The proportion of bacteriologically confirmed cases was lower than the proportion reported in 2017, which was 61% of pulmonary TB cases.[8] One possible reason for the discrepancy in the proportion of bacteriologically confirmed cases between the reported national proportion and the proportion in the five cities in this study could be that the capacity to diagnose non-pulmonary presumptive TB is low in peripheral health centers, and cases are referred to secondary and tertiary hospitals in the cities. As a result, most of the referred cases diagnoses would be either extrapulmonary TB or smear-negative clinically diagnosed pulmonary TB cases.

The private sector is usually attracted to TB diagnosis because patients can pay for registration and investigations, and it is in the interest of the private sector to provide comprehensive clinical services to retain clients who have the ability to pay. The challenge comes in administering daily DOT and following up with patients, which are free services. In Afghanistan the treatment success rate in the private sector is equivalent to the rate in the public sector, which was 85% in 2018. In Kenya the private-sector treatment success rate ranged from 74% to 85% in a four-year report, and the reason for such a high rate was that the patients seen in the private sector can pay for registration and diagnostic tests, while the government provides drugs free of charge.[12] We recommend research on incentives for the private sector to provide free TB prevention and care services in Afghanistan.

5. Conclusions

We found that the private-sector contribution to case detection in Afghanistan was high and the treatment success rate was also high and equivalent to that of the public sector. Although the contribution is high, what we do not

know is that what factors motivate private practitioners to give full or partial free services to patients. Studying these factors will help to include more private health facilities, including those at the lower level, such as pharmacies, in TB screening.

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Authors' contributions

Designed the study and led the research: A. Hammim, M.K. Seddiq, S.M Sayedi, K.M. Rashidi, G.Q. Qader, L. Manzoor, M. Melese, P. G. Suarez. All others participated in the data analysis and writing. All authors have approved the manuscript for submission.

Conflicts of interest

The authors have none to declare.

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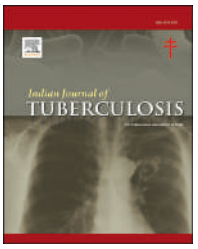

Review article

Comparison of IGRA and TST in the diagnosis of latent tuberculosis among women of reproductive age in South India

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ABSTRACT

Background: Latent tuberculosis infection (LTBI) is a mycobacterial infection defined on the basis of cellular immune response to mycobacterial antigens. The tuberculin skin test (TST) and the Interferon-Gamma Release Assay (IGRA) are the two tests currently used to establish the diagnosis of LTB. Literature suggests that a study regarding tuberculosis (TB) infection among women of reproductive age group is limited.

Methods: Female household contact, married, aged 18–49 years underwent written consent form and are screened for LTBI using the TST and IGRA. Participants are injected with TST [5 tuberculin unit (TU), purified protein derivative (PPD)] and IGRA [QuantiFERON®-TB Gold Plus kit (QFT-Plus)]. All the household contacts were followed-up for one year for incident TB cases. Statistical analysis was done using STATA version 14 (StataCorp., Texas, USA). Cohen's kappa test was used to determine the agreement between two tests.

Results: The prevalence of LTBI was found to be 69% (either TST or IGRA positive). Positivity rate of IGRA was higher when compared to that of TST. Out of 139 participants, 68 (49%) tested positive for TST, 80 (57.6%) tested positive for IGRA and 52 (37.4%) tested positive for both. Discordant results were observed in about two fifth of the study population and there was poor agreement between the two tests.

Conclusion: Longitudinal studies are required to detect incident TB cases to evaluate the usefulness of these tests. The study was found that IGRA is more consistent to diagnosis of latent tuberculosis infection than the TST. Such studies can also be performed in varied

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settings among different populations which would help us to improve the diagnosis of LTBI and consequently help in TB control.

1. Introduction

Latent Tuberculosis Infection (LTBI) is defined as "a state of persistent immune response to *Mycobacterium tuberculosis* without clinically manifested evidence of active TB disease". It is estimated that about one fourth of the world's population (1.7 billion people) has LTBI.[1] Persons with LTBI are asymptomatic and do not spread the infection. About 5%–15% of the people with LTBI go on to develop active TB and with poor immunity and existing co-morbidities, the risk of active TB increases.[2] The detection and treatment of LTBI is one of the pivotal steps in tuberculosis control.[3] India is a high TB burden country accounting for about one-fourth of the global TB cases.[1] With intensification of TB elimination activities in India, the diagnosis and management of LTBI should be focused upon in the coming years. Tuberculin Skin Test (TST) and Interferon-Gamma Release Assay (IGRA) are the two tests which are available to ascertain LTBI. Both are indirect tests which detects the body's immune response to TB bacilli exposure. As there are no gold standard tests for diagnosis of LTBI, it is difficult to assess the performance of these two tests.

TST is based on a delayed-type hypersensitivity reaction that occurs when those infected with *M. tuberculosis* are exposed to certain antigenic components present in extracts of culture filtrates, the "tuberculins".[4] A meta-analysis reported a pooled sensitivity and specificity of 70% and 60% respectively for TST for detection of infection with *M. tuberculosis*.[5,6] The sensitivity of TST results could be affected by malnutrition, immunodeficiency, TB disease and HIV[7,8] and the specificity may be influenced by BCG vaccination status, subjective interpretation of results and non-tuberculous environmental mycobacteria.[9–11] On the grounds of reliability and accuracy TST has its limitations[12] IGRA is an indirect test for *M.tuberculosis* infection which measures the cell mediated immune response to peptide antigens that simulate mycobacterial proteins. It has high specificity compared with TST because the former measure cellular response of T-lymphocytes to antigens of *M. tuberculosis* found in BCG and most non-TB mycobacteria.[2]

Studies have reported pooled sensitivity and specificity of up to 93% and 99% respectively.[5,6] But in areas with high TB prevalence including India, the sensitivity of IGRAs has not shown superiority over the conventional TST.[13] However, in some populations, a higher sensitivity and specificity of more than 90% were reported in which suggests that the IGRA may be utilized in selected populations.[14–19] The specificity of IGRA is affected with false conversions and reversions and peripheral lymphocyte counts.[20,21] Further indeterminate IGRA results have been associated with extremes of age.[22] Recently acquired TB infection is recognized as a major risk factor for progression to active TB. The high rate of TB exposure among women of reproductive age is of particular concern, since TB is a leading cause of maternal mortality in low-income countries.[23]

The aim of this study is to evaluate the agreement between these two tests in diagnosing LTBI in the absence of confounding factors such as gender, age, HIV status and behavioral characteristics like substance abuse, smoking and alcohol use for a reproductive age group of women. All of the study participants were recruited from the same TB endemic community. The results of the study may help in deciding the diagnostic modality for LTBI.

2. Materials & methods

2.1. Study population and recruitment

The data was collected as a part of a study titled "Impact of Pregnancy on Tuberculosis" conducted between September 2016 to October 2019 under Regional Prospective Observational Research for Tuberculosis (RePORT) India. We aimed to study TB exposure patterns in women of reproductive age enrolled in the RePORT cohort of Puducherry and Tamil Nadu. For this purpose, the participants included in the analysis were the household contacts (HHC) of new smear positive, culture confirmed persons with pulmonary TB (index case) enrolled up to 8 weeks following the index case enrollment. Inclusion criteria: female household contact, married, aged 18–49 years. A household contact is defined as anyone who on average had significant contact with the index case for at least 3 months before study enrollment as defined by: sleeps under the same roof as the index case on average at least 5 days per week or shares at least one meal per day with the index case on average at least 5 days per week or watches television (or equivalent) with index case on average at least 5 days per week. Participants with the history of tuberculosis and those with history of hysterectomy or tubal ligation were excluded. The study was approved by the Boston University Medical Campus Institutional Review Board (USA) and the JIPMER Institutional Review Board in India. Written informed consent was obtained from all the study participants.

2.2. Study procedure

A pre-designed structured questionnaire was used to collect the socio-demographic characteristics, alcohol use, tobacco use, details regarding comorbidities and details regarding nature of contact with index cases (time spent with the index cases, sleeping arrangements). Presence of Bacille Calmette-Guerin (BCG) scar and body mass index (BMI) were recorded at the time of enrolment. Blood collection for IGRA was done prior to TST. In this study none of the participants with LTBI were given any prophylactic treatment for TB.

2.3. *Assessment of LTBI*

a) **Tuberculin skin test (TST)**

Participants were injected with 5 TU (0.1 ml) of purified protein derivative (PPD) intradermally on the forearm by trained healthcare workers. (Span Diag India) and the size of induration (in millimeters) was measured 48–72 hours after injection. The cut-off size for positive TST was an induration of ≥5 mm.

b) **IGRA [QuantiFERON®-TB Gold Plus kit (QFT-Plus)]**

QuantiFERON®-TB Gold Plus kit (QFT-Plus) (Qiagen, Germantown, MD, USA) which detects interferon-γ (IFN-γ) by enzyme-linked immunosorbent assay (ELISA) was used for IGRA. The kit contains four color tubes namely Nil, TB Antigen Tube 1(TB1), TB Antigen 2(TB2) & Mitogen. TB1 and TB2 contain peptide antigens from the *M. tuberculosis* complex associated antigens ESAT-6 and CFP-10. Nil and mitogen tubes serve as negative and positive control respectively. About 1 ml of peripheral venous blood was collected through venipuncture in each tube. Following sample collection, the tubes are incubated for 16–24 hours and centrifuged. The plasma is then removed and IFN-γ is measured by ELISA. The results were then interpretated as positive, negative or indeterminate using manufacturer's instructions.

Statistical analysis was done using STATA version 14 (StataCorp., Texas, USA). Percentages were used to describe the characteristics of the study population. Assessment of agreement between TST & IGRA was done using Cohens kappa test. k value ≥0.75 denoted excellent agreement beyond chance, 0.4 to 0.75 denoted moderate agreement and ≤0.4 denoted poor agreement.

3. Results

Of the 139 participants included in the study about two-fifths were between 31 and 40 years of age and half of the participants spent at least six hours a day with the index TB cases. About half of the study participants were spouses of the index cases. BCG scar was present in about 121 (88%) participants 19 (13.7%) participants were underweight and 42 (30.2%) were overweight. None of the participants reported alcohol use whereas tobacco and smoking was reported by four participants (see Table 1).

Among the 139 participants, 68 (49%) tested positive for TST, 80 (57.6%) tested positive for IGRA and 52 (37.4%) tested positive for both. The prevalence of LTBI as determined by either positive TST or IGRA was 69% (n = 96; 95% CI 61.3%–76.8%). Overall agreement between QFT-Plus assay and TST was 66% (k = 0.36; 95% CI = 0.21–0.51) and the strength of agreement was poor. About 43 (30.9% or 31.8%) participants had discordant results, the higher proportion (n = 28, 65%) of which were due to negative TST and positive QFT-Plus assay (see Table 2).

The positivity rate for TST and QFT-Plus was nearly 60% among 41–49 year age category. Among those with BCG scar about half tested positive for TST and nearly 60% (n = 68) tested positive for QFT-Plus. Among those who spent >18 hours/day with the index case positivity rate for TST and QFT-Plus were 78% (n = 7) and 89% (n = 8) respectively. Positivity rates for TST and QFT-Plus was nearly 60% among those who shared the same room (see Table 3).

4. Discussion

The utility of TST and QFT-Plus assay have been evaluated in detecting LTBI among a cohort of women of reproductive age

Table 1 – Characteristics of the household contacts stratified by tuberculin skin test and QuantiFERON-TB Gold Plus results (N = 139).

Characteristics	Total N (%)	TST		[a]QuantiFERON-TB Gold	
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Total	139	68 (48.9)	71 (51.1)	80 (59.3)	55 (40.7)
Age (in years)					
20–30	36 (25.9)	14 (38.9)	22 (61.1)	20 (57.1)	15 (42.9)
31–40	62 (44.6)	30 (48.4)	32 (51.6)	38 (62.3)	23 (37.7)
41–49	41 (29.5)	24 (58.5)	17 (41.5)	22 (56.4)	17 (43.6)
BCG scar					
Present	121 (87.1)	58 (47.9)	63 (52.1)	68 (57.1)	51 (42.9)
Absent	18 (12.9)	10 (55.6)	8 (44.4)	12 (75)	4 (25)
BMI (kg/m^2)[b]					
Underweight	19 (13.7)	10 (52.6)	9 (47.4)	10 (55.6)	8 (44.4)
Normal	60 (43.4)	25 (41.7)	35 (58.3)	30 (52.6)	27 (47.4)
Overweight	42 (30.4)	25 (59.5)	17 (40.5)	28 (66.7)	14 (33.3)
Obese	17 (12.3)	8 (47.1)	9 (52.9)	11 (64.7)	6 (35.3)
Smoking	4 (2.9)	4 (100)	0	3 (75)	1 (25)
Comorbid conditions					
Diabetes	5 (3.6)	1 (20)	4 (80)	2 (40)	3 (60)
Other comorbid conditions[c]	6 (4.3)	3 (50)	3 (50)	3 (50)	3 (50)

[a] Indeterminate results of QuantiFERON-TB Gold are not included.
[b] Body mass index classification based on Asia Pacific guidelines.
[c] Asthma, Hepatitis, kidney failure.

Table 2 – Factors affecting exposure of the household contacts to the index cases and the results of tuberculin skin test and QuantiFERON-TB Gold (N = 139).

Characteristic	Total	TST		[a]QuantiFERON-TB Gold	
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Total	139	68 (48.9)	71 (51.1)	80 (59.3)	55 (40.7)
Relationship to the index case					
Spouse	69 (49.6)	34 (49.3)	35 (50.7)	39 (60)	26 (40)
Daughter in law	25 (18)	9 (36)	16 (64)	12 (48)	13 (52)
Mother	15 (10.8)	11 (73.3)	4 (26.7)	9 (60)	6 (40)
Daughter	14 (10.1)	6 (42.9)	8 (57.1)	8 (57.1)	6 (42.9)
Sibling	8 (5.8)	4 (50)	4 (50)	6 (75)	2 (25)
Others[b]	8 (5.8)	4 (50)	4 (50)	6 (75)	2 (25)
Sleeping location					
Same room, same bed	9 (6.5)	5 (55.6)	4 (44.4)	6 (66.7)	3 (33.3)
Same room, different bed	64 (46)	36 (56.3)	28 (43.7)	36 (58.1)	26 (41.9)
Same building, different room	57 (41)	25 (43.9)	32 (56.1)	34 (61.8)	21 (38.2)
Different building that is part of the same household	7 (5)	2 (28.6)	5 (71.4)	3 (42.9)	4 (57.1)
Others[‡]	2 (1.4)	0	2 (100)	1 (50)	1 (50)
Time spent with index case					
<1 hour	9 (6.5)	4 (44.4)	5 (55.6)	4 (44.4)	5 (55.6)
1 to <6 hours	26 (18.7)	9 (34.6)	17 (65.4)	14 (56)	11 (44)
6 to <12 hours	80 (57.6)	42 (52.5)	38 (47.5)	48 (61.5)	30 (38.5)
12 to <18 hours	15 (10.8)	6 (40)	9 (60)	6 (42.9)	8 (57.1)
>18 hours	9 (6.5)	7 (77.8)	2 (22.2)	8 (88.9)	1 (11.1)

[a] Indeterminate results of QuantiFERON-TB Gold are not included.
[b] Aunt, uncle, cousin, grandchild relative house.

group who were household contacts of smear positive culture confirmed TB patients. The prevalence of LTBI in this study was found to be 69% (either TST or IGRA positive). Positivity rate of IGRA was higher when compared to that of TST. Discordant results were observed in about two fifth of the study population and there was poor agreement between the two tests.

The prevalence of LTBI was found to be similar to the rate observed in a study conducted in southern part of India.[24] IGRA positivity was higher than TST positivity in our study population and this echoes the study findings from India and South Africa.[25,26] However, in another study conducted in India the positivity rates were similar for both the tests. Positivity rate increased with increasing age for TST, while for QFT-Plus it was similar across age groups. Similar results were observed in a study conducted in Peru.[27] But this was in contrast to the study conducted in India where there was a strong association with QFT-Plus positivity and increasing age.[26] The higher cut off level used in the study may have contributed to contradicting results.

Among those who have BCG scar the positivity rate was higher in QFT-Plus assay when compared to TST. Positivity rates of both the tests were higher among malnourished participants compared to those with normal BMI. This shows malnourishment may increase the chance of latent TB infection. This is contradictory to the findings observed in the studies conducted in other parts of India where the positivity was higher among those with normal BMI.[24–27] QFT-Plus positivity was higher when compared to TST across BMI categories and the difference was pronounced among obese participants (65% vs 47%).

IGRA positivity was higher than TST across various relationship categories except among mothers of index cases where TST positivity was higher. This could be because mothers of the index cases were older and there was increase in TST positivity rate with increasing age as observed earlier. Overall, the prevalence of LTBI was higher among mothers of the index cases. This could be because of the close nature of relationship with the index cases and the higher age which could have increased the chances of exposure to TB bacilli. Among the HHCs who shared the same room as index cases for sleeping the positivity rate was higher than those sleeping in separate rooms or buildings. The positivity rates increased with the increase in the time spent with the index cases. This is in line with the study finding that longer duration of exposure correlating with LTBI diagnosis and suggest that exposure time could be a vital component in LTBI diagnosis.[28] However, the positivity rates were lower among those who spent between 12 and 18 hours day with the index cases. The reason for this could not be clearly understood. Other factors like ventilation of the accommodation, severity of the TB disease in index cases and the proximity with the index cases could have influenced the results. The prevalence of smoking

Table 3 – Distribution of tuberculin skin test and QuantiFERON-TB Gold test results in the study population (N = 139).

TST	QuantiFERON-TB Gold			Total N (%)
	Positive n (%)	Negative n (%)	Indeterminate n (%)	
Positive	52 (37.4)	15 (10.8)	1 (0.7)	68 (48.9)
Negative	28 (20.1)	40 (28.8)	3 (2.2)	71 (51.1)
Total	80 (57.5)	55 (39.6)	4 (2.9)	139 (100)

among this study population was very low (n = 4) and amongst them all were TST positive and three fourth were IGRA positive. However, this sample size is too small to arrive at a conclusion. A further study on the influence of smoking and LTBI diagnostics is warranted. In this study none of the HHCs developed active TB at the end of follow up period of one year in spite living in a TB endemic community and being exposed to smear positive index cases in their homes. As the specificity of IGRA is better than TST.

5. Conclusion

This study has shown that the comparison of IGRA and TST in the diagnosis of LTBI has poor agreement between the two in the absence of confounding factors like gender, age and HIV infection. With no gold standard tests to compare with, longitudinal studies to detect incident TB cases are required to evaluate the usefulness of these tests. Such studies in varied settings and among different populations can help us improve the diagnosis of LTBI and consequently help in TB control.

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Conflicts of interest

The authors have none to declare.

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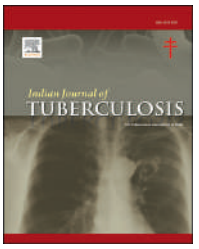

Review article

Tuberculosis of hand and wrist: Varied clinical presentation and functional outcome by surgical intervention in 13 cases

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ABSTRACT

A prospective study of 13 cases with tuberculosis of hand and wrist was presented with mean age of patients being 42.7 years (range 18 months to 84 years). Pain, swelling and difficulty in movement of adjacent joints were the most common presenting complaints. The discharging sinus, abscess & nerve compression were also observed in some cases. Out of 13 cases, bone involvement was seen in one case, joint involvement in five cases, soft tissue involvement in five cases and two cases had both soft tissue and joint involvement.

All patients had undergone operative intervention for confirmation of diagnosis and improvement in function. Surgeries like open biopsy, debridement and tenosynovectomy were performed. Depending upon drug sensitivity on culture and histopathology report, standard anti-tuberculous treatment (ATT) was commenced under supervision of Infection Disease expert. Hand function was evaluated by modified Green and O'Brian score. The mean score was 58.84 (35–70) before any intervention and it improved to 89.23 (60–100) at 6 months follow up after surgical intervention and ATT.

In conclusion, surgery may help for early functional recovery and for encouraging patient to use their hand for activities of daily living.

1. Introduction

Tuberculosis (TB) of bone is a common form of extrapulmonary tuberculosis mainly affecting long bones, joints and spine.[1] Diagnosis of extrapulmonary bony manifestation of TB is always challenging due to its variable and atypical presentation.[2] The hand and wrist involvement are seen in only 1%–2% cases of extraarticular TB.[1,3] Clinical presentation of TB in hand and wrist is varied most of the time and has its own

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symptomatology. Wrist joint TB may originate from the carpal bones, capsule or synovium.[4] Capitate is the most commonly involved carpal bone reported. Wrist TB may spread contiguously from the tenosynovitis of flexor and extensor tendon around wrist and digits. In hand, interphalangeal joint or palmar spaces or phalanges have variety of manifestation of TB.

The purpose of this study is to recommend surgical intervention in the form of extensive debridement, tenosynovectomy and achieving primary closure in cases of hand and wrist TB. We have found considering only biopsy and starting ATT may take longer duration to control the infection which may lead to stiffness or disability in function of hand.

2. Material and method

This was a prospective study undertaken between January 2018 to January 2020 in tertiary care hospital. Patient who were suspected to have TB of hand and wrist included in the study. We had twelve patients of TB of hand and wrist in which one patient had involvement of bilateral wrists. There were three males and nine female patients. The youngest patient was 18 months old child and eldest was 84-years with mean age of 42.7 years. The anatomical distribution of disease was as follow.

Based on radiological findings, the anatomical distribution was as follow.
Involvement of proximal phalanx in one patient.
Involvement of proximal interphalangeal joint (PIPJ) in 3 cases (Fig. 1)
Involvement of metacarpophalangeal (MCP) joint in 2 case
Involvement of radiocarpal joint in 1 case (Fig. 2)
Involvement of extensor tendons in 1 case
Involvement of flexor tendons in 3 cases (Fig. 3)
Involvement of both radiocarpal joint and extensor tendons in 2 cases

The common presentation was pain, swelling or soft tissue lump, abscess, infection, painful & restricted movement or deformity in and around joints of hand and wrist. They were suspected to have tuberculosis. Relevant blood investigations which suggest markers of infection, x-rays were performed. MRI was advised in cases of soft tissue involvement like tenosynovitis. MRI also helped in localizing and study the extension of the disease. Chest X-ray was done in all patients to rule out pulmonary Koch's.

Surgery was performed in all cases for diagnosis, functional improvement and infection control in hand and wrist. Open biopsy, extensive debridement, tenosynovectomy & primary complete or partial closure was considered depending up on the lesion. The diagnosis was made on the basis of histopathology and culture sensitivity of Acid-fast bacilli (AFB). The Gene Xpert was studied in two patients to detect mycobacterium tuberculosis and rifampicin resistant. In one case, the patient had palmar abscess and in other case, patient had bilateral involvement of TB infection. All patients were treated with Antitubercular treatment (ATT) using 4 drugs (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol) for 2

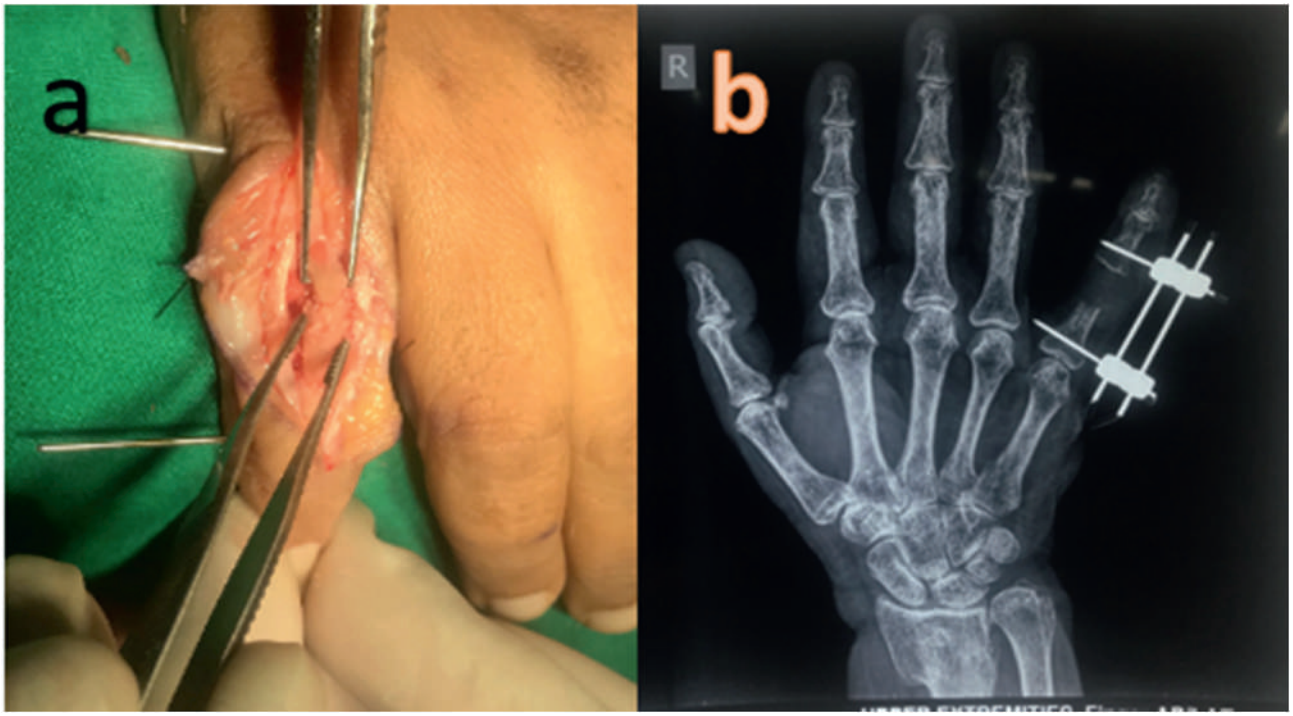

Fig. 1 – (a &b) A 58 years old female had right little figure pain and difficulty in movement without any trauma. Dorsal open debridement of infected tissue and external fixator application was performed. Later arthrodesis of PIP joint was done for stability and grip.

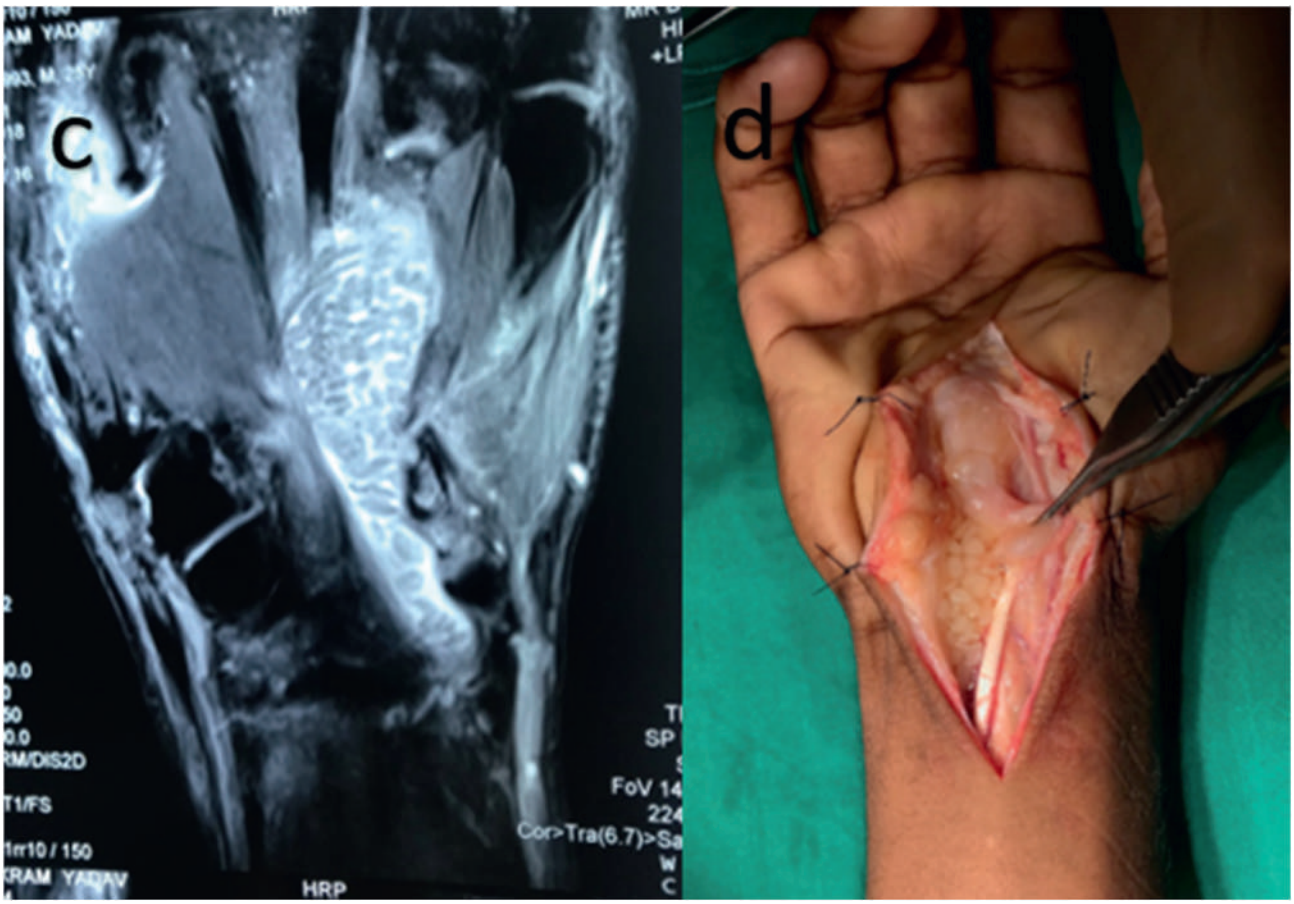

Fig. 2 – (c) MRI of right wrist was suggestive of hyperintense signal at carpel tunnel region. (d) Rice granules in carpel tunnel region.

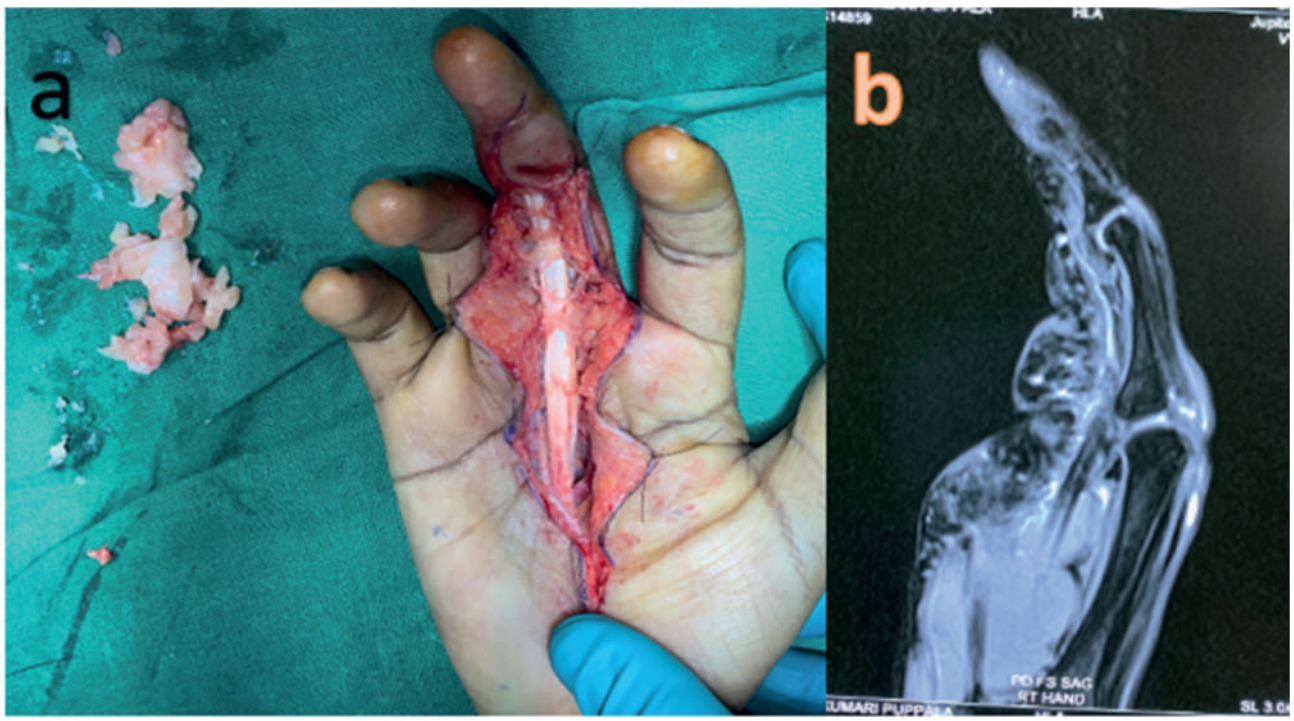

Fig. 3 – (a) A 29 years old female had middle finger pain and difficulty in movement. Extensive debridement and tenosynovectomy was done. (b) MRI suggestive of extensive enhancing soft tissue changes in synovial sheath of flexor tendons of middle finger.

months and Isoniazid, Rifampicin and Pyrazinamide for 4 months with periodic investigations like ESR, Liver Function test under the guidance of infection disease expert. The follow up was studied in all patients was at 3 weeks, 8 weeks, 3 months and 6 months (see Table 1).

3. Results

The hand function was evaluated by Green and O'Brien score before surgery. The mean score before treatment was 58.84 (Normal range is 35–70).

Patients who presented with palmar abscess had lowest score Green and O'Brien score preoperatively. In our case study, none of the patients had past history of pulmonary Koch's. The hand function score was recorded post operatively at 6 months from surgery. The mean score recorded was 89.23 (Range is from 60 to 100). We had excellent result in 7 patients, good result in 3 patients, fair result in 2 patients and poor result in 1 patient. Modified Green and O'Brian score[5] was used to evaluate function in paediatric cases.

4. Discussion

Tuberculosis is an infection caused by mycobacterium tuberculosis which may lead to multisystemic involvement. As the main portal of entry is by respiratory system, the pulmonary Koch's is more common. But gastrointestinal route or direct inoculation through skin is also noted and proved in literature. The Progression of TB as a disease depends upon immune response of patient.

A prevalence of skeletal TB is around 10–35% worldwide.[6] Around half cases of skeletal TB comprise of involvement of spine.[7] An Immunocompromised status like HIV infection, diabetes mellitus, chronic renal failure and malnutrition predispose for often to monoarticular TB involvement.[8]

Even though, TB of wrist or hand is presented less commonly, it should be certainly considered as a differential diagnosis for chronic wrist and hand swelling especially in countries where TB is prevalent. The diagnosis of TB has to be confirmed by histopathology and culture of infective material. Nucleic acid amplification test such as gene Xpert MTB/RIF test allow rapid identification of amplified specific RNA and DNA sequence via nucleic acid molecules along with detection of rifampicin resistance.[9] The newer method of faster detection of tuberculosis are available by methods like purified ESAT-6 by magnetic bead-coupled gold nanoparticle -based immune PCR assay[10] and identification of mycolic acid using surface enhanced Raman scattering.[11]

MRI is commonly used as a diagnostic tool for local extent of disease and effect on soft tissue involvement and association with neurovascular bundle, tendons in wrist and hand. The findings like bone erosion, synovial fluid effusion with thickening of synovium and osteomyelitis are captured on MRI.[12]

Prakash and Mahtani[13] have reported in their study of 44 paediatric patients that the diagnosis was done by biopsy in most of the cases. Out of 44, they found 29 patients (65%) malnourished as per Rainey-McDonald nutrition index. There were 13 patients who did not respond to 8 weeks of ATT. Those patients were managed with surgical intervention. The proximal phalanx of 4th digit and metacarpal of 5th digit were the most common site of involvement. The capitate & scaphoid involvement was found to be more common in wrist. The mean initial Green and O'Brian functional score was 52.7 and final was 93.4. They observed excellent results

Table 1 – Patient profile, diagnosis, complications and Green & O'Brian score.

Case No.	Age	Gender	Diagnosis	Surgery	Green & O'Brian score	
					1st visit	6 months postoperative follow up
1	19	F	Chronic osteomyelitis of metacarpal head of middle finger	Dorsal Open biopsy	65	100
2	22	F	Wrist infected extensor tenosynovitis with ? COM carpus	Tenosynovectomy	70	85
3	26	F	Index finger PIP joint septic arthritis	open biopsy	65	100
4	58	F	Little finger PIP joint septic arthritis	open biopsy	60	100
5	35	M	Right Wrist infected flexor tenosynovitis with CTS	Tenosynovectomy	60	85
6	35	M	Left wrist COM of capitate and lunate with ? Discharging sinus	open biopsy	65	75
7	9	F	Little finger proximal phalanx ? COM	Dorsal open curettage	55	95
8	29	F	Middle finger flexor tenosynovitis	Tenosynovectomy	55	60
9	30	F	Hand palmar abscess with infected flexor tenosynovitis	open biopsy	35	85
10	31	M	Middle finger PIP joint synovitis	open biopsy	55	100
11	1.5	F	Distal forearm/wrist level 1st extensor infected tenosynovitis with abscess	Debridement and biopsy	55	100
12	47	F	Post polydactyly excision MCP region painful soft tissue lump	Excision & biopsy	60	100
13	84	M	Wrist carpal bone? COM with extensor tenosynovitis	open biopsy	65	75

Table 2 – Comparison of present study with previous study of minimum 10 cases.

References	No of Patients	Diagnosis	Treatment	No of surgery	Complication	MDR
Benchakroun et al.[15]	11	surgical biopsy	ATT + Surgery in failure	–	Wrist stiffness	None
Benkeddache and Gottesman[16]	27	Biopsy	ATT + Surgery in failure	2	Wrist stiffness	None
Kotwal and khan[14]	32	core biopsy, open biopsy,PCR	ATT	8	None mention	None
Prakash and Mehtani[13]	44	Core biopsy	ATT + Surgery in failure	13	Wrist stiffness in 7, pathological fracture 1	3
Our study	12	surgical biopsy	ATT + Surgery	13	Wrist stiffness in 4	2

in 18 patients, good results in 15 patients, fair in 3 patients and poor in 8 patients. Out of 13 patients who had undergone surgical intervention, 5 had favourable results (Green and O'Brian score >80) and 8 patients had unfavourable results (Green and O'Brian score <80). The Surgery was performed within mean period of 9.2 weeks of development of symptoms (favourable group) having significantly better result than surgery performed after mean period of 11.6 weeks (unfavourable group).

Kotwal and Khan[14] have published their study of 32 cases of TB hand and wrist. Out of 32, 12 cases had bone & 20 patients had soft tissue involvement. Pain and swelling were the most presenting complaints. In their study, 8 patients required surgical intervention who did not respond up to 8 weeks of ATT. The mean Green and O'Brian functional score was 58.3 pre-operatively and final was 90.5 observed after surgery.

Table 2 compares the published series of greater than 10 patients with the present series.

Brashear and Winfield[17] have mentioned in their study that 10 patients required surgery in the form of synovectomy in 3 patients, arthrodesis in 7 patients and debridement in 1 patient. Solid fusion observed in 4 patients. In all patients, the disease was inactive after treatment with ATT.

Prakash and Mahtani[13] and kotwal and khan[14] have reported 70.45% (31 out of 44 patients) and 75% (24 out of 32 patients) outcome by conservative management with ATT respectively.

5. Conclusion

MRI and open surgical biopsy are the good diagnostic modality for extent of involvement and false negative reports for tuberculosis for wrist and hand. Early diagnosis and early commencement of ATT is very important for better functional outcome. But ATT takes at least 8 weeks to show some improvement in infection and reduction of lump or swelling or infection. As some cases show frank infection like abscess or sinuses, usage of hand and acceptance of that person in social environment remains major issue. Therefore, author recommends early surgical intervention in the form of debridement and tenosynovectomy for abscess and discharging sinus. In cases which are not responding to ATT and causing non-healing of wounds, the possibility of Multi-Drug-Resistance (MDR) tuberculosis should be kept in mind & get sorted out with help of infection disease expert.

Code availability

Not applicable.

Authors' contributions

Data acquisition and surgery performed by Dr. Parag Lad and Dr. Ashish Phadnis, Assistant surgeon and manuscript writing by Dr. Sanket Tanpure.

Ethics approval

The procedures were performed in this study after ethical approval from institutional ethical committee.

Consent to participate

Informed consent was obtained from all patients for being included in the study.

Consent for publication

Consent was obtained from all authors for publication of study.

Conflicts of interest

The authors have none to declare.

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Review article

Need to reinvigorateTuberculosis research in India – A review of studies registered under clinical trial registry of India

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Tuberculosis

ABSTRACT

Tuberculosis (TB) is one of the most serious public health issues in India. According to the global TB report 2020, India accounts for about one-quarter of the global TB burden. Despite considerable advances in mandatory notification of all TB cases, incorporation of the national health programmes with general health services (National Health Mission), and national drug resistance surveillance and many other accomplishments, much more needs to be considered in India to significantly decrease TB incidence. Research is the foundation for medical breakthroughs. In this study, all Tuberculosis-related studies registered under Clinical Trial Registry of India from its inception in July 2007 to February 2021 were reviewed and analysed using the keyword ''Tuberculosis'' in the 'Trial Search' section. A total of 31,196 studies were registered in CTRI, with 180 studies (0.58%) being related to tuberculosis. Of these studies, 76 (42.2%) were interventional in nature. These consisted of evaluating different management or treatment TB (50%, n = 90), diagnostic studies (19.4%, n = 35) and studies related to screening and prevention of TB (7.8%, n = 14). Maximum studies were conducted to evaluate safety and efficacy of anti-TB drugs (10%, n = 18) and to evaluate efficacy of shortening of duration of treatment (8.9%, n = 16). The studies related to extra pulmonary TB, MDR TB and TB in special populations and sources of funding and locations of the study sites were also analysed. These indicate that only minimal TB-related researches are conducted in India. It is indispensable to promote tuberculosis research in India in order to eradicate this infectious disease.

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1. Introduction

Tuberculosis (TB) is one of India's most serious health problems. India accounts for approximately a quarter of the global TB burden based on global TB report 2020. The estimated TB incidence in 2019 was 2,640,000. Tuberculosis had led to the deaths of 9,500 HIV-positive people in 2019, while 436,000 HIV-negative people died. After South Africa, India has the highest estimated number of HIV-related TB cases. India has the highest prevalence of tuberculosis and multidrug-resistant tuberculosis worldwide.[1,2,3]

Several initiatives have been launched by the Indian Council of Medical Research (ICMR) with the goal of bringing together all major national and international stakeholders in order to develop new tools (drugs, diagnostics, and vaccines) for the management of TB.[4] The ICMR has considered TB as a national priority. Research should be accelerated in order to improve cure rates and reduce the number of new cases. The implementation of research should centre on identifying and developing newer and easier diagnostic methods and treatment strategies in collaboration with all stakeholders; which in turn will ensure high-quality diagnosis and care for all TB patients.

Despite significant progresses in stringent notification of all TB cases, incorporation of the national TB elimination programme with general health services (National Health Mission), and national drug resistance monitoring systems, more remains to be accomplished to significantly reduce TB incidence in India. Hence it is imperative to revitalise research in this domain, particularly related to new drugs, fixed drug combinations and newer regimes. In this study, we have attempted to analyse the type of TB related research being conducted in India which are registered under the Clinical Trial Registry of India (CTRI) to identify the gap areas.

2. Methods

All TB-related studies registered under CTRI from its inception in July 2007 to February 2021 (13.5 years) were reviewed and analysed. The studies were found using the keyword "Tuberculosis" in the 'Trial Search' section. The studies being conducted in patients with TB, studies on other factors related to TB, and studies mentioning TB as an inclusion criterion were chosen for further analysis from all of the search results obtained. The variables used to collect data included the type of TB trials, trials conducted in special populations, studies on MDR-TB, pulmonary and extra pulmonary TB, the sources of funding for the studies (pharmaceutical sponsored/academic/government funded/PG thesis), and the geographical distribution of studies. The variables were analysed using descriptive statistics using SPSS for Windows, Version 20.0, SPSS Inc.

3. Results

A total of 31,196 studies were registered in CTRI from 2007 to February 2021, among which a total of 180 studies (0.58%) were related to TB. Out of the 180 TB-related studies, 76 (42.2%) were interventional in nature. The distribution of different areas of research in TB is depicted in Fig. 1. Maximum number of studies were conducted in evaluating different treatment strategies for TB (50%, n = 90). This was followed by diagnostic studies (19.4%, n = 35) and studies related to screening and prevention of TB (7.8%, n = 14). It is also worthwhile to note that while the International Clinical Trial Registry Platform (ICTRP) had 1963 TB-related studies registered, India had only 180, less than one-tenth of the international, in contrast to the burden of disease in the country.

Upon analysing the therapy areas maximum studies were conducted to evaluate safety and efficacy of anti-TB drugs (10%, n = 18) and to evaluate the efficacy of shortening of duration of treatment (8.9%, n = 16). We noted that studies related to evaluation of add on therapies (10%, n = 10) like Vitamin D, Vitamin C or antimicrobials and evaluation of new FDC or molecule (n = 9) and process of care change (n = 9) were among most preferred areas of research. The different therapy areas related to TB research is depicted in Fig. 2.

Out of the 180 TB-related studies in India, 70 of them had elderly population in the inclusion criteria. The studies that

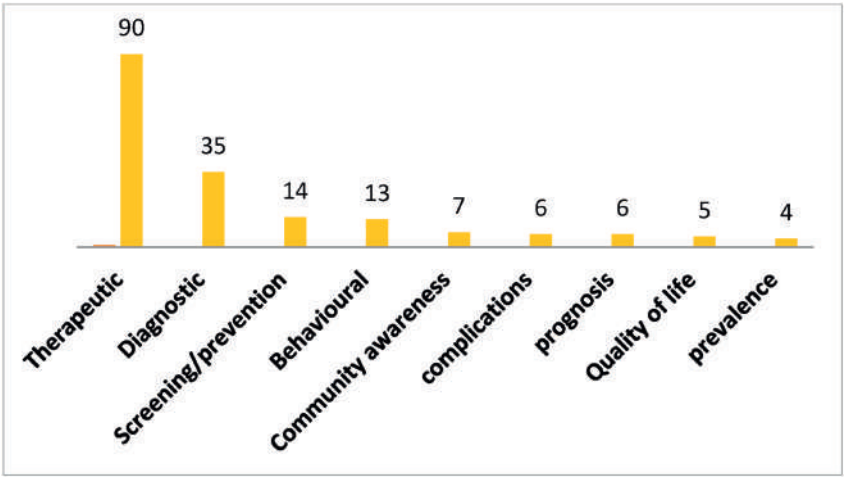

Fig. 1 – Different areas of Tuberculosis-related research in India.

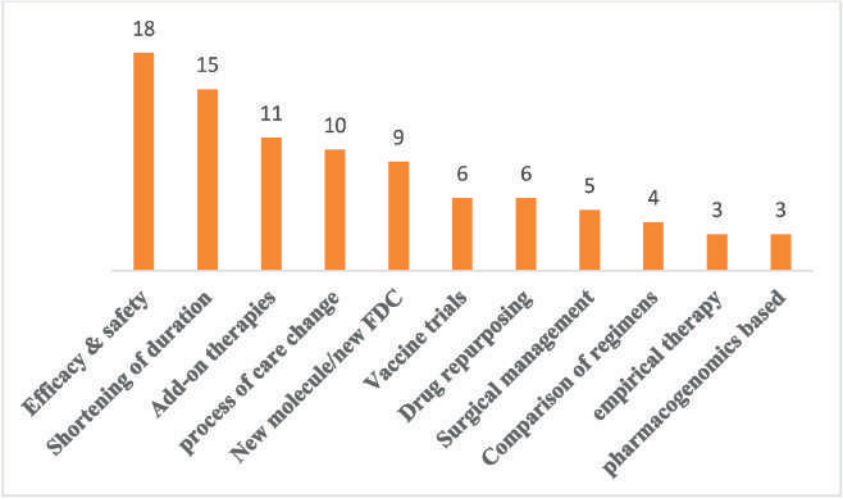

Fig. 2 – Types of therapeutic trials related to tuberculosis.

were conducted in other special population included those that were conducted in children (n = 17), HIV patients (n = 12) and neonates (n = 3) (Fig. 3).

Majority of the studies dealt with pulmonary TB (78.4%, n = 145) and rest of the studies were related to extra pulmonary TB like intestinal TB, TB of the spine and TB of the genital and urethral tract (19.4%, n = 35) (Fig. 4). It was also observed that 13 studies out of the 180 studies (7.2%) were conducted on MDR-TB cases exclusively.

A large number of studies (47.2%, n = 85) were sponsor funded from government funding agencies like ICMR, Non-Governmental Organisations (NGOs) and other international foundations. Post graduate thesis (n = 48) were mostly self-funded. The academic clinical trials and other investigator initiated trials were also self-funded (n = 32). The clinical trials conducted and sponsored by pharma companies accounted only 8.3% (n = 15) (Fig. 5).

Several studies were multi-centric and were conducted in several parts of India. It was observed that majority of studies were conducted in south India (n = 88), mainly in the state of Tamilnadu and Karnataka. Among north Indian states most of the studies were conducted in the states of Delhi, Chandigarh, Haryana and Rajasthan (n = 61). In Central India majority of the studies were conducted in Maharashtra (n = 25). Both western India and eastern India accounted for very few studies (Fig. 6).

4. Discussion

Several million new cases of TB and millions of death are reported every year, TB imposes a high burden on human suffering and loss, reflected predominately on poor and vulnerable people in low and middle income countries. It is also noteworthy in this situation that the national systems are missing several millions cases every year. Emergence of resistance to anti-TB drugs is also a serious concern. All these are the potential hurdles to achieve the end –TB strategy. Hence, research is the critical pillar in the journey to achieving the ultimate end-TB Strategy. To transform the way tuberculosis is diagnosed, treated, and prevented, it is necessary to improve the effectiveness of existing tools and

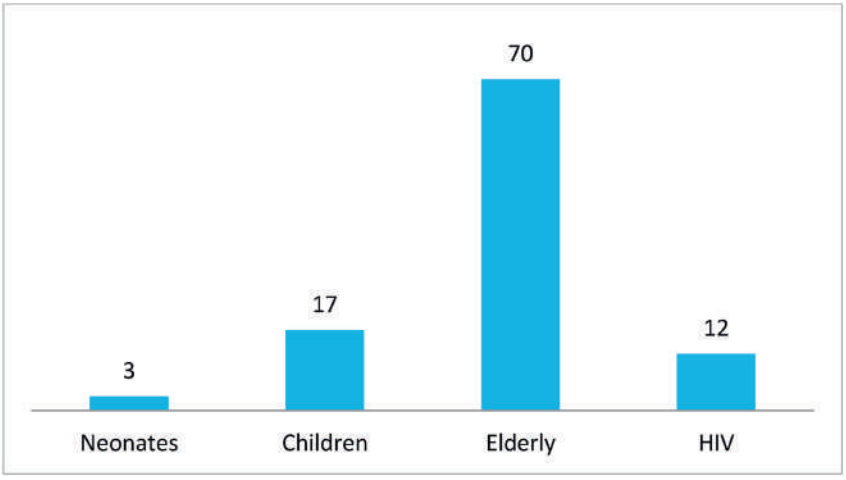

Fig. 3 – Tuberculosis-related research in special population.

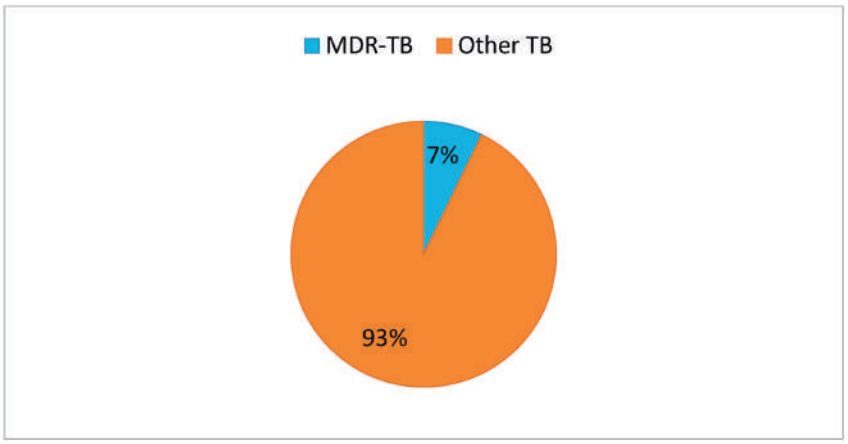

Fig. 4 – Clinical trials related to Multi Drug Resistant TB in India.

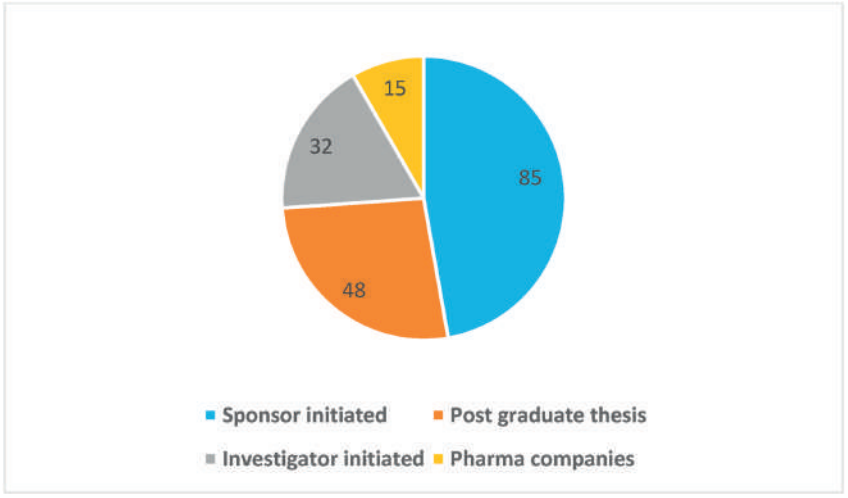

Fig. 5 – Sources of funding for TB-related research in India.

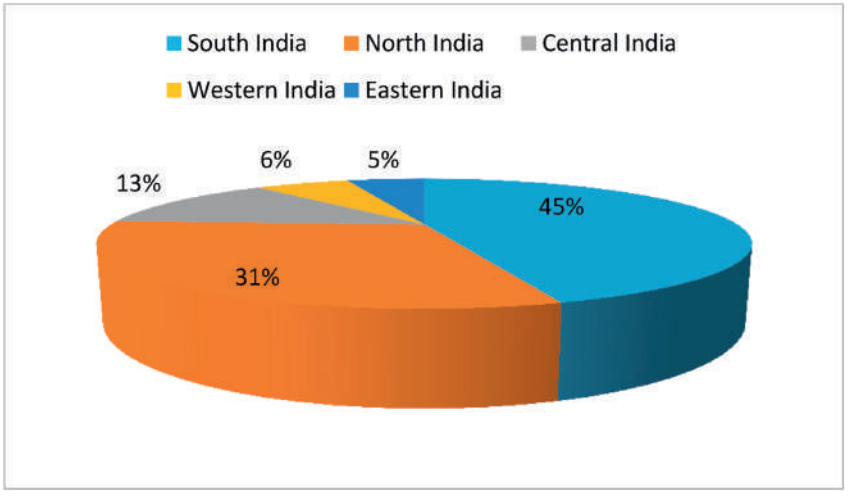

Fig. 6 – Location of TB-related study sites in India.

develop revolutionary new technologies, which is possible only through strengthening of research activities in this field.

The results of our study indicate that even though India carries a major burden of TB cases, the proportion of research conducted in this field in the country is comparatively very

less. In a study conducted by Thakur et al in 2017 among major stakeholders regarding the challenges of TB control in India, lack of public and patient awareness, limitations of resources and infrastructure, inadequate notification and general negligence were observed as the major challenges in TB elimination in India. These findings call for reinforcing of research activities pertaining to all aspects related to TB.[5]

The major areas of research that should be focussed in the target to eliminate TB are in the research of therapeutic drugs and screening and prevention. In our study it was observed that maximum studies were conducted in the field of drug related researches. Among these, majority of studies were conducted to assess the efficacy and safety of new molecules and fixed drug combinations (FDCs) and studies to evaluate the efficacy of shortening of anti-TB regimens. Even though this suggests a good trend, the researches on epidemiological aspects related to prevalence and other parameters as well as screening and prevention and behavioural modifications need to be further intensified.

Individuals who are HIV-positive are 20–40 times substantially more prone than people who are not HIV-positive and live in the same country to develop TB Disease. TB is the most common HIV-associated opportunistic disease in the world, contributing for more than a quarter of the 2 million AIDS deaths in 2008; it accelerates HIV disease progression by raising infectivity and decreasing HIV treatment efficacy.[6] As TB is a risk factor in HIV-positive individuals, more efforts should be driven to management of TB in HIV patients. The study by Dravid et al conducted in western India highlights the need for TB prevention and management in HIV positive individuals.[7] Similarly management and prevention of TB in pregnancy, neonates and elderly population should receive urgent consideration and measures should be implemented to foster studies in such special populations.

The deployment of drug-resistant tuberculosis (DR-TB) continuous monitoring systems improves access to adequate and accurate treatment and care. In the systematic review and meta-analysis carried out by Lohiya et al on prevalence and patterns of drug resistant pulmonary tuberculosis in India, the significant burden of drug resistant TB in Indian population was unveiled and the importance of deployment of universal drug susceptibility testing in all districts of the country, as well as continuous DR-TB surveillance.[8]

Since 2006, funding for tuberculosis detection, treatment and prevention has almost doubled, but it still falls far short of what is required. Still majority of these funding sources emerged from domestic agencies like ICMR and NGOs.[1] The study by Sagili et al highlights the importance of operational research for global fund supported projects to facilitate research in areas of national priority that influence policy and practice.[9]

According to our findings, there are very few pharmaceutical-sponsored drug intervention studies. The pharma company sponsored trials included few new molecules and rest of the studies were new about new FDCs and new diagnostic methods. Hence it is critical to promote interests of pharmaceuticals companies in this domain to propel us forward to achieve our strategy.

It was also observed that there is a gradual increase in the number of studies registered each year. This is due to mandatory registration of all studies with the CTRI as well as WHO efforts to strengthen TB-related researches in high TB burden countries. However, our research shows that India is still lagging in TB-related research, and several milestones to the end TB strategy can only be achieved by promoting research in this field.

The COVID-19 pandemic threatened to undo the recent progress in lowering the global burden of tuberculosis disease, especially in India. The reallocation of TB-related human, financial, and other resources to the COVID-19 response had a negative impact on critical TB services. In accordance with WHO recommendations, countries have reported expanding the use of digital technologies for remote care and monitoring services and lessening the requirement for visits to health centres by prioritising home-based treatment and providing transportation to TB patients.[2]

In order to ensure that patients are properly diagnosed and started on the most effective treatment regimen as soon as possible, efforts should be intensified to improve TB diagnosis and treatment and reduce the disparities between incidence and notification. Hence the following years are very critical. It is also equally important to embolden and indulge the TB infected community. The TB patients should not be viewed as passive recipients of services, but rather as key stakeholders in all process of design, strategic planning, execution, and tracking.[10]

These findings uphold the need to discover new entities for management of MDR TB, new FDCs and regimes, diagnostic and preventive measures. This can be achieved by facilitating the pharmaceutical industries to drive research and development activities for new entities focussed on TB diagnosis and treatment as well as prevention in the form of vaccines. Also academic research activities in this field should be promoted by the research institutions by providing incentives for carrying out the research activities. It is equally essential to promote research related to community practices and improvement of awareness regarding diagnosis, treatment and prevention of TB. Post graduates and research scholars have immense potential and should be encouraged to conduct researches in this field to add to our resources.

Even though India has made several progresses in managing TB burden through effective policy formation, use of technology and in partnership with WHO and BRICS, we have identified several gap areas which our country should focus in the coming years to improve the outcome of TB patients. First and the foremost efforts in case finding, diagnosis and notification should be strengthened. Appropriate treatment and ensuring adherence is another is another stumble block. The private hospitals should also be involved in the care of TB patients. Public health education strategies should be reinforced to overcome the social prejudice and public ignorance regarding TB. Also, social media and telemedicine should be utilized in delivery of services to all places and populations. Bold and innovative methods should be adopted focussed to the above areas to achieve the end-TB strategy.[11,12] Even though our findings suggests India's significant lack of TB-related research, the study emphasises the importance of expanding studies in all parts of India, particularly in areas with a high prevalence of tuberculosis. Furthermore, epidemiological research and studies on TB prevention and screening, as well as the effects of add-on therapy with other

anti-microbial drugs or other entities, must be prioritised in order to achieve our goal of eliminating TB in India.

The limitations of our study were manifold. CTRI registration was made mandatory for all clinical studies including post graduate thesis only in the 2017. Hence many studies that were not registered with CTRI would have been missed. However, based on the registered studies, we attempted to obtain an overview of the current state of research in tuberculosis.

Conflicts of interest

The author have none to declare.

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Review article

Effectiveness of home visiting on tuberculosis case detection: Systematic review and meta-analysis

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ABSTRACT

Background: Tuberculosis (TB) is a public health agenda globally. Most TB cases are detected using the usual passive method. Starting a decade, cases are detected using an active detection strategy. The home-visiting strategy is one of the active case findings approaches. However, no study shows the pooled effect of home visiting on tuberculosis case detection rate. Thus, we conducted this study to evaluate the effectiveness of home visiting on tuberculosis case detection.

Methods: In this systematic review and meta-analysis, the PRISMA checklist was used to report findings. A systematic comprehensive search was done to address all possible search databases. We used to search databases such as PubMed/MEDLINE, Scopus, and Science Direct to identify relevant articles. Data were extracted by two authors and consistency was checked by two co-authors. Cochrane risk of bias tool was used to assess the quality of studies. Data were extracted using a Microsoft Excel spreadsheet then; data were transferred to Stata version 16 for further analysis. Heterogeneity across studies was checked using the Q statistics (I2).

Results: Overall, 4174 articles were found. Two thousand one hundred seventy-five (2175) articles were excluded due to duplications. One thousand nine hundred twenty-four articles were excluded after reviewing titles and abstracts. Seventy-five articles were assessed using their full texts articles and 70 articles were excluded with unclear outcomes and poor methodological quality. Finally, 5 articles were selected for the final analysis. In all studies, the case-notification rate was significantly increased in the intervention arm than the control arm. The pooled effect size was RR: 1.65 (95% CI: 0.92, 2.39). This study showed that there was a significant heterogeneity ($I2 = 98.9\%$, $P < 0.001$). Visual examination of the funnel plot showed asymmetric distribution. However, the egger's and bigger tests showed there was no significant publication bias (($P = 0.313$).

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Conclusion: Home to home visiting is an effective TB case finding method as compared to the usual passive detection methods. The health system should be strengthened home to home visiting to enhance TB case detection.

The protocol PROSPERO registration was CRD42021227860.

1. Background

TB is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (Mtb), which is transmitted between humans through the respiratory route and most commonly affects the lungs but can damage any tissue.[1] A total of 1.5 million people died from TB in 2020. Worldwide, TB is the 13th leading cause of death and the second leading infectious killer next to COVID-19.[2] Overall progress in global TB elimination was modest in 2017, consistent with that in recent years[3]; intensified efforts to improve TB diagnosis, treatment, and prevention are required to meet global targets for 2020–2035.[4] However, under-detection of active TB cases and delay in diagnosis and initiation of treatment are major challenges of TB programs in low- and middle-income countries (LMICs) to achieve the end TB targets. It has been estimated that almost one-third of the global TB cases were undetected and had not received treatment. For instance, the WHO estimated that three million cases of TB were not detected globally in 2019.[5]

The WHO endorses two complementary approaches to improve tuberculosis case detection: active case finding (ACF) and systematic screening of household contacts.[6,7] Active case finding in groups at risk during home visits increases the case detection rate in the population and permits the identification of cases that may not be detected through passive case finding at the health facility level.[8] To enhance the case detection rate in TB high burden countries home to home visiting interventions were conducted in various countries.[9–15] A study confirms that integrated active case finding during home visits scheduled may help to identify cases that may not be detected through passive case finding at the health facility level (8).

Findings from interventions revealed that the case detection rate was increased; however, the effectiveness of the interventions was varied across studies. Furthermore, there is no evidence on the pooled effect of TB case detection using home-to-home visiting. Therefore, the main purpose of this systematic review and meta-analysis was to compute the pooled effect of home-to-home visiting on TB case detection.

2. Methods

2.1. Protocol design and registration

The review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P).[16,17] The protocol was registered in PROSPERO with registration number CRD42021227860.

2.2. Eligibility criteria

Studies included if and only if they had fulfilled the following criteria:

- Studies that have both intervention and parallel control groups
- Studies that have clear intervention outcomes
- Implementation of the intervention should be used a home to home visiting strategy
- Articles published in the English

2.2.1. Information sources

Electronic databases (PubMed/Medline, Scopus, Cochran, and science direct) and additional search engines such as Google scholar were used as information sources. The information was gathered from 1 December 2020 up to 15 March 2021.

2.2.2. Search strategy

A systematic comprehensive search was applied to address all possible search databases. We used search databases like PubMed/MEDLINE, Scopus, Science Direct, and Google scholar to access relevant articles. The search terms were defined according to Medical Subject Headings (MeSH). The search strategy was used to terms: "randomized control trial" OR "intervention study" AND "tuberculosis" OR "*M. tuberculosis*" OR "mycobacterial tuberculosis" AND "home to home visiting" OR "home visiting" OR "household visiting" AND "tuberculosis case detection" OR "case detection" OR "case notification" OR "case findings" OR "tuberculosis case detection rate" AND "tuberculosis high burden setting". Search terms were connected by using the Boolean operator "OR" and "AND", "OR" is used to connect synonyms words or phrases, and the operator "AND" is used to combine dissimilar terms. The search strategy applied to the PubMed database is annexed at the end of the manuscript.

2.3. Data abstraction

Data were extracted by the principal investigator (DA and GD) and reliability was checked by the two co-authors (MBS and EWM). Discrepancies between the reviewers were resolved after a serious discussion with the other co-author (FAG). Data abstraction included: name of first author/year of publication, study design, place of study, type of participant, type of intervention, and outcomes (case detection rate). Data abstraction was done using an EXCEL spreadsheet. Articles were assessed based on their title, abstract, research questions and objectives, method, and main findings.

Inappropriate articles to our study were excluded at this stage using their titles. Articles fitted to our purpose were retrieved for further evaluation using their abstract and the full text. Articles that fulfilled the eligibility criteria were used as a source of data for the final analysis.

2.4. Outcome measurement

The primary outcome of this review was the pooled effect of home visiting on tuberculosis case detection in TB prevalent settings. The effect of home visiting against the detection of TB cases was assessed by assuming the relative risk (RR) to evaluate the association between the dependent variables (CDR) and independent variables.

2.5. Quality assessment

There are many quality assessment tools such as Cochrane risk of bias (ROB), Critical Appraisal Skills Program (CASP), the Jadad scale, Centre for Evidence-Based Medicine (CEBM), Joanna Briggs Institute (JBI). For our purpose, we used the Cochrane risk of bias tool (ROB) to assess random sequence generation and concealment of allocation. Random sequence generation was considered inadequate if there was no sufficient controlling mechanism to protect biased allocation to interventions due to inadequate generation of a randomized sequence. Generation of the allocation sequence and concealment of allocation as adequate, inadequate, or unclear was assessed for each randomized controlled trial study.[18]

The risk of bias was considered high if the selective outcome reporting by authors and low risk of selective outcome reporting bias was not presented. The bias is considered "unclear" if the information obtained from authors is insufficient to permit judgment. Bias could be also considered when both participants and researchers were blinded. This can be considered as adequate if steps were taken to ensure that those recording the main outcome of the study were blind to the assigned interventions and inadequate if this was not the case. Completeness of follow-up was assessed as adequate if steps to the handling of incomplete outcome data were complete and unlikely to have produced bias, or inadequate if the attrition amount or nature or handling of incomplete outcome data was not maintained.

2.6. Data analysis

Data were extracted using a Microsoft Excel spreadsheet template. Then, data were transferred to Stata version 16 for further analysis. Heterogeneity across studies was checked using the inverse variance (I2) and Cochran Q statistics. I2 can be computed as $I2 = 100\% \times (Q-df)/Q$, where Q is Cochran's heterogeneity statistic and df the degrees of freedom.[19] The cutoffs of value were 25, 50, and 75% were declared the heterogeneity was low, moderate, and severe respectively ([19]). Since a heterogeneous study population was included in the study, we used a random-effects model to estimate the pooled effects of case detection with a 95% confidence interval (CI). Although I2 (I2 = 98.9%) was substantially high across the study, subgroup analysis was not performed because only a few studies were included in the study. The funnel plot was asymmetry; Egger's tests were applied to check a publication bias. In Meta-regression, the statistical test was considered as significantly associated when the p-value is < 0.05.

2.6.1. Publication bias assessment

Publication bias was assessed by visual inspection of funnel plots. The symmetrical presentation was interpreted to suggest an absence of publication bias, whereas an asymmetry of the graph showed the presence of publication bias. Also, since the funnel plot was a subjective judgment, Egger's test was applied to evaluate whether the presence or absence of publication bias objectively.

2.6.2. Sensitivity analysis

Sensitivity analysis was also done to estimate the influence of a single study over the pooled effect size of included studies.

2.7. The implication of the review

This study aimed to calculate and generate evidence on the effect of home visiting intervention on case detection rate(CDR) in high prevalent settings. The finding of this study could bring a change and design a strategy to enhance the TB case detection process in the national TB programs and facilitate the engagement of healthcare providers and programmers on the active case detection approach. The findings may also be the baseline for prospective research on CDR among patients who poorly access the healthcare system.

3. Results

3.1. Study selection

Overall, we search 4174 articles, 2175 were excluded due to duplications, and 1924 were removed after reviewing the titles and abstracts. Seventy-five (75) articles were assessed using their full texts and 70 articles were excluded with unclear outcomes and poor methodological quality. Finally, 5 articles were selected for the final analysis (Fig. 1).

3.2. Study characteristics

Five studies were included in the study. Two studies were RCT and three studies were Non-RCT. Randomization sequence and allocation concealment were not described in three studies.[11,12,14] Two studies by Corbet (9) and Miller[15] revealed that random sequence and allocation concealment were performed. However, a study conducted by Corbet[9] showed that community health workers and cluster residents were not masked to intervention allocation, but investigators and laboratory staff were masked to allocation until final analysis. Two studies included in our study were found in Africa and two other countries were found in Asia. The maximum sample size was 3000,000 and the minimum sample size was 7823. In all studies, the case-notification rates were significantly

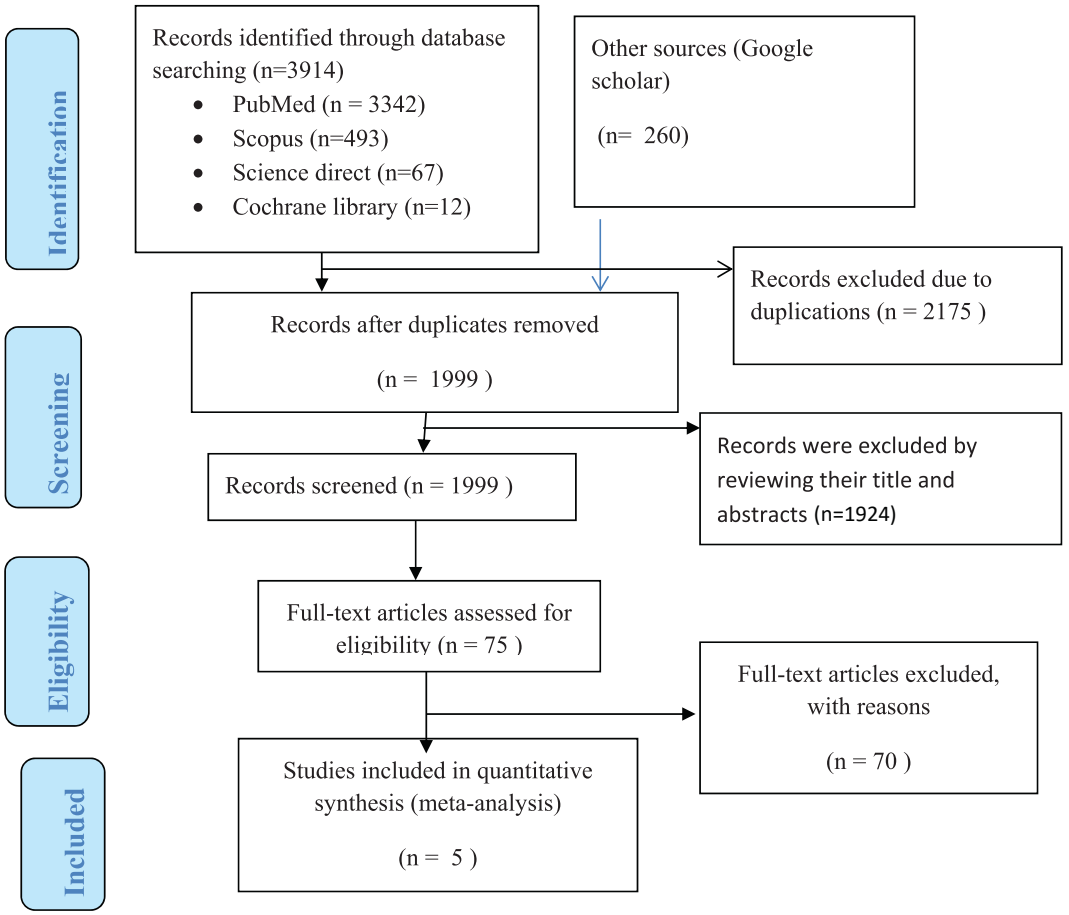

Fig. 1 – PRISMA flow diagram.

higher in intervention arms than in control groups (Table 1). Study participants, therapists, and outcomes assessors were not blind to treatment assignment and outcomes measurement. Baseline characteristics of studies were identified (Table 2).

3.3. *The pooled estimate of CDR*

The pooled effect size was RR: 1.65 (95% CI: 0.92, 2.39). This study showed that there was a significant heterogeneity (I^2 = 98.9%, P < 0.001) (Fig. 2). Even though substantial heterogeneity was observed in the studies, subgroup analysis was not done since the numbers of studies are a few. However, the Galbraith plot (Fig. 3) was used to identify the amount of heterogeneity from a meta-analysis.

3.4. *Publication bias*

Visual examination of the funnel plot showed the asymmetric distribution of studies. Most of the plots were presented asymmetrically to the right side of the (Fig. 4). However, the egger's test of intercept showed that there was a significant publication bias (P: 0.313). The sensitivity test (Fig. 5) examines the influence of a single study on the overall meta-analysis estimate.

4. Discussion

This study was aimed to compute the pooled effects of home visiting on tuberculosis case detection rate. TB case detection using the home visiting approach was found to be increased TB CDR in prevalent settings. Congruently, other systematic reviews and meta-analyses revealed that TB cases were significantly high among household contacts.[20,21] Also, active screening among household contacts is an effective way to improve TB case detection.[22] According to the STOP TB partnership explanation case notifications increased compared with expected case notifications based on historical trends.[23] However, the study confirmed that massive active case finding was not effective in the general population of a modest TB prevalence setting of China.[24] This may be due to the shorter time interval of ACF between TB symptoms onset and linkage to healthcare services that decrease the risk of TB community transmission.[24]

Table 1 – Characteristics of studies included in this systematic review and meta-analysis.

Author	Year	Country	Population	Study design	IG	Cases	CG	Cases	Type of Intervention	Outcome
Corbett EL et al.	2010	Zimbabwe	Adult	Cluster-RCT	55,741	5466	54,691	4711	Home visiting	Additional cases were detected
Miller AC et al.	2010	Brazil	All ages	Cluster-RCT	24,177	226	34,410	208	Home visiting	Case-notification rates were significantly higher in intervention arms than in control group
Yassin MA et al.	2013	Ethiopia	All ages	Non-RCT	3,000,000	7071	1,355,153	1133	Home visiting	All forms of TB, smear positive and negative cases detection rates were increased after implementation of the intervention
Morishita F et al.	2016	Cambodia	All ages	Non-RCT	9389	3767	7369	2701	Home visiting	Of all forms and bacteriological-confirmed cases were additionally notified in intervention districts.
Reddy KK et al.	2015	India	All ages	Non-RCT	7823	658	3961	255	Home visiting	Smear positive case detection was increased

Table 2 – Methodological quality assessment using Cochrane risk of bias (ROB) assessment tools.

Authors	Publication year	Randomization Sequence	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting bias	Recruitment bias
Corbett EL et al.[9]	2010	Yes	Yes	No	Yes	Yes	Yes	Yes
Miller AC et al.[15]	2013	Yes	Yes	No	Yes	Yes	Yes	Yes
Yassin MA et al.[12]	2009	No	No	No	Yes	Yes	Yes	Yes
Morishita F et al.[11]	2017	No	No	No	Yes	Yes	Yes	Yes
Reddy KK et al.[14]	2015	No	No	No	Yes	Yes	Yes	Yes

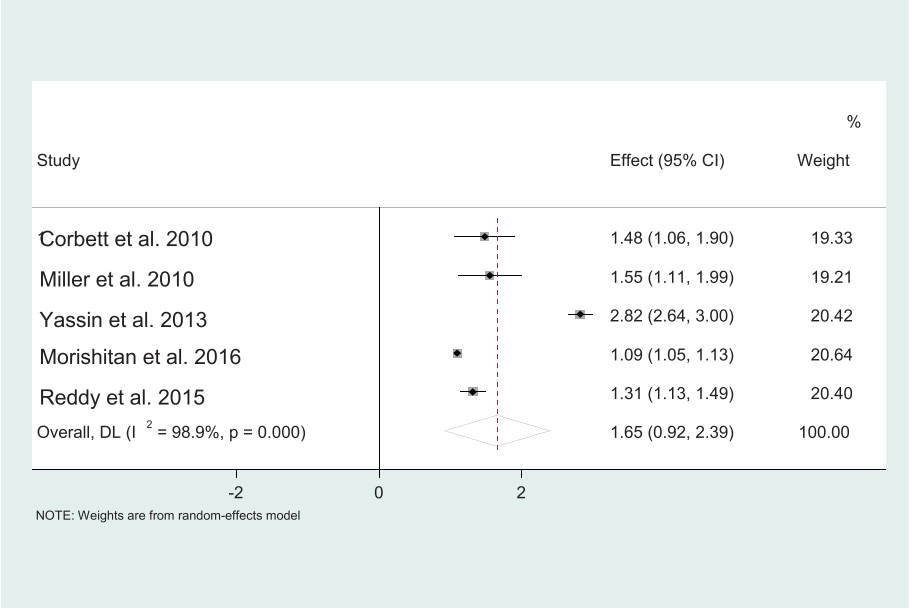

Fig. 2 – Forest plot of the effect size of home visiting on tuberculosis case detection in TB high burden settings.

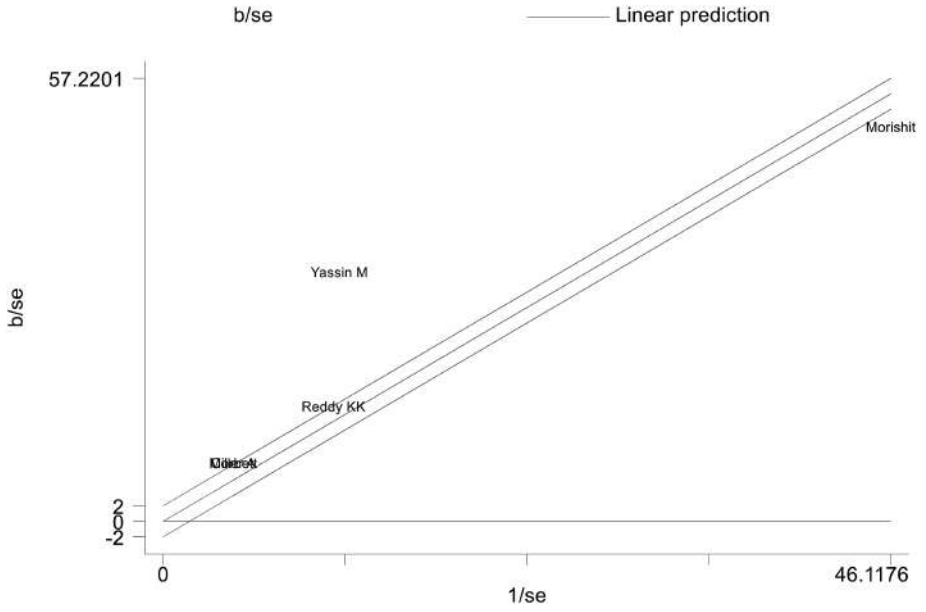

Fig. 3 – Galbraith plot on tuberculosis case detection in TB high burden settings.

This systematic review and meta-analysis showed that home-to-home visiting is very important to access the most remote area that is difficult to reach using the usual TB care approaches and increase the case detection rate significantly. The researcher identifies low TB case detection as one of the most important barriers to improving TB care but fails to propose interventions that would plausibly increase case detection.[25] This may also choose to do home visits only in high prevalence areas that have low access to care, such as rural districts.[26]

A major challenge of this meta-analysis is the substantial heterogeneity across the studies included in our study. Similarly, previous systematic reviews and meta-analyses conducted in low and middle-income countries showed that there was substantial heterogeneity among studies.[20] This heterogeneity may be due to differences in the included studies in geography, setting, sample size, and type of population.

This systematic review and meta-analysis had several strengths and limitations. The use of multiple databases and the involvement of several countries' data were the

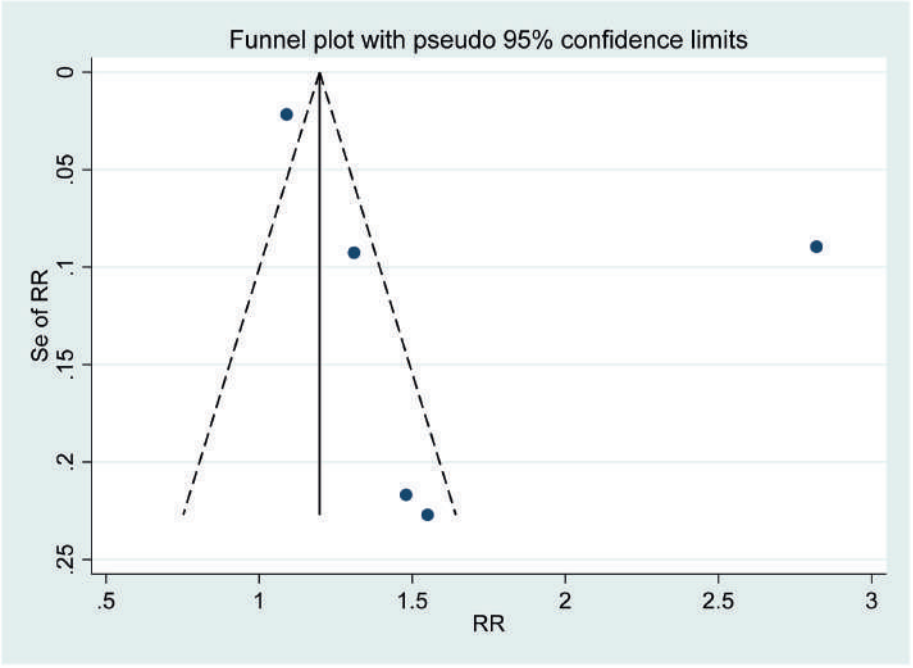

Fig. 4 – Funnel plot analysis of publication bias.

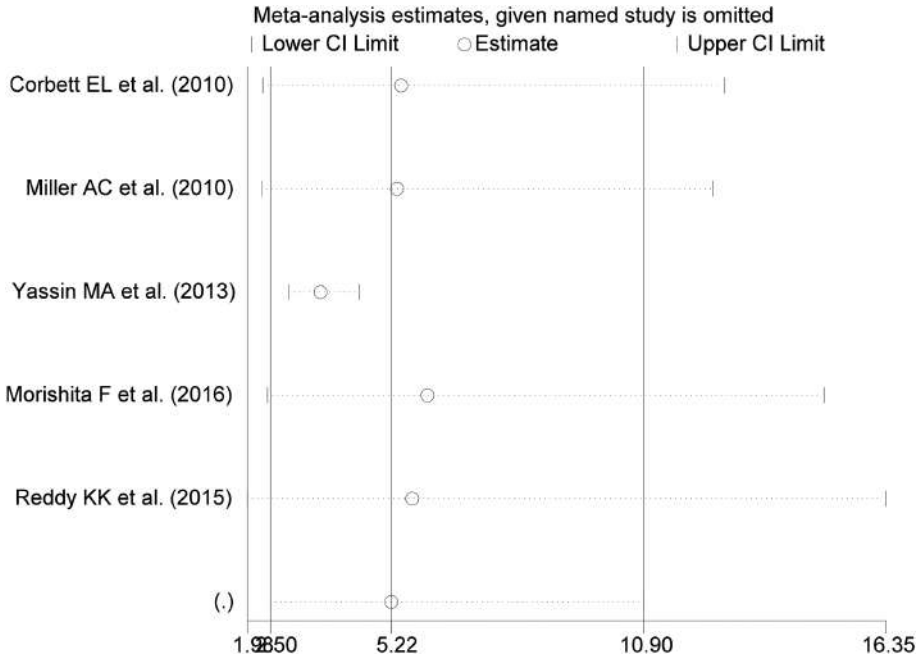

Fig. 5 – Sensitivity test.

strong side of the study. However, the number of studies included in this study was limited which may not show a clear picture of the impact of the intervention on TB case detection. This study is also limited to access to important databases such as EMBASE databases. A limited number of systematic review and meta-analysis studies in the field were obstacles for us to compare and contrast our findings elsewhere.

5. Conclusion

Home to home visiting is effective to increase TB case detection as compared to passive case detection method. In the future, we recommend for researchers to conduct further systematic reviews and meta-analyses by including all low and middle-income countries to increase the number of

studies and measure the true effect of home visiting on tuberculosis case detection.

Ethics approval and consent to participate

Ethical approval was not applicable because the ethics of published studies assured at the stage of primary investigation.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Authors' contributions

DA has done topic selection and conceptualization, proposal writing, study selection, data extraction, data analysis, and manuscript writing and approval. EWM has done study selection, data extraction, and data analysis. GD has done data extraction, data analysis, report writing, and manuscript writing and approval. Similarly, MBS participated in data analysis, and approval. Finally, FAG participated in data analysis, manuscript writing, editing, and approval.

Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2022.04.007.

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Original article

Urinary excretion of metformin in diabetic patients with and without tuberculosis

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ABSTRACT

Background: Patients with concurrent diabetes mellitus (DM) and tuberculosis (TB) pose an increased risk of treatment failure in TB and management of DM is complicated. Anti-diabetic and anti-TB drugs may interact with on another other when co-administered. The role of anti-TB drugs on the excretion of metformin in urine has not been studied. Therefore, we carried out a study in DM patients with and without TB to compare the percentage of metformin excreted in urine.

Methods: A total of 52 DMTB and 17 DM patients were recruited in this study from the Chennai Corporation Centres. DM and DM - TB patients were administered the prescribed anti-TB and anti-diabetic drugs (metformin (MET), glipizide (GLP),glimepiride (GLM),glibenclamide (GLB),rifampicin (RMP),isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB). DM and DMTB patients received metformin (MET) alone and in combination with sulphonylureas as diabetic drugs. The urine samples were collected from 0 to 8 hours after drug administration. Urine MET excreted in DM and DMTB patients were estimated by high performance liquid chromatography (HPLC) and percent dose was calculated.

Results: The percent dose of MET excreted in urine in DMTB patients was significantly higher when compared to DM patients. There is significant difference in the percent dose of MET excreted among DM patients with and without sulphonylureas, values being 23.3 and 17.7% respectively ($p = 0.044$).

Conclusion: This is the first study to report on the percent dose of MET excretion in urine in patients with DM and DMTB receiving MET along with anti-TB drugs.

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1. Introduction

India is hitting the dual trouble of the highest TB burden with a large diabetic population, and this is posing a serious threat to the health system.[1] There has been an escalating epidemic of Diabetes Mellitus (DM) with a prevalence of 7.8%. of population be Diabetic.[2] Mohan V et al reported diabetic prevalence of about 25% in TB patients in Tamil Nadu, which was higher in comparison with prevalence of diabetes in general population.[3] Patients on anti-diabetic therapy may also take treatment for tuberculosis, and it is likely that both these classes of drugs might interact with each other.

Rifampin and isoniazid, the first line anti-TB drugs are responsible for drug interactions. Many commonly used drugs such as anticoagulants, anticonvulsants, other antimicrobials, antihypertensives, oral contraceptives, glucocorticoids, immunosuppressants, sulfonylureas, and theophylline have pharmacokinetic interaction with rifampicin.[4]

Metformin is an oral antidiabetic medication that enhances glycemic regulation by inhibiting hepatic gluconeogenesis and Glycogenolysis. It is the only biguanide available for clinical use to treat non-insulin-dependent diabetes.[5] Metformin is absorbed slowly and after 24 hours, about 60% of an oral dose is excreted in the urine as an unchanged substance. Around 30% of the dose is excreted unchanged in the faeces.[6] It has a higher renal clearance than creatinine, reflecting that tubular secretion aids in its removal.[7]

Patients with TB and DM have a higher risk of TB treatment failure, and DM management may be difficult.[8] Rifampicin has been shown to improve the clearance of most of the oral antidiabetic drugs used in low- and middle-income countries.[9,10] Sulphohonylureas are metabolized in the liver by cytochrome P450 enzymes, which are induced by RMP.[10,11] Organic cation transporters (OCTs) play a major role in metformin ingestion, hepatic uptake, and renal excretion.[12]

Although there are reports on the excretion of MET in urine in DM patients, no study to date has been done on the effect of anti-TB drugs on metformin excretion in urine. Therefore, a study was undertaken to compare the percent dose of metformin excreted in urine between DM and DMTB patients.

2. Methods

2.1. Patients

A prospective study was undertaken in two groups of adult patients, namely, DM & DMTB; during May 2018 to January 2020. Patients were recruited from Chennai Corporation Centres (DM TB patients from TB units and DM patients from NCD clinics). Patients aged 18–60 years of both gender, with a body weight >35kg, HIV negative, and willing to give informed written consent were recruited in the study. Diagnosis and treatment of DM and DM TB groups were in accordance with the existing programme guidelines. Both pulmonary and extra pulmonary forms of TB patients were recruited. DMTB patients were receiving anti-TB treatment regularly at the Chennai Corporation treatment centres for a minimum period of two weeks.

Known cases of DM was confirmed by anti-diabetic medications received by the patients in the NCD register. Patients with DM in both groups received treatment with either MET alone and grouped as DM 1, MET along with anti-TB drugs grouped as DMTB 1 and in combination with sulphonylurea's (GLM/GLP/GLB) were grouped as DM 3 and DMTB 3. Patients received standard doses of the medications as prescribed by the treating physicians. The study design is illustrated in the figures (Fig. 1A and B).

2.2. Conduct of the study

This study was approved by the Institutional Ethics Committee (IEC No.2015021) and was conducted at the out-patient clinic of the National Institute for Research in Tuberculosis (NIRT). Eligible DM and DM - TB patients were requested to report at NIRT in the morning, without taking anti-TB or diabetic drugs on that day. On the day of the study, in the morning, before administration of food and drugs, patients were requested to void urine. The DM and DMTB patients were administered with the prescribed anti-TB and anti-diabetic drugs (MET, GLP, GLM, GLB, RMP, INH, PZA and EMB), with 200ml water under direct supervision of medical officer.

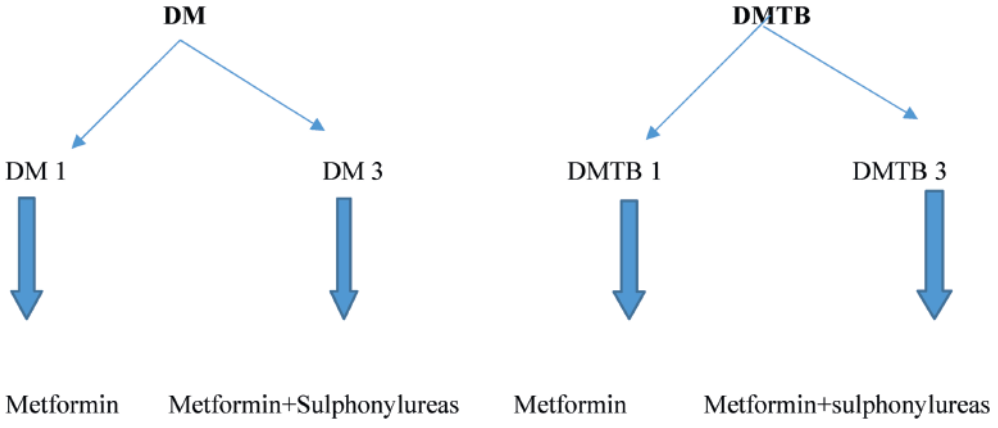

Fig. 1 – A and B: Study design A-: DM group of patients receiving MET with and without sulphonylureas and B represents DMTB group of patients receiving Metformin with and without sulphonylureas.

The patients were provided with a wide mouthed glass bottle and a container (2.5 liter) with lid along with a funnel to collect urine sample. The urine samples were collected between 0 and 8 hours after the drug administration. The study participants were provided with breakfast and lunch during the study period. The volume of urine voided was mixed well and measured. Aliquots of urine in duplicate were stored at −20 °C until assay.

2.3. Drug estimation

Urine concentrations of MET in both groups and sub-groups of patients, were estimated by HPLC (Shimadzu Corporation, Kyoto, Japan) according to a previously validated and published method.[13]

2.4. Statistical analysis

All the data were entered into a Microsoft excel sheet and verified before analyzing using the STATA 15.1 (StataCorp, Texas, USA). Shapiro–Wilks test shows that the data were not normally distributed and the values were tabulated using frequency, percentage, median and Interquartile range [IQR]. Z-proportion test was used to compare the proportion between the groups. Kruskal–Wallis test was used to compare the observation between groups and Dunn-test was used for the sub-group comparison. A p value ≤ 0.05 was considered statistically significant. The percent dose of MET excreted in unchanged form was calculated on the basis of their concentrations in urine.

$$\text{Total concentration of MET excreted in urine} = \frac{\text{Volume of urine excreted} \times \text{Concentration of MET}}{\text{Drug Dose}} \times 1000$$

3. Results

The demographic details of the patients are given (Table 1). 52 patients and 17 Patients with DMTB and DM respectively participated in this study. The mean age of patients in DM 1, DM3, DMTB 1 and DMTB 3 were 51.5, 45.5, 47.5 and 48 years, respectively. A high proportion of the patients were males. Patients with DMTB had lower BMI when compared to DM patients. However, it was not statistically significant. Both the groups of patients received MET 500 mg twice daily. It was observed that the percent dose of MET excreted in urine of DM 1, DM 3, DMTB 1 and DM-TB 3 groups were 23.3, 17.7, 42.6 and 27.4% respectively (Fig. 2).

The percent dose of MET excreted in urine in DMTB patients (both DMTB 1 & 3) were significantly higher when compared to DM patients (DM 1 & 3). There was a significant difference in the percent dose of MET excreted in DM patients with and without sulphonylureas, the values being 23.3 and 17.7% respectively ($p = 0.044$).

4. Discussion

Excretion of unchanged drug in urine is the major mode of elimination of MET. No metabolites of MET have been found in urine. Troja et al had observed the average amount of unchanged MET excreted was 41.17% for 850 mg tablet of the administered drug within 24 hours after oral administration.[14] G.T.Tucker et al, reported an average of 79% of the dose was recovered as unchanged drug in the urine of DM patients after 72h, 95% of this total appearing in the first 8h. MET has relatively poor oral absorption with an absolute bioavailability of 50–60% of the dose.[15]

Sub groups in DM patients receiving MET alone and in combination with sulphonylureas were compared. The percent dose excreted in DM patients receiving MET along with SU are lower and the differences were statistically significant ($p = 0.044$).

Not much is known about the role of anti-TB drugs on MET excretion in urine. Our study reported that MET excretion is increased in the presence of anti-TB drugs in DMTB patients. Limitation of this study is that the sample size of DM patients are much lesser when compared to DMTB patients. However, sub groups of DM and DMTB patients had similar number of patients.

Venkatesan et al (1992) observed that INH decreases the metabolism of oral glycemic agents and increase their levels in plasma, by means of cytochrome P2C9 involved in the metabolism of sulphonylureas; however, it was observed that the induction effect of RMP far outweighs this inhibitory effect.[16] Singhal et al (2014) reported that metformin has lack of clinical relevant interaction with RMP and aids in potential benefit on TB treatment.[17] In contrary, Williams et al observed pharmacokinetic interaction of MET when co-administered with RMP.[18] Brake et al observed that RMP increased MET plasma exposure in DMTB patients.[19]

Table 1 – Demographic parameters of DM and DMTB patients-Demographic parameters like age, BMI and gender were compared between DM and DMTB patients.

Parameters	DM 1 (n = 9)	DM 3 (n = 8)	DMTB 1 (n = 22)	DMTB 3 (n = 30)
[a]Age (Years)	50 (46–52)	51.5 (47.5–57.5)	45.5 (42–55)	47.5 (40–55)
[a]BMI (kg/m2)	25.8 (22.9–28.9)	25.4 (24.7–27.2)	22.1 (20.0–24.8)	21.1 (19.8–22.8)
<18.5 (kg/m2)	0	0	3 (13.6)	4 (13.3)
≥18.5 (kg/m2)	9 (100)	8 (100)	19 (86.4)	26 (86.7)
Gender				
Male	3 (33.3%)	5 (62.5%)	15 (68.2%)	20 (66.7%)
Female	6 (66.7%)	3 (37.5%)	7 (31.8%)	10 (33.3%)

BMI-Body Mass Index.
[a] Values are expressed as Median (IQR).

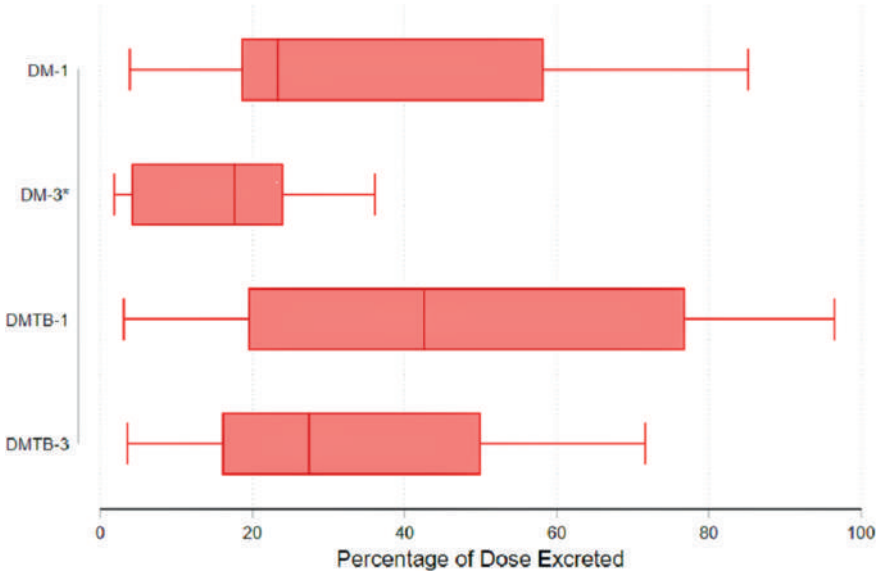

Fig. 2 – Percent dose of Metformin excreted in urine in DM and DMTB Patients receiving MET with and without sulphonylureas.

This is the first study to report on the percent dose of MET excretion in urine in patients with DM and DMTB. Though DMTB patients receive RMP, INH, PZA and EMB as first line anti-TB drugs for treatment of TB, several studies provided evidence that RMP plays a key role in the interaction with MET.[19] This study has provided information on the role of anti-TB drugs in the urinary excretion of MET in DMTB patients thereby suggesting to monitor glucose level in these patients. Further studies are required to understand the cause for increased MET excretion in urine during ATT.

Conflicts of interest

The authors have none to declare.

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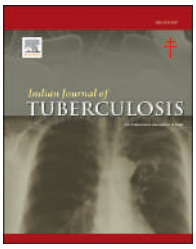

Original article

The clinician, the lab and the patient: Understanding lab diagnostics to eradicate tuberculosis

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MOTT/NTM

ABSTRACT

Background: Diagnostic modalities for diagnosing Tuberculosis caused by Mycobacterium group of organisms include mainly AFB smear by Ziehl Neelsen carbol fuchsin smear microscopy, GeneXpert (CB NAAT) molecular method, Line probe assay (Molecular method) and AFB culture (Liquid automated systems and solid media) methods.

Methods: This study was initiated to understand and prioritize TB lab diagnosis, with reference to selection of lab diagnostic tests and its order of preference for MTC and NTM/MOTT closely associating it with the TB irradication program initiated by the Government of India.

Result and conclusion: The results and discussion bring to light the importance of each test and the purpose of their requisition. When diagnosis is handled half heartedly eradication of TB becomes a challenge. All the efforts including planning, resources in the form of manpower, infrastructure, finances, education, time etc., would be hampered. This challenge is not only for India but the globe. For countries harboring TB, Correct diagnostic request and timely diagnosis and treatment is the key to eradication of tuberculosis.

1. Introduction

Tuberculosis (TB) is a transmissible bacteriological ailment triggered by *Mycobacterium tuberculosis* (MTB) and is still one of the biggest challenges for developing countries.[1] Main reasons for using the Ziehl-Neelsen (ZN) smear microscopy is its low cost, high specificity, does not require sophisticated equipment or high laboratory standards. It is one of the screening tests. AFB Culture is the gold standard for diagnosis of TB, but is unable to provide early results and necessarily 4–6 weeks are required for final diagnosis. CBNAAT or GeneXpert MTB assay takes only two hours to provide the final results[10] and indirectly differentiates MTB and Mycobacteria other than tuberculosis/Non tuberculosis mycobacteria (MOTT/NTM) and the test, also provides rifampicin resistance status simultaneously.[1]

Tuberculosis remains one of the deadliest communicable diseases, that is caused by the Bacterium *Mycobacterium*

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tuberculosis (MTB).[2] TB spreads by inhaling minute droplets produced by coughing or sneezing from infected patients. The disease usually affects the lungs (pulmonary TB) and spreads by air transmission from people with pulmonary TB.[2] Further the study was to compare and emphasis the clinical utility of Genexpert MTB assay for direct detection of *Mycobacterium tuberculosis* complex with conventional method Ziehl-Neelsen (Zn) smear microscopy and AFB culture method.[2]

Mycobacterium tuberculosis (Acid Fast Bacilli: AFB) can be stained by using a simple staining method called Ziehl Neelsen carbol fuchsin (ZNCF). The Acid fast bacilli in the smear (*Mycobacterium tuberculosis*) stains up bright red while nonacid fast Bacilli as blue, when methylene blue is used as counter stain, making this a popular method of choice for prompt diagnosis of MTB in most of the countries.[1] There are leading reasons for using the Ziehl-Neelsen (ZN) smear microscopy having low cost, high specificity, does not require intricate equipment or high laboratory standards. Provisional results can be achieved usually within two hours; however test is less sensitive[7, 8] and normally requires bacilli high as 10,000 per ml of specimen for the smear to be positive in comparison to GeneXpert which requires 133 bacilli per ml to be positive (The study has not used ULTRA cartridges). So even an AFB smear negative can be GeneXpert positive for MTB, however this test can't be used to distinguish between MTB and *MOTT/NTM*. AFB Culture is the gold standard for diagnosis of TB, but is unable to provide early results and necessarily 4–6 weeks are required for final diagnosis. Relatively faster radiometric (BACTEC) and non-radiometric methods like fluorescent labeled mycobacterium growth indicator test (MGIT) methods are being used which provides results in 7–10 days depending on the load of Acid Fast Bacilli. Both these methods are time consuming to some extent.[1] AFB culture by automated system grows both MTB and MOTT/NTM. AFB culture also helps to differentiate between viable/live and dead bacilli.[1]

GeneXpert (*Mycobacterium tuberculosis* (MTB)/rifampicin (RIF)) is a cartridge based automated and rapid molecular diagnostic device that performs sample processing and semi-nested real-time PCR analysis in a single, hands-free step for identifying *Mycobacterium tuberculosis* and rapid detection of rifampicin (RIF) resistance in samples.[4] Utilization of polymerase chain reaction (PCR) test for MTB diagnosis has been evaluated to an appreciable extent and has already initiated to influence clinical microbiology.[1–4]

Aim of this study was to assess the importance, relevance and need of TB diagnosis, with reference to selection of lab diagnostic tests and its order of preference GeneXpert MTB Rif Assay in diagnosis of *Mycobacterium tuberculosis* complex in comparison with Ziehl Neelsen smear microscopy and AFB culture methods.

2. Materials and methods

This retrospective data with descriptive study was carried out at Neuberg Supratech Reference laboratories, Ahmedabad, Gujarat, India from 01st January 2019 to 31st December 2019.

All the specimens were studied by the following tests as requested by the physicians.

1) Smear for AFB by ZNCF (Zeil Neelson Carbol Fuchsin) Manual Stain
2) GeneXpert (CB NAAT Testing) for MTB and Rifampicin (Molecular method)
3) AFB culture (By Automated MGIT culture)

ZNCF (Zeil Neelson Carbol Fuchsin) Stain assay: AFB smear microscopy is a rapid and economical method that is very useful to identify Acid-fast bacilli. Members of the mycobacterium genus have a unique cell wall structure that contains a high concentration, over 60%, of mycolic acid. The cell wall is the major reason for the bacterium virulence; it is associated with impermeability to stains and dyes, antibiotic resistance and resistance to human defense mechanism.[3] The bacilli are classified as Acid Fast Bacilli (AFB) due to their resistance to decolourization by acids during staining procedures.[11]

A thin smear is prepared from the specimen. The smear is air-dried and fixed by gentle heating. For hot staining: A filter paper is placed on top of the smear (optional), as this does not allow the smear to dry while heating. The slide is flooded with carbol Fuchsin and heat is applied from below till the steam rises. This is repeated for 2 to 3 times. The stain is allowed to remain on the smear for 4–5 minutes. For cold staining: The slide is flooded with carbol Fuchsin and allowed to stay for 15–20 minutes. Now for both hot and cold ZNCF staining, slide is washed under running tap water. The smear is decolorized with 4% acid alcohol (4 mL conc. HCl in 96 mL absolute alcohol), till no more stain appears. Methylene blue is poured over the smear and allowed it to remain for 15–20 minutes. The slide is washed with water and allowed to air dry. Slide is examined under oil immersion lens.

GeneXpert MTB assay: Xpert assay was performed following the manufacture's suggested protocol. The Xpert MTB (Cepheid, Sunnyvale, CA, USA), rapid, automated cartridge-based nucleic acid amplification test using RT-PCR for the TB-specific *rpoB* gene can detect TB and rifampin resistance simultaneously, and the result is available in less than 2 hours.

Each specimen is kept in container with screw cap. The sample reagent is added to the sample at 2:1 (v/v) ratio, using separate plastic disposable pipette. Mix the specimen cup properly 10–20 times using back and forth movements. The sample is incubated in the cup for 15 minutes at room temperature. During the incubation period, shake the cup at least once, as described above. The specimen sample is liquefied with no visible clumps of specimen after incubation. The mixture is transferred into the Xpert MTB/RIF cartridge using the sterile pipette provided until the meniscus is above the minimum mark. The inoculated cartridge is placed into the GeneXpert instrument. Results are available within two hours and interpreted by the system automatically.[5,9]

AFB Culture (Manual) assay: LJ medium (Lowenstein-Jensen's medium) and Middlebrook solid medium are selective medium for isolation and cultivation of Mycobacterium. Addition of antibiotics in the medium inhibits the growth of non acid-fast bacilli. Selective Growth factors enrich the growth of mycobacterium.

Proceed for culture from the sediment of petroff's concentration method. Inoculate 3 slopes of LJ medium

(Lowenstein-Jensen's medium) and 3 slopes of Middlebrook solid medium with the sediment of the centrifugation procedure.

Incubate at 35°–37 °C in the incubator. Incubation of Mycobacterium is always done on a different shelf. Lay the inoculated tubes horizontally for a couple of hours for the specimen to adhere firmly to the medium. Examine daily for one week for rapid growers, or for contamination, indicated by bluing, digestion of media etc. and thereafter at weekly intervals for evidence of growth. Examine all positive cultures microscopically to be certain that acid-fast bacilli have grown and not a non-acid-fast contaminant that may form somewhat similar colonies.[6]

Final report is released either on day 56, if culture is negative or whenever culture is positive in between. These results have not been included in the analysis.

AFB Culture (Automation) assay: A clinical sample may or may not contain Acid-fast bacilli. Demonstration of acid-fast bacilli (AFB) in a smear made from a clinical specimen provides a preliminary diagnosis of mycobacterial disease, while the isolation of mycobacteria on culture provides a definite diagnosis of tuberculosis or disease due to mycobacteria other than *M. tuberculosis* (MOTT bacilli) or non-mycobacteria (NTM). As much as 50–60% of AFB culture-positive clinical specimens may fail to reveal AFB on smear made from the specimen. As a consequence, culture techniques play a key role in the diagnosis of mycobacterial disease.

In this procedure, the initial concentration of NaOH is 4%. This 4% NaOH solution is mixed with an equal quantity of sodium citrate solution (2.9%) to make a working solution (NaOH concentration in this solution is 2%). When an equal quantity of NaOH-NALC-citrate and specimens are mixed, the final concentration of NaOH in the specimen is 1%. Add NaOH-NALC-sodium citrate solution in a volume equal to the quantity of specimen. Vortex lightly or hand mix for about 15–30 seconds. Invert the tube so the whole tube is exposed to the NaOH-NALC solution. Wait for 13 minutes (up to 15 minutes maximum) after adding the NaOH-NALC solution. Vortex lightly or hand mix/invert every 5–10 minutes or put the tubes on a shaker and shake lightly during the whole time. Make sure the specimen is completely liquefied. If still mucoid, add a small quantity of NALC powder (one pinch) directly to the specimen tube. Mix well. At the end of 13 minutes, add phosphate buffer (pH 6.8) up to the top ring mark on the centrifuge tube. Mix well (lightly vortex or invert several times). Addition of sterile water is not a suitable alternative for the phosphate buffer. The specimen is centrifuged at a speed of 3000 g for 15–20 minutes. After centrifugation, tubes are allowed to sit for 5 minutes and aerosols are allowed to settle. Then carefully decant the supernatant into a suitable container containing a mycobactericidal disinfectant. Make sure the sediment is not lost during decanting of the supernatant fluid. Add a small quantity (1–2 ml) phosphate buffer (pH 6.8) and resuspend the sediment with the help of a pipette or vortex mixer. Use the resuspended pellet for making smears and for inoculation of 7 mL MGIT tubes with liquid Middlebrook medium. The inoculated tubes are then loaded in MGIT 960.

3. Results

3.1. *Column A*

Out of 5473 total samples, 513 (9.37%) specimens were genexpert positive with Zn negative smear. These 513 specimens included 57 fluids (9 specimens were AFB culture positive for MTB, 13 specimens were AFB culture negative and 35 specimens were not requested for AFB culture), 144 (28.07%) respiratory samples (31 specimens were AFB culture positive for MTB, 2 specimens were AFB culture positive for NTM, 38 specimens were AFB culture negative and 73 specimens were not requested for AFB culture), 25 CSF (1 specimen was AFB culture positive for MTB, 1 specimen was AFB culture negative and 23 specimens were not requested for AFB culture), 82 pus (25 specimens were AFB culture positive for MTB, 23 specimens were AFB culture negative and 34 specimens were not requested for AFB culture), 1 swab (1 specimen was AFB culture positive for MTB), 202 tissues (98 specimens were AFB culture positive for MTB, 67 specimens were AFB culture negative and 37 specimens were not requested for AFB culture) and 2 urine (2 specimens were not requested for AFB culture) (Table 1).

3.2. *Column B*

3306 (60.41%) specimens were genexpert negative with Zn negative smear. These 3306 specimens included 807 fluids (20 specimens were AFB culture positive for MTB, 251 specimens were AFB culture negative and 536 specimens were not requested for AFB culture), 940 respiratory samples (22 specimens were AFB culture positive for MTB, 5 specimens were AFB culture positive for NTM, 332 specimens were AFB culture negative and 581 specimens were not requested for AFB culture), 369 CSF (5 specimens were AFB culture positive for MTB, 49 specimens were AFB culture negative and 315 specimens were not requested for AFB culture), 157 pus (1 specimen was AFB culture positive for MTB, 1 specimen was AFB culture positive for NTM, 83 specimens were AFB culture negative and 72 specimens were not requested for AFB culture), 5 swab (1 specimen was AFB culture positive for MTB, 2 specimens were AFB culture negative and 2 specimens were not requested for AFB culture), 895 tissues (33 specimens were AFB culture positive for MTB, 4 specimens were AFB culture positive for NTM 412 specimens were AFB culture negative and 446 specimens were not requested for AFB culture), 126 urine (10 specimens were AFB culture negative and 116 specimens were not requested for AFB culture), 5 bone marrow (2 specimens were AFB culture negative and 3 specimens were not requested for AFB culture), 1 stool (1 specimen was not requested for AFB culture) and 1 semen (1 specimen was AFB culture negative (Table 1).

3.3. *Column C*

751 (13.72%) specimens were genexpert positive with Zn positive smear. These 751 specimens included 20 fluids (8 specimens were AFB culture positive for MTB, 3 specimens were AFB culture negative and 9 specimens were not requested for

Table 1 – Distribution of specimen in our study.

Total number of Samples: 5473												
A			B			C			D			E
Smear Negative GeneX Positive: 513 (9.37%)			Smear Negative, GeneX Negative: 3306 (60.41%)			Smear Positive, GeneX Positive: 751 (13.72%)			Smear Positive, GeneX Negative: 71 (1.30%)			No smear and culture. GeneX Positive: 832 (15.20%)
AFB Culture			AFB Culture			AFB Culture			AFB Culture			AFB Culture
Positive	Negative	No culture	Positive	Negative	No Cultu re	Positive	Negative	No Culture	Positive	Negative	No culture	Positive	Negative
167	142	204	92	1142	2072	290	67	394	36	12	23	188	644
32.55%	27.68%	39.77%	2.78%	34.5	62.6	38.6	8.92%	52.4	50.7	16.9	32.	22.60%	77.40%
				2%	7%	2%		6%	0%	0%	39 %		

AFB culture), 563 respiratory samples (207 specimens were AFB culture positive for MTB, 19 specimens were AFB culture negative and 337 specimens were not requested for AFB culture), 96 pus (40 specimens were AFB culture positive for MTB, 19 specimens were AFB culture negative and 37 specimens were not requested for AFB culture), 65 tissues (33 specimens were AFB culture positive for MTB, 26 specimens were AFB culture negative and 6 specimens were not requested for AFB culture) and 7 urine (2 specimens were AFB culture positive and 5 specimens were not requested for AFB culture (Table 1).

3.4. *Column D*

71 (1.30%) specimens were genexpert negative with Zn positive smear. These 71 specimens included 6 fluids (1 specimen was AFB culture positive for MTB, 2 specimens were AFB culture negative and 3 specimens were not requested for AFB culture), 42 respiratory samples (16 specimens were AFB culture positive for MTB, 9 specimens were AFB culture positive for NTM, 4 specimens were AFB culture negative and 13 specimens were not requested for AFB culture), 10 pus (2 specimens were AFB culture positive for MTB, 2 specimens were AFB culture positive for NTM, 1 specimen was culture AFB negative and 5 specimens were not requested for AFB culture) and 13 tissues (3 specimens were AFB culture positive for MTB, 3 specimens were AFB culture positive for NTM, 5 specimens were AFB culture negative and 2 specimens were not requested for AFB culture).

3.5. *Column E*

There were a total of 832 specimens which were processed only for genexpert. Out of there 188 specimens were positive for MTB and 644 specimens were negative. The total 832 specimens were consisted of 144 fluids, 285 respiratory samples, 14 CSF, 65 pus, 1 swab, 200 tissues, 16 urine, 1 bone marrow, 2 stool, 02 semen and 2 pure culture of mycobacterium (Table 1).

4. Discussion

In case of Indian scenario we need to consider 04 different popular clinical diagnostic practices:-

1. **AFB smear (ZN Stain and fluorescence stain)**
2. **GeneXpert (CB NAAT Testing)**
3. **AFB Culture (LIQUID rapid and solid culture)**
4. **Line probe assay (MDR and XDR)**

As we have studied 03 modalities, we shall compare and discuss the same.

With the above Technologies there are different possibilities of request:

1. Only AFB smear study requested
2. Only geneXpert requested
3. Only AFB culture requested
4. AFB smear and geneXpert requested
5. AFB smear and AFB culture requested

6. GeneXpert and AFB culture requested
7. All three modalities requested.

Column A: Out of 5473 total samples, 513 (9.37%) specimens were genexpert positive with Zn negative smear. These 513 specimens included 57 fluids (9 specimens were AFB culture positive for MTB, 13 specimens were AFB culture negative and 35 specimens were not requested for AFB culture), 144 (28.07%) respiratory samples (31 specimens were AFB culture positive for MTB, 2 specimens were AFB culture positive for NTM, 38 specimens were AFB culture negative and 73 specimens were not requested for AFB culture), 25 CSF (1 specimen was AFB culture positive for MTB, 1 specimen was AFB culture negative and 23 specimens were not requested for AFB culture), 82 pus (25 specimens were AFB culture positive for MTB, 23 specimens were AFB culture negative and 34 specimens were not requested for AFB culture), 1 swab (1 specimen was AFB culture positive for MTB), 202 tissues (98 specimens were AFB culture positive for MTB, 67 specimens were AFB culture negative and 37 specimens were not requested for AFB culture) and 2 urine (2 specimens were not requested for AFB culture).

The most important point of discussion here is:

1. **India is working hard on the program of TB irradiation. In March 2017, the government of India announced that the new aim of elimination of TB by 2025.**[12]
2. Even after marathon efforts from the government, The reason this does not seem probable is the point of discussion
3. Diagnosis of Tuberculosis is a challenge as it involves **the patient, the clinician and the lab. All of the three need to be clear on the requirement for being disease free nation (TB).**
4. The government of India provides free treatment in the public sector but as per government data 50% of India's TB patients seek treatment in the private sector. Understanding TB diagnostics is not very common amongst all population of Clinicians.
5. The Focused approach: Right clinician approach, Right Diagnostic requests, Right technology for testing and a compliant patient is all we need to eradicate TB.
6. The Diagnostic knowledge on priority of which tests should be ordered for best probability of diagnosis of tuberculosis.

Table 1 shows the bifurgation of total samples received and the break up of positives and negative results with reference to ZNCF staining, GeneXpert (CB NAAT testing), and AFB culture.

4.1. *Column A (Table 1)*

In the total 5473 samples, 513 (9.37%) samples were smear negative and GeneXpert (CBNAAT) Positive.

1. The point of discussion here is that in almost 10% (9.37%) of the cases we could miss TB diagnosis if only AFB smear is requested.
2. Second important point here is that CBNAAT along with MTB also gives rifampicin status. In case rifampicin is sensitive the patient can immediately be put on first line and if rifampicin is resistant, a second line LPA can be requested which allows treatment to begin in maximum 72 hours
3. Requesting only for AFB smear for primary TB diagnosis is misleading and should never be done.
4. If only smear is requested, and its positive, the probability of NTM/MOTT also should be ruled out.
5. Looking at the AFB cultures for the total 513 geneXpert positive cases in this group, 167 were positive, 142 Negative and there was no request for AFB cultures in 204 (40%).

The possibilities here that cause concern are the

a. We could be dealing with dead bacilli and if culture is not requested specially for patients on treatment there would be abuse of drugs.
b. If smear is Positive or geneXpert is positive then AFB culture is not requested
c. AFB culture allows space for DST in case there is therapeutic failure even after geneXpert is Positive for MTB with rifampicin sensitive. Therapeutic drug levels may not be reached in numerous patients.
d. LPA can also be done from Positive AFB culture in nonresponding patients.

4.2. *Column B (Table 1)*

In the total 5473 samples, 3306 (60.41%) samples were smear negative and GeneXpert (CBNAAT) negative. AFB culture was requested in 37% of the cases where 2.78% were Positive. These could be patients on treatment or patients with primary diagnosis and low load of Mycobacteria.

The point of concern in this data is:

1. In 60% of the cases AFB culture was not requested. From the existing data close to 7% of the 60% could have been AFB culture positive.
2. AFB smear and GeneXpert negative cannot rule out infection with viable Mycobacteria.
3. The possibility of missing out MDR and XDR mycobacterial infections also is very likely.
4. This is not going to help us eradicate TB.

4.3. *Column C (Table 1)*

In the total 5473 samples, 751 (13.72%) samples were smear Positive and GeneXpert (CBNAAT) Positive. Of these 290 (38.62%) were AFB culture positive and 67 (08.92%) were AFB culture negative. In 394 (52.46%) patients AFB culture was not requested, which is more than half the total cases.

The point of Discussion and concern here is:

1. In more than 50% AFB cultures are not requested
2. When AFB smear and geneXpert are positive, there are two possibilities:
   a. Primary TB diagnosis.
   b. Patient on treatment.
3. When its primary diagnosis, AFB culture is very much recommended as geneXpert gives only rifampicin results. Even if Rifampicin is sensitive, INH resistance would be

missed out if there is no AFB culture is requested and DST is therefore not possible.
4. When patient is on treatment, at times smear and geneXpert both could definitely be Positive but if AFB culture is negative it would clearly indicate dead bacilli and free from disease. Not requesting AFB culture in almost 50% of the cases is not to going to help eradicate TB program.
5. INH monoresistance would be missed out and patients who do not need treatment would continue having drugs.

4.4. *Column D (Table 1)*

In the total 5473 samples, 71 (1.30%) samples were smear Positive and GeneXpert (CBNAAT) negative. 36 (50.70%) were positive for AFB culture, all NTM's. 12 (16.90%) were negative for AFB culture. These 12 cases could be:

a. Fastidious NTM/MOTT unable to grow in MGIT
b. On Treatment, Dead NTM/MOTT where smear could be positive but geneXpert is negative

In 23 patients (32.39%) of the cases, AFB culture was not requested.

The points of concern here:

1. When AFB culture is not requested the evidence of getting viable/nonviable bacteria is lost
2. Drugs for NTM therapy may continue increasing irrational use of drugs
3. The possibility of speciation in NTM/MOTT and MIC testing is lost when cultures are not requested.
4. This has gradually increased the load and burden of NTM/MOTT in the society

4.5. *Column E (Table 1)*

In the total 5473 samples, GeneXpert alone was requested in 832 (15.20%) samples. There was no request for AFB smear or AFB culture.

The points of concern with this data reflects:

1. GeneXpert Negative does not mean no TB. A big inhibition in the program of eradication of TB.
2. For a patient on treatment, If geneXpert is positive with low or trace load, the probability of dead bacilli cannot be ruled out. The patient would be on treatment when it might not be necessary at all. Again irrational use of drugs
3. No AFB culture means no DST. Monoresistance to INH is missed if present.
4. Heteroresistance and mixed population is also missed. LPA could be done from positive AFB culture if there is a suspicion of heteroresistance or mixed population.

Understanding TB Diagnostics by both the clinician and the lab is the key to TB eradication. There are multiple reasons why requests are inadequate.

a. Financial aspect of testing
b. Lack of clarity on priority of Tests.
c. Patient's resistance on diagnostics
d. Nonavailability of tests in some regions

The reason the title says "The Clinician, The Lab and the patient: Understanding lab diagnostics to eradicate tuberculosis "It is the responsibility of all three: The Clinicians, the labs and the patients to understand and select tests wisely if our common goal is to eradicate tuberculosis from our Nation. A revision in TB national guidelines also appears to be the need of the hour.

5. Conclusions

AFB culture remains the gold standard for Diagnosing TB. The advantages are:

a. Low load is detected
b. Viability of TB bacilli is confirmed
c. DST is possible from positive AFB culture
d. Differentiation between MTB and NTM can be done from pure growth of AFB culture.

GeneXpert (CBNAAT testing).

a. This is the second test which can be helpful for rapid diagnosis. The results of MTB and Rifampicin status are ready within 02 hours. Excellent modality for a clear case.
b. Indirect evidence of NTM may be got when smear is Positive for AFB and geneXpert is Negative.
c. The disadvantage is that it does not differentiate between dead and live TB bacilli.
d. If rifampicin is resistant, the probability of status of other drugs is lost if AFB culture is not requested.

AFB smear by ZNCF stain:

This method is one of the oldest basic methods of TB diagnosis.

1. Rapid and economical method to detect AFB
2. Disadvantage is of course quality of sample, operator skill in picking up the right material, operator skill in staining being a manual method.
3. The test is less sensitive and normally requires bacilli as high as 10,000 per ml of specimen for the smear to be positive in comparison to GeneXpert which requires 133 bacilli per ml to be positive. (The new cartridge Ultra requires only 13 bacilli/ml)
4. Only skilled microbiologist can theoretically differentiate between MTB and NTM/MOTT on smear when there is a suspicion.

The one year data very clearly proves that all the above 03 tests should be requested for TB diagnosis. In case due to any reason fewer tests have to be ordered, then the priority should go as follows:

1. Only one test then AFB Culture (Liquid)
2. Two tests to be requested: AFB Culture and geneXpert for MTB

3. All three tests is ideal and can definitely help the TB irradication program.

Discussing the 03 methods, it is important to touch the fourth method which is very commonly tested in the public sector but not very frequently requested in the private sector. This test is the Line probe assay. The advantage of LPA is that the results give both Rifampicin and INH status along with MTB band, Mixed population and heteroresistance but the disadvantage is that the Turn around time is 48–72 hours compared to 02 hours of geneXpert so clinicians get attracted as results can come in early in outpatient department.

The challenge of Eradication is definitely there. But if the public and private sector come to a consensus of choosing the right diagnostics wisely with compulsory educational programs for all the three: Labs, Clinicians and patients, irrelevant of public or private sector. The process will definitely support and strengthen the optimistic program of TB Eradication by government of India. This challenge is not only for India but the whole world where TB is prevalent. For countries harboring TB, Correct diagnostic request and timely diagnosis and treatment is the key to eradication of tuberculosis.

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Conflicts of interest

The authors have none to declare.

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Original article

Formulation, characterization and evaluation of inhalable effervescent dry powder of Rifampicin nanoparticles

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Pulmonary tuberculosis

ABSTRACT

Background: Dry powder inhaler is a popular approach to pulmonary drug delivery to treat tuberculosis. Spray dried Nanoparticles using lactose carrier is extensively used for pulmonary drug delivery. Though lactose nanoparticles show deep lung deposition, they fail to uniformly disperse nanoparticles in its original form in alveoli. Rifampicin is one of the first line drugs in tuberculosis treatment. Lung targeted drug delivery system is an approach to reduce dose related side effects of rifampicin. Inhalable nanoparticles also help to target alveolar macrophages, thus improving treatment efficiency.

Methodology: This study focuses on rifampicin nanosuspension formulation and optimization using nano-precipitation method followed by characterizing effervescent DPI of rifampicin nanoparticles with effervescent pair (citric acid and sodium bicarbonate). Preliminary studies showed suitability of 4:5 solvent: antisolvent ratio and lecithin (1%) as stabilizer. The drug and stabilizer concentration in nanoparticles was successfully optimized using 3 * 2 factorial design using DESIGN EXPERT software. The rifampicin nanoparticles were further converted to spray dried powder using effervescent carrier.

Result: The effervescent pair formulation was monodisperse and had a particle size of 1.5 microns (polydispersity index 0.289), thus showing better redispersibility than lactose nanoparticles. The mass median aerodynamic diameter and fine particle diameter of both spray dried formulations were similar and suitable for deep lung deposition.

Conclusion: These findings are suggestive that effervescent technique can be successfully employed to improve redispersibility of rifampicin nanoparticles.

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1. Introduction

The pulmonary route of administration has been used for many years for the local treatment of lung diseases like asthma, tuberculosis (TB), cystic fibrosis, lung cancer etc. There has been growing interest in exploring pulmonary route for systemic administration of various active pharmaceutical ingredients especially in treatment of cancer, diabetes mellitus and pain treatment. In recent years, the respiratory tract has become an attractive route of administration for a large range of molecules and drug substances. Pulmonary delivery is becoming an important route of drug administration for the treatment of intra and extra pulmonary diseases because of unique characteristics of lungs such as large surface area, thin epithelial layer, high vascularization, avoidance of first-pass metabolism and rapid drug deposition in the target organ using a lower dose, which results in fewer systemic side effects than other routes of administration.[1] There are three approaches to pulmonary drug delivery *viz.* nebulizers, pressurized metered-dose inhalers (pMDIs) and dry-powder inhalers (DPIs). DPIs are becoming more popular because they offer high formulation stability due to their dry state and can provide deep lung deposition.[1]

TB is one of the leading causes of preventable deaths in the world today. TB is treated with a multidrug regimen including Rifampicin (RIF), Isoniazid, Ethambutol, Pyrazinamide, and is thus exceptionally vulnerable to incidences of side effects such as nausea, vomiting, and epigastric pain, skin reactions, hepatotoxicity etc.[2] One of the major problems is noncompliance to prescribed regimens, primarily because treatment of TB involves continuous, frequent multiple drug dosing. In addition to that, resistance of *M. tuberculosis (M.tb)* to anti TB agents is a worldwide problem in both immune-competent and HIV infected population. To minimize toxicity and improve patient compliance, extensive efforts have been made to develop particulate and colloidal carrier baseddrug delivery system to either target the site of *M. tb* infection or reduce dosing frequency.[3] *M.tb* is known to infect Alveolar Macrophages (AM) and affect pathogenesis of TB. *M.tb* hidden in AMs is the cause of secondary tuberculosis.[4]

Nanoparticles (NPs) are widely used for pulmonary drug delivery. But due to their smaller size i.e. 100–1000 nm, they are exhaled and do not give deep lung deposition. In case of deep lung deposition of particulates their particle size plays an important role. Larger particles do not reach deep in the lung and smaller particles tend to be exhaled. To get desired particle size for deep lung deposition (1–5 microns), NP agglomerates are formed using carrier. Spray drying or spray-freeze drying results in variable amounts of NP aggregation and thus delay redispersibility process and causes an increase in NP size. An approach developed by Ely et al[5] depend on adding an effervescent pair in the formulation during the spray drying process. On spray drying, microcarrier particles that contain NPs will acquire effervescent properties due to which they release NPs actively once they are in contact with any source of water or humidity, such as the physiological fluids in the lungs.[5] There are number of excipients used as carriers in spray drying such as mannitol, sucrose, trehalose, sodium chloride and lactose.[6,7] Lactose is one of the excipients extensively used as carrier for spray dried powders due to its inert properties and ability to increase bulk and surface properties of NPs.[8,9] After inhalation of dry powder of lactose NP agglomerates, lactose gets dissolved and releases NPs in lung fluid for absorption. Though such NP agglomerates shows deep lung deposition but fails to redisperse the NPs in its original form. Therefore, excipients such as a fast dissolving matrix (spray-dried lactose and mannitol and cyclodextrins), water soluble polymers (polyvinyl alcohol and PEG 6000) or different surfactants (pulmonary surfactant components and polysorbate-80) were used to enhance redispersibility. Some excipients (e.g., polysorbate-80) that demonstrated *in vitro* efficacy might be associated with *in vivo* toxicity.[7]

RIF is one of the first line drug used in treatment of TB. The dose of RIF when orally given is 10mg/kg (maximum 600mg/day) daily. Since the minimum inhibitory concentration of RIF is only 0.005–0.2 μg/ml after a usual 600mg dose,[2] there is a need to decrease the dose and thus decreasing the high dose related side effects such as skin rashes, immunological reactions, hepatotoxicity etc. Lung targeted drug delivery system is an approach to reduce dose related side effects of RIF. In addition to that, inhalable NPs will help to target AM.[2,4]

Narumon changsan et al,[10] have performed the physicochemical characterization and stability studies of RIF liposome dry powder formulations for inhalation. Sung et al,[11] have formulated and conducted studies on pharmacokinetics of self-Assembled RIFNPs systems for pulmonary delivery. Chuan et al,[12] have prepared solid lipid nanoparticles of RIF for the enhanced delivery to alveolar macrophages.

Song et al,[13] have formulated RIF loaded mannosylated cationic nanostructured lipid carriers for alveolar macrophage-specific delivery. However no studies have been conducted so far on effervescent NPs agglomerates targeting the alveolar macrophages. Highlighting the above aspects, the work undertaken relates to formulation of effervescent nanoaggregates of RIF. In the present study, an attempt has been made by the authors to formulate nanosuspension of RIF using nanoprecipitation method and its optimization followed by development of DPI of RIF NPs with lactose and effervescent pair and their characterization.

2. Materials and methods

2.1. Materials

Rifampicin (RIF) was kindly gifted by Lupin (Mumbai, India), lecithin was obtained from Alfa Aesar (India), and lactose monohydrate (Inhalac 70) was purchased from Meggle Pharma (Mumbai, India). Citric acid and sodium bicarbonate were purchased from SDFCL (Mumbai, India). All other solvents and reagents employed in the current study were of analytical grade and were procured locally.

2.2. Preparation of RIF nanosuspension

RIF nanosuspension was prepared by liquid antisolvent precipitation method.[14] Briefly, a weighed quantity of RIF was dissolved in methanol AR. The resultant methanolic solution was added dropwise with flow rate of 0.5ml/min to an

aqueous phase containing 1% w/v lecithin and the stirring was continued (800 rpm) for 1 h to remove excess of organic phase. The resultant nanosuspension was then sonicated using probe sonicator (VCX500, Sonic and material, USA) for 3 min at 40% amplitude and particle size and zeta potential were measured using Zetasizer Nano ZS (Malvern, US).

2.3. Experimental design

In order to get an optimized formulation with best size, concept of Design of experiment (DOE) was used. There were two major factors affecting the particle size of final product viz. drug concentration and stabilizer concentration. Thus, in the current study, effect of these two variables on particle size of nanosuspension was evaluated using Design Expert 7 software (statease). The best fitting model for optimization design was 'Response surface 2FI model'. The independent variables selected are shown in Table 1.

The polynomial equation generated by model is as follows:

$$Y = b0 + b1X1 + b2X2 + b3X1X2$$

in which Y is the measured response (particle size nm), b0 is intercept, X1 and X2 are independent variables, b1to b3 are linear coefficients and X1 X2 represent interaction between two independent variables. Ten batches were formulated as suggested by software shown in Table 2 and observed responses were measured.

2.4. Development of DPI of RIF nanosuspension

The optimized nanosuspension was formulated into DPI by spray drying. Citric acid, sodium bicarbonate (effervescent pair) and lactose (Inhalac 70) were selected as carriers for pulmonary delivery of nanosuspension.

Table 1 – Variables in design.

Factor	Level (% w/v)		
Independent variables	−1	0	+1
X_1 = Drug concentration	0.25	0.5	0.75
X_2 = stabilizer concentration	0.5	1	1.5

Table 2 – Observed response in design of nanosuspension.

Batch number	Independent variables		Dependent variables
	X_1	X_2	Y = particle size (nm)
1	0.25	0.5	693.5 ± 66.4
2	0.25	1	822.4 ± 36.6
3	0.25	1.5	715.7 ± 29.7
4	0.5	0.5	1299 ± 42.3
5	0.5	1	1198 ± 31.7
6	0.5	1	1156 ± 43.1
7	0.5	1.5	1052 ± 37.3
8	0.75	0.5	1783 ± 29.5
9	0.75	1	1609 ± 23.6
10	0.75	1.5	1310 ± 46.2

(n = 3).

2.4.1. Incorporation of RIF nanosuspension into effervescent carrier particles

Citric acid and sodium bicarbonate (1:1) was used as effervescent pair. The total concentration of effervescent pair in formulation was optimized on the basis of presence of effervescence observed visually and 3.5% w/v of effervescent pair was selected for effervescence generation (Table 3). For the preparation of dry powder, briefly citric acid was dissolved in the prepared nanosuspension and immediately pH of resultant mixture was adjusted to 8 by addition of ammonia solution (30%). This was followed by addition of sodium bicarbonate to the resultant mixture. The mixture was then spray dried using Spray drier (Labultima, Mumbai, India). The instrumental conditions were maintained as: inlet temperature 150°C–160 °C, outlet temperature 60°C–70 °C, atomization pressure 1.5 bars, aspiration rate 55–65%, and vacuum 135–140 mm of Hg. The dried product was then cooled to 40 °C and stored in vials.

2.4.2. Incorporation of RIF nanosuspension into lactose

Inhalac 70 was used as carrier in the present study. Briefly 3.5% w/v of lactose was dissolved in prepared nanosuspension and resultant mixture was spray dried using the above mentioned parameters. The obtained dry powder was cooled to 40 °C and stored in glass vials.

2.5. Characterization of spray dried product

Effervescent NP agglomerates and lactose agglomerates were compared with respect to their particle size, zeta potential, surface characteristics, surface morphology, preliminary lung deposition studies and *in vitro* drug release study.

2.5.1. Particle size, polydispersity index and zeta potential

Weighed quantity of spray dried product was redispersed in Millipore water and particle size, polydispersity index and zeta potential were measured using Zetasizer Nano ZS (Malvern, UK).

2.5.2. Surface morphology

Surface morphology of both spray dried formulation and RIF sample were studied and compared using scanning electron microscope (FEI Quanta 250, USA). Samples were mounted on aluminum stub with carbon adhesive tape and sputter coated by means of palladium prior to assessment.

2.5.3. In vitro release study of RIF

In vitro release study of RIF from spray dried formulations were performed by means of the dialysis tube diffusion technique. Phosphate buffer pH 7.4 was used as medium for current study. Dialysis tubing (cellulose membrane) with mol. wt. of 12,000 g/mol (Himedia). was soaked overnight in deionized water prior to the release study. RIF loaded spray dried formulations i.e. effervescent RIF NP agglomerates and RIF NP agglomerates with lactose, each containing 4 mg equivalent of RIF were redispersed in 2 ml of phosphate buffer pH 7.4 and positioned in dialysis membrane separately. Both

Table 3 – Concentration of citric acid monohydrate and sodium bicarbonate as carrier for effervescence generation.

Batch number	Citric acid Monohydrate (%)	Sodium bicarbonate (%)	Effervescence generation
1	1.25	1.25	Not seen
2	1.5	1.5	Slightly seen
3	1.75	1.75	Good amt. of effervescence

ends of the tube were secured through nylon thread and placed in glass beaker containing 100 ml phosphate buffer pH 7.4 maintained at 37 °C ± 0.5 °C with continuous agitation at 100 rpm using magnetic stirrer (Remi1MLH, Mumbai, India). At predetermined time intervals, 5 ml of aliquots were withdrawn and equal volumes were replaced with RIF free phosphate buffer pH 7.4 to maintained sink condition. The samples were analyzed for RIF content by UV spectrometry (V-530, jasco, Japan) at 234 nm after suitable dilution and % cumulative release of RIF was calculated.

2.5.4. *Preliminary in-vitro lung deposition studies*

Lung deposition of spray dried nanosuspension was preliminarily determined using twin stage impactor (Coopley scientific, UK) by the following procedure. Both the spray dried formulations were weighed and filled in HPMC capsules (size 3). Liquid impinger was charged with methanol AR, 7 ml for stage 1, and 30 ml for stage 2. The air flow was adjusted to 60 ± 5 L/min. Rotahaler was used to puncture the capsules and content aerosolized for 4 seconds was determined. Methanol AR was used to rinse particles deposited in each stage; and RIF content in each stage was determined using UV spectrometry at 234 nm. Based on drug deposited in each stage, recovered dose, emitted dose, fine particle dose, % fine particle fraction and dispersibility were calculated.

Mass median aerodynamic diameter of spray dried formulations was determined using Anderson cascade impactor (ACI) (Cooply scientific, UK). Each stage of ACI has multi-oriface that displays progressively smaller values from top to bottom. For the present study ACI was applied with a vacuum pump under flow rate of 60 ± 5 L/min. HPMC capsules containing spray dried NP was punctured and aerosolized for 4 seconds using Rotahaler. Methanol AR was use to rinse particles deposited on each stage and RIF content in each stage was determined using UV spectrometry at 234 nm. Amount of RIF deposited in capsule, device, pre-separator, stage 0–7 was calculated. The data was evaluated for calculating MMAD, GSD, FPD using MMAD calculator. The particles deposited on stage 6 and 7 correspond to those present in the alveoli *in vivo*.

2.5.5. *Confocal microscopy*

The geometric diameter of the spray dried effervescent NP agglomerates and effervescent effect of carrier particles were investigated using confocal laser scanning microscope (Leica Map DCM 3D). The samples were observed before and after being exposed to humidity. The imaging prior to contact with humidity was performed by taking spray dried formulation on glass slide and was observed under the microscope with 20× magnification. The effervescent imaging was obtained by adding 3 drops of water on the same sample slide and observing under microscope with 5× magnification.

3. Result and discussion

3.1. *Preparation and characterization of RIF nano suspension*

RIF nanosuspension was successfully prepared by liquid antisolvent precipitation method. Effect of liquid: antisolvent ratio on particle size of nanosuspension was analyzed and results are shown in Table 4. The particle size of RIF nanosuspension with 4:5 ratio of solvent: antisolvent was found to be below 8 microns with PDI 0.66 ± 0.26 whereas solvent: antisolvent ratio 2:5 resulted in large particles with a particle size range of 8–15 microns with increased PDI 0.81 ± 0.11. It was observed that as solvent: antisolvent ratio was decreased from 4:5 to 2:5; particle size of RIF nanosuspension was increased. This increase in particle size could be due to high supersaturation of drug in 2:5 solvent: antisolvent ratio, which increases the mass transfer gradient of drug from solvent to nuclei and can enhance the particle growth. Thus NPs prepared with 4:5 ratio of solvent: antisolvent ratio showing smaller particle size was chosen for preparation of RIF nanosuspension for further studies.

3.2. *Experimental design*

Ten batches of nanosuspension formulations as suggested by software were prepared by solvent precipitation method. The observed values of response i.e. particle size for all batches are shown in Table 2.

The selected independent variables were found to influence the response measured. All batches show size range between 600 and 1300 nm. Using the ANOVA provision available in the software, the polynomial equations involving the main effects and interaction factors were determined based on estimation of various statistical parameters. The results of ANOVA study are shown Table 5. Accordingly, model F value for response was found to be 166.63 which indicate that the model selected was significant. Moreover, value of "probability > F" <0.05 indicates that the model terms are significant. Hence for response Y, X_1, X_2 and X_1X_2 were found to be significant model terms.

Table 4 – Effect of solvent: antisolvent ratio on particle size of nanosuspension.

Solvent:antisolvent	Particle size (micron)	Polydispersity Index (PDI)
2:5	9.20 ± 5.17	0.66 ± 0.26
4:5	4.37 ± 2.68	0.81 ± 0.11

(n = 3).

Table 5 – Analysis of variance (ANOVA) of optimization design.

Parameter	Model F- value	P > F for model	P > F for X_1	P > F for X_2	P > F for X_1 X_2	Lack of fit F value	Pred. R^2	Adjusted R^2	Adequate precision
Experimental value	166.63	0.0001	0.0001	0.001	0.0021	2.96	0.9353	0.9822	35.161

The "Lack of Fit F- Value" of 2.96 indicates the Lack of Fit is not significant relative to the pure error. There is a 41.39% chance that a "Lack of Fit F-Value" this large could occur due to noise. Non-significant lack of fit is good. The "Predicted R-Squared" of 0.9353 is in reasonable agreement with the "Adjusted R-Squared" of 0.9822 which indicates significance of model. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desired. Adequate precision was 35.161 indicates an adequate signal. This model can therefore be used to navigate the design space. The effect of independent variables on particle size could be quantified by the following equation.

$$\text{Particle size} = +77.79333 + 2637.33333 * X1 + 262.6 * X2 - 990.4 * X1 * X2$$

The positive values before a factor in the above regression equation indicate that the response increases with the factor and vice versa.[15] Three-dimensional response surface plots and two-dimensional contour plots were obtained from the data by using DESIGN EXPERT 7 (statease) to ascertain effects of independent variables on response and represented in Figs. 1 and 2 respectively. On the basis of Figs. 1 and 2 and Table 2 it was found that variables selected for formulation optimization had marked influence on particle size.

In Table 2, batch 3 showed smallest particle size when minimum drug concentration i.e. 0.25% was used and batch 10 showed largest particle size when maximum drug concentration i.e. 0.75% was used and the stabilizer concentration was kept same i.e. 0.5%. The resultant particle size was directly proportional to the drug concentration used. In Table 2 batch 3, 5 and 1 when drug concentration was kept minimum i.e. 0.25% and the stabilizer concentration was varied as 0.5%, 1% and 1.5%, results revealed that the particle size was not significantly dependent on stabilizer concentration used. In batch 9, 4, 8 when the drug concentration was kept intermediate i.e. 0.5% and the stabilizer concentration was increased from minimum to maximum. It was observed that as stabilizer concentration increased particle size was decreased. In batch 10, 7 and 2 the drug concentration was kept maximum i.e. 0.75% and the stabilizer concentration was increased from minimum to maximum. Similar effect was seen that as stabilizer concentration was increased the particle size was decreased. However, the effect on particle size was more pronounced in batches with higher drug concentration i.e. 0.75%. Thus based on above results it was observed that when drug concentration was minimum, stabilizer concentration doesn't have significant effect on particle size. However, at intermediate and maximum levels of drug, particle size was inversely proportional to stabilizer concentration.

3.3. *Interaction between drug and stabilizer concentration*

Interaction between the two factors X1 and X2 i.e. drug concentration and stabilizer concentration is shown in Fig. 3. Graphically, the interactions are visualized by lack of parallelism in the lines. The interaction between two variables suggests that the effect of one variable has been moderated or modified by the other variable. It was clear that when the drug concentration was minimum there was no correlation between the stabilizer concentration and the particle size whereas when the drug concentration was intermediate and

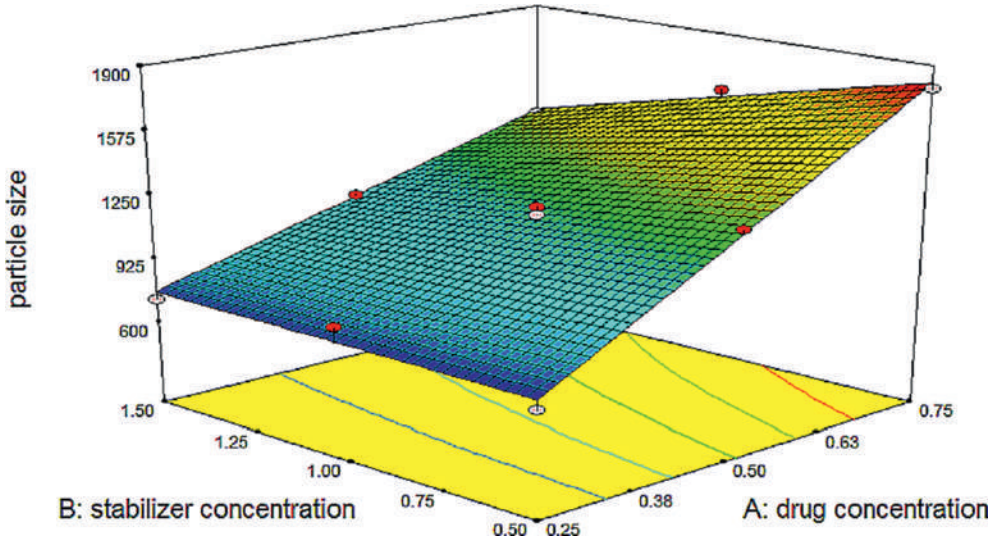

Fig. 1 – Response surface three dimension showing effect of drug concentration on particle size of NPs.

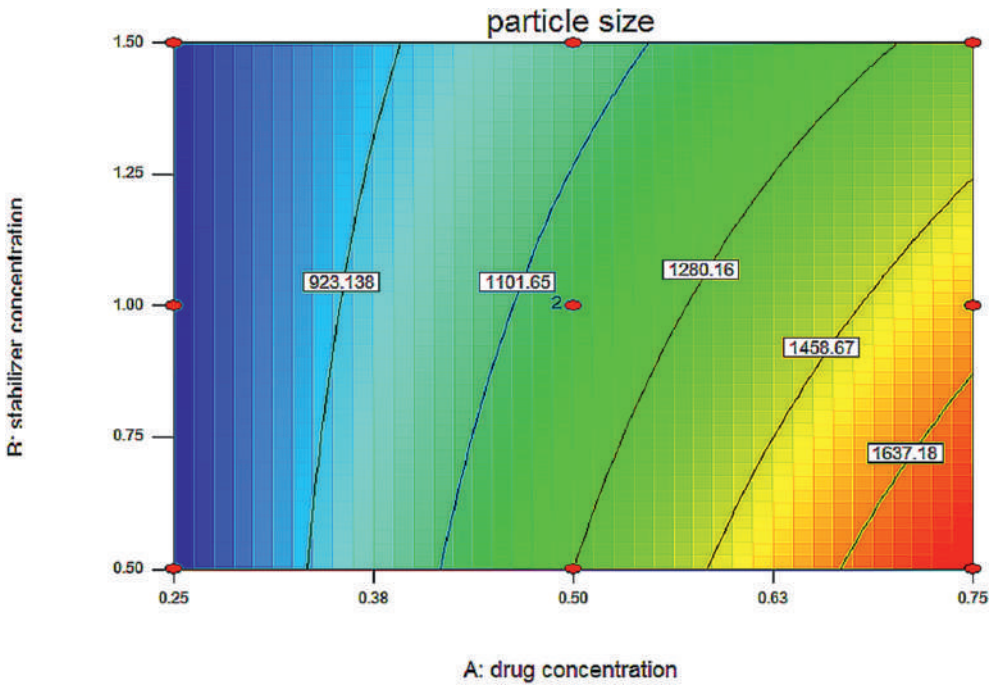

Fig. 2 – Two dimensional contour plot showing effect of variables on particle size.

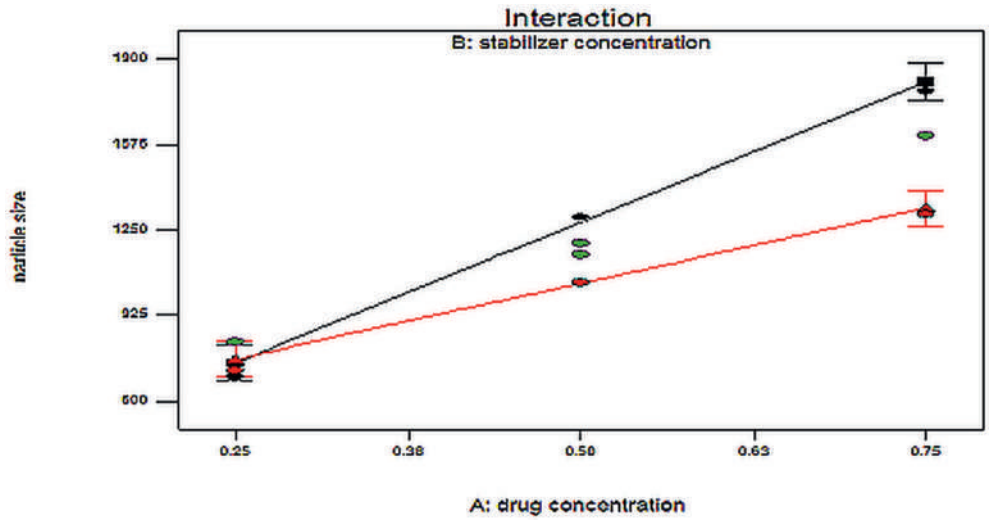

Fig. 3 – Interaction between the two factors X1 and X2.

maximum, as stabilizer concentration was increased the particle size was decreased. It showed that the effect of stabilizer concentration on particle size has been modified by the drug concentration which is interaction.

Validation of design was done by plotting three different actual (726.93nm, 1012.46nm, 1238nm) vs. predicted (750nm, 1000nm, 1250nm) particle sizes. Results are shown in Table 6 and graphically represented in Fig. 4. Predicted particle size

Table 6 – Validation batches for optimization.

Drug concentration (%)	Stabilizer concentration (%)	Particle size predicted (nm)	Particle size obtained (nm)	% Error
0.25	0.79	750	726.93 ± 17.87	3.076
0.39	0.68	1000	1012.46 ± 20.86	−0.012
0.52	0.81	1250	1238 ± 29.51	0.96

(n = 3).

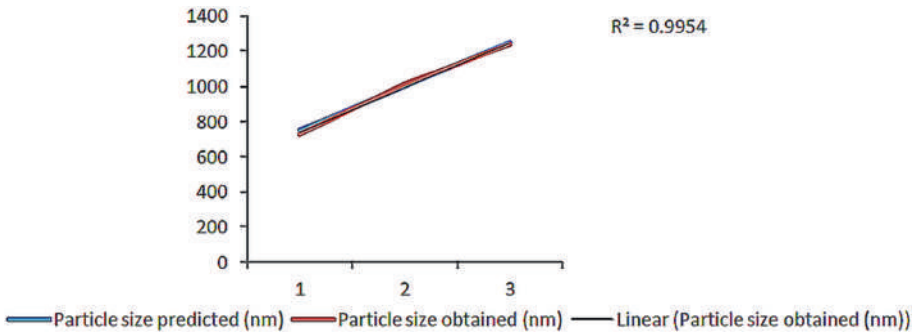

Fig. 4 – Graph of particle size predicted and particle size obtained experimentally.

of 750nm, 1000nm and 1250nm vs. experimentally obtained particle size of 726.93nm, 1012.46nm and 1238nm shows 3.076%, −0.012% and 0.96% error respectively and the regression coefficient was 0.9954. Percent error less than 5% demonstrated that that the experimental particle size was close to predicted particle size, which fits its acceptance criteria. Since drug delivery to alveolar macrophages (AMs) increases as particle size increases from 100 to 1000nm, the nanosuspension prepared with 0.39% drug concentration and 0.68% stabilizer concentration giving 1000nm particle size was chosen for further studies to target AMs which are the cause of secondary tuberculosis. After pulmonary administration, nanoparticles are exhaled due to their small size. Thus it is necessary to convert it into desired particle size (1–5 microns) using carrier. Thus optimized Nanosuspension was formulated into DPI by Spray drying.

3.4. *Characterization of spray dried product*

3.4.1. *Particle size, index and zeta potential determination*

The particle size of RIF NP agglomerates with lactose and effervescent RIF NP agglomerates after redispersion are shown Fig. 5 (a) and **(b)** respectively. Average particle size of RIF NPs agglomerates after redispersion was found to be increased irrespective of type of carrier used. The potential explanation for this is that carbohydrates can form a thick protective layer around the nanoparticles which protects them against the mechanical stress and heat stress during spray drying, when amount of carbohydrate decreases and is insufficient to protect the nanoparticles against the stress during spray drying, which could increase the tendency of particle aggregation, thus causing an increase in particle size.[16] In Fig. 5 (b) average particle size of agglomerates was found to be 1272 nm with PDL 0.284 indicating good redispersibility of effervescent NP agglomerates. Zeta potential of both formulations was −47 ± 2mV. Such high negative value of zeta potential indicates physical stability of formulation.

3.4.2. *Surface morphology assessment*

Surface morphology of RIF and both spray dried formulations were captured by SEM and shown in Fig. 6. The image obtained reveals that RIF NP agglomerates with effervescent pair Fig. 6(c) were highly porous and NP agglomerates with lactose Fig. 6(b) had smooth surface, with size range of 1–10 μm, which is suitable for deep lung deposition.[5] Porous particles with size 1–10 μm and density less than one show less cohesiveness, better flowability, deposit deeply in lung and redispersed properly in lung. SEM image of RIF drug show clear elongated crystals of approximate size range 10–20 μm, Fig. 6(a) which is not suitable for deep lung deposition.

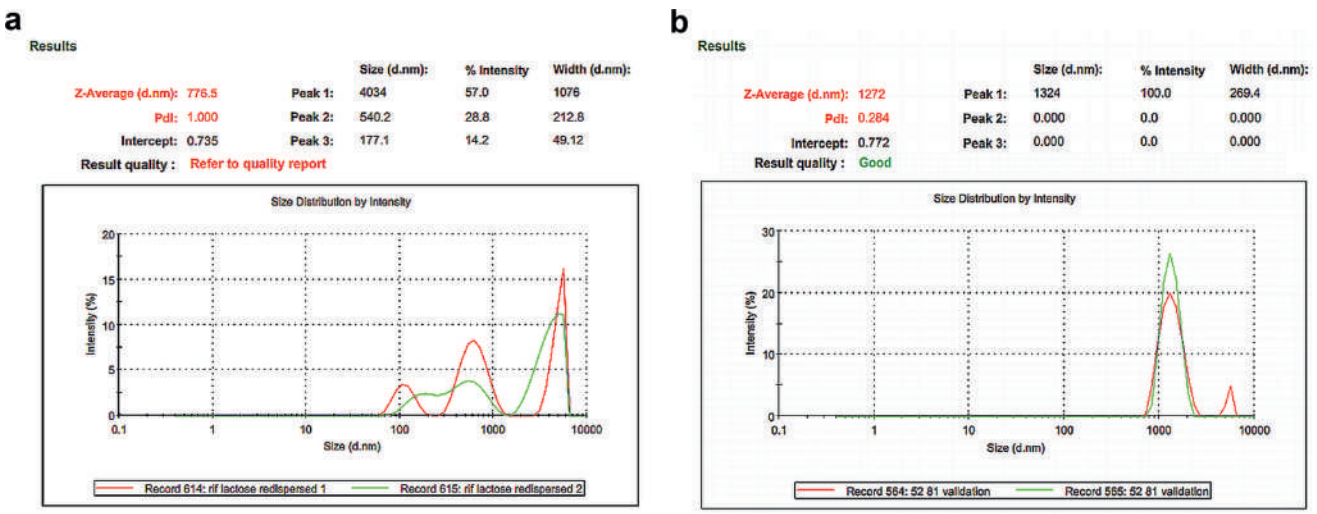

Fig. 5 – (a) Particle size of NP agglomerates with lactose. (b) Particle size of effervescent RIF NP agglomerates.

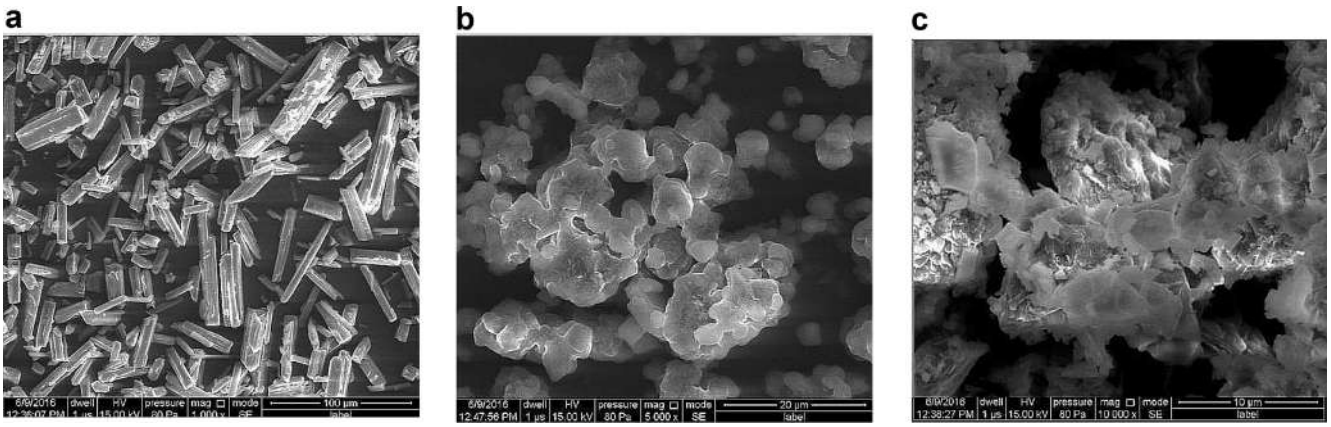

Fig. 6 – (a) SEM image of RIF pure drug. (b) SEM image of RIF NPs agglomerates with lactose. (c) SEM image of effervescent RIF NPs agglomerates.

3.4.3. In vitro *release study of RIF*

The results of *in vitro* release studies are illustrated in Fig. 7. Percent cumulative release (%CR) of RIF from effervescent NP agglomerates was found to be 47.42 ± 0.91 at the end of 10 hrs. which was lower compared to % CR of RIF solution (methanolic solution of RIF) i.e. 63.45 ± 2.04. This shows delayed release of RIF from effervescent NP agglomerates compared to that of solution. Percent cumulative release of RIF from NP lactose agglomerates was found to be 18.71 ± 0.47, which was very low compared to that of release of RIF from effervescent NP agglomerates. The result reveals that effervescent particles were able to increase the drug release by providing good redispersibility of the NPs.[5]

3.4.4. In vitro *lung deposition studies*

Lung deposition of Rifampicin and both spray dried formulations was preliminarily determined using Twin stage impactor. RD, ED, % FPF, FPD, dispersibility data are shown in

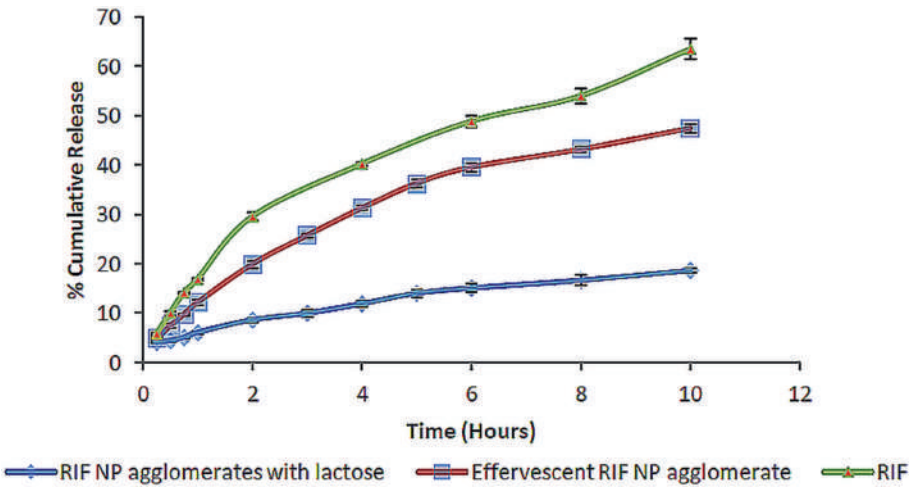

Fig. 7 – Drug release from dialysis bag containing RIF, effervescent RIF NP agglomerates and RIF NP agglomerates with lactose.

Table 7 – Parameters of RIF, effervescent RIF NPs agglomerates and RIF NPs agglomerates with lactose related to lung deposition.

	RIF (API)	Effervescent RIF NP agglomerates	RIF NP agglomerates with lactose
Total drug impinged (mg)	16	16	16
Recovered dose (mg)	15.94 ± 1.4	11.29 ± 2.1	11.05 ± 3.01
Emitted dose (mg)	14.20 ± 2.1	5.9 ± 0.98	4.81 ± 1.2
Fine particle fraction (mg)	0.08 ± 0.01	1.79 ± 0.63	0.58 ± 0.13
Fine particle fraction (%)	0.48 ± 0.02	15.86 ± 3.79	5.20 ± 2.13
Dispersibility (%)	0.54 ± 0.02	30.34 ± 4.18	11.94 ± 3.78

(n = 3).

Table 8 – Parameters of effervescent RIF NPs agglomerates and RIF NPs agglomerates with lactose related to lung deposition.

Formulation	Total RIF impinge (mg)	RD (mg)	ED (mg)	% FPF	MMAD (μm)	GSD
Effervescent RIF NP agglomerates	40	27.47 ± 0.59	16.46 ± 9.3	15.51 ± 8.01	4.23	1.41
RIF NP agglomerates with lactose	40	27.94 ± 0.42	11.59 ± 0.98	10.59 ± 1.39	5.29	2.03

(n = 3).

Table 7. RD and ED of both spray dried formulations were less compared to that of RIF (API). This was due to environmental humidity which may have caused particle agglomeration and thus inability to emit both formulations from capsule. The FPD of both spray dried formulations was found to be more as compared to RIF (API); which implies deep lung deposition. RIF crystals were longer in size compared to spray dried formulation thus deposited majorly in stage 1, thus showed lesser FPF, FPF, and dispersibility. Preliminary study using TSI showed that FPF, FPD and dispersibility of RIF (API) can be improved by formulating spray dried NP agglomerates.

MMAD was calculated by using Anderson cascade impactor; MMAD, % FPF, emitted dose and recovered dose, data of both spray dried formulation are shown in Table 8. MMAD and GSD of both spray dried formulations i.e. effervescent RIF NPs agglomerates and RIF NPs agglomerates with lactose was found to be 4.23μm, 5.29μm and 1.41, 2.03 respectively, which is suitable for deep lung deposition. MMAD of effervescent RIF NP agglomerates was low as

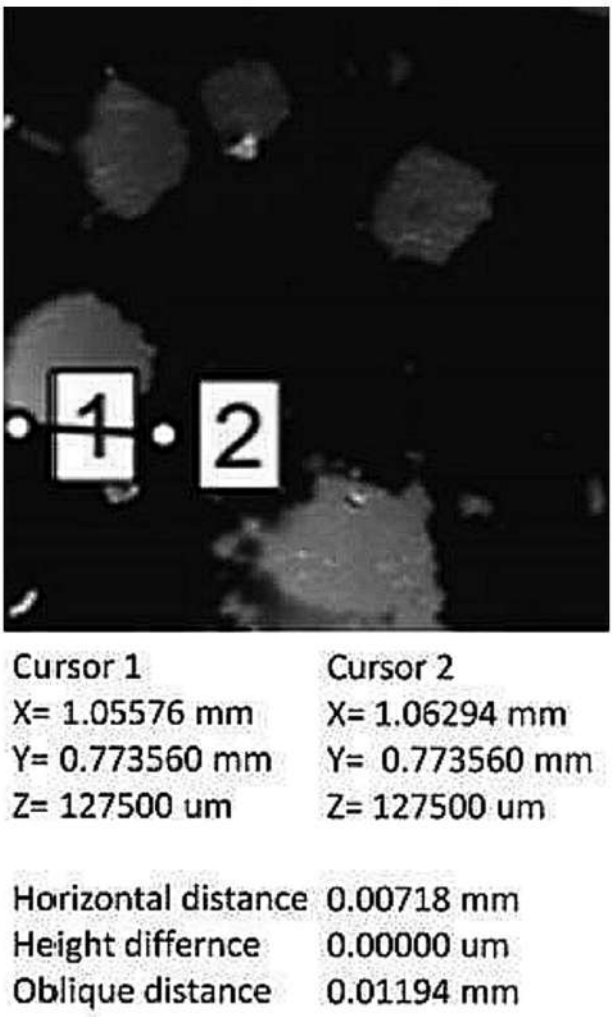

Fig. 8 – Confocal microscopy of effervescent RIF NP agglomerates before exposure to humidity.

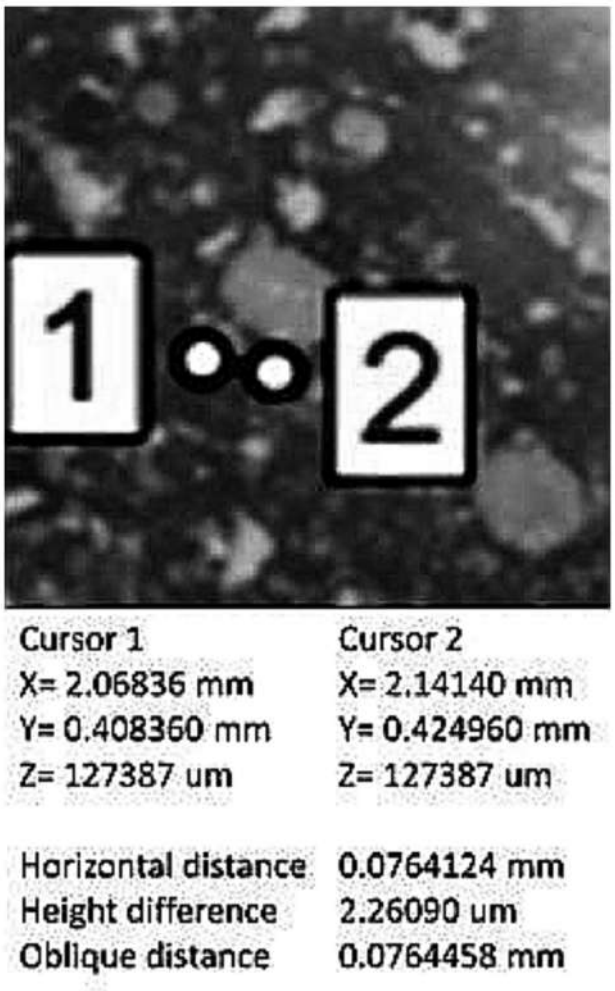

Fig. 9 – Confocal microscopy of effervescent RIF NP agglomerates after exposure to humidity.

compare to that of agglomerates with lactose. This indicate effervescent NP agglomerates deposit deep lung better than agglomerates with lactose.

3.4.5. *Confocal microscopy*

Images of effervescent RIF NP agglomerates before and after coming in contact with water were taken using confocal microscope and are shown in Figs. 8 and 9 respectively. In Fig. 8 the average geometric size of effervescent RIF NPs agglomerates was found to be 9 μm whereas after exposure to humidity small bubbles were seen visually on the slide and in Fig. 9, the nanoparticles were found to be uniformly distributed throughout the gas bubbles of different sizes under the microscopes. The size of largest bubble was found to be 76 μm. This shows that due to active mechanism the effervescence carrier particles were able to release nanoparticles with less particle agglomeration.[5]

4. Conclusion

There is need to develop easily redispersible NPs agglomerates for the effective treatment of TB. RIF NPs were prepared by nanoprecipitation method and formulation variables were studied by 3^2 factorial design by applying DESIGN EXPERT 7. Optimized formulation converted into DPI using citric acid and sodium bicarbonate (3.5%) showing good FPD, MMAD and redispersibility. SEM imaging show RIF NPs agglomerates are suitable for deep lung deposition. However, *in vivo* pharmacokinetic as well as pharmacodynamic studies are required for confirmation of improvement in bioavailability.

Conflicts of interest

The authors have none to declare.

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Original article

Correlation expression Toll-like receptor 4 with multidrugs resistant tuberculosis in diabetes mellitus condition

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ABSTRACT

Background: Toll-like receptor (TLR) are ligand homologous protein in the APC cell membrane that has functions as a receptor to triger leukocytes and innate immune responses. When there is a *Microbacterium tuberculosis* (MTB) infection enters from droplets to the lungs, the alveolar macrophages perform a phagocytic function. The interaction between *M. tuberculosis* and the TLR macrophage receptors produces chemokines which induce migration of monocytes and dendrite cells for destruction. Diabetes militus (DM) has become risk factor for developing tuberculosis. DM condition will reduce immunity and the ability of immune cell phagocytes bactery and triger severe infections. The consequences of more severe infection and metabolic disorders that occur make a person more likely to experience Multidrugs resistant MTB. Not much data that reports on the expression of TLR4 as a ligand that triggers an immune response in conditions of MDR and DM. We try to find out correlation between TLR-4 in MDR MTB, diabetes and level of MTB bacteria in experimental animals.

Methods: We conducted an experimental study on 30 experimental mice weighing 25 grams consisting of negative control grub, infected with MTB, infected with MDR MTB, negative control diabetes, MTB DM, MDR MTB DM. DM animals were induced by streptozosin to experience DM, then in the treatment of infection, intraperitoneal MTB and MDR MTB bacterial injections were given. Termination was carried out on day 14. We count number of bacteria level in the lungs and perform evaluation TLR4 from blood sampel.

Results: The negative control group had mean TLR value of 1.47 (± 0.46) while the MTB group showed an increase in TLR 9.22 (± 0.39) followed by MDR MTB 9.50 (± 0.29), DM negative

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control 9, 21 (± 0.24) and more increasing in conditions of DM MTB 13.36 (± 0.32) and DM MDR MTB 13.35 (± 0.34). ANOVA analysis showed a significant difference (P = 0.00). pearson correlation analysis find strong correlation TLR4 in MTB and MDR MTB with diabetes.
Conclusion: there were a significant difference level TLR4 between MTB and MDR TB infection with diabetes. higher TLR4 level higher in DM MTB, DM MDR MTB. TLR 4 strong correlates with an increase in the number of MTB bacteria.

1. Background

Toll-like receptor (TLR) is a homologous protein in the APC cell membrane that has functions as a ligand receptor to activates leukocytes and trigger innate immune responses against pathogens. This protein was first discovered in Drosophila as a Toll protein. These receptors consist of leucine-rich areas in the extracellum and in the cytoplasmic tail region which are receptors for IL-1 and IL-8 and are called Toll/IL-1 receptors (TIR).[1]

Activation of the TLR ligand will trigger pathogenic phagocytosis and inflammatory responses that release phagosome content. Several TLRs, namely TLR2 and TLR4, are capable of assisting the placement of phagosomes, then become as immune system's that earliest contact with potentially damaging microbial antigens. The most important characteristic of TLR activation is the formation of proinflammatory conditions represented by certain cytokines and chemokines, dominated by TNF-α and IL-12 on (NF) -kB and α/β IFN on the IRF-3 TLR ligand marker.[1,2]

When there is an MTB infection that enters from droplets in the lungs, the alveolar macrophages perform a phagocytic function. The interaction between *Mycobacterium tuberculosis* and receptors for macrophages (Toll-like receptors/TLRs) produces chemokines that induce the migration of monocytes and dendrite cells from the bloodstream to the infected lung. The dendritic cells will then include bacteria, then mature and are destroyed by CD4 and CD8 T cells. There were a phenomenon of granuloma formation due to the accumulation of macrophages, T cells, B cells, endothelial cells, dendritic cells, and epithelium, as well as other cells that are useful for limiting infection, but can become a residence for *M. tuberculosis* in a relatively long period of time latent bacteria and reactivated when there is a cytokine imbalance.[3–5]

Diabetes mellitus (DM) is a major risk factor for developing active pulmonary TB. Hyperglycemia in diabetic patients will lead decrease ability of cell phagocytes that has correlation with wider spread of infection, granuloma formation and p reactivation caused by the lagging and missing granulocytic phase of inflammation. In addition, individuals with diabetes has low elastic recoil reserves in the airways accompanied by thinning of the alveolar epithelium and capillary basement membrane, centrilobar emphysema and microangiopathy. These changes will affect low lung defense mechanism.[6,7]

The condition of decreased immune response, delay in diagnosis and reactivation of TB in individuals with diabetes will increase the risk of developing MDR which affects patient outcomes. In this study, we wanted to determine the relationship between TLR-4 in MDR TB and diabetes mellitus and its correlation to the level of MTB bacteria in experimental animals.

2. Methods and materials

The research was carried out at the molecular biology and immunology laboratory Faculty of Medicine Hasanuddin University. This experimental research was carried out on 30 experimental mice weighing 25 grams that divided into 6 groups. Group 1 was a negative control animal, group 2 animals with MTB bacterial infection, group 3 animals with MDR MTB bacterial infection, group 4 animals with negative control animals with diabetes condition, group 5 animals with MTB diabetes conditions and group 6 MDR MTB with diabetes. To trigger diabetes, an intraperitoneal injection of Streptozosin 40mg/kgbw was carried out to damage pancreas then trigger hyperglycemia. 3 days after blood glucose sticks were examined that taken from the tails of the mice. An increase in fasting blood glucose above 126mg/dl was an indicator of diabetes. 3 days after the adaptation of diabetes treatment and negative control, animals treated with MTB infection and MDR MTB were given an injection intraperitoneal of 0.2 ml of normal saline mixture with culture of bacterial MTB and MDR MTB bacterial colonies after 14 days, animal get termination, venous blood sampling was carried out to measure TLR-4 level and examination of MTB bacterial levels from lung tissue. The TLR-4 examination was carried out by isolation of Peripheral Blood Mononuclear Cells (PBMC) from blood and flow cytometry staining buffer. Examination of the number of bacteria is done by counting the number of levels of bacteria per 100 microscope fields of view.

3. TLR expression

Peripheral Blood Mononuclear Cells (PBMC) was isolated from the blood. Then the blood sample was dissolved 1: 1 with PBS in a conical tube. Support the dissolved sample with an amount of Ficoll volume equal to the volume of the original sample. Centrifuge was performed for 20 minutes (1000×*g*) with the brake OFF position. The PBMC is located at the junction between the PBS and Ficoll layers into the newly extracted tube. Fill the tube with PBS to wash the cells. Again centrifugation of the cell suspension 4–5 minutes (300-400×*g*) at 4 0C, discard the supernatant. Next, re-suspend the cell pellets in the flow cytometry staining buffer and perform cell

counts and viability analysis. Centrifuge the cells as in the previous step, then re-suspend them with an appropriate volume of flow cytometry staining buffer so that the concentration is as needed.

4. Subject characteristics

Table 1 shows the current blood sugar profile and bacterial level per field of view in all groups. The blood glucose value shows that the mean blood sugar levels are increased in the streptozosin induced group as diabetes group. The highest blood glucose level value was obtained in the DM MDR TB group with level 408 (±20.59) followed by the DM MTB group 394.40 (±24.95) and diabetes negative control without MTB bacteria 394.20 (±13.19). Diabetes conditions in experimental animals with MDR TB have a higher blood sugar value than other groups. There was a significant difference in blood glucose level in all treatment groups (P = 0.00).

Table 1 – Characteristic blood glucose in experimental animals.

Animal profile	Mean	SD ±	Min–Max	Median	P
Blood glucose level g/dl					
Control negative	102.40	7.86	95–115	101	0.00
MTB	100.80	10.66	90–116	98	
MDR TB	104.00	10.22	89–114	106	
Control negative DM	394.20	13.19	380–414	390	
DM MTB	394.40	24.95	370–423	403	
DM MDR MTD	408	20.59	379–437	409	

5. The correlation between TLR-4 and MTB conditions, MDR MTB with diabetes mellitus

Correlation analysis was carried out to assess the direction of the relationship between the TLR-4 level and the increase in the number of MTB bacteria in all treatment groups. In Fig. 1, we can see there is a direct relationship with the increase in TLR4 within each group and bacterial level. The increase in TLR will increase more number of bacterial level. Mostly found in the conditions of DM MTB and DM MDR TB. Table 2 shows the correlation between TLR in MTB conditions, MDR MTB and diabetes mellitus. The analysis showed a significant correlation between TLR and the amount of AFB with a strong correlation coefficient of R = 0.744 and P = 0.000.

6. Comparison TLR 4 gene expression on MDR MTB with diabetes militus

In this study, an analysis of TLR 4 gene expression was carried out in the treatment group of diabetic rats and then infected with MTB and MDR MTB bacteria. Evaluation was carried out for each experimental animal group, the TLR4 value can be seen in Fig. 2 and Table 3.

Table 2 – Correlation of TLR to the number of bacteria MTB and MDR MTB with diabetes.

Variable	R	P[a]
TLR4- groups	0.878	0.000
TLR4-bacterial level	0.744	0.000

[a] Pearson Correlation analysis.

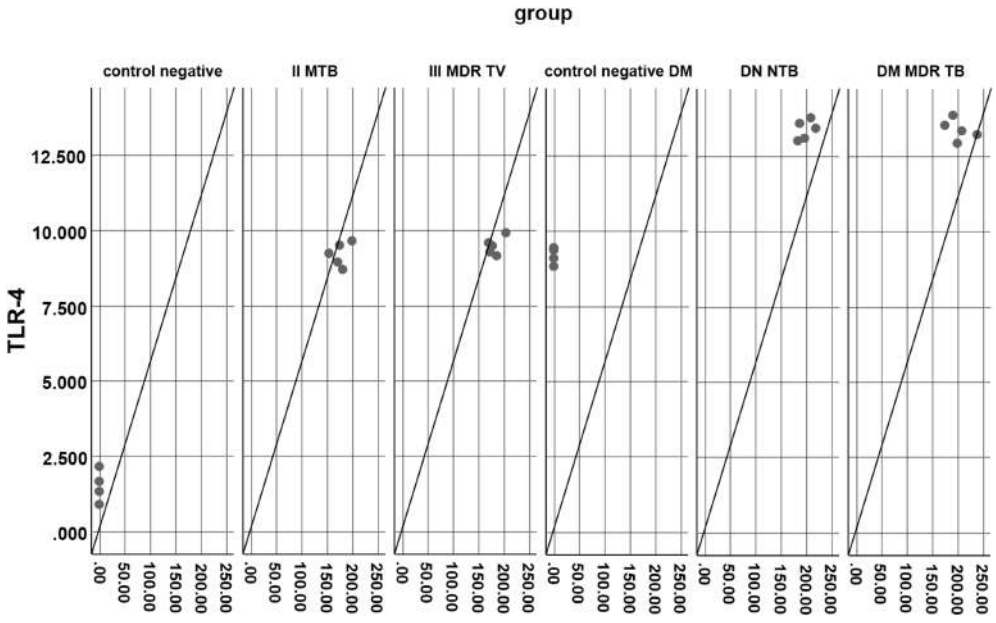

Fig. 1 – Bacterial level and expression of TLR-4 in each treatment group, there is a linear increase in the number of bacterial in MTB, MDR MTB and higher in diabetic condition.

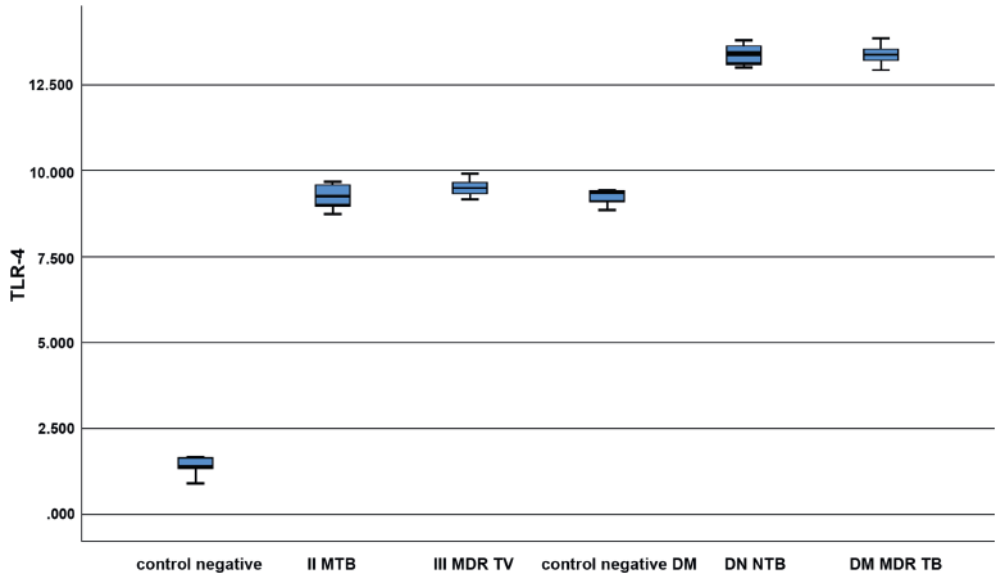

Fig. 2 – Expression of TLR 4 gene in diabetic MTB, MDR MTB and control groups. The highest expression value was obtained in the DM MTB and MDR MTB treatment groups.

TLR expression level in negative control group was 1.47 (±0.46) while the MTB group had higher level TLR 9.22 (±0.39) followed by MDR MTB 9.50 (±0.29), DM negative control 9, 21 (±0.24) and increasing in conditions of DM MTB 13.36 (±0.32) and DM MDR MTB 13.35 (±0.34). Diabetes conditions are able to give the effect of increasing TLR4. In the analysis of variance (ANOVA) there was a significant difference in the mean of TLR expression in the treatment group ($P = 0.00$) (see Table 4).

Based on the post hoc LSD analysis, there are significant differences in all groups except the MTB vs MDR MTB group, MDR vs negative control and DM MTB vs DM MDR MTB group which did not have a significant difference ($P > 0.05$). DM MDR MTB and DM MTB were the highest difference value on the expression value of TLR-4 compared to negative control.

7. Discussion

In this study, bacterial level in animals with diabetes has a higher number per field of view. The highest mean bacterial level was in the DM MDR MTB 201 group (±23.52), followed by the DM MTB group 197.60 (±14.31), the MDR MTB group 181.60 (±13.93) and MTB 174 (±15.95). There were significant differences in the number of bacterial level in the DM MTB MDR group compared to other groups. This suggests that diabetes triggers a higher number of bacterial level of MTB. The bacterial level in diabetic patients will also increase due to metabolic and the absorption problems of TB drugs, so patient cannot reach the therapeutic targets to eliminate bacteria. This will lead to an increase in the number of bacteria and the risk of drug resistance. Nijland et al. Reported that TB patients with diabetes had low level serum of rifampin 53% than non-diabetic TB patients. Failure to reach the correct dose will inhibit bacterial elimination and allow the risk of MDR. Antonia et al. Reported a significant relationship between diabetes mellitus and the development of MDR MTB.

Table 3 – TLR 4 expression level.

Treatment groups	Mean	SD ±	Min–max	P[b]
Control negative	1.47	0.46	0.881–2.15	0.00
MTB	9.22	0.39	8.17–8.66	
MDR MTB	9.50	0.29	9.16–9.92	
Control negative DM	9.21	0.24	8.84–9.41	
DM MTB	13.36	0.32	12.99–13.76	
DM MDR MTB	13.35	0.34	12.90–13.84	

[b] ANOVA test.

Table 4 – Comparison between treatment groups. By carrying out a Post-hoc LSD analysis, the differences between experimental animal treatments can be assessed.

Treatment groups	Mean differences	P[c]
Control negative vs MTB	−7.75	0.00
Control negative vs MDR TB	−8.03	0.00
Control negative vs control negative DM	−7.73	0.00
Control negative vs DM MTB	−11.89	0.00
Control negative vs DM MDR TB	−11.88	0.00
Control negative DM vs DM MTB	−4.15	0.00
Control negative DM vs DM MDR MTB	−4.14	0.00
MTB vs MDR MTB	−0.28	0.220
MTB vs control negative DM	−0.012	0.956
MTB vs DM MTB	−4.14	0.00
MTB vs DM MDR TB	−4.13	0.00
MDR TB vs control negative DM	−0.29	0.201
MDR MTB vs DM MTB	−3.86	0.00
MDR MTB vs DM MDR MTB	−3.85	0.00
DM MTB vs DM MDR MTB	0.0084	0.970

[c] Post Hoc LSD test.

Individuals with tuberculosis and diabetes mellitus have a 6.8 greater risk of developing MDR MTB.[8,9]

TB DM patients were also described as having more severe TB infection, requiring longer treatment and possibly developing MDR-TB than patients with TB alone. Jenn et al reported that TB patients with DM who were compared without DM, also experienced more severe infections, higher levels of mycobacterial load, 17% higher treatment failure rate and longer mycobacterial elimination than TB patients alone.[10]

Increased bacteria and a higher risk of MDR in patients with comorbid TB-DM. It has been reported that poor glucose control is often associated with phagocytic dysfunction, production of reactive oxygen species (ROS), chemotaxis and T-cell reactions in DM patients, thus triggering a stronger rate of bacterial development. On the other hand, a higher mycobacterial burden, changes in the pharmacokinetics of anti-TB drugs and lower adherence to treatment also encourage MDR TB to occur in diabetic patients.[11]

8. The correlation between TLR on MTB bacteria and MDR MTB diabetes conditions

TLRs are known to be homologous proteins on the APC cell membrane that function as functional receptors that activate leukocytes to trigger innate immune responses and responses against pathogens. This protein was first discovered in Drosophila as a Toll protein. These receptors consist of leucine-rich areas in the extracellum and in the cytoplasmic tail region which are receptors for IL-1 and IL-8 and are called Toll/IL-1 receptors (TIR). TLR activation will trigger immunity coding genes that cause changes in the immune response and influence the development of TB disease.[1]

Once activated the TLR ligand will trigger pathogenic phagocytosis and an inflammatory response to the phagosome content. Some of the most dominant TLRs in TB are TLR2 and TLR4 which are capable of assisting phagosome placement, as the immune system's earliest contact with potentially damaging microbial antigens. The most important characteristic of TLR activation is the formation of proinflammatory conditions represented by certain cytokines and chemokines, dominated by TNFα and IL-12 on (NF) -kB and α/β IFN on the IRF-3 TLR ligand marker. TLR4 recognizes mycobacterial lipopolysaccharides (LPS) and can trigger one of two innate immune response pathways, the dependent or independent MyD88 pathway. When there were an MTB infection that enters from droplets in the lungs, alveolar macrophages perform the function of phagocytosis. The interaction between *M. tuberculosis* and the TLR macrophage receptors produces chemokines which induce the migration of monocytes and dendritic cells from the bloodstream to the infected part of the lung. The dendrite cells will include bacteria, mature cell then destroyed by CD4 and CD8 T.[12–14]

In this study, wa can see the value of TLR4 expression in conditions of bacterial infection of MTB, MDR MTB, DM MTB, DM MDR MTB and negative control. When there is infection, the TLR4 value increases following MDR MTB. In conditions of heavier infection accompanied by diabetes status, the TLR4 value is also higher in both DM MTB and DM MDR MTB. then also there was a significant difference in TLR4 values in each group with significant strong correlation. The existence of a continuous increase from this is an indicator that TRL 4 can be a biomarker for TB infection conditions including during MDR MTB and diabetes. Previously has been known that patients with MDR TB will usually have worse clinical pathology than TB infection alone. Soedarsono et al. Reported that TLR was significantly associated with more severe clinical conditions of TB infection and MDR TB. This decrease in signaling ability against TLR4 can greatly affect the susceptibility of TB disease. TLR4 are of the most studied and shown to be associated with TB susceptibility and more severe disease conditions.[13,15]

The results of this study also explain that the conditions of TB and MDR TB infection accompanied by diabetes will have a higher TLR4 value. Previously, TLR4 was also reported to have correlation with diabetes condition and proinflammatory stage. Mohammad et al. Reported an increased expression of TLR2 and TLR4 in nonobese type 1 diabetic rats, then has correlation with increased activation of nuclear factor κB (NFKβ) in response to LPS TLR4 ligands resulting in increased proinflammatory cytokines. Sridevi et al. Also demonstrated the increased expression and activity of TLR2 and TLR4 in monocytes diabetic animals, also explaining that increased TLR4 expression contributes to the pro-inflammatory state of diabetes.[16,17]

9. Conclusion

There is a significant difference between TB and MDR TB infection with diabetes will have a higher TLR4 value. TLR 4 correlates with an increase in the number of MTB bacteria.

Author contribution

Heidy Agustin: first author, literature search, data collector, Muhammad Nasum Massi: supervisor in literature search, Irawati Djaharuddin: methods and study design, Agus D. Susanto: writing and literature search, Andi A. Islam: methods and study design, Mochammad Hatta: methods and study design, Agussalim Bukhari: data analysis, Nur A. Tabri: data analysis, Arif Santoso: data analysis, Erlina Burhan: literature search and statistic interpretation, Fathiyah Isbaniyah: literature search and statistic interpretation, Zulham Effendy: Data collecting and analysis

Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2022.03.012.

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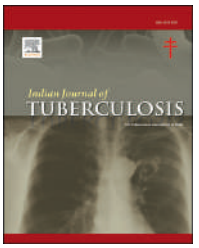

Original article

Application of vaginal tampon as an alternative to nasal swabs for higher recovery of DNA from sheep and goats for PCR based diagnosis of bovine tuberculosis

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ABSTRACT

A study was conducted to find the applicability of vaginal tampons as an alternative to regular cotton swabs as a nasal secretion collection tool for the higher recovery of DNA. Nasal secretions were collected from sheep and goats using regular cotton swab and tampon swab. The mean yield and purity of the DNA extracted from tampon were significantly higher than that of the DNA extracted from cotton swab. The tampon swabs resulted higher DNA recovery than the cotton swabs after they were allowed to absorb *M. bovis* culture. The tampon swab was also found to be more sensitive in detecting *M. bovis* by PCR. This study concluded that vaginal tampons are having a higher absorption capacity with more DNA yield and can be used as a nasal swab in the diagnosis of bovine tuberculosis.

1. Introduction

The use of swabs for the collection of samples from the nasal or oral regions is a routine sampling method for use in nucleic acid based detection tests such as polymerase chain reaction (PCR). Improper sampling or poor absorption capacity of the swabs used could lead to erroneous results and compromise disease diagnosis. For isolation of respiratory pathogens, especially *Mycobacteria*, it is important to use the best

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collection tool that would provide a higher yield of bacteria in nasal secretions. Traditionally cotton swabs are used for the collection of nasal exudates. The study aimed to determine the suitability of the tampon swabs for the collection of nasal secretion and isolation of DNA. Cotton and tampon swabs were used in sheep and goats for the collection of nasal secretion and the DNA recovery rates were compared. Mycobacterial culture fluid was allowed to absorb by cotton or tampon swabs and the DNA recovery rates were compared. PCR was done with the DNA recovered from both types of swabs and its sensitivity (detection limit) was estimated. In this study, tampon swabs have demonstrated better performance in the recovery of *Mycobacteria* from both nasal secretions and culture fluids.

2. Materials and methods

Tampons were used as a nasal secretion collection tool to assess their absorbing efficiency over the cotton swab. Regular cotton swab (HIMEDIA®) and O.B.®PRO COMFORT®(Johnson & Johnson) tampon were used in this study.

2.1. *Experiment 1—Absorption of nasal secretions onto swabs/tampons*

This study was conducted in a group of small ruminants comprising 14 sheep and 20 goats. Nasal secretions were collected from the nostrils ofthe animal using either a cotton swab or tampon swab from each nostril separately. The same weight of tampon as the weight of cotton fibers present in the cotton swab was taken for the study. Then each swab was kept in 15ml centrifuge tubes containing 5ml phosphate buffered saline (PBS) and stored at −20 °C till DNA extraction.

2.2. *Experiment 2—Absorption of* M. bovis *culture fluids onto swabs/tampons*

This study was conducted using a positive *M. bovis* culture available in our laboratory. This isolate was obtained from post mortem sample collected from skin test positive cattle that had died in a private farm. A total of 24 No's of cotton swab and tampon swab were allowed to absorb *M. bovis* culture available in the MGIT tubes after 42 days of growth, for 30

Fig. 1 – The yield (ng/µL) (A) and purity (A_{260}/A_{280} ratio) (B) of the DNA extracted from tampon swab were significantly higher ($p < 0.05$) than that extracted from cotton swab (Student's *t*-test) after collection of nasal secretion from sheep and goats.

seconds. Then each swab was kept in 15 ml centrifuge tubes and stored at −20 °C till DNA extraction.

2.3. *Experiment 3—estimation of limit of detection of* M.bovis *DNA in PCR (sensitivity test)*

4 sets of each type of swabs were soaked in 6 serially diluted *M. bovis* samples (Neat culture, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions) of decreasing bacterial concentrations. After 30 seconds of soaking, the swabs were kept in 15ml centrifuge tubes and stored at −20 °C for sensitivity test by PCR.

2.4. *DNA quantification and analysis*

DNA was extracted from both kinds of nasal swabs using XpressDNA™ Bacteria DNA Extraction Kit (MagGenome Technologies). The yield and purity of DNA extracted were measured using TECAN NanoQuant Plate™ Spectrophotometer. Differences between the two types of swabs for DNA yield and purity were determined using the Student's *t*-test. Statistical significance was set at $p < 0.05$.

2.5. *PCR for IS6110*

PCR assay was performed for the amplification of the 445bp fragment of *IS6110* to identify the presence of *Mycobacterium tuberculosis* complex (MTBC). The reaction was subjected to initial denaturation 95 °C for 10 minutes followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 58.9 °C for 1 minute and extension at 72 °C for 1 minute with a final extension at 72 °C for 5 minutes. PCR amplification was carried out in 15 μl reaction mixtures containing 1 μl of DNA template, 20pM of 2μl each of forward and reverse primers, 7.5 μl of GoTaq® Green Master Mix (Promega) and 2.5 μl of nuclease free water. The amplicons were analyzed by gel electrophoresis in a 1.7% agarose gel. The gels were visualized and documented using an automatic documentation system.[1]

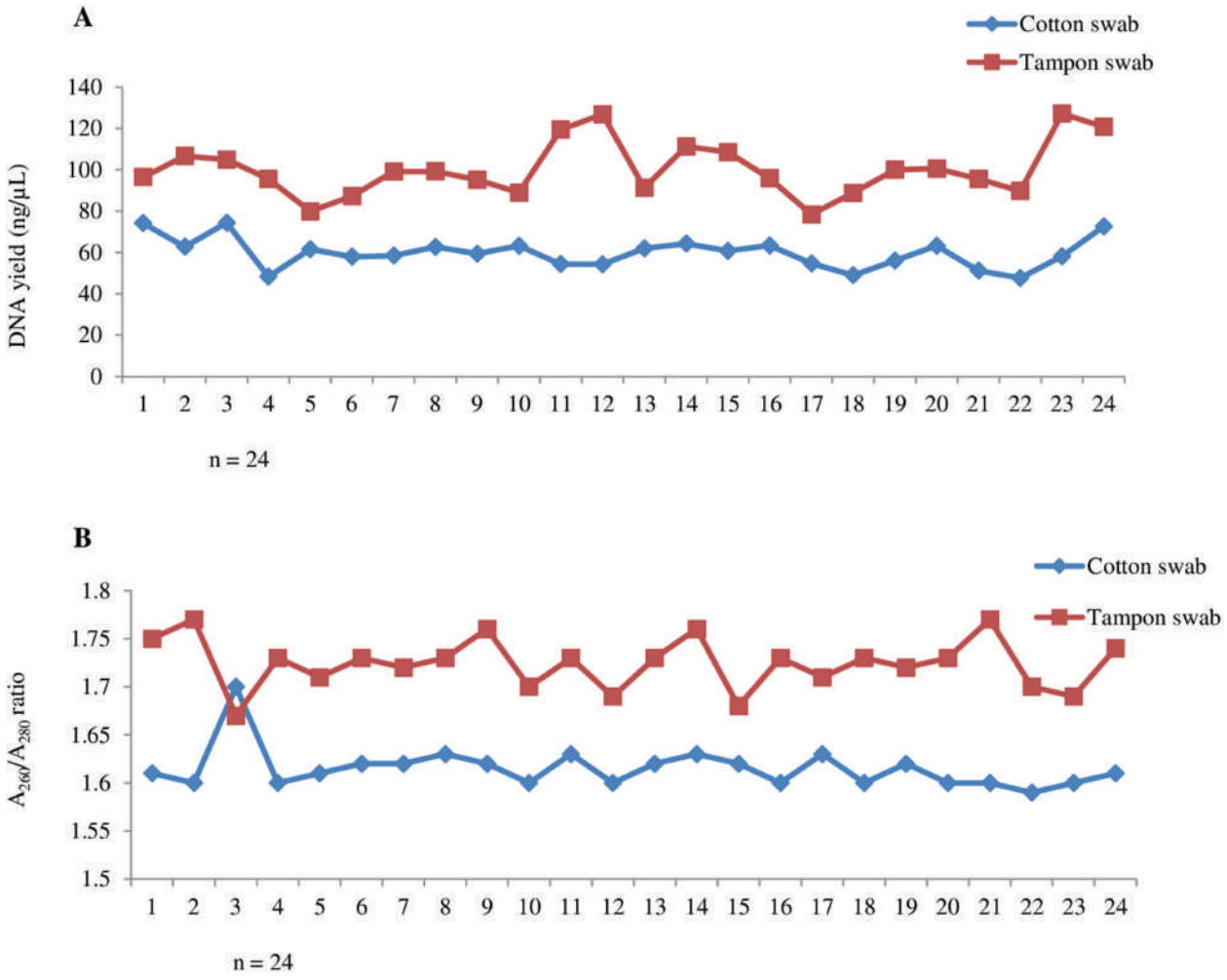

Fig. 2 – The yield (ng/μL) (A) and purity (A_{260}/A_{280} ratio) (B) of the DNA extracted from tampon swab were significantly higher ($p < 0.05$) than that extracted from cotton swab (Student's *t*-test) after soaking both the swabs in *M. bovis* culture.

The primers used were:

IS*6110* F - 5'-GACCACGACCGAAGAATCCGCTG
IS*6110* R - 5'- CGGACAGGCCGAGTTTGGTCATC

3. Results

3.1. *DNA yield and purity from swab and tampon used in animals*

In experiment 1, 34 No's of sheep and goats were used. When cotton swab and tampon swab were used as nasal swabs, the mean yield± SD of DNA recovered from cotton swab and tampon swab was 50.57 ± 3.77 ng/μL and 64.77 ± 5.65 ng/μLrespectively (Fig. 1A). The purity A_{260}/A_{280} ratio of DNA recovered was 1.72 ± 0.02 and 1.77 ± 0.01 respectively (Fig. 1B). The yield and purity of the DNA extracted from tampon swab were significantly higher ($p < 0.05$) than that extracted from cotton swab (Student's *t*-test).

3.2. *DNA yield and purity from swab and tampon used in* M. bovis *culture*

In experiment 2, 24 No's of each cotton swab and tampon swab were soaked in *Mycobacterium bovis* culture and DNA was recovered from the swabs. The mean yield ± SD of DNA recovered from cotton swab and tampon swab was 59.7 ± 7.36ng/μL and 100.26 ± 13.34ng/μLrespectively (Fig. 2A). The purity A_{260}/A_{280} ratio of DNA recovered from cotton swab and tampon swab was 1.62 ± 0.02 and 1.72 ± 0.03 respectively (Fig. 2B). The yield and purity of the DNA extracted from tampon swab were significantly higher ($p < 0.05$) than that extracted from cotton swab (Student's *t*-test).

3.3. *Sensitivity of PCR in serially diluted* M.bovis *culture*

In PCR, the lowest *M. bovis* concentration detectable was 1 in 10 dilutions when tampon swab used as collecting material. But when cotton swab used, PCR detected *M. bovis* concentration in neat culture only (Fig. 3).

4. Discussion

Nasal swabs are used to collect microbial agents from the respiratory tract. Vitale et al,[2] reported MTBC are often detected in nasal swabs by PCR with high specificity and Romero et al,[3] demonstrated that nasal-mucus samples give better results for the detection of the microorganism than other body fluids like blood or milk. Woolums[4] suggested that the nasal swabs might be capable to identify bacteria associated with bovine respiratory disease in clinically affected animals.

Reports have shown that rayon swabs yields higher DNA and RNA concentrations[5] with higher extraction efficiency[6] in swabs made of rayon. Since the tampons are made of compressed layers of highly absorbent rayon or cotton or a mixture of both, we compared the absorbing capacity of the tamopons and cotton swabs.

Our studies clearly demonstrated that a significantly higher DNA absorption occurred in tampons as compared to the traditional cotton swabs and found the quality of DNA was also significantly higher. However, since the animals from which swabs were taken, were all negative for MTBC PCR the effect of higher DNA yields in disease detection could not be assessed. To assess the contribution of increased DNA yields and quality on PCR for pathogen genome detection, similar comparisons were done on *M. bovis* positive culture fluids. The DNA yield and purity was high in tampon swab than cotton swab. In the sensitivity

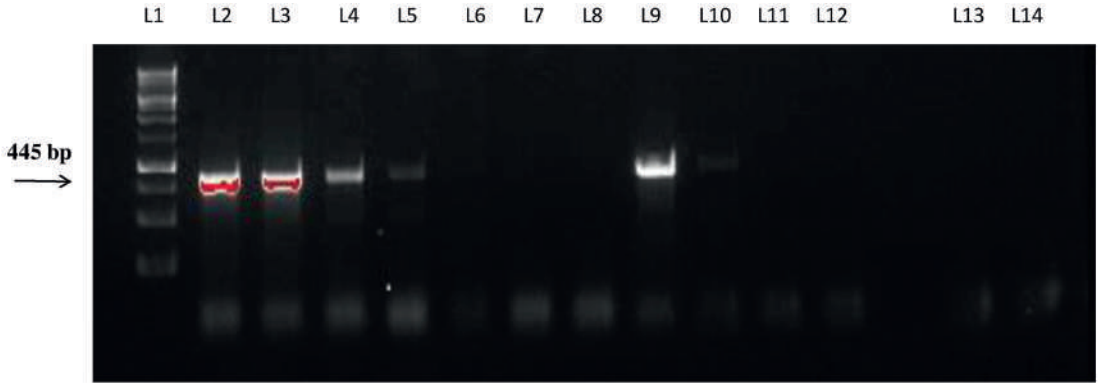

Fig. 3 – Detection of MTBC by PCR for IS*6110* in serially diluted *M. bovis* culture using cotton swab and tampon swab: Lane 1–100 bp DNA ladder, Lane 2 - *M. bovis* Positive Control, Lane 3 - (Tampon swab) Neat culture, Lane 4 - (Tampon swab) 10^{-1} dilution, Lane 5 - (Tampon swab) 10^{-2} dilution, Lane 6 - (Tampon swab) 10^{-3} dilution, Lane 7 - (Tampon swab) 10^{-4} dilution, Lane 8 - (Tampon swab) 10^{-5} dilution, Lane 9 - (Cotton swab) Neat culture, Lane 10 - (Cotton swab) 10^{-1} dilution, Lane 11 - (Cotton swab) 10^{-2} dilution, Lane 12 - (Cotton swab) 10^{-3} dilution, Lane 13 - (Cotton swab) 10^{-4} dilution, Lane 14 - (Cotton swab) 10^{-5} dilution. In PCR, the lowest MTBC concentration detectable was 1 in 10 dilutions (Lane 4) when tampon swab used. But PCR detected MTBC in neat culture (Lane 9) only when cotton swab used.

test also, tampon swab showed at least 10 times increased sensitivity in detection of *M. bovis* DNA in PCR. Similar findings of higher sample release and more DNA recovery were reported by Benschop[7] and Brownlow[8] in nylon flocked swabs than conventional cotton swabs.

5. Conclusion

Our studies concluded that the vaginal tampons are more efficient absorbing material than regular cotton swabs to collect nasal secretions for use in nucleic acid based assays such as polymerase chain reaction. Hence it is recommended to use tampons for collection of nasal swabs for detection of mycobacterial DNA, which would result in increased sensitivity of pathogen genome detection. This may also be extrapolated to other respiratory pathogen genome detection.

Conflicts of interest

The authors have none to declare.

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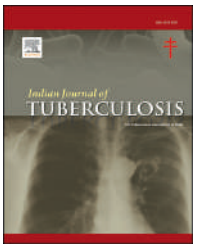

Original article

An evaluation of Composite Reference Standard (CRS) for diagnosis of Female Genital Tuberculosis

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ABSTRACT

Background: Female genital tuberculosis (FGTB) is a common cause of infertility in developing countries. Its diagnosis is difficult due to its paucibacillary nature, with no single test having high sensitivity and specificity. This study is to share the experience of using Composite Reference Standard (CRS) for the diagnosis of FGTB.

Methods: This is a prospective study conducted between September 2017 to June 2019, over 100 infertile females found to have FGTB on composite reference standard which consisted of acid-fast bacilli on microscopy or culture, histopathological evidence of epithelioid granuloma, positive gene Xpert on endometrial sample or definite or probable finding of FGTB on laparoscopy.

Results: A total of 100 infertile women (78% primary, 22% secondary) found to have FGTB on CRS were enrolled in this study. Mean age, body mass index, parity and duration of infertility were 28.2 years, 23.17 kg/m^2, 0.24 ± 0.12 and 2.41 years respectively. Various symptoms were scanty menses (16%), irregular cycle (7%), dysmenorrhea (11%), pelvic pain (11%). Various signs were vaginal discharge (65%), adnexal mass (6%), tubo-ovarian mass on ultrasound (15%), abnormal hysterosalpingography findings (57.14%), positive polymerase chain reaction test (65%) and abnormal hysteroscopy (82.2%). The positive findings on CRS were positive AFB on microscopy or culture (3%), positive gene Xpert (28%) (done in some cases), epithelioid granuloma on histopathology (13%), definite findings on laparoscopy like tubercles, caseous nodules and beaded tubes in (57.19%) patients while probable findings of FGTB like straw colored fluid in POD, extensive dense pelvic, peri-tubal, peri-ovarian adhesions; hydrosalpinx; tubo-ovarian mass; thick fibrosed tubes; mid tubal block; peri hepatic adhesions (Fitz Hugh Curtis Syndrome); hyperemia of tubes/blue uterus on

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chromotubation were seen in (48.8%) patients. All patients found to be positive on CRS were given 6 months of anti-tubercular therapy.

Conclusion: This study demonstrates the high reliability of use of composite reference standard for diagnosis of FGTB.

1. Introduction

Tuberculosis continues to be a major public health problem globally with 10 million new cases annually in the world and 2.7 million new cases in India.[1,2] Liberal immigration, concomitant HIV infection and emergence of drug resistant TB has further fueled the epidemic of TB in the world.[1,2]

Female genital tuberculosis (FGTB) is a type of extrapulmonary TB which causes morbidity like infertility accounting for about 10% cases in India.[3,4] The prevalence of FGTB in infertile women is higher in referral hospitals and in women attending the assisted reproductive services clinics.[3] In a meta-analysis, Chaman-Ara et al observed the prevalence of FGTB in infertile women to be 24.2%.[5] Presentation of FGTB is vague with most patients presenting with menstrual problems especially oligomenorrhea and hypomenorrhea; others being poor general health, vaginal discharge, abdominal pain and most importantly infertility.[3,4,6]

Being pauci-bacillary in nature, the diagnosis of FGTB is difficult. Although gold standard is detection of acid-fast bacilli (AFB) on microscopy or culture of endometrial aspirate or biopsy or peritoneal biopsy, they are positive in small percentage of cases with risk of missing the diagnosis. Polymerase chain reaction, though a sensitive test has high false positivity and hence is not used for the diagnosis.[3,4] Radiological imaging (ultrasound, CT scan, MRI and PET-CT scan) are useful in only tubo-ovarian masses and are non-specific in diagnosis of FGTB.[3,4] Cartridge based nucleic acid amplification (CB-NAAT) also called gene Xpert on endometrial aspirate or biopsy has high specificity but low sensitivity in detecting FGTB.[7] Recently Sethi et al observed promising results with loop mediated isothermal amplification (LAMP) assay on endometrial biopsy but it is still not validated and is not easily available at most centers.[8] Endoscopy like laparoscopy and hysteroscopy have been useful in diagnosing FGTB.[9] In fact, laparoscopy can detect FGTB in early stages by finding definite findings of FGTB like tubercles, caseous nodules and beaded tubes and by probable findings of FGTB like straw colored fluid in POD; extensive dense pelvic, peri-tubal, peri-ovarian adhesions; hydrosalpinx; tubo-ovarian mass; thick fibrosed tubes; mid tubal block; peri hepatic adhesions (Fitz Hugh Curtis Syndrome); hyperemia of tubes and blue uterus on chromotubation.[3,4,9,10]

In conditions like extra-pulmonary TB, especially FGTB where gold standard diagnostic tests like demonstration of acid-fast bacilli on microscopy or culture or epithelioid granuloma on histopathology is obtained infrequently with the risk of missing the diagnosis and risk of over diagnosing and over-treatment with tests like PCR with high false positivity, composite reference standard (CRS) has been used for better picking up of cases. CRS combines many tests which are in use for diagnosis of that condition with chance of higher diagnosis of cases. However, we should not use tests like PCR, ultrasound, hysterosalpingography etc. in CRS as they have poor specificity. Tyagi et al have recommended use of CRS for diagnosis of pleural TB, another type of EPTB.[11] Sharma et al developed Index TB Guidelines for various EPTB cases including FGTB by using CRS.[12] We performed this study to evaluate the usefulness of using CRS in diagnosis of FGTB by combining AFB on microscopy or culture, epithelioid granuloma on histopathology, gene Xpert and definite and probable findings of FGTB on laparoscopy.

2. Materials and methods

It was a prospective study conducted between September 2017 to June 2019 in a tertiary care center. All infertile patients attending the gynecological outpatient department were screened after taking consent for female genital TB on the basis of detailed history taking, general physical and gynecological examination, baseline investigations, ultrasound, hysterosalpingography (wherever possible), endometrial biopsy or aspirate for AFB microscopy or culture, gene Xpert (in some cases), histopathology, polymerase chain reaction, Gene pert could be done on only 50 cases as it was not available in first half of study. Diagnostic laparoscopy and hysteroscopy were done in selected cases with high probability of FGTB on the basis of family history of TB or positive findings of TB with tests like PCR and definite and probable findings of FGTB were noted. The inclusion criteria were all patients of infertility willing to participate in the study and found to have FGTB on CRS which included five parameters; acid-fast bacilli positivity on microscopy or culture, positive gene Xpert, positive epithelioid granuloma on histopathology on endometrial biopsy or aspirate and definite findings of FGTB on laparoscopy like tubercles, caseous nodules and beaded tubes or probable findings of FGTB like straw colored fluid in POD; extensive dense pelvic, peri-tubal, peri-ovarian adhesions; hydrosalpinx; tubo-ovarian mass; thick fibrosed tissue; mid tubal block; peri hepatic adhesions (Fitz Hugh Curtis Syndrome); hyperemia of tubes/blue uterus on chromotubation. The sensitivity and specificity of various tests were calculated. Patients not willing to participate in study, with concomitant HIV infection, with malignancies and past history of any TB were excluded from the study.

PCR, ultrasound, hysterosalpingography (HSG) and hysteroscopy were done wherever possible but were not used in CRS. These tests along with contact history of TB or other

Table 1 – Characteristics of patients with FGTB (n = 100). Total women screened = 569. FGTB on composite reference standard = 100. Incidence of FGTB in infertile women = 17.57%.

S.No.	Characteristic	Mean	Range
1.	Age (years)	28.27 ± 3.67	22–40
2.	Body mass index (kg/m^2)	23.17	18.33–31.93
3.	Parity	0.24 ± 0.12	0–3
4.	Duration of infertility (years)	2.41 ± 1.44	1–7
		No. (n = 100)	**Percentage (%)**
5.	History of TB contact	39	39
6.	History of BCG vaccination	77	77
7.	History of consumption of unpasteurized milk	4	4
8.	Type of Infertility		
	a) Primary	78	78
	b) Secondary	22	22
9.	Menstrual pattern		
	a) Normal menses	64	64
	b) Heavy flow	2	2
	c) Scanty menses	16	16
	d) Irregular cycles	7	7
	e) Dysmenorrhea	11	11
10.	Weight loss	4	4
11.	Dyspareunia	7	7
12.	Vaginal discharge	4	4
13.	Chronic pelvic pain	11	11

symptoms suggestive of FGTB were only used to screen patients for laparoscopy and not for diagnosis. The findings of ultrasound like adnexal mass, findings of HSG like beaded tubes, unilateral or bilateral blockage, hydrosalpinx, intravasation or extravasation of dye were observed but not included in CRS. Hysteroscopy was performed to look for any abnormal findings like thin endometrium (pale cavity), any tubercles and any intra-uterine adhesions.

All patients found to have FGTB on CRS were started on anti-tubercular therapy using directly observed treatment short course (DOTS) under National Tuberculosis Elimination Program of India (NTEP) for 6 months using 4 drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol (HRZE) daily in the intensive phase for 2 months followed by using 3 drugs (Rifampicin, Isoniazid and Ethambutol (HRE) continuation phase for 4 months daily. All patients were followed up for any adverse effects of drugs and liver function tests were done in selected cases.

2.1. *Statistical analysis*

Data Analysis was carried out using STATA software v 12.0. Continuous variables were tested for normality assumption using Kolmogorov-Smirnov test. Descriptive statistics such as mean, standard deviation, range values were carried for normally distributed dates. 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. Sensitivity and specificity of various tests were calculated using 95% Confidence Intervals.

3. Results

Out of 569 infertile women attending gynecological outpatient department of a tertiary care center screened for female genital tuberculosis, a total of 100 women were found to have FGTB on composite reference standard (acid-fast bacilli on microscopy or culture, evidence of epithelioid granuloma on histopathology, positive gene Xpert on endometrial biopsy, definite or probable findings of FGTB on laparoscopy). The characteristics of the women are shown in Table 1.

The mean age, body mass index, parity and duration of infertility was 28.2 years, 23.17 kg/m^2,0.24 ± 0.12 and 2.41 years respectively. History of TB contact, BCG vaccination and use of unpasteurized milk were seen in 39%, 77% and 5% cases respectively. Primary infertility was seen in 78% and secondary infertility in 22% cases. Various menstrual disorders and symptoms are also shown in Table 1. Normal menstrual cycles were seen in 64% women, while menstrual disorders seen were heavy flow (2%), scanty menses (16%), irregular cycles (7%) and dysmenorrhea (11%). Weight loss was seen in 4%, dyspareunia in 7%, vaginal discharge in 4% and lower abdominal pain in 11% patients.

Clinical signs and baseline investigations not included in composite reference standard are shown in Table 2. Various signs were pallor (10%), lymphadenopathy (4%), chest crepitations (6%), abdominal tenderness or fullness (6%). On speculum examination, vaginal discharge was seen in 65% women and on bimanual examination, normal findings were seen in 93% women while adnexal tenderness and adnexal mass were seen in 6% and 1% women respectively. The investigations shown in Table 2 were hemoglobin (12.2 ± 0.99 g/dL), mean

Table 2 – Clinical signs and investigations in FGTB patients.

S.No.	Characteristic	Mean	Range
1.	Hemoglobin (g/dL)	12.21 ± 0.99	8.7–14.0
2.	ESR	32.10 ± 12.78	13–67
3.	Random blood sugar	114.77 ± 15.0	78–204
		No.	**Percentage (%)**
4.	Pallor	10	10
5.	Lymphadenopathy	4	4
6.	Chest crepitations	6	6
7.	Speculum examination		
	Normal findings	35	35
	Vaginal discharge	65	65
8.	Vaginal examination		
	Normal findings	93	93
	Adnexal tenderness	6	6
	Adnexal mass	1	1
9.	Chest Xray		
	Normal	94	94
	Mediastinal lymphadenopathy	6	6
10.	Ultrasound Pelvis		
	Normal	82	82
	Tubo-ovarian mass	15	15
	Thin endometrium	3	3
11.	Hysterosalpingography (n = 84)		
	Normal	36	42.8
	Unilateral block	15	17.8
	Bilateral block	24	28.5
	Beaded tubes	8	9.5
	Extravasation of dye	5	5.9
	Intravasation of dye	1	1.1
	Hydrosalpinx	11	13.0
12.	Polymerase chain reaction (n = 100)		
	Positive	65	65
	Negative	35	35
13.	Hysteroscopy findings (n = 84)		
	Normal	15	17.8
	Pale endometrium	54	64.2
	Grade I-II adhesions	14	16.6
	Grade III adhesions	1	1.1

ESR (32.10 ± 12.28), mean random blood sugar (114.77 ± 15.0 mg/dL), abnormal Chest X ray showing mediastinal lymphadenopathy (6%). Ultrasound was normal in 82% patients and showed adnexal masses in 15% women and thin endometrium in 3% women. Hysterosalpingography was not taken in CRS but was done in 84 women. It was normal in 36 (42.8%) women, while others had either unilateral free spill (17.8%), bilateral no spill (28.5%), beaded tubes (9.5%), extravasation of dye (5.9%), intravasation of dye (1.1%) and hydrosalpinx(13.0%). Polymerase chain reaction on endometrial biopsy was done in all the screened women and was positive in 376 (66.4%) women and negative in 191 (33.8%). It was not included in CRS. In the 100 women found positive for FGTB on CRS, PCR was positive in 65% women and had a sensitivity of 69.15% to detect FGTB but a specificity of only 34.18%. Hysteroscopy was done in 84 patients (who were negative for AFB or epithelioid granuloma). It was not included in CRS. Normal findings were seen in 15 (17.8%) women, pale endometrium was seen in 54 (64.2%) cases, grade I or grade II adhesions in 14 (16.6%) cases and grade III adhesions were seen in 1 (1.1%) case.

Table 3 shows the results of components of composite reference standard (CRS) used by us in the study. Acid-fast bacilli on microscopy or culture on endometrial aspirate or

Table 3 – Composite reference standard (CRS) parameters in the study (n = 100).

S.No.	Component of CRS	No.	Percentage (%)	Sensitivity (%)	Specificity (%)
1.	AFB microscopy or culture	3	3	3	100 (CI 21–38.5)
2.	Gene Xpert (done in only last 50 cases)	14	14	28	100 (CI- 99.1–100)
3.	Histopathological evidence of epithelioid granuloma	13	13	13	100
4.	Definite findings of FGTB on laparoscopy	43	51.19	–	–
5.	Probable findings of FGTB on laparoscopy	41	48.8	–	–

Table 4 – Laparoscopic findings in FGTB patients.

Laparoscopy Findings	No. of Subjects	Percentage (%)
Definite findings		
Tubercles	22	26.1
Beaded Tubes	11	13.0
Caseous Nodules	10	11.9
Probable findings		
Pelvic Adhesions	65	77.3
Fitz Hugh Curtis Syndrome	31	36.9
Bilateral tubal block	24	28.5
Unilateral tubal block	15	17.8
Hydrosalpinx	12	14.2
Tubo-ovarian Mass	19	22.6

biopsy was seen in only 3 (3%) cases. Gene Xpert was positive in 14 out of 50 (28%) cases with a sensitivity of 28% and specificity of 100%. Histopathological evidence of epithelioid granuloma on endometrial aspirate or biopsy was positive in 13% cases (Sensitivity 13%, specificity 100%).

Out of 84 cases where laparoscopy was done, definite findings of FGTB were seen in 43 (51.19%) cases with sensitivity of 51.19% while probable findings of FGTB were seen in 41 (48.8%) cases with sensitivity of 48.8%. On combination of definite and probable findings of FGTB on laparoscopy, the sensitivity of laparoscopy was almost 100% in detecting FGTB.

Table 4 shows various laparoscopic findings in the study patients. The definite findings seen were tubercles (Fig. 1) in 22 (26.1%) cases, beaded tubes in 11 (13%) cases, caseous nodules in 10 (11.9%) cases. Probable findings of FGTB were pelvic adhesions in 65 (77.3%) cases, perihepatic adhesions (Fitz Hugh Curtis Syndrome) in 31 (36.9%) cases (Fig. 2), hydrosalpinx in 12 (14.2%) cases, unilateral tubal block in 15 (17.8%) cases and bilateral tubal block in 24 (28.5%) cases. All patients found to have FGTB on CRS were started anti-tubercular therapy using Directly Observed Treatment Short Course (DOTS) under National Tuberculosis Elimination Program of India (NTEP) for 6 months.

Hence, composite reference standard (CRS) is a reliable method of diagnosis of FGTB.

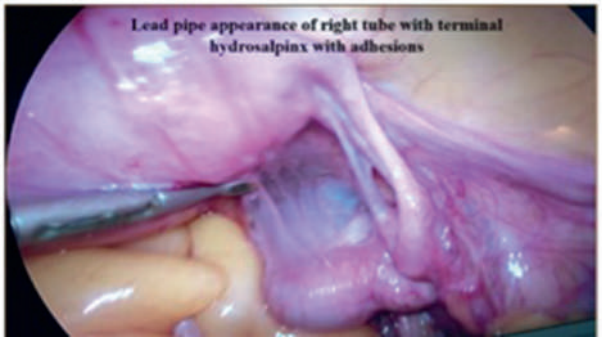

Fig. 2 – Laparoscopic pictures showing definite and probable findings (pelvic adhesions, lead pipe appearance with terminal hydrosalpinx) in FGTB.

4. Discussion

Female genital tuberculosis is an important type of EPTB causing significant morbidity especially infertility especially in developing countries like India.[3,4,6,10,13–16] It causes infertility through effect on fallopian tubes (tuberculous exosalpingitis, TB endo-salpingitis, interstitial TB salpingitis and salpingitis isthmica nodosa), endometrium (causing thin endometrium, adhesions and Asherman's syndrome) and ovaries (decreased ovarian reserve and poor quality of ovum).[17–19] The damage of genital organs is more in advanced cases.[3,4] If diagnosis and treatment are done in early stage before permanent damage to tubes, endometrium and ovaries, prognosis for fertility can be improved.[3,4] Hence timely detection and early treatment with antitubercular treatment can improve pregnancy outcome in these cases. Though, the gold standard in diagnosis of FGTB remains microbiological evidence of *Mycobacterium tuberculosis* on microscopy or culture of endometrial biopsy or aspirate or peritoneal biopsy, they are positive in few cases only and thus missing the diagnosis in many women.[3,4,20,21] Agrawal et al

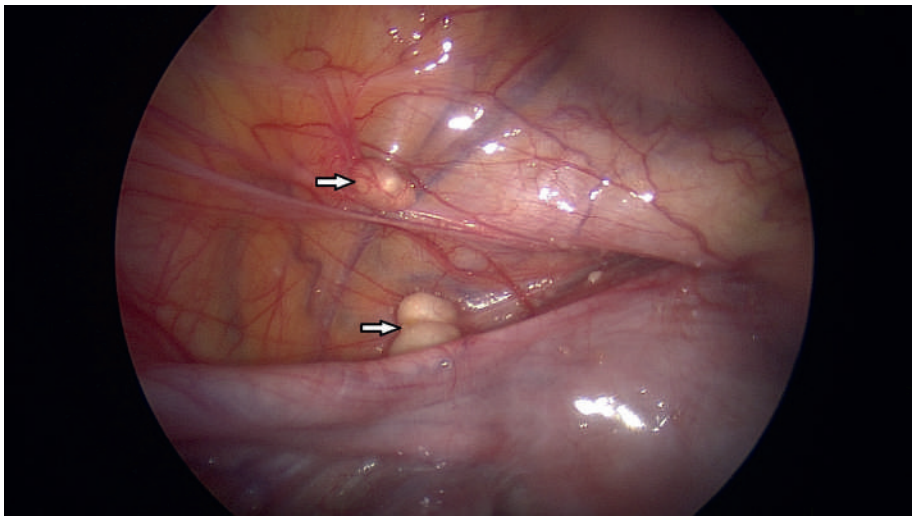

Fig. 1 – Laparoscopy showing definite findings of FGTB in the form of caseous nodules (arrow).

observed 18 positive samples out of 438 samples of endometrial aspirate in infertile patients with TB-PCR positivity of 3.6% as compared to 1.59% of culture.[21] Histopathological evidence of epithelioid granuloma on endometrial biopsy can also be used as gold standard in diagnosis of FGTB but is again positive in very few cases.[3,4,22,23] Cartridge based nucleic acid amplification tests (CB-NAAT) also called as gene Xpert has been used successfully for diagnosis of both pulmonary and extra pulmonary TB including FGTB and has been endorsed by World Health Organization for use.[1,3,4,6,7,24–26] However, although specificity of gene Xpert is very high, its sensitivity is low (about 30–35% in FGTB) and hence it may also miss cases of FGTB.[7]

Radiological imaging technologies like ultrasound, computed tomography (CT Scan), magnetic resonance imaging (MRI) and positron emission tomography (PET-CT) are of use mainly in tubo-ovarian masses but may not be able to differentiate between tuberculosis and malignancy.[27–29] Ultrasound, CT and MRI can also be used to diagnose concomitant FGTB in pulmonary TB or EPTB patients presenting with infertility to see infection of genital organs.[29] However, diagnosis of FGTB cannot be definitely made by radiological methods, they can mostly guide us to do microbiological testing or to perform laparoscopy to confirm the diagnosis of FGTB. Endoscopy (laparoscopy and hysteroscopy) have been used in diagnosis of FGTB.[9,17,30–32] Hysteroscopy can show pale endometrium, tubercles, caseous nodules and varying grades of adhesions in FGTB.[3,4,17] Diagnostic laparoscopy is more reliable in diagnosing FGTB as we can directly visualize whole of pelvic and abdominal cavity and its organs, it can be used in infertility, chronic pelvic pain, tubo-ovarian masses (on clinical or radiological examination) and on clinical suspicion of FGTB in patients with positive contact history or past history of pulmonary or EPTB with positive PCR or to differentiate between tuberculosis and ovarian malignancy.[3,4,9,30–32] On laparoscopy, there can be definite findings of FGTB on laparoscopy like tubercles, caseous nodules and beaded tubes or probable findings of FGTB like straw colored fluid in POD; extensive dense pelvic, peri-tubal, peri-ovarian adhesions; hydrosalpinx; tubo-ovarian mass; thick fibrosed tubes; mid tubal block; peri hepatic adhesions (Fitz Hugh Curtis Syndrome); hyperemia of tubes or blue uterus on chromotubation.[3,4,9] Other findings in FGTB include Sharma's hanging gall bladder sign, Sharma's blue python sign, Sharma's parachute sign, Sharma's ascending colonic adhesion sign, Sharma's kissing fallopian tubes sign.[33–37]

As, no single test is diagnostic of FGTB due to its paucibacillary nature, a combination of various reliable tests can be done to make a composite reference standard for higher pick up of TB in its early stages for timely detection and treatment. It has been used in pleural TB and other EPTB including FGTB.[11,12]

In the present study, CRS was used and out of 569 screened patients of infertility, FGTB could be diagnosed on CRS in 100 cases while individually AFB on microscopy or culture was positive in only 3% cases, epithelioid granulomas in 13% cases, gene Xpert in 28% cases (done in only last 50 cases), but addition of definite findings of FGTB on laparoscopy could detect 57.19% more cases and addition of probable findings of FGTB on laparoscopy could detect another 48.8% cases. Hence, out of all components of CRS, laparoscopy picked up highest number of cases in comparison to AFB, histopathology and gene Xpert. PCR was positive in 65% cases but was not included in CRS due to its high false positivity, but was used to select patients for laparoscopy. Diagnostic laparoscopy and hysteroscopy were not done in culture or histopathology positive cases to avoid flare up of disease and also because they themselves are gold standard for diagnosis of FGTB. Gene Xpert can even detect rifampicin resistance also, as MDR FGTB can occur.[38] All patients of FGTB were treated with 6 months of anti-tubercular therapy.

5. Conclusion

To conclude composite reference standard by combining AFB microscopy, histopathology, gene Xpert and definite and probable findings of FGTB, can diagnose maximum number of FGTB cases. However, large multi-centric studies are recommended before its routine use in clinical practice.

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Conflicts of interest

The authors have none to declare.

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Original article

Knowledge about tuberculosis among tribal population in Kerala in the backdrop of TB elimination goal by 2025

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ABSTRACT

Introduction: Kerala is one among the States in India with least prevalence of tuberculosis and is reportedly aiming to be the first State to reach the target of 'Zero TB' by 2025. But knowledge about TB among the vulnerable groups plays a critical role in controlling the spread and achieving the target of eliminating TB.

Materials and methods: Drawing on a collaborative research program in India to estimate the burden of TB among tribal population, the level of knowledge and its possible links between life style of tribals, their customs and practices is examined Multi stage cluster sampling technique was adopted and 3 wards were selected in three districts in Kerala: Wayanad, Idukki and Palakkad which encompasses major share of the tribal population by probability proportional to size sampling method to draw a sample of 2600 individuals.

Results: Awareness about TB among Tribal population in Kerala is impressive. However, in-depth knowledge on how TB is caused and spread, the symptoms, place of treatment and the cost are not so appreciable. Misconceptions and also lack of knowledge still prevail on who is prone to TB, how TB is spread and the causative agent. The IEC activities have had its effect in sensitizing the tribal population on how to identify the symptoms of TB. The average knowledge score was 5.06 points (72.2 percent, SD: 1.81) out of a total possible score of 7 points. The individual mean knowledge score is 0.65 overall considering all the knowledge domains where the maximum value is 1 and minimum is 0. The mean knowledge score among the Malayarayan Christians and Hindus is relatively higher but poor among Kattunayaka and Irular tribes. Mean knowledge score decreases significantly with increasing age. Gender differential in mean knowledge score is absent but greater educational attainment is associated with higher knowledge scores. However knowledge is not translated to practice of all preventive aspects of TB.

Conclusion: Knowledge deficit poses challenges in the efforts to eliminate TB in Kerala because the State is progressing towards zero TB target. Hence spreading awareness on these vital aspects need better focus among the tribal population.

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1. Introduction

Tuberculosis has long been considered as a public health problem in India due to higher incidence of TB at 177 per 100,000 (notification rate). Inter state variation is evident with half of the TB cases being reported from 5 States of Uttar Pradesh (20 percent), Maharashtra (9 percent), Madhya Pradesh (8 percent), Rajasthan (7 percent) and Bihar (7 percent) whereas in Kerala, TB incidence notified is estimated to be 1.1 percent which is 75 cases per 100,000 population.[1] In India, there has been a step by step progress in implementation of programmes to eliminate TB ever since the National TB programme was launched by Government of India in 1962. Recent initiative of implementing the web enabled patient management system for TB control NI-KSHAY-(Ni = End, Kshay = TB), under the RNTCP had been a major step which applied to the private sector as well as the public sector. Of late the RNTCP has been renamed as National Tuberculosis Elimination Program (NTEP) in 2020 aiming at elimination TB by 2025.

Kerala has been successful among the other States in reducing TB incidence. The number of notified TB cases in Kerala is 2.56 lakh with cases being double among men than women as per the India TB Report 2020. About 64 percent of the notified cases are Pulmonary TB. District wise variations are evident from the India TB Report 2019 wherein Thrissur, Thiruvananthapuram, Kozhikode, Ernakulam and Malappuram districts shows have higher burden while Idukki, Wayanad and Kasaragod have lesser burden. Kerala employed a humanistic approach in controlling the diseases integrating the RNTCP programme with the State's health system. Using conventional techniques, the vulnerability mapping and surveillance has been completed and the treatment of latent TB cases is in progress following the revised RNTCP guidelines. Moving a step forward quite early, in 2019, the Government joined hands with private hospitals to treat and manage latent TB. This comes as an aftermath of annual 4 percent decline in TB incidence in the State following the joint initiative of Government and Public sector in controlling TB.[2] Apart from implementing the national level programmes as per guidelines the State has laid specific focus on diabetes-TB comorbidity due to the higher prevalence of diabetes.

TB among tribal population is high owing to their social and geographic vulnerability and it has been a challenging task ensuring adherence to TB treatment owing to the sociocultural and environmental factors.[3] Since the life of tribals are closely linked to their environment, habitat and sociocultural and religious beliefs and customs, implementation of prevention and control activities in this regard was also specific in Kerala. Tribal population is concentrated in three districts Idukki, Wayanad and Palakkad. The close proximity of the tribal population groups in these districts have brought about tremendous changes in lifestyle. New schemes and programmes have tended to change their life style. A shift from agriculture and involvement of tribes in work provided under the Mahatma Gandhi National Rural Employment Guarantee Scheme (MGNREGS) is a noted feature. The literacy levels have also laid its mark in the tribal population with a substantial proportion literate. Educated unemployment is now a noted feature among tribal population which is usually a phenomenon noted among the general population. These developments have also influenced the acceptance of the TB elimination programmes. Little is known about these various aspects among the tribals. Here we focus on the knowledge of TB among tribal population in the three tribal dominated districts of Kerala, their life style and practices that influence the their knowledge of the disease.

1.1. Objectives

The primary objective of the study is to understanding the knowledge about TB among the tribal population in Kerala.

The secondary objective is to understand the determinants of levels of knowledge and possibly explain how the living conditions of the tribes, their customs and practices are likely to influence knowledge of TB among the tribes.

2. Materials and methods

2.1. Data

Data pertain to the collaborative research project "Estimate the Burden of TB Among the Tribal Population and Develop an Innovative Health System Model to Strengthen TB Control" conducted by Indian Council of Medical Research (ICMR) New Delhi with the technical support from National Institute for Research in Tuberculosis (NIRT) Chennai. The project was implemented in Kerala by the School of Public Health, SRM University, Kancheepuram and carried out by the Population Research Centre, Kerala in 2018. The study involved qualitative assessment of the tribes, quantitative data collection and also intervention activities that were made in spreading knowledge on prevention and control of TB. The present study uses he data so collected in Kerala.

2.2. Sampling technique

The study population was drawn using multi stage cluster sampling technique. The three tribal dominated districts in Kerala: Wayanad, Palakkad and Idukki were included in the ICMR supported study. Villages with more than 50 percent tribal population were selected at the second stage which lead to the selection of Noolpuzha in Wayanad, Sholayur in Palakkad and Arakkulam in Idukki respectively. Since the basic unit is a 'ward' in Kerala one ward each from the three areas with greater representation of tribal population selected were: Muthanga in Noolpuzha, Chundakulam in Sholayur and Pathipally in Arakkulam. All the households in the study areas were first listed and mapped and the basic household profile were recorded.

2.3. Sample size

The study considered only usual residents and hence included 2609 tribal respondents aged 15 years and above from 952 households from three study areas: 373 households from Noolpuzha (Wayanad), 326 households from Arakkulam (Idukki) and 389 households from Sholayur (Palakkad). Noolpuzha, a large village located in Sulthan Bathery Taluk of Wayanad district, is a tribal dominated one with Schedule

Tribe (ST) constituting 51.6 percent of the population. Muthanga ward (the study area), with 89.6 percent of its population as Tribes is dominated by Kurumas, Urali, Kattunayakka and Paniya tribes. Arakkulam, a village located in Thodupuzha Taluk of Idukki district, has a substantial representation of Malayarayan Hindu, Malayaraya Christian and Urali community. Pathipally ward (the study area) has 86.6 percent of its population as Scheduled Tribes. Malayarayans out class all the other factions in socio-economic and educational aspects. Sholayur (the study area), village located in Mannarkad Taluk of Palakkad district, is a tribal dominated district with Schedule Tribes (ST) constituting 52.17 percent of the population. Irular community dominates the area selected.

2.4. *Methods*

Univariate, bivariate and multivariate analysis derives the details of the study population, their demographic, socio-economic characteristics, the knowledge about tuberculosis and the association with habits and negative health behaviours. Descriptive statistics was performed to summarize the data. Thereafter 'Mean knowledge score' is estimated by considering 7 domains of knowledge which were knowledge on cure, transmission, cause, symptoms, place of diagnosis, group at risk and cost of treatment. So the total unweighted score varies between 0 and 7 when the respondent's correct answer to questions on knowledge is assigned a value of 1 and wrong answer or 'don't know' response is assigned a value of '0'. A single summary score for each topic was estimated and averaged by the number of variables. Mean score for each individual was correlated to the factors that influence the knowledge of respondent. Respondents were asked about the prevention strategies they usually adopted in getting TB infection. Their response to this practice of preventing TB is linked with knowledge about TB to understand the importance of having good knowledge about the various aspects of TB. Univariate and multivariate regression analysis is employed to derive the association between knowledge about TB and the socio-demographic variables. The dependent variable id 'knowledge about TB' classified as 'poor' and 'good' knowledge. Age converted to a dichotomous variable based on mean age of the population, sex, education (in years of schooling) and occupation (, no job, skilled and unskilled job), district of origin, prevalence of symptoms of TB and substance use ware introduced in the model as independent variables. The findings from qualitative assessment through focus group discussions are used to substantiate the quantitative assessment findings.

2.5. *Ethical approval*

Ethical approval for carrying out the Project "Estimate the Burden of TB among the Tribal Population and Develop an Innovative Health System Model to Strengthen TB Control" was obtained from the Ethics Committee, SRM MCH & RC, SRM Institute of Science and Technology, Kattankulathur. All the subjects included in the research study had given informed consent in writing before participation.

3. Results

The district wise distribution of tribal population out of the total sample was 31.1 percent from Palakkad, 38.3 percent from Wayanad and 30.7 percent from Idukki district. The distribution of Tribes in the sample population as depicted in Fig. 1 shows one in three tribes under study to be Irular. Malayarayan (Hindu and Christrian) and the Kuruma tribes have almost equal representation in the study population. The

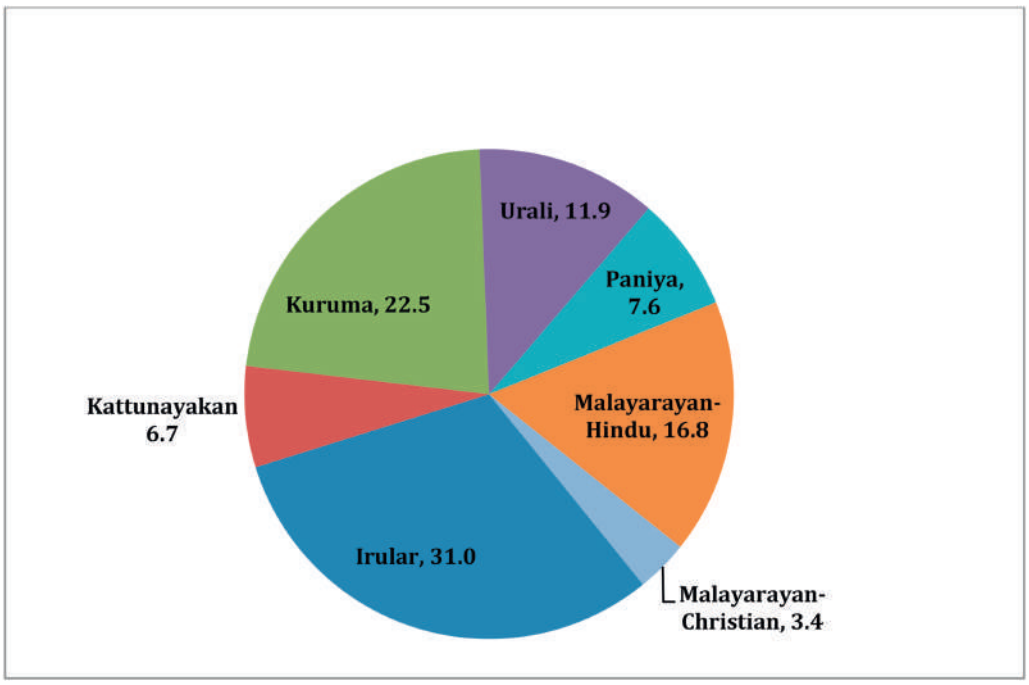

Fig. 1 – Percentage distribution of tribes in the sample population.

Urali tribes constitute about 12 percent and Paniya and Kattunayaka represent less than ten percent of the sample population.

A breakup of age of tribal population under study reveal 12.7 percent to be the elderly and those in the younger cohort of 15–30 years is almost thrice this proportion (35 percent) (Table 1). Almost one in three respondents are 31–45 years and one in five are aged 46–60 years.

The slightly higher representation of elderly when compared to the total elderly population of Kerala (12.6 percent) is partially due to the longer periods of absence of the adult population among the tribal groups who often go for work like collection of forest produce, while the elderly population remain at home.

Females slightly outnumber males in the sample population as we observe in the general Kerala population. The distribution of population by marital status shows nearly 65 percent of the tribal population to be currently married, 23.6 percent unmarried and one in ten to be widowed.

Revamping the educational sector has been the top priority in Kerala and tribal population too receive due focus. There have been remarkable changes in the educational status of the tribal population in Kerala which is the resultant of conscious Governmental efforts for the upliftment of the tribal community and those living in remote areas. Compulsory schooling, special concessions, tribal hostels, reservations granting more intake of students in schools/colleges etc for tribals has had its effect as we find three in five tribals completing 10 years of schooling and one in ten going for higher educational levels. .

One in three tribals are reported to be currently working although engaged in small income generating activities. The non working group includes house wives and students. Again the impact of the various programmes in the state and also the special reservations in occupation extended to the ST population in an attempt to bring them to the main stream is visible in the proportion of the study population holding Government or Private jobs (10.1 percent). Over one in four earn their livelihood by own cultivation and just about 20 percent are engaged in agricultural/Live stock. MGNREA has absorbed 8.8 percent of the population living in the tribal areas and 7.4 percent earn from Construction work. These observations highlight how the life of tribal population shifted drastically.

3.1. *Awareness about TB*

The journey towards Zero TB by 2025 is the country's goal and Kerala is reported to be in the forefront. Tribal population have good awareness about TB. About 92 percent of the population had heard about TB. The source of information is from multiple sources. Friends/neighbours/peers have been the most important source (77.9 percent). The efforts of the State in sensitizing the tribal population are evident as over one in three respondents report the source of information to be Posters/pamphlets/Propaganda. One in four received awareness about TB from Television. Doctors/Health Staff and Village Health Nurse provided information to 21.4 percent of the respondents. Students report hearing about TB from School/College (see Table 2).

Although the basic awareness of tribal population on TB is impressive, in-depth knowledge on how TB is caused and spread, the symptoms, place of treatment and the cost are not so appreciable.

The fact that TB is curable is known to 84 percent of the respondents (Table 3). But 22 percent of the respondents are totally ignorant on who is prone to TB and also half of them do not know how TB is spread. What is more striking is the ignorance on how TB is caused. Knowledge that the causative agent is a microorganism is known to just 29 percent of the respondents. Misconceptions that TB is an inherited disease still prevail. Others feel malnutrition, smoking and alcoholism causes TB although they are risk factors (see Fig. 2).

One in three tribals interviewed do not know how TB is spread. Only 64.6 percent are aware that it is an airborne disease. So spreading awareness on these vital aspects need better focus among the tribals (see Table 3).

Table 1 – Percentage distribution of study population by background characteristics.

	Percentage		Percentage
Age		**Marital Status**	
15–30	35.0 (905)	Currently married	64.9 (1681)
31–45	32.1 (832)	Never Married	23.6 (610)
46–60	20.2 (523)	Divorced/Separated	1.5 (39)
60+	12.7 (329)	Widowed/Widower	10.0 (259)
Gender		**Current Work Status**	
Male	47.9 (1240)	Working	69.3 (1794)
Female	52.1 (1349)	Non Working (includes Housewife, Students)	30.7 (795)
Education [a]		**Occupation**	
Illiterate	19.9 (515)	Govt/Pvt	9.1 (64)
1–9 years	40.9 (1059)	Own cultivation	26.6 (478)
10 years	19.5 (505)	Agriculture/Livestock	19.7 (353)
>10 years	19.7 (510)	Construction work	7.4 (133)
	2589	MGNREA	8.8 (157)
		Forest labour and product	5.2 (93)
		Other	23.2 (416)
			1794

[a] Expressed as no. of years of schooling.

Table 2 – Percentage distribution of tribal population by source of awareness about TB.

Source of Knowledge[a]	Percentage
Doctor/Health Staff	13.2
Village Health Nurse	8.2
Television	26.1
Radio	2.1
Newspaper	14.6
Posters/Pamphlets/Propaganda	36.4
Health Program at Work Place	7.8
Self Help Group Meetings	6.6
Friends/neighbours/peers	77.9
Self/TB History	9.0
School/College	17
Others	3.7

[a] Multiple response.

Table 3 – Percentage distribution of tribal population by knowledge about tuberculosis.

Know that TB is Curable	Percentage	Know the symptoms of TB	Percentage
Yes	83.6	Cough that lasts>2 weeks	87.7
No	1.3	Expectoration	26.6
Don't know	15.1	Chest pain	29.1
Know who is prone to TB[a]		Blood in sputum	17.8
Anybody	65.7	Shortness of breath	10.6
Women/Men/Children	3.8	Loss of appetite	7.5
Smokers	11.2	Fever	15.5
Alcoholics	5.9	Weight loss	36.2
Other	4.4	Night sweats	3.9
Don't Know	22.3	Tiredness	26.6
Know how TB is caused		Others	2.4
Microorganisms	28.8	Don't know	7.7
Hereditary	1.8	**Know where TB is diagnosed**	
Malnutrition	2.1	Govt. health facility	90.3
Smoking/Alcoholism	18.9	Private health facility	14.7
Other	3.7	Other	0.8
Don't Know	50.5	Don't Know	9.1
Know how TB is spread		**Know TB treatment is free**	
Air	64.6	Yes	83.4
Food	7.4	No/Don't Know	16.6
Other	4.9		
Don't Know	33.5		

[a] Multiple responses.

Around 83 percent of the respondents are aware about the free treatment for TB. The tribal population are always in close contact with the primary health care institutions and their health issues are strictly followed up by the grassroot level workers. So majority of the respondents are aware of the place of treatment for TB. However the IEC activities seem to have had it effect in sensitizing the tribal population on how to identify the symptoms of TB. The most common and foremost symptom of TB, 'Cough that lasts more than 2 weeks', is known to about 88 percent of the respondents. One in three tribals associates 'weight loss' along with cough as a symptom of TB. More than a quarter of them are aware that 'tiredness', 'expectoration' and 'chest pain' are symptoms of TB. Symptoms like 'blood in sputum', 'fever' etc are known to less than 20 percent of the tribal population.

Table 4 presents the results of Univariate and multivariate analyses that was employed to understand the level of significance and association of socio-demographic variables on the respondents knowledge about TB.

Age and sex of respondent fails to significantly explain the respondent's knowledge in the multivariate model although both variables showed association in the Univariate analysis. Respondents who have attained formal schooling are significantly at greater odds of having good knowledge about TB (AOR = 4.83 (CI: 2.07–11.26) for <10 years and (CI: 23.31 9.95–54.62) for ≥10 years of schooling)). Respondents who are doing unskilled jobs have better odds of having good knowledge than those without jobs (AOR = 1.68 (CI: 1.27–2.21). Prevalence of symptoms of TB, interpreted here as chest symptomatic respondents, do not show significant association with knowledge. However those who had reported substance use in the form of use of alcohol/drugs/tobacco are at significantly lesser odds of having good knowledge that those who do not report substance use.

The average knowledge score was 5.06 points (72.2 percent, SD: 1.81) out of a total possible score of 7 points. The individual mean knowledge score is 0.65 overall considering all the knowledge domains where the maximum value is 1 and minimum is 0. We find that the mean score varies by districts, type of tribes, age, gender and education.

The study population includes 7 major tribes in the State. The mean knowledge score among the Malayarayan Christians and Hindus is relatively higher (around 0.90). Ethnographic descriptions and anthropological observations speak of the Malayarayan tribes to be more civilized,[4] occupied better positions in social hierarchy among tribes[5] all of which contributes to better understanding of programmes implemented on TB. The Kuruma and the Urali tribes too fare better with mean knowledge scores around 0.70 and 0.80. Paniya tribe of Wayanad district depicts mean knowledge score of around 0.60. Knowledge about TB is poor among Kattunayaka tribes especially with regard to the mode of transmission, cure, cause etc although most of them have heard about TB.

The mean knowledge score is highest among tribals in Idukki district as this district has greater representation of Urali and Malayarayan tribes who are better informed about TB. Wayanad district is dominated by Kurumas, Kattunayakas, Paniya and also Urali and hence the knowledge level is only average as we found Kattunayakas to be having lesser in-depth knowledge about TB. Since Palakkad district is dominated by Irular community and the study sample too represent the same, this district has the least mean knowledge score.

The higher knowledge levels in Idukki is not surprising given the specific focus on TB diagnosis and treatment in Idukki which is all set to being declared as a TB free district. Spreading awareness, vulnerability mapping, active case

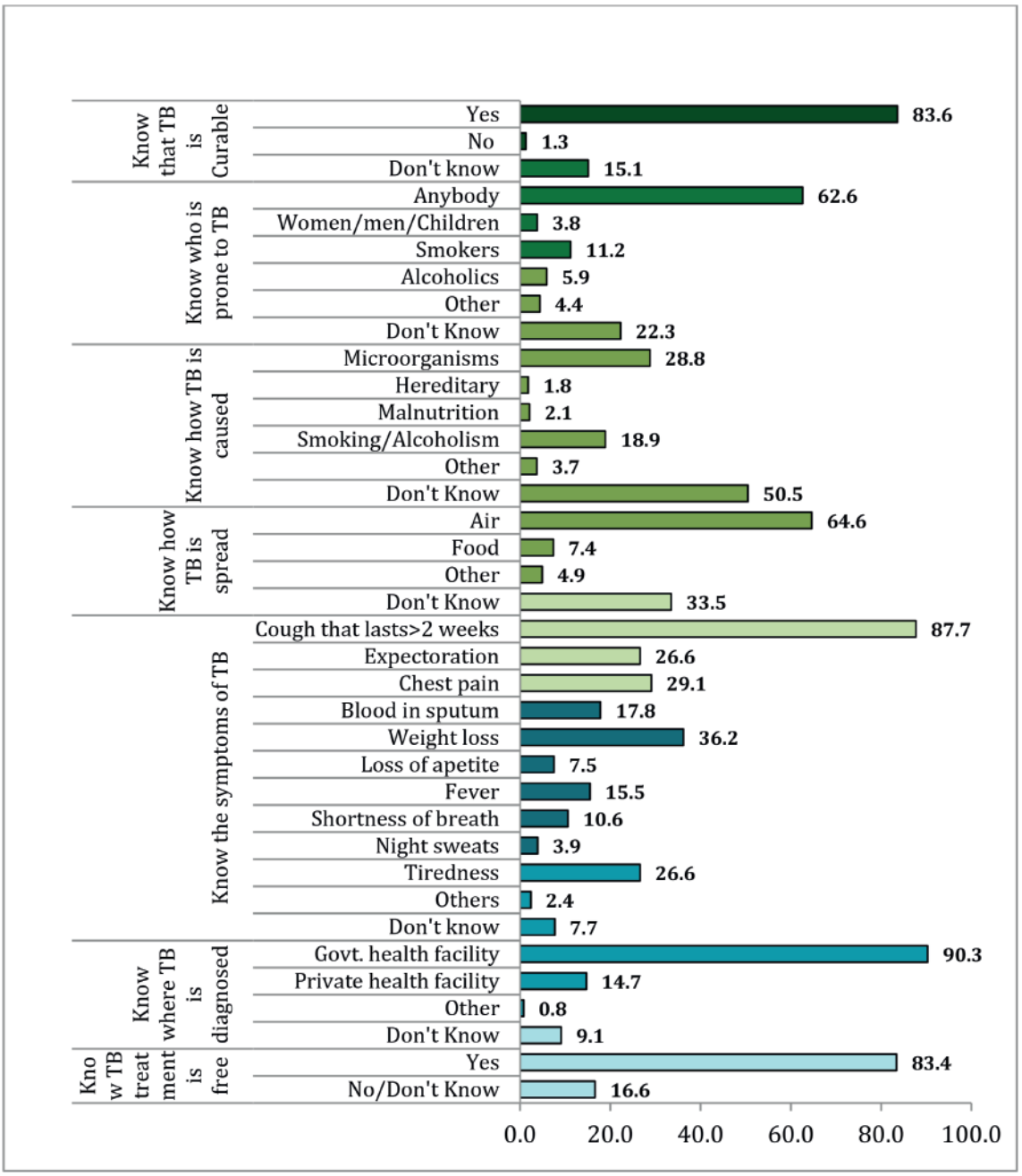

Fig. 2 – Knowledge among tribal population about various aspects of tuberculosis.

finding to regularly monitor, test and treat, financial support to patients to access treatment, door-to door screening by health staff and better diagnostic tools to speed up treatment paved the way to this achievement in Idukki and the other districts are not far behind in the goal.

Mean knowledge score (Fig. 3) differs significantly by age as the younger cohorts are well informed about the various aspects of TB and mean score decreases clearly with increasing age of the population. Gender differential in mean knowledge score is absent. Greater educational attainment levels are associated with higher knowledge scores. In-depth knowledge about TB is poor among illiterates. Respondents who had attended secondary school and above are well informed of the mode of transmission, causes, symptoms and cost of treatment of TB.

Kerala is aiming at achieving the goal of 'zero TB' well ahead of the target year 2025. Through the Nikshay portal of Government of Kerala, every notified TB patient is tracked in real time for adherence to treatment protocol.

3.2. *Ignorance about TB in the context of TB elimination*

Tribal population forms a vulnerable group and knowledge deficit poses challenges in the efforts to eliminate TB and the finding that around 9 percent of the sample population have

Table 4 – Association between socio-demographic variables and knowledge about TB in Univariate and multivariate analysis.

Variable	Univariate			Multivariate		
	OR	95% CI	P value	AOR	95% CI	P value
Age (<40 years)[a]						
≥40 years	0.54	0.44–0.65	<0.000	0.85	0.65–1.10	0.216
Sex (Male)						
Female	1.28	1.06–1.55	0.010	1.26	0.97–1.63	0.08
Education[b] (Illiterate)						
<10 years	0.02	0.01–0.05	<0.001	4.83	2.07–11.26	<0.001
≥10 years	0.17	0.13–0.21	<0.001	23.31	9.95–54.62	<0.001
Occupation (No job)						
Unskilled Job	1.39	1.11–1.75	0.004	1.68	1.27–2.21	<0.001
Skilled Job	0.70	0.49–1.00	0.052	1.33	0.87–2.04	0.193
Chest Symptomatic (No)						
Yes	3.01	1.28–7.03	0.011	1.53	0.59–3.99	0.384
Substance Use (No)						
Yes	0.21	0.16–0.25	<0.001	0.52	0.39–0.68	<0.001
District (Wayanad)						
Idukki	2.64	2.14–3.26	<0.001	2.59	2.01–3.33	<0.001
Palakkad	0.42	0.31–0.56	<0.001	0.52	0.37–0.73	<0.001
N	2354			2354		
-2Log likelihood				2002.66		

Reference categories given in parentheses against each variable.
AOR – Adjusted Odds Ratio.
[a] Mean age of the population is 39.8 years.
[b] Education expressed in years of schooling.

Table 5 – Percentage distribution of study population who do not have knowledge about TB by behaviour aspects.

Behavioural Aspects	Percentage
Ever use of Smokeless Tobacco	83.4 (196)
Ever Smoked (cigarette, beedi etc)	21.7 (51)
Ever used Alcohol	31.1 (73)
Total	**235**

not even heard of TB is a matter of concern in the States progress towards zero TB drive. The lack of in-depth complete knowledge on causes of TB, spread and mode of transmission is also observed even among those who have heard of the disease. So we focus on the group of respondents who have not even heard of TB due to the importance accorded to 'zero TB drive'.

Ignorance about TB is prevalent among all age groups among those who are unaware of the disease. Gender differentials are evident but not substantial. Two in three respondents are illiterate. Among the tribal groups who have not heard of TB, Irular tribes form the major share (71.5 percent) which necessitates specific focus especially in Palakkad district where Irular tribes are concentrated. Substantial share of the Kattunayaka tribes either have very low mean knowledge scores among those who have heard of TB or have not at all heard about the disease.

Smoking, alcoholism, use of drugs have been shown to be significantly associated with tuberculosis in studies done across the country.[6–9] Without proper knowledge about TB the tribals continue to be the victims of such negative behaviours increasing their risk of TB.

Ever use of smokeless tobacco has been found among 83 percent of the tribal group who are ignorant about TB, history of smoking is found among 22 percent and alcohol use was reported by 31 percent (see Table 5). One in five have ever smoked and ever use of alcohol is reported by nearly one-third of the respondents. Overall 88 percent of this ignorant group have atleast one negative behaviour exposing them to the risk of TB. Malnutrition is also found among this tribal group as evident from lower BMI values. One in 10 respondents are under-nourished.

When respondents were asked what they do to prevent themselves from being infected with TB, the positive responses were 'cover the mouth while coughing', 'not spitting in open', 'maintaining personal hygiene', 'keeping surrounding clean' and 'good nutrition'. Those who knew about it practised the preventive aspects. Very few respondents knew that BCG vaccination was to prevent TB. Mean practice score developed varied between 0 and 1. Only few people practised all the prevention aspects. Maximum response was only for 'cover the mouth while coughing'. So the mean practise score was only 0.22 with very few respondents with mean score above 0.50. However knowledge is found to be linked to practices followed in preventing the disease (see Fig. 4).

Though high mean practice scores are associated with high knowledge scores, the mean practice score hovers between values 0.15 and 0.20 among majority which indicates knowledge is not translated to practice of all preventive aspects of TB. Most of them prevent infection by just covering mouth while coughing which places the mean score below 0.2.

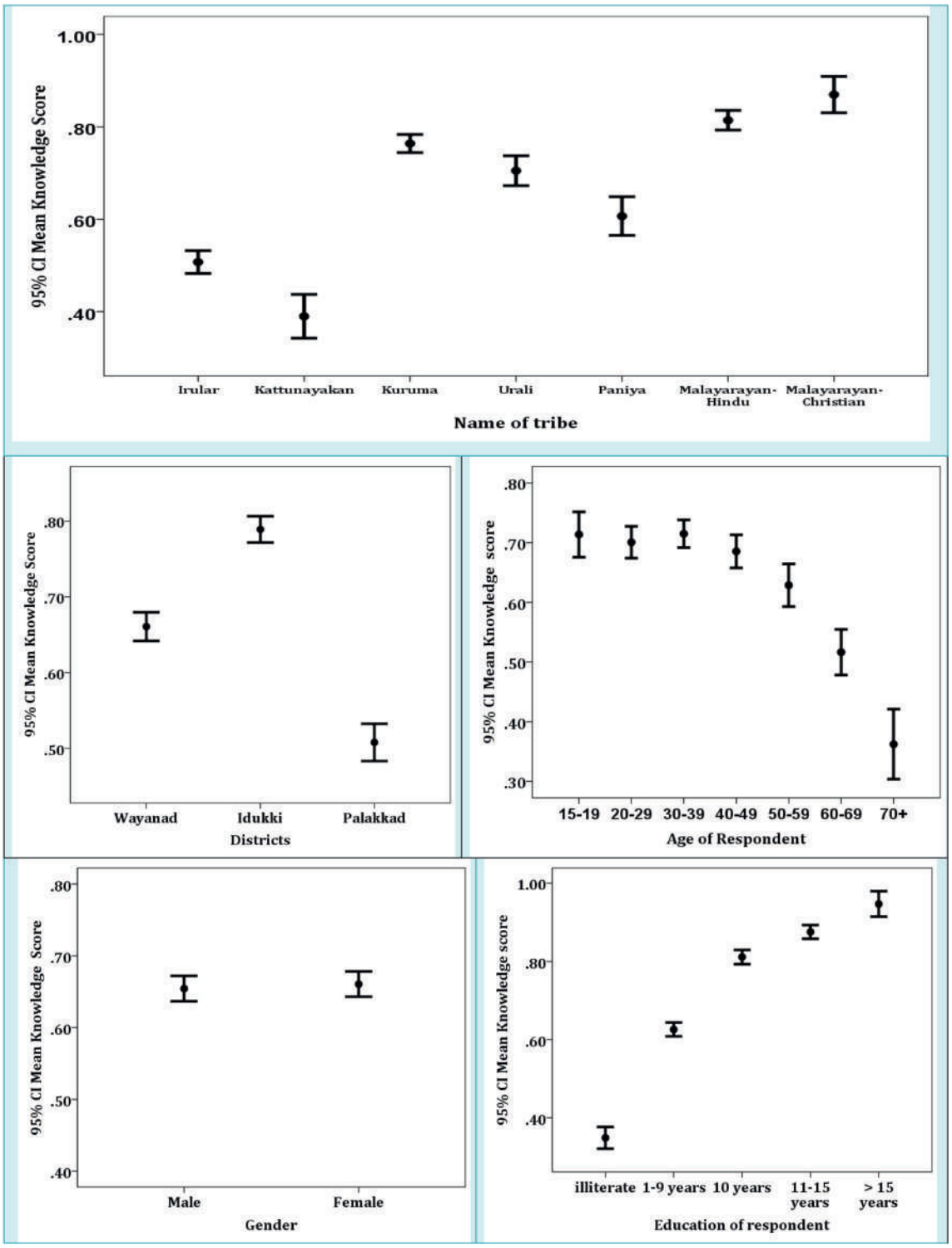

Fig. 3 – Mean knowledge Score against 1. Tribes, 2. Districts, 3.Age, 4. Gender and 5. Education of respondent. Error bars: 95% Confidence Interval.

4. Discussion

An important aspect of inquiry is the question of whether knowledge is linked to the lifestyle of the tribal population. Qualitative assessment through focus group discussions and key informant interviews in the study portrayed the life and traditions of the tribes in the three districts under study which substantiates the findings on level of knowledge about TB. Social exclusion due to rigid customs, isolated settlements in hilly interior parts of the districts continue to hinder programmes aiming at development of tribes.[10] Among the tribals under

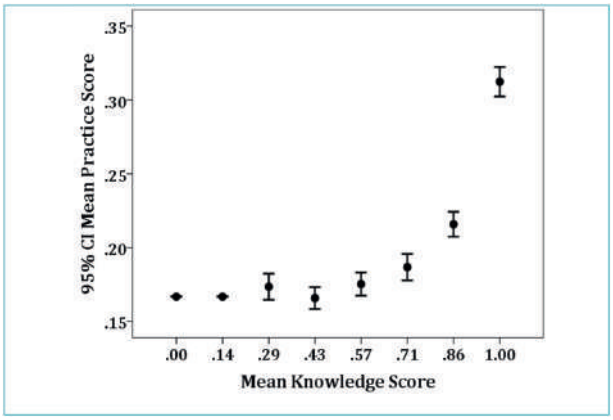

Fig. 4 – Mean knowledge score against mean practise score. Error Bars: 95% Confidence Interval.

study, traditionally Kurumas were engaged in hunting. But now they are engaged in animal husbandry, agriculture etc. and they move out of their place and take up MGNREGA jobs. Many of them pursue Government jobs. Urali tribes, concentrated in Idukki district reside in the woods, showed little interest in hunting, engages in cultivation of different crops and are not strangers to the towns and its developments. Paniya community is engaged as coolies or manual workers. Majority of them are servants in well to do families and staying with those families. Malayarayan tribes are hill tribes who are more civilized, superior in appearance and self sufficient. Unlike these tribes, Kattunayakas and Irular tribes continue to have rigid customs and practices. Irular tries, concentrated in Palakkad district and one of the larger tribal groups are marginalized tribes, Kattunayakas are primitive tribes and like Irulars, marriages outside the community are not allowed. Most of them are honey dwellers in deep forest and also collect forest products. Usually females do not do any job. So the Kattunayakas and to some extent the Irular tribes exhibit a more or less closed community without much exposure to the outside world which limits opportunities to create awareness. True to these variations in exposure to the outside world, the level of knowledge about TB are higher among Malayarayans, Urali, Kurumas and to a substantial extent the Paniyas but rather poor among Irular and Kattunayakas.

Among those who have not heard about TB the Irular tribes form the largest share, seclusion and rigid customs posing to be the barriers in educating them although the State has no dearth of awareness programmes. The words of a village or hamlet 'head' or 'Moopan' as they are called substantiate their beliefs: "*I believe that TB* (better known as 'Ellurukki Novu' among tribals) *patients do not live longer and can be cured only if our 'God' cures them*". Such beliefs deter them from ideal practices to prevent TB as is evident in the low mean practice scores. Traditional practices like keeping a dead body in a separate place in the hamlet, singing and dancing around it, practice of a young girl spending one month following menarche in a separate hut outside the house, offerings made during burial whatever the person liked the most including even alcohol, fruits and food etc. speak of their customs practised even now. A tribal woman remarked in one of the FGDs: "*We prefer natural herbs and medicines for treatment of any illness and only when it is not gone, we seek care at the hospital*". A young man expressed his wish of going back to old practice of cultivating crops of their choice in their house premises rather than being victims of life style diseases like diabetes and hypertension due to dependence on food supplied through the Pubic Delivery System (PDS) and Community Kitchens (a programme for providing meals within the locality to manage malnourishment). Breaking through such rigid customs and spreading knowledge about TB and transcribing into good practices, treatment of TB and its follow up etc are major challenges atleast among certain tribal communities.

Findings of multivariate analysis show the significant association of education on knowledge levels and hence better focus could be laid on improving detailed awareness on mode of spreading, free treatment and practising methods to avoid spread. So the observed variability in knowledge among different tribal groups is partially due to the geographic location which isolates certain groups and varying customs and traditional beliefs of the different tribal groups. Implementation of TB prevention and control activities would be more effective if the tribal promoters, hamlet ASHAs and educated youth within the tribal community are used for intervention activities to increase awareness on TB. Inspite of these efforts there is lot to be done in creating better knowledge about the various aspects related to TB among certain tribal communities inorder to achieve the goal of zero TB by 2025.

Declaration of consent

The authors certify that they have obtained all appropriate consent wherein the study participants has/have given his/her consent for being interviewed and understand that their names will not be published and due efforts will be made to conceal their identity.

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Conflicts of interest

The authors have none to declare.

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Original article

Tuberculosis burden in India and its control from 1990 to 2019: Evidence from global burden of disease study 2019

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ABSTRACT

Background: Tuberculosis is still a major public health problem in India. This study aims to assess trends in the burden of tuberculosis from 1990 to 2019 for tracking success of tuberculosis control programme in India.

Methods: In this study, the 2019 global burden of disease study data were used to measure the incidence, prevalence, mortality, and disability-adjusted life years lost (DALY)rates of Tuberculosis during 1990–2019 for India and its states. Age and gender-specific rates were also analyzed for India. All rates were age-standardized and 95% uncertainty intervals (UIs) were computed.

Result: Overall incidence, prevalence, death and DALY of TB decreased in India from 1990 to 2019. Tuberculosis morbidity and mortality was higher in males as compared to females. Incidence of TB was low in children up to 14 years of age. Prevalence of TB was higher in females as compared to males till 29 years of age, whereas higher prevalence was reported in males as compared to females in adults aged 30 years and more. Death rate of TB was low in children and young adults up to 29 years of age.

Conclusion: This study shows that overall incidence, prevalence, death and DALY of tuberculosis decreased from 1990 to 2019 in India. The burden of TB was higher among males as compared to females during study period. TB affects all the age groups but deaths were higher in older age groups.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease and one of the leading public health problems in India.[1] A total of 1.4 million people died and 10 million people fell ill from TB in 2019 around the globe.[2] Worldwide, TB is one of the top 10 causes of death.[3] Eight countries account for two thirds of the total TB burden, with India leading the count. India has more than 27%

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of the world's burden of both TB and drug resistant TB.[2] In the year 2019, TB incidence in India was 2.6 million cases, of which 59% were in men, 34% in women and rest in children 0–14 years.[4] Risk of TB is attributable to HIV, diabetes, undernutrition, smoking, harmful use of alcohol, poverty and indoor air pollution.[2] As per a review 7–32% of drug sensitive TB and 68% of drug resistant TB patients in India experience catastrophic costs during the diagnosis and treatment of TB.[5]A loss of nearly $300 million due to lost wages and a $3 billion indirect cost per year to the Indian economy is attributable to TB due to staff absenteeism and lost productivity.[6]

Global public health and TB community is shifting its focus from control of the TB epidemic towards elimination. WHO has set an ambitious goal of ending global TB epidemic by 2030 under the End TB Strategy, which targets to reduce TB deaths by 95% and new cases by 80%, and to ensure that no family is burdened with catastrophic expenses due to TB. As per the strategy, 20% reduction in the global TB Incidence was expected between 2015 and 2020, but only 9% cumulative reduction in TB incidence was seen between 2015 and 2019at the rate of 2% fall per year.[2] India has set an even more ambitious target of ending TB by 2025.[7] TB Incidence in the country has declined nearly 11% from year 2015 (217per 100,000population) to 2019 (193 per 100,000 population).[4]

Nationwide TB prevalence survey was done in 1955–58,[8]after that Government of India launched National TB Control Program in 1962.Till date no other nationwide study was conducted; TB continued to have high burden and wide variation in the prevalence across different parts of the country as per a number of district/sub-district level surveys conducted by various investigators.[9,10]Prevalence of bacteriologically positive pulmonary TB in adults was found to vary from 170.8 (123.5218.2) to 528.4 (433.5, 623.3) per 100,000 populations in a pooled estimate of eight different sites of India during the year 2005–2011. Prevalence was found to be higher in rural areas, among males and increased with age.[11] TB counts are mainly obtained as part of the routine surveillance under the Program, but real picture is not visible as the large numbers of patients seek treatment from the private providers. To tackle this problem notification of all TB cases was made mandatory through Nikshay (National Digital Information System) in the year 2017 despite that 540,000 TB affected persons remain uncaptured by TB surveillance/notification and services.[7] Paucity of nationwide prevalence, incidence studies was a rationale to conduct an analysis for understanding the real scenario.

Though incidence, prevalence, mortality and DALY of tuberculosis decreased over past few decades but it is still a major public health problem in India. This study aims to assess trends in the burden of tuberculosis from 1990 to 2019 for tracking success of tuberculosis control programme in India.

2. Material and methods

The Global Burden of Disease study provides valuable resources for understanding the evolving health problems that people around the world face in the twenty-first century. The Global burden of disease study is one of the most systematic global observational epidemiological studies to date, led by the Institute for Health Metrics and Evaluation (IHME).GBD provides comparable estimates of incidence, prevalence, mortality and DALYs rate for various diseases including TB.

2.1. *Data sources*

GBD 2019 estimated various epidemiological measures such as prevalence, incidence, mortality, disability-adjusted life-years (DALYs), years lived with disability (YLDs) and years of life lost (YLLs)for 23 age groups; both sexes combined as well as separately for males and females. GBD provides these estimates for 21 regions and seven super-regions and also separately for 204 countries.[12] Data sources used to model non-fatal health outcomes for tuberculosis in India includes annual case notifications, prevalence surveys conducted by Government of India under revised national tuberculosis control programme and published scientific articles. The main sources of data used to model the cause of death for Tuberculosis in India was vital statistics, medical certification of cause of death for the country and various states, cause of death studies by verbal autopsy (VA), other surveys on cause of death and published scientific articles.[12]

Cause of Death Ensemble model (CODEm) and spatiotemporal Gaussian process regression were used to calculate Cause-specific death rates and cause fractions. CODEm has been defined in detail elsewhere.[13–16] To measure YLLs, deaths were multiplied by standard life expectancy at each age. To ensure consistency between prevalence, incidence, remission, excess mortality, and cause-specific mortality for several causes a Bayesian meta-regression modelling tool, DisMod-MR 2.1, was used. To measure YLDs, prevalence estimates were multiplied by disability weights for mutually exclusive sequelae of diseases and injuries. The 25th and 975th ordered 1000 draw values of the posterior distribution were used to measure uncertainty intervals (UIs) for each metric.[12]

The case definition includes all forms of TB, including pulmonary TB and extrapulmonary TB, which are bacteriologically confirmed or clinically diagnosed. In this study we considered TB with corresponding ICD 10 codes: A10-A19.9, B90–B90.9, K67.3, K93.0, M49.0 and P37.0.

We have extracted the incidence, prevalence, Death and DALYs rate of TB for 1990 to 2019 from a publicly available online tool provided by the IHME which is called the GHDx (Global Health Data Exchange) query tool (http://ghdx.healthdata.org/gbd-results-tool).[17]We have calculated the percentage change and annualized rate of change in the estimates of tuberculosis for the aforesaid period.

3. Results

There is decrease in the incidence of TB in all the age groups in both the sexes. Over all the incidence of TB changed from 320.13 (95% UI, 262.21–387.69) in 1990 to 218.83 (95% UI,186.24–256.62) in 2019 per 100,000 persons i.e. TB incidence fell by −31.64% from 1990 to 2019. The incidence of TB in females (−40.02%) fell more vis-a-vis males (−23.15%). Overall, the age-standardized incidence fell by −42.85% from 1990 to 2019, it fell more in females (−47.74%) as compared to male (−37.81%). In both the sexes the maximum decrease in the incidence of TB were reported in Under 5 years followed by age

group of 5–14 Years i.e. −66.89% and −66.2% respectively, In males −65.17% and −60.46% respectively, whereas In females maximum decrease in the incidence of TB were reported in the age group of 5–14 Years followed by Under 5 years i.e. −68.92% and −67.96% respectively from 1990 to 2019. The minimum decrease in the incidence of TB was reported in the age group of 15–49 Years in both sexes and in males i.e. −37.67% and −28.01% respectively from 1990 to 2019 whereas in female's the minimum decrease in the incidence of TB was reported in the age group of 50–69 Years i.e. −40.63%. There is increase in the incidence of TB in the age group of 5–14 Years in both the sexes as well as in females, whereas in males increased incidence is reported in the age group of all ages from 2015 to 2019. In males increased incidence of TB is reported in the age group of 15–49 Years and all ages from 1995 to 2000, age standardized incidence also increased during same period in males. In females increased incidence of TB is reported in the age group of 15–49 Years and all ages from 1995 to 2000 & in the age group of 50–69 Years from 2010 to 2015 [Table 1].

There is decrease in the prevalence of TB in all the age groups in both the sexes from 1990 to 2019. Overall, the prevalence of TB changed from 564.24 in 1990 to 374.22 in 2019 per 100,000 persons i.e., TB prevalence fell by −33.68% from 1990 to 2019, whereas the age-standardized prevalence fell by −44.91% during same period. The age-standardized prevalence fell more in females (−51.15%) vis-à-vis males (−38.17%). In both the sexes the maximum decrease in the prevalence of TB were reported in the age group of 5–14 Years followed by Under 5 years i.e. −66.07% and 64.29% respectively, In males −63.43% and −62.63% respectively, whereas in females −67.04% and −65.18% respectively from 1990 to 2019. The minimum decrease in the prevalence of TB was reported in the age group of 15–49 Years in both sexes and males i.e., −39.07% and −27.39% respectively from 1990 to 2019, however, in females the minimum decrease in the prevalence of TB was reported in the age group of 50–69 Years i.e., −44.74%. There is increase in the prevalence of TB in the age group of 5–14 Years from 2015 to 2019 in both sexes, in males as well as in females [Table 2].

There is decrease in the mortality due to TB in all the age groups in both the sexes. Over all the mortality of TB changed from 71.76 (63.53–79.70) in 1990 to 30.39 (25.77–35.86) in 2019 per 100,000 persons i.e., TB mortality fell by −57.65% from 1990 to 2019. The maximum decrease in the mortality of TB was reported in the age group of Under 5 years in both the sexes, males and females i.e., −88.27%, −88.13% and −89.47% respectively from 1990 to 2019. The minimum decrease in the mortality of TB was reported in the age group of 15–49 Years in both sexes and in males i.e., −65.64% and −61.09% respectively, whereas in females the minimum decrease in the mortality of TB was reported in the age group of 50–69 Years i.e. −69.28% from 1990 to 2019. In males increase in the mortality due to TB is reported in the age group of 5–14 Years from 1995 to 2000 [Table 3].

There is decrease in the DALY of TB in all the age groups in both the sexes. Over all the DALY of TB changed from 3105.43 (2780.10–3440.63) in 1990 to 1125.42 (971.85–1307.52) in 2019 per 100,000 persons i.e., TB DALY fell by −63.76% from 1990 to 2019. The maximum decrease in the DALY of TB were reported in the age group of Under 5 years in both the sexes, males and females i.e., −88.43%, −87.72% and −88.96% respectively from 1990 to 2019. The minimum decrease in the DALY of TB was reported in the age group of 15–49 Years in both sexes and males i.e., −64.65% and −59.66% respectively, from 1990 to 2019. In females slight increase in the DALY of TB is reported in the age group of 50–69 Years from 2010 to 2015 (Table 4).

Incidence rate of TB was higher in males as compared to females from 1990 to 2019; However, prevalence was higher in females from 1990 to 1995, after that prevalence was reported higher in males as compared to females. There is a decrease in the trend of age-standardized death and DALY rates of TB in males as well as in females from 1992 to 2019; however, age-standardized death and DALY rates of TB were always higher in males as compared to females (Fig. 1).

Incidence of TB was low in children up to 14 years of age and more in 15 years and above age group. Incidence of TB was higher in females as compared to males up to 24 years of age, whereas 25 & above years population incidence of TB was higher in males as compared to females. Prevalence of TB was higher in females as compared to males till 29 years of age, whereas higher prevalence was reported in males as compared to females in adults aged 30 years and more. Death rate of TB was low in children and young adults up to 29 years of age & higher in adults aged 30 years and more. Death rate was higher in males as compared to females in all the age groups except in infants (Fig. 2).

Highest incidence of TB was reported in Northern States of India i.e., Rajasthan, Gujarat and Uttar Pradesh & lowest incidence was reported from Kerala; Highest Prevalence was reported from Rajasthan, Uttar Pradesh, Madhya Pradesh Assam and Gujrat & lowest were reported from Goa, Kerala and Tripura. Highest death rate was reported in Rajasthan, Gujarat, Uttar Pradesh, Chhattisgarh, Assam and Meghalaya & lowest in Kerala. DALYs was highest in Uttar Pradesh and high in Rajasthan, Gujarat, Chhattisgarh, Assam and Uttarakhand and lowest in Kerala (Fig. 3).

4. Discussion

India has made a remarkable progress to reach Millennium Development Goals (MDGs) and started its journey towards Sustainable Development Goals (SGDs) in relation to TB as per our study and same has been confirmed by few other different studies.[18] Our study showed that incidence, prevalence, mortality and DALY of TB all decreased from 1990 to 2019 in all the age groups and among both sexes in India. Overall decrease in incidence, prevalence, mortality and DALY was found to be 31.64%, 33.68%, 57.65% and 63.76% respectively. Although, the reduction in TB deaths in India during 2015–2019 has been very less (4%) as compared to other SEAR countries.[19] Annual surveys conducted by Tuberculosis Research Centre, Chennai in Thiruvellur district of Tamil Nadu between 1999 and 2005 showed a decline in prevalence of TB at the rate of about 12% per year,[20] However, our study showed 7.3% decline in prevalence during the same period. Decline in the TB incidence, prevalence, and mortality among under five and 5–15years was noticeable in our findings which

Table 1 – Incidence of TB by age-sex from 1990 to 2019 in India (per 100,000 persons, 95% UI).

Age Sex	1990	1995	2000	2005	2010	2015	2019	% Δ (1990–2019)
Both Sexes								
Under 5	85.84 (60.10–116.32)	75.73 (52.39–102.60)	64.68 (45.99–86.39)	57.87 (41.67–76.84)	44.02 (31.77–58.16)	33.83 (24.22–45.05)	28.42 (20.55–37.77)	−66.89
5–14 Years	104.64 (70.68–142.57)	89.82 (60.03–124.33)	83.52 (56.79–115.37)	71.97 (48.63–99.41)	50.81 (34.44–70.65)	34.90 (23.93–49.11)	35.37 (24.50–48.79)	−66.2
15–49 Years	403.48 (302.55–517.83)	362.26 (276.97–462.63)	379.08 (290.18–481.32)	346.73 (269.35–436.55)	313.49 (244.73–388.97)	254.66 (202.34–313.47)	251.47 (201.72–309.77)	−37.67
50–69 Years	672.15 (466.73–920.25)	598.52 (428.31–812.24)	576.15 (411.42–778.18)	510.83 (370.23–682.72)	443.95 (322.51–587.62)	432.94 (319.13–566.40)	396.80 (290.33–522.83)	−40.97
70+ Years	783.45 (512.29–1104.81)	685.94 (456.01–939.20)	664.39 (456.03–902.45)	579.63 (404.78–770.59)	456.60 (326.55–600.36)	421.13 (312.37–551.15)	390.62 (289.83–508.95)	−50.14
All ages	320.13 (262.21–387.69)	289.87 (239.04–349.20)	299.30 (247.54–358.56)	277.78 (231.01–330.61)	250.13 (208.79–296.93)	220.21 (186.28–258.93)	218.83 (186.24–256.62)	−31.64
Age Standardized	390.22 (323.28–467.69)	346.33 (288.29–412.17)	347.24 (291.09–412.43)	313.10 (264.45–368.29)	271.88 (228.82–318.06)	234.16 (199.73–272.64)	223.01 (191.37–259.72)	−42.85
Male								
Under 5	66.70 (46.23–89.73)	59.31 (41.00–81.12)	49.38 (34.86–65.49)	43.60 (30.90–58.38)	33.70 (23.97–44.99)	26.79 (19.12–35.94)	23.23 (16.86–30.65)	−65.17
5–14 Years	65.22 (45.18–88.62)	55.71 (37.95–78.08)	53.76 (36.88–74.40)	48.61 (32.61–67.74)	39.56 (26.37–55.27)	26.30 (17.74–37.13)	25.79 (17.85–35.97)	−60.46
15–49 Years	373.50 (283.78–473.50)	349.10 (267.52–441.02)	376.38 (290.57–473.81)	362.10 (282.73–454.11)	329.51 (258.40–407.65)	269.37 (214.86–331.25)	268.90 (214.77–329.66)	−28.01
50–69 Years	793.14 (551.68–1078.51)	713.15 (511.36–961.29)	700.19 (506.86–940.07)	623.81 (451.77–829.33)	548.20 (401.26–722.89)	510.17 (379.37–669.52)	475.83 (349.58–623.75)	−40.01
70+ Years	887.01 (578.83–1255.64)	784.36 (523.90–1068.15)	770.14 (521.84–1048.05)	676.05 (477.16–902.99)	538.55 (385.45–714.09)	484.07 (357.26–631.74)	452.71 (333.65–588.72)	−48.96
All ages	308.47 (253.06–372.33)	285.66 (235.13–342.17)	302.02 (250.70–361.41)	291.48 (242.76–345.32)	267.74 (224.11–316.07)	235.42 (198.61–274.74)	237.06 (201.70–278.12)	−23.15
Age Standardized	398.04 (329.37–477.52)	360.70 (300.10–429.14)	370.50 (310.61–439.11)	343.88 (289.22–405.33)	301.70 (255.44–352.70)	257.45 (219.36–300.19)	247.55 (212.26–288.32)	−37.81
Female								
Under 5	106.52 (74.31–144.59)	93.60 (64.24–126.89)	81.40 (58.28–109.16)	73.52 (53.50–97.12)	55.33 (40.02–72.77)	41.56 (29.73–55.24)	34.13 (24.59–45.55)	−67.96
5–14 Years	147.59 (99.06–200.90)	127.86 (85.08–176.27)	116.97 (80.04–160.44)	97.93 (66.55–135.51)	63.19 (42.87–87.70)	44.33 (30.27–62.64)	45.87 (31.48–64.12)	−68.92
15–49 Years	433.98 (320.98–570.21)	376.42 (281.99–489.61)	381.98 (286.38–493.16)	330.34 (251.05–419.95)	296.47 (229.88–374.13)	239.05 (187.93–296.58)	232.97 (184.14–288.74)	−46.32
50–69 Years	536.56 (367.93–741.98)	474.11 (335.25–650.29)	447.64 (317.06–613.01)	395.32 (282.83–532.10)	337.57 (242.17–448.40)	355.61 (260.44–468.64)	318.54 (232.34–423.11)	−40.63
70+ Years	682.13 (445.10–957.80)	588.10 (391.16–809.84)	559.46 (376.91–757.79)	489.86 (337.46–657.98)	385.20 (272.86–511.01)	366.66 (267.19–482.99)	335.96 (249.35–439.69)	−50.75
All ages	332.80 (270.47–405.54)	294.45 (240.63–357.76)	296.36 (243.33–359.92)	263.11 (217.89–313.74)	231.44 (192.12–276.92)	204.15 (172.68–239.72)	199.63 (169.11–234.58)	−40.02
Age Standardized	381.20 (312.50–463.21)	331.11 (274.63–395.66)	323.79 (268.51–384.55)	282.42 (237.81–332.88)	242.43 (203.97–286.34)	211.55 (180.45–245.90)	199.20 (169.84–231.97)	−47.74

TB: Tuberculosis; UI: uncertainty intervals; % Δ: the percent changes of Incidence rate from 1990 to 2019.

Table 2 – Prevalence of TB by age-sex from 1990 to 2019 in India (per 100,000 persons).

Age Sex	1990	1995	2000	2005	2010	2015	2019	% Δ (1990–2019)
Both Sexes								
Under 5	167.37	154.36	128.94	111.64	90.24	69.45	59.77	−64.29
5–14 Years	270.53	246.21	221.94	188.81	141.26	88.69	91.78	−66.07
15–49 Years	642.58	597.95	596.76	472.57	434.86	395.93	391.52	−39.07
50–69 Years	1125.44	1041.84	965.75	808.24	743.12	716.56	652.46	−42.03
70+ Years	2231.70	2061.89	1936.39	1568.50	1303.91	1207.57	1110.69	−50.23
All ages	564.24	531.28	523.00	436.61	401.85	376.60	374.22	−33.68
Age Standardized	707.94	655.19	628.78	512.03	453.70	410.62	390.03	−44.91
Male								
Under 5	122.09	113.05	91.20	78.55	63.42	51.82	45.62	−62.63
5–14 Years	146.14	128.82	118.21	106.12	83.52	50.55	53.44	−63.43
15–49 Years	572.73	549.60	558.05	470.97	446.26	426.64	415.86	−27.39
50–69 Years	1305.52	1227.48	1169.05	1008.75	943.95	878.42	795.91	−39.04
70+ Years	2432.79	2284.10	2199.48	1789.76	1513.68	1386.88	1272.24	−47.7
All ages	514.64	494.41	496.89	436.47	417.50	404.98	399.57	−22.36
Age Standardized	693.67	654.66	641.05	543.40	495.01	457.50	428.92	−38.17
Female								
Under 5	216.27	199.34	170.18	147.91	119.65	88.81	75.31	−65.18
5–14 Years	406.06	377.11	338.56	280.70	204.77	130.49	133.82	−67.04
15–49 Years	718.17	649.96	638.17	474.28	422.73	363.35	365.67	−49.08
50–69 Years	923.62	840.35	755.10	603.24	538.20	554.48	510.42	−44.74
70+ Years	2034.95	1840.96	1675.37	1362.52	1121.11	1052.43	968.48	−52.41
All ages	618.08	571.32	551.19	436.77	385.23	346.65	347.53	−43.77
Age Standardized	722.74	656.53	618.37	482.69	414.61	365.61	353.03	−51.15

TB: Tuberculosis; **% Δ**: the percent changes of Prevalence rate from 1990 to 2019.

could be due to the expansion of BCG vaccination under universal immunization program. While looking at State wide distribution (Fig. 3) Northern States like Rajasthan, Uttar Pradesh and Gujarat, and North eastern states mainly, Arunachal Pradesh, Assam have high TB morbidity and mortality while northern states have larger population and north eastern states lack behind in providing good health care services. Southern States like Karnataka, Kerala have higher prevalence of TB but lower mortality and DALYs which may be due to better health care services in these states. With the mandate of notification by the Private Providers through Nikshay.

India's fight for TB started with the launch of National TB Control Program (NTCP) (1962–1992), which relied mainly on chemoprophylaxis and BCG vaccination. By 1993, it was realized that NTCP failed in reducing TB burden, BCG was used without clear role in TB control and there was no other alternate for primary prevention, prevalence of drug resistance was increasing and HIV pandemic had set in21. This was the time when WHO declared TB as the Global emergency, DOTS was launched and NTCP was redesigned during the same year as Revised National TB Control Program (RNTCP) which was rolled all over India by 2005. Prior to its launch case fatality rate of TB was 25% which declined to 5% in the post RNTCP era as reported under the Program, though WHO estimates of 2019 still reports it to be 17% as Program mentions only the registered cases barring the missing cases.[4,22] In 2007, Programmatic Management of Drug Resistant TB (PMDT) was initiated under RNTCP and made it pan-national in 2013, as the studies reported MDR prevalence to be 0.5–3% among new cases and 12% among retreatment cases.[23]Over the years an increase in the prevalence of drug resistance among new TB patients while decrease among previously treated TB patients has been noted by a recent meta-analysis. A number of factors have been shown to be associated with DR-TB in this analysis like male sex, younger age, history of previous treatment, delay in initiating treatment, one or more treatment regimens being advised, treatment side effects, irrational prescriptions, poorly formulated medications and insufficient dosage and length of treatment, poor knowledge and financial burden are some of them. Lack of affordable access to the reliable testing solutions has remained as the biggest challenge in the control of DR-TB.[24] Also, past few years treatment success rates of MDR TB under our national programme has been found to be low (46%).[25] In the year 2012, India mandated notification of TB cases through Nikshay portal and newer initiatives like Patient Provider Support Agency channelled by a third party agency usually an NGO to rope in the missing TB cases within the Program.[26] As a result of which increase in the incidence and prevalence during the year 2015–2019 was noticed in our study. Also, by adoption of "End TB Strategy" WHO has set an ambitious goal of ending the global TB epidemic by 2030, with targets to reduce TB deaths by 95% and new cases by 80%, and to ensure that no family is burdened with catastrophic expenses due to TB. The global discourse has brought impetus to the program with more resource allocation and increased political commitment, universal drug sensitivity testing by GeneXpert, fixed dose combination therapy for drug sensitive cases and use of newer drugs for drug resistant cases like Bedaquiline and Delamanid, ICT-based adherence support, enhanced monitoring and pharmacovigilance, enhanced surveillance through an updated Nikshay platform it seems country has geared up to detect the missing one, treat the diagnosed.

Table 3 – Mortality of TB by age-sex from 1990 to 2019 in India (per 100,000 persons, 95% UI).

Age Sex	1990	1995	2000	2005	2010	2015	2019	% Δ (1990–2019)
Both Sexes								
Under 5	36.65 (30.51–47.61)	30.70 (24.52–37.85)	21.60 (17.021–26.26)	15.02 (11.79–18.55)	10.64 (8.51–13.17)	7.08 (5.62–8.77)	4.30 (3.22–5.50)	−88.27
5–14 Years	7.67 (5.87–9.68)	6.002473 (4.75–7.32)	5.19 (4.17–6.20)	3.58 (2.88–4.27)	2.21 (1.74–2.69)	1.41 (1.01–1.73)	1.15 (0.87–1.45)	−85.01
15–49 Years	49.44 (44.58–54.56)	42.38 (38.74–46.03)	40.59 (37.49–43.68)	29.91 (27.76–32.28)	24.84 (23.04–26.77)	18.98 (17.22–20.74)	16.99 (14.36–19.92)	−65.64
50–69 Years	263.79 (229.57–297.31)	225.10 (201.89–249.38)	192.63 (175.02–210.15)	135.63 (124.52–147.01)	114.62 (105.42–124.60)	101.93 (91.07–114.41)	83.55 (69.39–100.53)	−68.33
70+ Years	681.07 (575.39–790.90)	583.76 (506.65–670.50)	528.17 (464.39–588.26)	387.89 (346.65–425.41)	273.19 (246.05–299.81)	246.85 (218.77–276.78)	211.92 (178.36–251.89)	−68.88
All ages	71.76 (63.53–79.70)	62.52 (56.66–68.63)	55.772 (53.06–62.36)	44.12 (40.61–47.54)	37.35 (34.72–40.24)	33.66 (30.40–36.81)	30.39 (25.77–35.86)	−57.65
Male								
Under 5	32.85 (25.74–41.28)	26.38 (20.54–33.05)	18.53 (14.47–22.80)	12.60 (9.87–15.72)	8.95 (6.99–11.24)	6.27 (4.85–7.99)	3.90 (2.88–5.04)	−88.13
5–14 Years	5.19 (3.91–6.52)	3.10 (2.99–4.95)	3.69 (2.86–4.45)	2.75 (2.11–3.33)	1.10 (1.54–2.42)	1.27 (0.97–1.56)	0.97 (0.73–1.23)	−81.31
15–49 Years	56.56 (49.96–63.43)	49.41 (44.45–54.56)	48.46 (44.048–52.90)	37.12 (33.82–40.72)	31.58 (28.60–34.72)	24.41 (21.73–27.16)	22.01 (17.66–26.61)	−61.09
50–69 Years	339.66 (285.73–389.56)	294.98 (255.55–332.16)	256.80 (227.82–283.42)	182.18 (162.34–201.89)	158.05 (141.15–176.11)	134.67 (119.18–150.62)	112.47 (88.01–138.63)	−66.89
70+ Years	821.01 (646.11–959.22)	712.62 (586.44–818.26)	653.83 (554.21–731.13)	491.32 (423.76–541.30)	357.45 (313.01–396.52)	322.88 (278.70–361.90)	272.23 (221.28–327.16)	−66.84
All ages	84.02 (72.18–94.24)	74.30 (65.74–82.35)	69.61 (62.26–75.85)	54.15 (49.11–59.24)	47.22 (42.69–51.75)	42.05 (37.37–46.33)	38.27 (30.64–46.05)	−54.45
Female								
Under 5	44.91 (33.10–56.82)	35.40 (26.94–44.60)	24.95 (19.25–31.02)	17.66 (13.70–21.88)	12.50 (9.93–15.88)	7.98 (5.99–10.29)	4.73 (3.35–6.42)	−89.47
5–14 Years	10.38 (7.60–13.63)	8.24 (6.38–10.35)	6.88 (5.34–8.50)	4.51 (3.54–5.51)	2.45 (1.87–3.07)	1.56 (1.15–2.01)	1.34 (0.97–1.74)	−87.09
15–49 Years	41.74 (34.51–48.91)	34.82 (29.62–39.89)	32.17 (28.20–36.20)	22.22 (19.74–24.84)	17.68 (15.52–19.71)	13.22 (10.97–15.22)	11.65 (8.70–15.05)	−72.09
50–69 Years	178.78 (137.02–216.84)	149.25 (120.19–177.46)	126.14 (103.85–146.01)	88.03 (75.79–101.07)	70.29 (60.23–79.16)	69.15 (54.89–85.32)	54.92 (40.82–73.66)	−69.28
70+ Years	544.15 (382.03–716.86)	455.66 (336.48–588.92)	403.50 (311.01–490.58)	291.61 (233.15–345.58)	199.76 (160.13–235.72)	181.07 (144.45–220.16)	158.83 (119.53–213.32)	−70.81
All ages	58.46 (46.51–69.17)	49.72 (41.43–58.06)	44.89 (38.16–50.96)	33.38 (29.03–37.88)	26.87 (23.22–30.06)	24.80 (20.74–29.28)	22.10 (16.85–28.54)	−62.2

TB: Tuberculosis; UI: uncertainty intervals; % Δ: the percent changes of Mortality rate from 1990 to 2019.

Table 4 – DALYs of TB by age-sex from 1990 to 2019 in India (per 100,000 persons, 95% UI).

Age Sex	1990	1995	2000	2005	2010	2015	2019	% Δ (1990–2019)
Both Sexes								
Under 5	3415.68 (2700.70 –4200.93)	2720.37 (2163.82 –3334.78)	1923.86 (1532.75 –2328.84)	1345.79 (1058.45 –1655.35)	958.23 (769.45 –1177.65	641.70 (512.98 –785.48)	395.33 (304.90 –502.50)	−88.43
5–14 Years	698.68 (550.43 –868.86)	556.62 (444.09 –674.39)	482.19 (392.37 –573.50)	344.26 (281.29 –408.11)	219.72 (177.61 –264.86)	138.78 (109.74 –167.53)	119.53 (93.36–147.17)	−82.89
15–49 Years	2826.97 (2548.06 –3111.22)	2433.85 (2221.14 –2645.96)	2349.32 (2168.34 –2539.28)	1735.14 (1600.95 –1874.80)	1440.38 (1334.28 –1555.78)	1101.58 (999.88 –1206.14)	999.42 (863.31 –1152.26)	−64.65
50–69 Years	8264.33 (7224.77 –9285.18)	7024.92 (6302.91 –7739.33)	5984.75 (5448.92 –6508.58)	4252.38 (3907.53 –4601.47)	3615.45 (3324.88 –3927.95)	3289.24 (2949.32 –3665.38)	2695.30 (2263.96 –3232.67)	−67.39
70+ Years	11265.96 (9584.90 –13025.21)	9645.29 (8406.26 –10996.15)	8764.39 (7769.55 –9694.68)	6401.66 (5744.98 –7007.71)	4582.72 (4128.20 –5047.17)	3981.36 (3548.66 –4427.28)	3429.92 (2908.38 –3995.36)	−69.56
All ages	3105.43 (2780.10 –3440.63)	2647.10 (2414.77 –2890.21)	2399.07 (2210.44 –2584.85)	1792.37 (1654.71 –1919.15)	1494.30 (1391.11 –1604.84)	1262.20 (1148.24 –1374.51)	1125.42 (971.85 –1307.52)	−63.76
Male								
Under 5	2899.04 (2276.71 –3643.82)	2333.63 (1821.09 –2910.16)	1645.68 (1293.44 –2016.02)	1125.06 (883.79 –1392.58)	801.93 (629.75 –999.15)	564.75 (442.78 –716.32)	356.04 (265.63 –456.72)	−87.72
5–14 Years	459.49 (358.31 –568.96)	357.88 (276.07 –435.07)	328.86 (258.80 –390.51)	250.72 (197.74 –299.43)	183.82 (144.18 –220.15)	115.41 (89.93–140.19)	93.17 (71.94–115.49)	−79.72
15–49 Years	3100.72 (2746.88 –3472.59)	2726.29 (2445.85 –3002.16)	2694.58 (2450.64 –2944.17)	2077.04 (1896.97 –2270.89)	1768.70 (1607.93 –1948.08)	1375.31 (1226.05 –1527.08)	1250.98 (1028.38 –1488.30)	−59.66
50–69 Years	10600.88 (8967.94 –12096.51)	9159.46 (8006.92 –10278.86)	7962.84 (7084.91 –8734.23)	5714.26 (5138.37 –6322.12)	4972.42 (4451.65 –5521.80)	4317.03 (3840.52 –4805.22	3602.64 (2861.53 –4438.54)	−66.02
70+ Years	13577.98 (10892.96 –15787.92)	11792.76 (9828.14 –13570.37	10885.33 (9346.36 –12131.63)	8099.54 (7058.23 –8949.13)	5949.62 (5272.29 –6596.18)	5186.80 (4495.85 –5786.41)	4421.33 (3598.72 –5290.05)	−67.44
All ages	3393.91 (2978.00 –3786.75)	2948.55 (2641.26 –3214.50)	2737.11 (2499.28 –2953.62)	2108.27 (1917.26 –2296.82)	1825.19 (1665.74 –1994.26)	1548.85 (1391.13 –1700.004)	1397.28 (1142.57 –1670.75)	−58.83
Female								
Under 5	3973.64 (2947.75 –5007.66)	3141.33 (2403.74 –3942.66)	2227.87 (1731.40 –2751.45)	1587.74 (1238.69 –1955.02)	1129.59 (900.62 –1417.82)	726.18 (550.22 –923.33)	438.50 (324.07 –586.22)	−88.96
5–14 Years	959.28 (724.47 –1233.14)	778.26 (610.59 –969.93)	654.55 (519.35 –803.83)	448.21 (357.90 –544.89)	259.21 (202.37 –319.47)	164.40 (126.48 –208.54)	148.44 (112.99 –189.15)	−84.53
15–49 Years	2530.70 (2094.11 –2922.84)	2119.30 (1827.61 –2413.80)	1980.01 (1734.81 –2223.88)	1370.27 (1221.83 –1524.75)	1091.44 (965.06 –1209.61)	811.14 (687.85 –927.43)	732.30 (573.62 –920.56)	−71.06
50–69 Years	5645.71 (4361.15 –6793.63	4708.26 (3861.89 –5574.67)	3935.12 (3278.10 –4529.34)	2757.71 (2390.36 –3140.39)	2230.90 (1923.98 –2512.65)	2260.04 (1827.33 –2788.14)	1796.88 (1368.62 –2373.61)	−68.17
70+ Years	9003.82 (6391.29 –11668.22)	7510.21 (5673.59 –9515.77)	6660.06 (5205.94 –8035.22)	4821.06 (3916.28 –5648.68)	3391.64 (2794.77 –3956.23)	2938.32 (2370.47 –3525.28)	2557.21 (1977.36 –3357.23)	−71.6
All ages	2792.28 (2300.49 –3227.83)	2319.69 (1988.72 –2643.79)	2034.18 (1777.45 –2270.68)	1454.09 (1297.81 –1612.01)	1142.89 (1013.86 –1263.71)	959.70 (801.96 –1104.80)	839.26 (662.35 –1043.87)	−69.94

TB: Tuberculosis; UI: uncertainty intervals; % Δ: the percent changes of DALYs rate from 1990 to 2019.

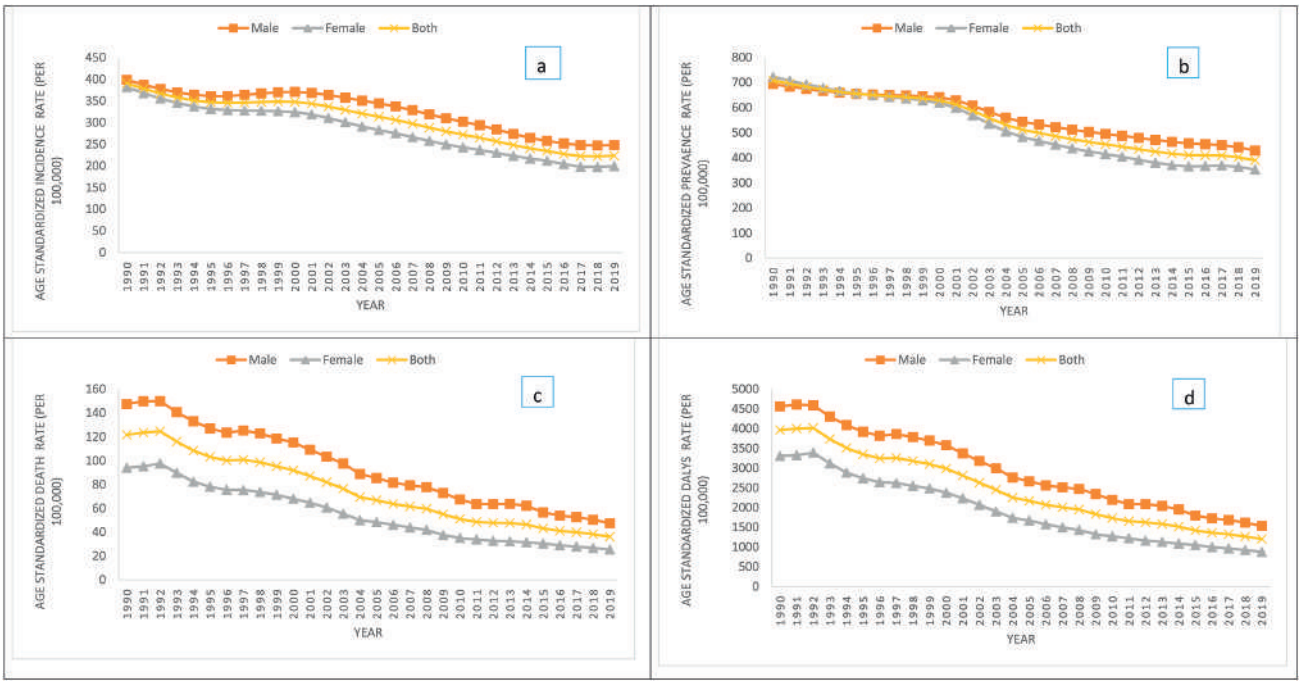

Fig. 1 – Gender wise trends of incidence, prevalence, death and DALY rates of Tuberculosis for India; 1990 to 2019. (a) Incidence, (b) Prevalence, (c) Death, (d) DALYs.

Policy makers realized that TB can't be controlled unless the attributable risk factors like HIV, malnutrition, smoking, diabetes are managed well. The population attributable fraction for various risk factors in relation to TB in India has been estimated to be 31.6% due to undernutrition, 11.3% to smoking, 9.1%, diabetes, 5.0% to HIV[27] But amongst all highest relative risk of having TB is associated with HIV.[19] During the year 1992 first HIV-TB co-infection was reported.[21] Since, the year 2001 TB-HIV coordination activities have been implemented in the country and they were intensified to cover the entire country by 2012. That included offering HIV testing to TB patients, intensified TB case finding at Integrated

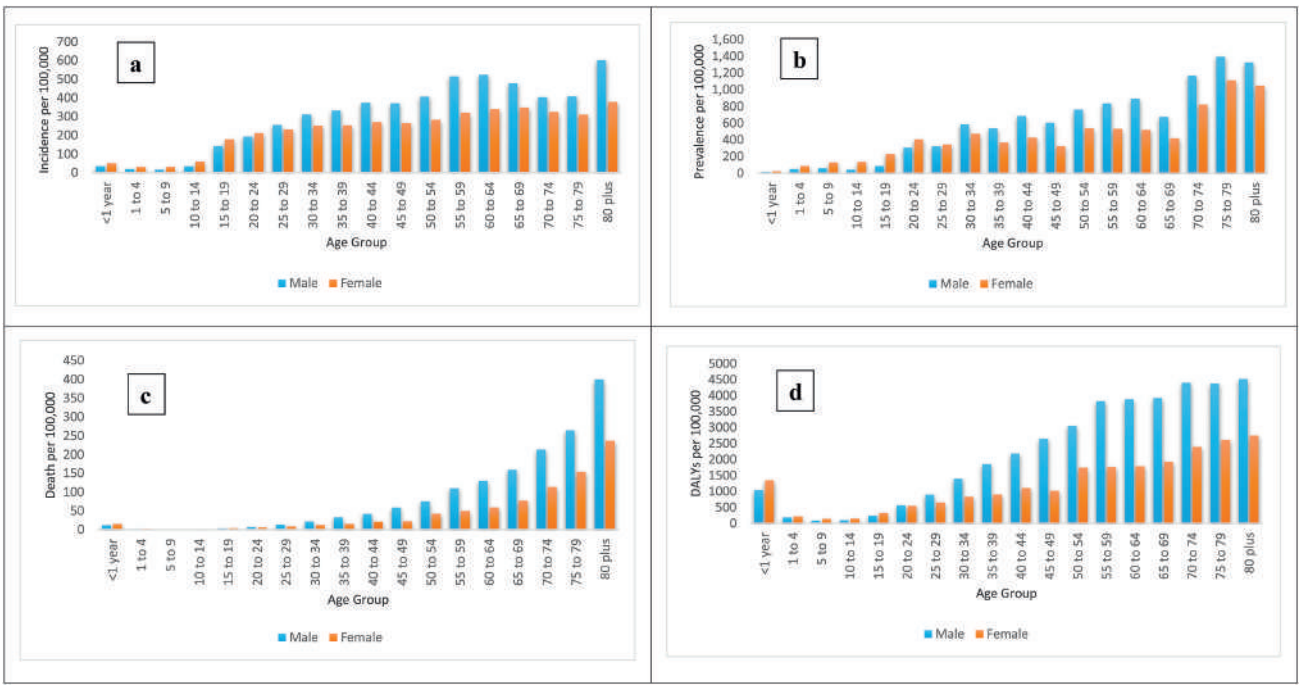

Fig. 2 – Age and Gender wise distribution of Incidence, Prevalence, Death and DALY rates of Tuberculosis for India in 2019. (a) Incidence, (b) Prevalence, (c) Death, (d) DALY.

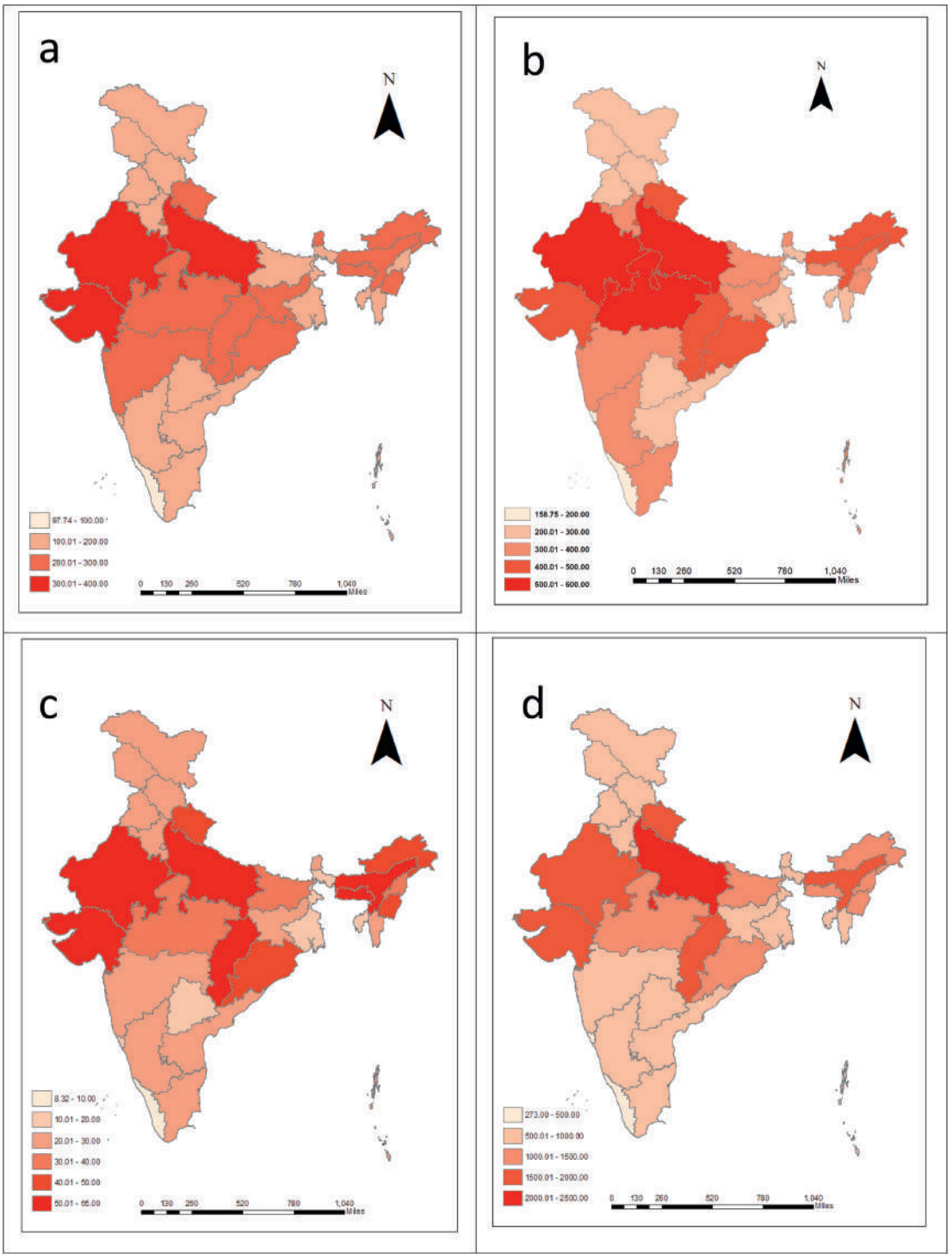

Fig. 3 – State wide distribution of age standardized rates of TB in India for 2019. (a) Incidence, (b) Prevalence, (c) Death, (d) DALY.

Counselling and Testing Centers (ICTC), Antiretroviral Therapy (ART), and community care centers and linking of HIV positive TB patients to National AIDS Control Programme and RNTCP for care and support.[28] All this resulted in increased HIV testing overtime from around 11% in 2008 to more than 81% tested in 2019. And proportion of TB Patients who are HIV positive has decreased from 18% to 2% due to rigorous efforts in controlling the disease.[7]

Risk of TB has found to be 3fold higher among tobacco smokers than non-smokers[29] and 2–7fold higher odds of treatment failure among smokers has been seen in two cohort studies conducted in Malaysia and Morocco.[30] Risk of Second

Hand Smoke (SHS) associated active TB (pooled RR 3.41, 95%CI 1.81–6.45) among children is 3 fold higher than, the risk in adults exposed to SHS (summary RR 1.32, 95%CI 1.04–1.68).[31] An example of strong political advocacy was seen with enactment of Cigarette and other tobacco products act (COTPA) during 2003 which exercised ban on all kind of tobacco products to the minors (<18years), and ban on smoking in the public places. From 2009 to 2016 overall smokers in India reduced from 14% to 10.7%. Prevalence of smoking was higher among men as compared to women during all these years.[32]

Diabetes Mellitus is associated with higher the risk of TB infection, TB disease, adverse impacts on TB treatment outcomes like mortality during TB treatment, TB relapse, and possibly multi-drug resistant TB. In 1990, 11.4%of new TB incident cases and 14.5% of TB-related deaths were attributed to DM. While in 2017, this proportion increased to 21.9% cases and 28.9% deaths respectively. Higher proportion may be attributed to the better reporting following inclusion of the mandatory screening for DM in the program during 2017.[33]

Undernutrition and TB share a chick and egg kind of relationship. An increased risk of TB is associated with undernutrition, TB itself can lead to malnutrition. Risk of progression from latent TB infection to active disease, drug toxicity, relapse and death are increased due to undernutrition. Nearly 20–22% of adults and 35% of children are undernourished in India as per NFHS-4.[34] One of the secondary data analysis mentioned that half of all TB cases among adolescents and adults in India could be attributable to undernutrition. The analysis highlighted the need to address undernutrition among adolescents and adult men in India, given the marginal difference in their prevalence of undernutrition among women and children who always have been the focus of attention in studies on undernutrition.[27] Poverty and undernutrition and are interlinked, more than half of the yearly income in Low Middle Income Countries is spend on TB care by the patients and their affected families. Large proportion of this economic burden is contributed by wage loss (60%) and non-medical expenses (20%), therefore even when free treatment is availed in majority of these countries it doesn't guarantee the financial protection. 'Social protection' schemes' can cover such indirect costs and out of pocket expenditure and will also improve access to the quality TB services as recommended by the End TB strategy. In 2018 'Nikshay Poshan Yojana' (NPY) was launched by Government of India to fulfil this aim. It is a cash assistance scheme to support TB patients during treatment. Overall main focus of the NPY is to help the TB patients to complete their treatment by giving social protection and nutritional support.[35]

India accounts for 27% of the global childhood tuberculosis (TB) burden and 6% of all notified cases in India are paediatric cases. Our study found incidence (−31.64%), prevalence (−33.68%) and mortality (−57.65%) have decreased in India from 1990 to 2019. But still there are lot of challenges in diagnosing paediatric TB as symptoms in children are non-specific and symptoms may even resemble to other diseases commonly found in such settings like malaria, asthma and pneumonia, paucibacillary disease and dearth of sensitive diagnostic tests. Often poor outcomes in children are seen due to such delays in TB diagnosis and treatment initiation.[36]

We can thus conclude that India has made significant progress towards reduction in TB morbidity and mortality. But, the present rate of ~3% annual decline in TB incidence would need to be accelerated to ~11% to achieve the 2030 Sustainable development goal targets by 2025. The major challenges that hinder the progress to reach the goal are -missing TB Cases due to lack of Notification, large number of unregulated informal providers in the Private health system, poor access to quality services in public health system, social stigma leading to the lack of seeking treatment, insufficient supplies of drugs and diagnostics to meet demands, deficient human resources at the required positions within programme, incomplete utilization and implementation of newer diagnostics and advanced systems, large paediatric case load.[7] This study has some limitations. Global Burden of Disease study has developed methods for improving the quality of data by adjusting for incomplete or missing data, but there might be possibility of some inaccuracy in the estimates. Also, results obtained from this study are true at population levels but might not hold for individuals as this is an ecological study.

This study shows that overall incidence, prevalence, death and DALY of tuberculosis decreased from 1990 to 2019 in India. The burden of TB was higher among males as compared to females during the study period. TB affects all the age groups but deaths were higher in older age groups. Though the prevalence of TB was high in Kerala and Karnataka but they have reported lowest deaths and DALY. Government needs to strengthen existing infrastructure for TB control and improve case notification and surveillance under national tuberculosis elimination programme. Experiences of states like Kerala and Karnataka can be utilized at the national level to reduce deaths and DALY of TB. Intensify information, education and communication activities regarding TB control at peripheral level for further reduction in TB morbidity and mortality.

Authors' contributions

Conceptualization: DD, RPJ; Formal analysis: DD, RPJ; Methodology: DD, RPJ; Writing-original draft: DD, RPJ, SA; Writing-review & editing: DD, RPJ, SA.

Availability of data and material

Data is publicly available on http://ghdx.healthdata.org/gbd-results-tool and free to use.

Code availability

None.

Conflicts of interest

The authors have none to declare.

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Original article

Perception of DOTS providers on changes in tuberculosis case management: Comparison of alternate day and daily treatment regimens using mixed method design in Mandya district

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ABSTRACT

Background: Tuberculosis case management has undergone various changes in recent years. Most of the decisions have been taken based on provider intelligence or perspective. It is essential to know the beneficiary perspective about the changes in RNTCP. DOTS providers are active witnesses for the series of changes and they have first hand experience of effect of these changes on TB patients. Thus it is ideal to learn from DOTS providers, about comparative strengths and limitations of the new strategy and effects of the changes.

Methods: Study design Mixed method using survey and matrix ranking method.

Study population: DOTS providers who have supervised at least one patient before the daily regimen and completed at-least intensive phase of one patient in daily regimen.

Study was conducted in Mandya district between May–June 2019 involving 25 DOTS providers selected through snowball sampling technique. Data collection was done using proforma and Matrix ranking technique using 10 stones. Thematic analysis, wilcoxon sign rank test were used for analysis.

Results: Significant improvement was reported by DOTS providers in new regimen with respect to compliance, treatment response, ease of supervision and patient attitude towards outcome. Related to drug supply system, there was no significant difference.

Conclusions: There was significant improvement in compliance, response, ease of supervision and patient attitude. Significant reduction in side effects in daily regimen compared to alternate day regimen. NPY is helping poor and elderly patients but irregular payment is an issue. Remuneration of DOTS providers was not consistent.

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1. Introduction

India has been fighting tuberculosis from decades and from time to time it is making changes to strengthen its fight against the disease which has killed millions from centuries. Moving on from sanatorium approach, India started its first National Tuberculosis program in 1962, to diagnose as many cases as possible and cure most of them through domiciliary approach. Based on the operational research findings, it was revised and strengthened program with DOTS as its main weapon was launched in 1995. The patient categorization was changes into only two categories in 2013 to reduce the risk of resistance due to under-treatment. Despite wide publicity and multipronged efforts, resistance went on increasing and alternate day regimen was criticized, as it was not the best regimen available. As a result of private health sector's criticism for DOTS, public forum discussions about effectiveness of daily regimen compared to alternate day regimen, lack of drug sensitivity testing for every patient, persuasion from operational research findings which suggested better patient outcome in daily regimen and increasing problem of drug resistance in intermittent regimen, daily regimen was launched under RNTCP from November 2017. Government brought out new standards for TB care in India with vision of providing highest standard of TB care to TB patients and an option to receive care from healthcare providers of their choice.[1–4]

Alternate day regimen had reduction of stigma associated with tuberculosis treatment and giving a breather of one day to the patient, which was helping in reducing adverse effects of antitubercular drugs, in its strategy.[1,3] This is no more as daily regimen has been adopted. The decision has been taken largely from provider perspective and very limited published literature is available regarding perspective of patients.

Tuberculosis case management has undergone numerous changes in recent years. Alternate day regimen has been changed to daily regimen; multi drug blister packs being replaced by fixed dose combinations (FDCs) are few among those. These decisions have been taken largely from **'Provider (policymaker)'** intelligence or perspective. What are the effects of these changes on the treatment compliance, drug toxicity in patients, national program functioning, drug supply, DOTS provider workload are yet to be found out.

It is desirable to know the effects of new regimen and strategies from patient's perspective. As the patients will not be able to provide comparative experience of both the regimens, DOTS providers are ideal as they would have seen these patients from close association. Thus this study was planned with following objectives.

1. To compare daily and alternate day regimens from DOTS providers perspective.
2. To explore strengths and lacunae of new case treatment guidelines as perceived by DOTS providers

2. Methods

Study design: Community based cross sectional mixed method design was adopted using a quantitative survey and Qualitative technique using matrix ranking method.

Study population: DOTS providers who have supervised at least one patient before the daily regimen and completed atleast intensive phase of one patient in daily regimen.

Study area and period: present study was conducted in Mandya district between May–June 2019.

Sample size: 25 DOTS providers were included in the study who satisfied the eligibility criteria. Number was restricted once the data saturation was obtained.

Sampling technique: snowball sampling technique was used to identify the eligible participants till data saturation was obtained.

2.1. *Data collection*

Statutory permissions were taken from District tuberculosis office and Nodal officer for Tuberculosis before starting the study. After explaining the study objectives and methodology, consent was taken from selected DOTS provider and details related to her education, work experience, number of patients supervised, remuneration received, outcome of patient supervised, were collected using a structured pretested proforma.

Perception of DOTS providers related to compliance side effects, ease of supervision, drug supply from DTC, change in patient attitude towards treatment was assessed using matrix ranking method. For comparative ranking, 10 stones were given for each component to DOTS providers and were asked to share between old and new regimens according to merit of the two regimens as per their experience or perception based on patient response. At the end of matrix ranking exercise, participants were briefed about interpretation of their scoring and were asked to make changes if they wished to. Photograph of the scoring system was captured for future analysis. Suggestions were also taken from participants for effective program implementation.

2.2. *Statistical analysis*

Impressions and suggestions provided by participants were clubbed with their interpretations to draw conclusions. Thus grounded theory was used and Thematic analysis was done to draw final conclusions. Scores provided for each component were analyzed using *Wilcoxon sign rank* test. Other variables were described using frequency, percentage, mean and standard deviation.

3. Results

Out of 25 study participants, 15 were ASHAs and 10 were Anganwadi workers. Mean age of participants was 41.04 ± 8.20 mean years of schooling was 10.12 ± 1.56.

Experience of participants regarding old regimen was as mentioned below. 16 (56%) DOTS providers had supervised less than 5 TB cases.19 (76%)DOTS providers had 100% treatment completion rate. Among the remaining, 5 cases had defaulted and 2 were lost to follow up. Participants had not got their due remuneration in 6 cases(24%). 11 (44%) had got Partial remuneration.

New regimen experience was limited as study was carried out in less than a year of launching the daily regimen. Out of 25 participants, 80% had supervised two or less tuberculosis cases.

10 (40%) had successfully completed course supervision. Two had seen their patients default and one case was lost to follow up. 80% participants had not received DOTS provider designated remuneration yet(June 2019). Two providers had experience of supervising TB-HiV co-infection cases(one each).

As seen in Table 1, score awarded by the DOTS providers for determinants of treatment outcome observed better performance by daily regimen. Statistically significant difference in scores were observed in favour of daily regimen with regard to compliance of patient to treatment, symptomatic evidence of treatment response, ease of treatment supervision by providers and change in patient attitude (positive change) towards outcome.

As seen in Table 2, significant improvement was reported by DOTS providers in new regimen with respect to compliance, treatment response, ease of supervision and patient attitude towards outcome. Related to drug supply system, there was no significant difference.

17(68%) were aware of Nikshay Poshan Yojana but the payment was not regular. 14(56%) DOTS providers were undergone formal training for new DOTS regimen, remaining were informally briefed about daily regimen by Medical officer, before starting the treatment.

Suggestions given by participants were:

- Responsible family member as DOTS provider as it is difficult to visit the patients every day. If not visited, same level of motivation is difficult to sustain among patients.
- Instead of monetary incentive, food products like protein powder should be given to patients under national program. Many times money will not be used for valid reasons.
- NPY amount should be increased as cost of materials is increasing and should be disbursed regularly.
- Timely incentive for DOTS providers to keep them interested in the program.

4. Discussion

Present study mixed method study tried to ascertain beneficiary perspectives regarding the determinants of treatment outcome, through 25 DOTS providers. There were no published literature which focussed on beneficiary perspective, especially after change of regimen. It was observed that patient compliance, treatment outcome were significantly better in daily regimen. An RCT by Gopalan had noted that among HiV-TB coinfection cases of South India, negative cultures reached 98% or better within 2 months of daily regimen treatment while at 2 months only 87% of the patients treated with the intermittent schedule had achieved negative cultures. Overall favourable outcome was 91% for daily regimen compared to 77% in intermittent regimen.[3] Another Meta-analysis which reviewed more than 27 articles from across the world also reaffirmed this finding with higher success rate with daily regimen.[2] Better compliance is explained further by the study participants; '*in old regimen, it was difficult to consume 7 different tablets on one day. Now they feel it is single drug but number of tablets vary. So they don't give reasons and adhere to treatment, which is improving compliance and outcome*'. Participants opined this has an effect on reduced adverse effects also.

There were no studies which had studied ease of supervision and other qualitative perspectives like patient attitude towards disease outcome. But it is clearly observed with significant difference in the ranking given by participants that daily regimen is better in this aspect. One of the participants quoted '*earlier patients used to forget to take the tablets on exact alternate days. To compensate they used to take it continuously, which meant there was a gap of 2 to 3 days and suddenly it was taken on consecutive days. This was more in continuation phase*". But they also expressed the difficulty faced by the DOTS providers saying, '*Earlier we used to visit them on alternate days and supervise tablet consumption. Now we have to visit everyday and our other works get hampered. Especially when the houses are far*'. They also suggested '*as the tablets have been simplified (FDC), now family members can be given the responsibility as it hardly requires any expertise. This avoids stigma of we daily visiting their houses*'. They also opined, patients feel better early with daily regimen and apatite improves early in daily regimen. This is also supported by quantitative study findings which suggest early culture negative results with daily regimen.

Table 1 – Comparison of Perception of DOTS providers about two regimens.

Variable	Perception of DOTS providers (n = 25)				Wilcoxon sign rank test 'P' value
	Alternate day Regimen		Daily regimen		
	Mean score	SD	Mean score	SD	
Compliance	4.16	1.74	5.84	1.74	0.036
Side effects	6.20	1.41	3.80	1.41	0.001
Treatment response	3.20	1.15	6.80	1.15	<0.001
Ease of supervision	3.92	2.25	6.08	2.25	0.032
Drug supply	5.08	0.27	4.92	0.27	0.157
Change in patient attitude	3.92	1.91	6.08	1.91	0.014

Table 2 – Perception of DOTS providers about treatment outcome determinants (Grounded theory).

Sl no	Theme	Subthemes
1	Compliance	• Difficult to take 7 tablets at once as in case of alternate day regimen • Chances of forgetting tablet consumption are low in daily regimen. • Patients of daily regimen expressed more fear of side effects.
2	Adverse effects	• Taste alteration, gastritis, vomiting, weakness are routine side effects • Side effects are less in daily regimen.
3	Patient response	• Time to increase apatite and weight gain was 5–7 days in old regimen • Same is achieved in 2–3 days in daily regimen
4	Ease of supervision	• Chances of forgetting, confusion and missing dose was more in old regimen. • In daily regimen visiting patient for supervising daily is a problem.
5	Drug supply	Equally good in both regimen

Present study observed that adverse events were significantly less with daily regimen. This is contrary to other studies which reported higher incidence. RCT in South India observed incidence of adverse effects among daily, partially daily and intermittent treatment groups to be 27%, 21% and 17% respectively.[3] Another prospective study from Pune and meta-analysis study also reported higher chances of defaults in daily regimen.[2,4] Explanation of study participants for this contrary observation was, "*As the patients' apatite improves fast with new regimen, they feel better and start eating well early. Single type of pills (FDC) also has psychological effect in reduction of side effects.*" Participants also observed that Nikshay Poshan Yojana(NPY) has helped the patients especially in below poverty line families and where earning adults are suffering. They observed, "*when earning member of the family is bed ridden, it proves handy for whole family. But the amount of money is not sufficient to feed the dependant members and definitely parents spare food for their children and eat less. So it will be helpful if the amount is increased or if nutritional supplements are given instead of money; as drawing money and purchasing ration though look simple work to us, it is difficult to the patient and their family in the initial days of treatment*". They also mentioned that NPY amount is not disbursed regularly and thus it does not serve it's purpose.

Many providers also complained about the delay in release of DOTS provider remuneration. Many had not received their due amount even after 6 months of treatment completion. A report on Joint TB Monitoring Mission in India also observed delayed payment and old rate payment in some districts in its report submitted to Govt in 2015. It also made a recommendation that 'All community DOT providers to be paid honorarium regularly and use e-transfer mechanism linking to NIKSHAY'.[5]

Thus our study observed that patients are being benefitted with daily regimen and DOTS providers are finding it easy for the patients to comply with.

5. Conclusion

- There was significant improvement in compliance, response, ease of supervision and patient attitude.
- Significant reduction in side effects in daily regimen compared to alternate day regimen.
- NPY is helping poor and old patients but irregular payment is an issue.
- Remuneration of DOTS providers was irregular.

Ethical approval

Permission for the study has been obtained from District Tuberculosis officer of Mandya.

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Conflicts of interest

The authors have none to declare.

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Original article

Outcomes and adherence of shorter MDR TB regimen in patients with multidrug resistant tuberculosis

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ABSTRACT

Background: In 2016 WHO guidelines conditionally recommended standardized shorter 9–12 months regimen for MDR-TB treatment. The objective is to study outcome analysis of cured, lost to follow-up, treatment completed, treatment failure and mortality of MDR Patients on shorter standardized MDR TB regimen.

Methods: In this prospective study, 360 adults with confirmed Rifampicin Resistant pulmonary TB were studied between March 2018 to February 2020 at Department of Pulmonary Medicine, Guntur Medical College, Govt. Fever Hospital, Guntur.

Results: Among 360 confirmed MDR Patients, 42.50% patients were cured, 41.60% completed treatment, 6.11% of them were lost to follow-up, 0.50% were considered as treatment failure and 9.10% of them were died.

Conclusion: Overall success with a standardized shorter MDR regimen was high with low treatment failure. When introducing shorter regimens base line drug susceptibility testing and minimizing missed doses are critical.

1. Introduction

Rifampicin-resistant (RR) and multidrug-resistant (MDR) tuberculosis (TB) remain a public health emergency, with an estimated 4 84 000 cases occurring worldwide in 2018.[1] Diagnosis and management are expensive and resource-intensive, with only 32% of the estimated globally incident MDR-TB cases able to access treatment to international standards.[1]

By definition, MDR TB is a TB patient whose biological specimen is resistant to both Rifampicin and isoniazid with or without resistance to other anti-TB drugs.[2]

Rifampicin Resistant TB(RR-TB) is defined as is a TB patient whose biological specimen is Rifampicin resistant detected genotypically or phenotypically with or without resistance to other anti tuberculosis drugs.[2]

Due to poor management, drug resistant TB (MDR TB/RR TB) has been emerging as a major problem. Global TB report of

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2019 estimated that 2.84% newly diagnosed and 11.62% of previously treated TB cases had MDR-TB.[2]

Worldwide report on poor treatment outcomes was reported in most of the 2 year long conventional MDR treatment. The probable reasons include, poor treatment adherence due to lengthy, expensive and toxic regimens leading to poor compliance. This prompted a shorter regimen of MDR-TB regimen of 9–12months instead of conventional 2 years duration.[4]

In 2016, WHO guidelines conditionally recommended a shorter 9–12-month MDR-TB regimen for patients meeting specific criteria, based on results of a systematic review and individual patient data meta-analysis.[3,4] However, uncertainty remains about the regimen's effectiveness in the presence of resistance to constituent drugs, including fluoroquinolones, ethambutol, pyrazinamide and prothionamide.[5–9]

2. Aims and objectives

To study adherence and treatment outcome of shorter MDR TB regimen in patients with MDR-TB.

3. Materials and methods

A 2 years (March 2018 to February 2020) prospective study of MDR-TB conducted among 360 patients, whose sputum samples are positive for AFB and RR detected in CBNAAT are included in the study and subjected to shorter MDR-TB regimen as per diagnostic algorithm of Programmatic Management of Drug Resistant TB under National Tuberculosis Elimination Programme in Department of Pulmonary Medicine, Guntur Medical College, Govt. fever hospital, Guntur. All these 360 RR resistance samples sent to LPA to confirm the resistance of the other first line and second line TB drugs and excluded if other drug resistance present.

4. Inclusion criteria

All patients with pulmonary TB and newly identified Rifampicin Resistant/MDR-TB [Rifampicin+ Isoniazid (katG mutation)] and who did not have a history of prior treatment with second-line anti-TB drugs for more than 1 month, and for whom informed consent could be obtained, were eligible for inclusion.

5. Exclusion criteria

DST based criteria:

- If DST result for FQ or SLI is resistant or
- Presence of InhA mutation (for Eto) or
- Resistence to pyrazinamide.

Non DST based criteria:

- Pregnancy
- Any extrapulmonary disease in PLHIV.
- Disseminated meningeal or CNS TB.
- Intolerance and risk of toxicity to any drug in shorter MDR regimen.

Shorter MDR TB Regimen under PMDT 2019 Guidelines[2] IP Phase (4–6 months) Mfx^h, Km/Am, Eto, Cfs, Z, H^h, E and CP Phase is (5 months) Mfx^h,Cfs, Z, E. Culture conversion done in 4th month.

6. Results

This is a prospective study involving 360 MDR-TB patients which were subjected to shorter MDR regimen. Among these 268 are males and 92 are females, Median age was 41yrs, 2.8% (10/360) of Patients having BMI<18, 10.28% (37/360) are diabetics, 13.5% (47/360) cases were HIV reactive.

Baseline characteristics and outcomes for standardized shorter MDR-TB regimen

Characteristic	Total	Cured	Treatment completed
Total	360	153 (42.50%)	150 (41.60%)
AGE			
15–44 yrs	214	90 (42.05%)	88 (41.50%)
45–64 yrs	109	47 (43.11%)	47 (43.11%)
≥65yrs	37	16 (43.24%)	15 (40.54%)
GENDER			
Male	268	109 (40.67%)	112 (41.79%)
Female	92	44 (47.82%)	38 (41.30%)
HIV STATUS			
Non reactive	313	135 (43.13%)	135 (43.13%)
Reactive	47	18 (38.29%)	15 (31.91%)
DIABETES			
NO	323	136 (42.10%)	134 (41.48%)
YES	37	17 (45.94%)	16 (43.24%)
BMI			
<18	10	8 (80%)	0
≥18	350	145 (41.42%)	150 (42.85%)
FIRST LINE DST			
ISONIAZIDE (KAT G)			
Resistance	8	3 (37.50%)	2 (25%)
Susceptible	348	150 (42.97%)	146 (41.83%)
Unknown	4	0	4 (100%)
ISONIAZIDE (Inh A)			
Resistance	7	3 (42.85%)	2 (28.57%)
Susceptible	349	150 (42.97%)	146 (41.95%)
Unknown	4	0	4 (100%)
WEIGHT CHANGE (Intensive phase)			
No change	10	8 (80%)	0
Any loss	0	0	0
Any gain	350	145 (41.42%)	150 (42.85%)
WEIGHT CHANGE (CP)			
No change	0	0	0
Any loss	0	0	0
Any gain	360	153 (42.50%)	150 (41.66%)
TREATMENT ADHERENCE (IP)	**38**	**12 (31.57%)**	**20 (52.63%)**
<7 missed doses	26	10 (38.46%)	14 (53.84%)
≥7 missed doses	12	2 (16.66%)	6 (50%)
TREATMENT ADHERENCE (CP)	**72**	**23 (31.94%)**	**36 (50%)**
<7 missed doses	43	16 (37.20%)	20 (46.51%)
≥7 missed doses	29	7 (24.13%)	16 (55.17%)

Treatment outcomes at the end of treatment among patients treated with standardized shorter MDR-TB regimen:	
Successful outcomes	Outcome evaluated at the end of treatment
Cured	153 (42.50%)
Treatment completed	150 (41.60%)
UNSUCCESSFUL OUTCOMES	
Died	33 (9.10%)
Lost to follow-up	22 (6.11%)
Treatment failure	02 (0.50%)

6.1. *Adverse events*

Adverse events were common with 78% patients reporting atleast one adverse event. 30% of the patients have 5 or more adverse events. The majority of the adverse events were nausea and vomiting (23.6%), weakness (10.5%), abdominal pain (10.5%), headache (8.1%), arthralgia (7.3%), Renal failure (6.8%), ototoxcity (4.8%), anorexia (6.4%), diarrhea (3.4%), itching (2.9%), hepatitis (2.5%), anemia (1.5%).

7. Discussion

India constitutes a considerable burden of MDR TB patients. However, in a resource poor country like India, performing frequent cultures, arranging daily treatment for 2 years, particularly adherence and managing adverse events for prolonged period is very difficult, so study on shorter MDR TB treatment regimen and evaluating adherence and outcome is very essential.

In this study we found that a Shorter MDR TB treatment regimen achieved good success with a low failure rate. Previously published studies of similar Shorter MDR treatment regimens have reported end-of-treatment success between 81.6 and 89.2% which is similar to our outcome (84.1%).[10–13] Most of the radiological lesions are infiltrative and bilateral, right upper lobe is more common than left.

In 2010, VanDeun et al reported an observational study undertaken in Bangladesh in which sequential cohorts of MDR-TB patients were treated with standardised regimens. The most effective regimen (known as Bangladesh regimen) was 9–11 months in duration used Kanamycin, high dose Gatifloxacin, prothionamide, high dose isoniazid, clofazimine, pyrazinamide and ethambutol and achieved recurrence free cure in 88% of Patients which is nearly similar to our outcome (84.1%).[14]

Unsuccessful outcomes was 15.7%, which is slightly higher than internationally reported pooled rates of 11% (10–12%), this may be due to severity of the by the time of diagnosis and missed pills. Culture conversions seen in 78% at the end of 3rd month, 72% at the end of 6th month and 62% at the end of 9th month. At the end of 9th month 2 patients were culture positive with LPA showing Fluroquinolone resistance and they were converted to All oral longer regimen.Initial successful short course observation studies from Bangladesh has led to proposal of 9–12 months.[14]

The present study has reported successful outcome in 84.1%, which is higher than the successful treatment outcome in 72.5% of patients treated with modified DOTS plus strategy (duration of 24–27 months) in a study conducted by Abhijeet et al.[15] Unsuccessful treatment were seen in 15.71%, which is lower when compared to that of modified DOTs plus strategy which has 27.5%[15] and Raghu et al study[16] on conventional 2 years TB regimen successful outcome was 53.7% and high defaulters.

Lost to follow up in 6.11% of the cases which is lower than the Prasanta kumar Das et al study,[17] which is 13.7% during the study period due to migration and symptomatic relief and adverse reactions even though patients suffered with minor side effects like nausea and vomiting (23.6%), followed by weakness and abdominal pain (10.5%), headache (8.1%), arthralgia (7.3%), Renal failure (6.8%), ototoxcity (4.8%), anorexia (6.4%). Mortality was seen in 33 patients (9.10%), during the study, on which 17 are died in intensive phase and remaining 16 patients died during continuation phase. Among these 17 patients 8 patients died within 14 days of initiation of treatment, 7 patients less than 1 month, 2 patients less than 2 months), due to extensive lesions in chest X ray and less BMI. Another 16 died in continuation phase, in that most of the patients missed pills and extensive disease.

Further meta-analysis have shown that key determinants of success of these regimens include resistance to fluoroquinolones, pyrazinamide and injectable.[11] Initial concerns of FQ and Kanamycin resistance were shown to be important only in high FQ resistance patients as shown in follow up studies, anyhow this was overcome by prolonging the treatment duration after monitoring cultures.[18]

These findings suggest/propose for scaling up for DST.[11] The drawback of observation study has been nullified by the non inferiority of this regimen as shown in recent STREAM trial.[19]

The main disadvantages of short course regimen include usage of injectables, drug toxicity and availability of molecular methods. It will be exciting to see whether inclusion of newer drugs like Bedaquiline and Delaminid can shorten the duration even further limiting to 6 months as it is equal to drug sensitive tuberculosis regimen.

8. Conclusion

Overall success with a standardized shorter MDR-TB regimen was high with low treatment failure with less adverse events. Further improvements in this regimen may include avoidance of injectables making complete oral regimens. Hopefully one day we may have tailored regimens with minimum toxicities and short duration for all drug resistant cases as drug sensitive regimen.

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Original article

Clinician perspectives of drug-resistant tuberculosis care services in the Philippines

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ABSTRACT

Background/Objectives: In the Philippines, treatment success rates for drug-resistant tuberculosis (DR-TB) remains low and little is known about the quality of DR-TB services. This study aimed to explore clinician's perspectives of DR-TB care services.

Methods: We conducted semi-structured in-depth interviews from January–March 2018 with 11 providers selected purposively to explore the barriers associated with DR-TB care service delivery, best practices, and recommendations for enhancing patient care. Emerging themes were organized according to the socio-ecological framework.

Results: Five major themes were identified: (1) nurses do not feel empowered; (2) particular patients are left behind and more vulnerable than others; (3) infection control practices, fear, and limited capacity in rural health centers; (4) financial insecurity due to program reimbursement mechanisms; and (5) local government support is limited and requires more involvement in support of DR-TB elimination activities. Best practices focused on tailored approaches that eliminated structural, economic, and motivational barriers for patients. Participants recommended financial support from local government units, nutritional assistance for patients, and refresher training for healthcare workers.

Conclusion: The findings provide additional understanding regarding the barriers that limit successful DR-TB care delivery and provide critical information to improve clinical practice and develop public health interventions for frontline staff including nurses in the Philippines. These strategies could ultimately reduce disparities associated with access to care and treatment adherence, if implemented.

1. Introduction

Disparities in tuberculosis (TB) treatment outcomes for patients with drug-resistant TB (DR-TB) remains a significant roadblock to TB eradication worldwide.[1] The Philippines continues to rank among countries with the highest burden of multidrug-resistant TB (MDR-TB).[2] Although deaths attributed to TB infection are preventable, factors driving low treatment outcomes for patients with DR-TB include social, economic, health system, and programmatic barriers that generate patient vulnerability, especially in patients who are financially

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insecure, and those with limited psychosocial support.[3] These include limited access to healthcare and support services, weak health insurance schemes and financial protection policies, and structural barriers including TB stigma, poverty, discrimination, unemployment, and geographical barriers.[4,5]

A core pillar of the End TB Strategy developed by the World Health Organization (WHO) explicitly advocates for a person-centered approach that eliminates the risk of patients having to incur catastrophic costs for the care delivered.[6] At the primary care level, the implementation and organization of the National Tuberculosis Program is largely dependent on nurses who are responsible for diagnosis, treatment and management of MDR-TB.[7,8] However, human resource capacity at the facility and community levels is limited (e.g., funding restraints, difficulties retaining staff), despite the increasing numbers of patients affected by DR-TB.[7] Recent efforts to introduce new anti-TB drugs and novel regimens in the Philippines to address low treatment outcomes in patients with DR-TB have shown promise.[9] However, substantial efforts to address the unmet need for coordination of DR-TB and other priority services to reach those most impacted are still needed to ensure a truly person-centered model that reduces unnecessary burdens for individuals and the health system.[10,11]

Information about the factors associated with the quality of care delivered to patients with DR-TB and its impact on poor treatment outcomes is limited in the Philippines. By describing how frontline workers themselves experience barriers to providing care, we seek to fill knowledge gaps around the individual, interpersonal, community, health-system, and policy contexts impacting their ability to deliver care, and the additional supports they may need to promote medication adherence among their patients. These insights could help guide the successful implementation of Programmatic Management of Drug-resistant Tuberculosis (PMDT).

2. Methods

2.1. Purpose

This study sought to explore the perspectives of frontline staff dedicated to providing care to patients with DR-TB, understand the perceptions about facility strengths that enabled effective service delivery to patients, and obtain suggestions for improving DR-TB care services.

2.2. Setting and participants

Study participants were recruited across all 18 regions of the Philippines from 146 DR-TB treatment facilities. From December 2017 and March 2018, 272 participants completed a self-administered online survey to identify the patient- and health-system-related barriers associated with care and treatment delivery to patients with DR-TB (unpublished). In-depth interviews with a subset of 11 healthcare workers selected through purposive sampling methods (i.e., those who had provided complete and informative responses, were available, and represented different geographical areas of the Philippines) were conducted to further understand their perceptions.

2.3. Theoretical framework

We applied a socioecological model as the lens to analyze, interpret, and organize the interview data.[12] We structured our investigation around individual, interpersonal, community, health-system, and policy levels of influence to develop targeted programmatic recommendations. Across these levels of influence, we were able to capture the rich community context in which frontline staff were embedded and their shared community experiences.

2.4. Ethical considerations

IRB approval was waived for this study, and the findings were considered the result of a program evaluation. Findings of the evaluation were expected to directly affect the conduct of the national TB program and identify improvements. Before participation, the goals of the study were explained to the participants. Participants provided their consent to participate and to have their interview sessions recorded and were assured that their information would remain confidential.

2.5. Data collection

A semi-structured interview guide was developed based on a review of literature.[13,14] Interviews explored healthcare delivery to patients with DR-TB and the structural factors that health workers thought influenced patient access to and retention in DR-TB care services. Questions also focused on facility-based practices and strategies for engaging patients to foster capacity building. All interviews were conducted in English via telephone by JJ or YE. Notes were taken and all conversations were digitally recorded with permission.

2.6. Data analysis

Audio-recordings were anonymized, reviewed, and were transcribed verbatim through an independent transcription service. Data were analyzed using framework analysis, a systematic approach to organizing and elucidating themes from textual data.[15] After reviewing all interview transcripts, JJ drafted an initial analytic framework (i.e. codebook) containing descriptive categories, which is considered a crucial component for judging the validity, trustworthiness and quality of research findings.[16] The framework was subsequently refined with the guidance of the WHO TB Medical Officer for the Philippines. The team discussed divergent coding, revised codes, and added new codes if needed, to ensure findings were representative across the health system. JJ used the updated framework to code all transcripts. NVivo 11 qualitative software was used to manage, organize, and analyze the coded transcripts.

3. Results

3.1. Respondent characteristics

From January–March 2018, eleven in-depth interviews were carried out with 9 nurses and 2 physicians (Table 1). Staff represented 6 out of the 18 regions in the Philippines and

those with the largest population centers including Region IV-A: Calabarzon, National Capital Region, and Region III: Central Luzon, respectively (Table 2). Most worked in an NGO or public facility (N = 8), and more than half of the participants had under 7 years of experience providing care to patients with DR-TB (N = 7). Interview duration ranged from 1:30–3 hours. Across interviews, five key themes emerged and were organized below (Table 3).

3.2. *Nurses do not feel empowered*

Nurses felt disempowered by their limited communication with patients and with other staff. Many nurses did not feel confident in providing psycho-social counseling to patients experiencing adverse drug reactions (ADRs), nor did they feel supported within their facilities. Nurses described the professional development opportunities afforded to TB project-hired staff which were not extended to organic staff (i.e., permanent staff) and impacted morale.

> Our employer is PBSP [Philippines Business for Social Progress], but we are in the compound of the hospital … we felt that we … don't belong. Even if we like to do something for the program, it is not easy … the hospital is not supportive. (Participant (P)11).

Some nurses described successful patient-provider relationships where tailored counseling techniques were employed throughout the care continuum, and where effective teamwork within and across facilities existed, which enabled more productive work environments.

Table 1 – Participant demographic data.

Demographic Characteristic	Overall (N = 11)
Sex	
Female	6 (54.6%)
Male	5 (45.4%)
Age Group (in years)	
18–25	1 (9.1%)
26–35	6 (54.5%)
36–45	1 (9.1%)
46–55	3 (27.3%)
Profession	
Project-hired nurse	9 (81.8%)
Project-hired physician	1 (9.1%)
Permanent physician	1 (9.1%)
Experience (in years)	
1–3	4 (36.4%)
4–7	3 (27.3%)
8–10	2 (18.1%)
11–15	1 (9.1%)
> 16	1 (9.1%)
Facility Type	
Pubic	8 (72.7%)
Private	3 (27.3%)
Region	
NCR: National Capital Region	3 (27.3%)
Region VI: Western Visayas	3 (27.3%)
Region IV-A: Calabarzon	2 (18.1%)
Region III: Central Luzon	1 (9.1%)
Region X: Northern Mindanao	1 (9.1%)
Region XIII: Caraga	1 (9.1%)

Table 2 – Selected regions 2020 population estimates[a].

Regions	Population	Percentage
Region IV-A: Calabarzon	16,057,299	14.7%
NCR: National Capital Region	13,804,656	12.7%
Region III: Central Luzon	12,313,718	11.3%
Region VI: Western Visayas	7,904,899	7.3%
Region X: Northern Mindana	5,017,051	4.6%
Region XIII: Caraga	2,753,109	2.5%

[a] Population 2020 estimates for 6 of 18 regions according to DOH Population Projections by Region, Province, Cities and Municipalities, 2020–2025. For 2020, the projected Philippine population total were 108,771,978 inhabitants across the 18 regions.

> Counseling won't stop prior to treatment. Counseling would always be thorough until they end their treatment … even simple talks. It shouldn't be a blackboard [but] any setting (P6).

Additional trainings on therapeutic counseling techniques to better provide psychosocial support to patients, capacity-building opportunities for organic nurses to augment their skillsets in DR-TB care delivery, and visual job aids for ADRs were suggested.

3.3. *Particular patients are left behind and more vulnerable than others*

Lack of transportation and coordinated services were identified by both nurses and physicians as barriers disproportionately impacting their patients with co-morbidities. Quality service delivery was hindered by limited capacity for staff to conduct outreach to patients when they were considered lost to follow-up (i.e., program vehicle requests needed to be made days in advance) and by their ability to offer coordinated services to patients battling multiple diseases.

> I have people living with HIV … with cancer … people [with] substance abuse and yes, they're all problematic … I have one who had interrupted because he cannot sustain treating both two diseases. So eventually, he died (P6).

Person-centered approaches through personalized treatment plans and coordination of care for patients with co-morbidities were best practices that were highlighted by some respondents. To reach patients, staff "tapped" into existing resources such as the local ambulance if the service vehicle was unavailable, or took the bus, tricycle, taxi services (Uber/Grab), or their own cars.

> We use the transport vehicle of the LGU [local government unit] … [which is] an ambulance. We open the windows … If the ambulance is not there, then we ride the tricycle … or we use another car of the staff and we put gasoline in it. Even if it's a barrier, it could be remedied (P7).

Participants suggested developing partnerships with the local community to bring services closer to patients (e.g., whether at home or at their workplace), and trainings on the clinical management of patients with co-morbidities.

Table 3 – Major themes, barriers, best practices, and recommendations with representative quotes.

Theme	Barrier	Best practice	Recommendation
1. Nurses do not feel empowered	Communication with patients/staff Working environment	Tailored counseling Effective teamwork	Additional trainings; job aids *"The project-hired nurse is always the one that's being sent for validation and for training and for updates. To equalize their capacity and their skills, maybe [organic nurses] could also be included … all of them should be invited."* **P7**
2. Particular patients are left behind and more vulnerable than others	Transportation Patient outreach Limited care for patients with comorbidities	Tapping into local resources (e.g., ambulance) Personalized treatment plans	Partnerships with local community (e.g., midwives) *"If you go to the patient, then the patient … would not be absent … I had my midwife partner with him and he comes back only on [weekends] to the STC* [satellite treatment center) *and he's okay. I'm also monitoring the midwife if the patient goes to the barangay health station every day."* **P7**
3. Infection control practices, fear, and limited capacity in rural health centers	Facility stigma Limited knowledge	Mentorship	Professional development; oversight; info campaigns *"[An]information campaign would help … in the health centers … A misinformed healthcare worker is very tragic … The stigma comes from the barangay health workers or the informal health workers who are not trained properly."* **P1**
4. Financial insecurity due to program reimbursement mechanisms	Time lags Out-of-pocket costs	Patient volunteers/role models	Nutritional support *It would be better for the program to sustain or maintain the allowance or the enablers that they are giving to patients … like the food package … Since 2018, there is no more enabler like the food package … most of our patients are now interrupters. I think [the food package] is a motivational thing for patients to continue their treatment."* **P11**
5. Local government support is limited and requires more involvement in support of DR-TB elimination activities	Limited cooperation Limited financial support	TB advocates leveraged to engage local government	Economic opportunities; free transportation; advocacy *Whenever a patient is enrolled from that particular LGU, they will give 10,000 pesos [$206 USD] … They can actually [create] a small business … Right now, if they're just given the exact amount [of transportation] … what will happen to the family?.. It may not be monetary, but if [the LGU] can give, work for the mother or for some of the older children just to augment their living. That could also help these patients to continue with the treatment.* **P5**

3.4. *Infection control practices, fear, and limited capacity in rural health centers*

Many respondents reported having patients who requested to return to TB treatment centers that were located further away once they were decentralized closer to community/peripheral health centers due to negative experiences with staff. This prevented the use of their transportation allowance on other basic needs according to nurses and physicians.

> [Decentralized patients] … feel [staff] act like they're dirty or they're germs, 'nandidiri,' and that you should wear a mask just [for] touching her hand … our patients will say, 'Can we just continue here? I don't care if it's too far (P6).

To lessen stigma and reduce fear, some respondents mentioned working closely with rural health units to increase local staff knowledge about TB infection control and prevention measures.

> I tell them that … they have to practice necessary measures … I make sure that the patient doesn't feel judged … I want the patient to take their medicines in the most convenient way without compromising the quality of service." (P9, Project-hired nurse, Central Region).

Recommendations from respondents included professional development opportunities, oversight of personnel, and information campaigns to curb stigma in the community.

3.5. *Financial insecurity due to program reimbursement mechanisms*

Delays in program reimbursement mechanisms drove many health staff to support patients financially. One participant described paying 500 pesos (10USD) a month for the tuition fees of her patients' two daughters as an incentive to ensure that she remained in treatment.

> The barrier is budget because sometimes our revolving fund is depleted so we have to use our own money … [For the] cash advance, you must foresee, or we have to identify activities a month before … [so] it's about two to three weeks before it gets approved (P10).

In other facilities, respondents reported that patients who successfully completed treatment were employed as volunteers or were directly hired as clerks or janitors with help from the LGU.

> One patient who graduated from the category 4 treatment … we convinced [the mayor] to hire him … so that the population would know that there still is life after category 4 TB … I have two graduates. One is a clerk, and one is a janitor in the health center (P7).

Most respondents alluded to the nutritional support that was discontinued in 2018 and suggested similar supports for patients and their families to meet basic needs and maintain them in care.

3.6. *Local government support is limited and requires more involvement in support of DR-TB elimination activities*

Many respondents felt that local government cooperation was limited and that municipal political leaders did not prioritize the TB program, given the small budgets allocated for health.

> I haven't encountered a visit [from] LGU [local government unit] … I really need their help … I don't know if they have much budget for the health and I think they are more on the business side (P6).

Those that did enjoy some level of local government support, reported on the contexts in which the support developed, and how local data, advocacy, and TB champions in the community were leveraged to inspire local leaders to commit to financially supporting the program.

> The local chief executive or mayor, is very supportive of the program … we have four persons in the PMDT facility that only cater to DR-TB … The mayor had a clear vision of what could happen … since it was based on the data, the situational analysis of tuberculosis in the municipality (P7).

Participants advocated for LGU support through livelihood programs, LGU-sponsored community boards to support medication adherence, and the development of a memorandum for every local barangay to ensure patients accessed free transportation.

4. Discussion

This study explored individual healthcare worker perspectives across individual, interpersonal, community, health system, and policy levels, best practices, and suggestions for enhancing DR-TB care across six populous regions in the Philippines. We conclude with programmatic strategies for application in the field.

Our study findings provide further evidence that healthcare workers do not feel fully capable of providing therapeutic counseling to patients. In Metro Manila, the type of social support provided by nurses has shown to directly influence treatment outcomes among patients with TB.[5] Whether as a consequence of drug side-effects from an arduous treatment regime or the challenge of living with DR-TB, depression, and anxiety have been linked to patients with DR-TB.[17] Simple psychological support packages help patients and families and provide relief to those experiencing social isolation, stigma, and poverty.[17] However, nurses also need to be adequately trained on effective counseling techniques periodically through mentoring/peer coaching opportunities in order to be better equipped to provide holistic care to patients.[18]

In our study, we found that differences in role and job function between permanent and project-hired nurses created tensions around professional career progression. Limited professional support and staff disempowerment has been linked to lower staff retention rates, and low observance of infection control guidelines.[19] In South Africa, nurses' emotional distress due to fear of contagion, lack of material

resources, and poor infection control practices highlighted the need for in-service education and supervisory support.[20] While guideline training and visual job aides to target individual knowledge, staff motivation, and health worker stigma have been developed, their efficacy is unclear.[21] Targeted educational interventions for nurses and efforts to improve inter-professional communication among healthcare workers have shown promise in improving and sustaining TB infection control measures at health facilities.[19,22] Further research is required to better understand the needs of the staff in order to build comradery, encourage observance of infection prevention guidelines, and promote healthy work environments.

With the ongoing implementation of the Universal Health Care Act in the Philippines, and renewed commitment to strengthening primary health care, the scale-up of evidence-based person-centered approaches are currently needed and more relevant than ever.[23] Our findings suggested obstacles to decentralizing patients, coordinating care across multiple levels and sectors, and providing integrated care to patients with comorbidities.[11] With the support from midwives and community health workers, tailored treatment plans and personalized multi-component adherence packages of patient support were implemented by some providers in our study through community or home-based directly observed therapy (DOT) as advocated by WHO.[24] Other methods such as video DOT, non-daily DOT, unsupervised treatment, using only oral regimens against DR-TB, and digital adherence support technologies could allow for greater flexibility in the administration of treatment and help reduce financial constraints.[10] Nonetheless, programs need to monitor and evaluate their successes and challenges regarding innovations to identify which combinations work best for their patients.[24]

Catastrophic costs associated with TB are still considered a significant impediment to TB treatment completion in the Philippines as it further pushes patients into the cycle of poverty.[4] Our study adds to the growing body of literature on the policy-related barriers impacting care delivery to patients with DR-TB and the fundamental role that financial insecurity plays through reimbursement mechanisms that trigger patient and health worker vulnerability.[25,26] Administrative barriers relating to reimbursement processes and delays described in our study placed an unfair burden on providers to support patients with personal financial contributions. In the Philippines, strong multi-sectoral platforms for DR-TB have been suggested for achieving programmatic amendments to legislation for the provision and use of reimbursements with local government support.[25,27,28] However, such recommendations are unfeasible without strong advocacy efforts from local leaders, TB champions, and civil society to raise revenues for TB health services at local levels and evidence-informed decision-making rather than politically-motivating funding decisions.[28]

There are several limitations to this study. First, the study is specific to the lived experiences of its participants and is not generalizable to all clinicians. Because our sample was drawn from a third of the 18 regions of the country, findings may not be representative of the country at large. However, the healthcare workers interviewed represented 6 of the 18 regions in the Philippines, where over 50% of the population resides.[29] Of these six regions, the "Big Three" regions (i.e., those with the largest population centers including Regions III, IV-A and the NCR), were represented. Regions III, IV-A and the NCR are priority areas for the National TB Program, which has recently focused additional resources for scaling up TB interventions and initiatives, such as community-based screening campaigns.[30] Therefore, our findings have meaningful policy implications for guiding policy decisions in the areas observed and potentially others if additional data is collected in other regions. The addition of the voices of patients could have provided yet another layer to understand the factors impacting retention and adherence to medication. Second, although the sample size was restricted to 11 healthcare workers, saturation was reached (i.e., the point at which no new information or themes are observed in the data) at 11 interviews given the longer interview time (1.5–3 hours), which allowed for a greater and richer amount of information to be obtained per person. This increased the retrieval of salient items, and has been achievable in samples of at least 10 persons.[31–33] Our third limitation was that all interviews were conducted via phone rather than in-person, which could have affected the quality of data collected.

5. Conclusion

The increased vulnerability experienced by patients with DR-TB underscores the urgent need to develop person-centered care plans for patients. This study focused on qualitative interviews with frontline treatment providers. It revealed that perceptions related to patient care are multi-faceted and will require a multi-faceted targeted approach. Understanding the significant role that DR-TB frontline staff play in curbing the TB epidemic and how their needs and recommendations are impacted by daily interactions with patients, other care providers, health systems, and political leaders, provide critical information for researchers, medical administrators, and providers charged with implementing policy, interventions, and supports to ensure the well-being of patients with DR-TB in the Philippines.

Author contributions

Study conception and design: JJ.
Data collection: JJ and YE.
Data analysis and interpretation: JJ and YE.
Drafting of the article: JJ.
Critical input and revision of the article: All authors.

Conflicts of interest

The authors have none to declare.

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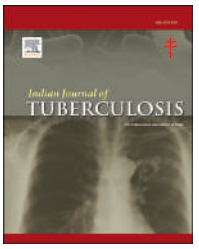

Case report

Can Oral TB develop in susceptible individuals after an oral surgical procedure? 3 case reports

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ABSTRACT

As opposed to the popular assumption, there have been an increase in the cases of Oral Tuberculosis as of late. Owing to increased drug resistance, there has been a change in the disease pattern leading to an upsurge in the Extra-pulmonary Oral Tuberculosis. According to the WHO, Diagnosis is the first step in the control of TB; but due to the lack of pathognomonic signs associated with Oral Tuberculosis and the rarity of these lesions, diagnosis is often difficult. So, to enable a timely diagnosis, we point out the occurrence of such lesions in the post-operative refractory lesions in susceptible individuals. However, a thorough search of literature did not yield any conclusive results. In this paper we present the clinical, radiographic and histopathological findings of three cases between the ages of 5 and 50 years old who were diagnosed of Oral Tuberculosis. These patients have undergone a recent oral surgical procedure prior to the development of Oral TB lesions. More research is required to increase the awareness of the pattern of this disease and to enable a quicker diagnosis so that the overall morbidity and mortality is reduced.

1. Introduction

Even in this era of antibiotics and advanced diagnostic methods, Tuberculosis is the leading infectious disease killer in the world, exceeding HIV (Human immunodeficiency Virus); claiming 1.8 million lives every year.[1] Oral TB (Tuberculosis) is extremely rare and accounts for 0.5–5% of Extrapulmonary TB.[2] The basic strategy to reduce the morbidity and mortality is to accurately diagnose and provide timely treatment to infected individuals.[1] Although the diagnostic criteria for Pulmonary TB is well established, diagnosis of Oral TB is difficult because it lacks pathognomonic features.[3] The aim of this paper is to enable a quicker diagnosis by answering the question whether Oral TB occurs following an invasive Oral Surgical procedure/breach in the mucous membrane in susceptible individuals.

Two predominant types of oral TB are recognized – Primary and Secondary. Patients with Primary Oral TB do not have an accompanying pulmonary TB while Secondary TB is believed to result from either hematogenous or lymphatic spread; autoinoculation by infected sputum or direct extension from nearby structures. However, a breach in the oral epithelium or trauma is alleged to cause either types of TB.[4–7]

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Although many case reports of Oral TB following oral surgical procedures have been published, no result could implicate a breach in the mucous membrane as a contributory factor which could lead to Oral TB. A thorough search of literature did not reveal any conclusive reviews.

We report three cases of patients between the ages of 5 and 50 years. Each of them presented with chronic refractory oral lesions which resembled post-surgical site infection following mandibular resection, mandibular osteomyelitis and submandibular space cellulitis/lymphoma respectively. These were eventually diagnosed as Oral Tuberculosis. We also noted that despite varied presentation, all the patients in our paper have had a previous history of Oral surgical procedure following which a refractory lesion developed. All the patients were treated with Surgery combined with Anti Tubercular treatment (ATT) before being declared completely free of TB.

2. Case 1

A 27-year-old male presented to the department of Oral and Maxillofacial Surgery department with a swelling in the left submental, submandibular and retromandibular region and history of febrility. History revealed that the swelling was sudden and gradual with no dysphagia and wasn't associated with any discharge. Three weeks prior, the patient underwent segmental resection of Left mandible and reconstruction with Anterior Iliac Crest following OKC (Odontogenic Keratocyst) (Fig. 1). On examination, the swelling was soft, mildly tender and warm to touch. Ultrasound revealed 15 ml of collection. Provisionally, the patient was diagnosed with Facial space infection secondary to graft failure. Incision and drainage were done and parenteral antibiotics were started but culture results were inconclusive. Since low grade fever persisted, thorough blood investigations were ordered which revealed an elevated ESR (Erythrocyte sedimentation rate) and Leukocytosis. Chest radiograph revealed right sided pleural effusion for which pleural tap was performed. He was diagnosed with Right sided Pleural TB based on the exudative and Lymphocytic predominant nature of pleural fluid analysis. PCR (Polymerase Chain reaction) of the pus sample from the oral lesion was positive for TB revealing a secondary lesion at the oral surgical site. He was initiated on antitubercular therapy with Rifampicin, Isoniazid, Ethambutol and Pyrazinamide following which a significant improvement was noted both in the Oral and Pulmonary lesions; he was declared disease free six months after the commencement of ATT. Thereafter, the patient was reviewed regularly for 2 years during which period He remained disease free; both the surgical site and graft healed well.

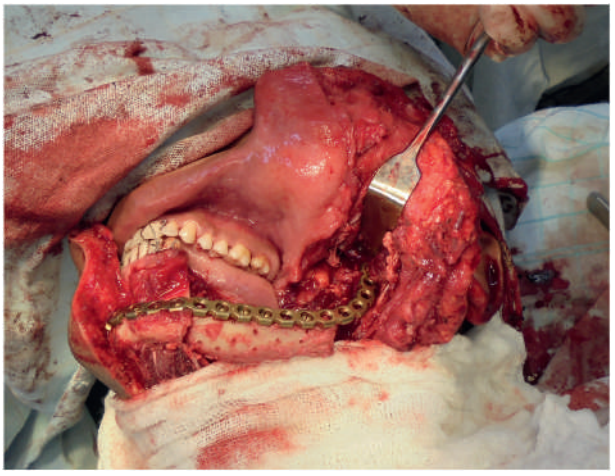

Fig. 1 – Mandibular reconstruction with Iliac Crest graft following Mandibular resection for OKC.

3. Case 2

A 50-year-old gentleman presented with the chief complaint of swelling over the right lower third of the face and severe continuous pain for 6–7 months. The swelling was gradual and associated with reduced mouth opening and paresthesia. A year back, he was diagnosed with Chronic suppurative osteomyelitis of the right mandible for which He underwent extraction of right mandibular first and second molar with sequestrectomy; histopathology report revealed acute bacterial osteomyelitis. The surgical site and extraction sockets healed well following surgery and remained symptom-free for 6–7 months. On clinical examination, a tender, diffuse and irregular expansion of both the buccal and lingual cortices of right mandibular body was palpated; first and second molars were missing. Extraorally, a diffuse hard and lumpy swelling over the right mandibular body was present; however, Lymph nodes could not be palpated. OPG revealed a mixed radiopaque radiolucent lesion with a continuity defect (Fig. 2). A provisional diagnosis of Osteomyelitis of the mandible with pathological fracture was made. The patient underwent segmental resection of the mandible and reinforcement with reconstruction plate under General Anesthesia (Fig. 3). Postoperatively, the surgical wound showed dehiscence and erythematous wound margins.

Histopathology of the resected specimen revealed Tubercular Osteomyelitis. The patient was immediately started on ATT under Directly observed treatment short course (DOTS) program. The surgical wound margins immediately improved in healing after starting the treatment; the patient is on regular follow-up and is currently disease free.

4. Case 3

A 5-year-old boy was referred to the department of Oral and Maxillofacial Surgery from the department of Pedodontics and Preventive Dentistry when he developed a painful swelling with punctum on the right submandibular region since a week. Two weeks back the patient underwent extraction of right mandibular deciduous first and second molars due to periapical abscess. Post extraction, the socket healed satisfactorily with no adverse sequelae. After a week, a swelling developed which gradually increased in size. Medical history did not reveal anything significant. Intraoral examination revealed a well healed extraction socket with no tenderness of the adjacent teeth and surrounding periodontium. Extraorally, a diffuse, tender, non-fluctuant swelling with localized

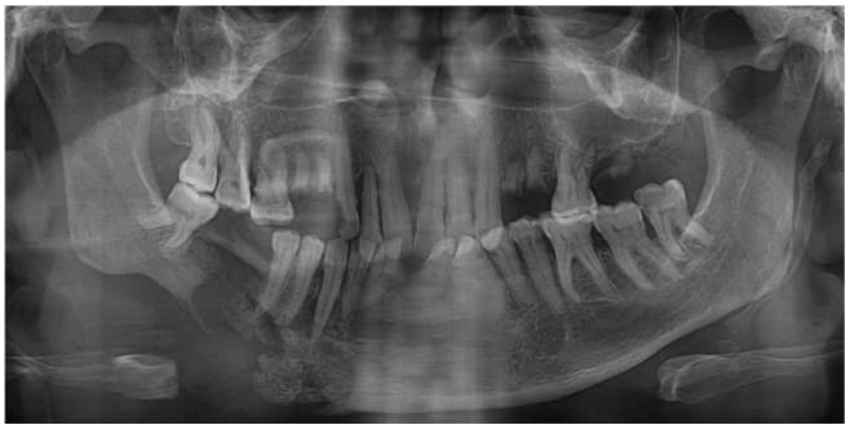

Fig. 2 – A diffuse radiopaque-radiolucent lesion with continuity defect and missing first and second molars of right mandibular body.

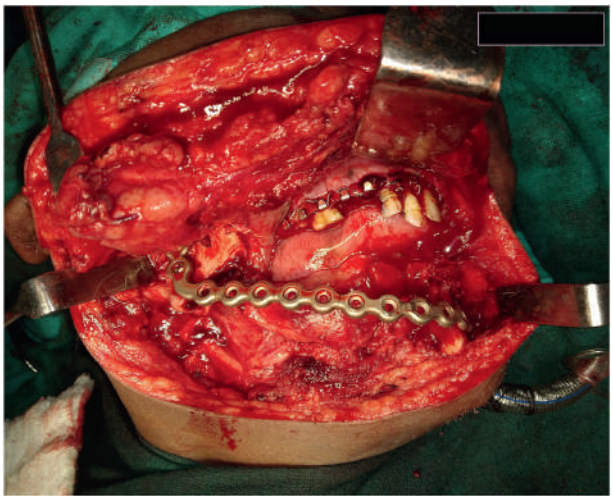

Fig. 3 – Mandibular resection followed by Reconstruction plate reinforcement for osteomyelitis.

raise in temperature without associated discharge was palpated (Fig. 4). Differential diagnosis of Lymphoma, submandibular space cellulitis and Tubercular lymphadenitis was made. The patient was advised oral antibiotics and analgesics. On persistence of the lesion despite local measures, ultrasound guided FNAC was performed which tested positive for TB-PCR. Patient was referred to TB center and was started on ATT under DOTS program. The patient is on regular follow up and the swelling has completely regressed post ATT.

5. Discussion

Tuberculosis is a communicable disease which is characterized as a chronic granulomatous infection caused by Mycobacterium Tuberculosis. According to the (World Health Organization) WHO, the incidence of TB in 2018 was approximately 10 million globally and the mortality was 1.3 million, however, Oral TB is very rare and accounts for only 0.05–5% of Extrapulmonary TB cases.[2,8] India is endemic for TB and bears 1/3rd of the Global TB burden which influences the global TB

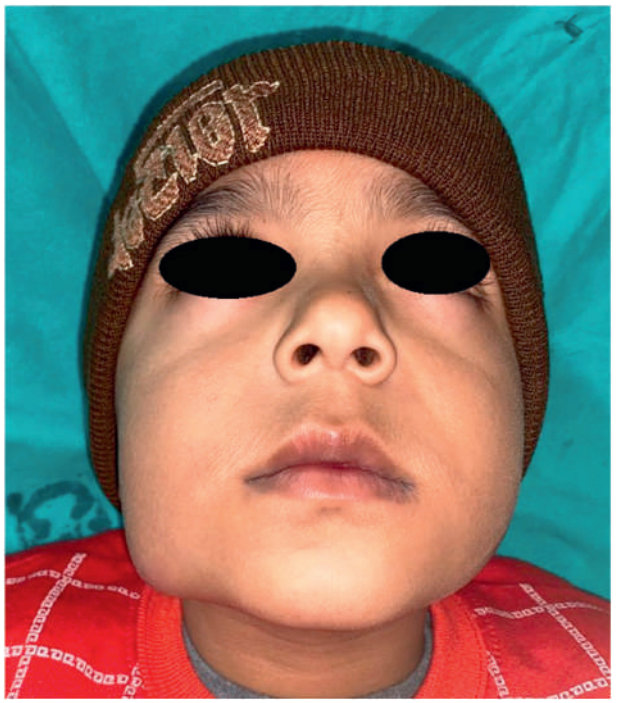

Fig. 4 – Diffuse swelling on the right submandibular region in a 5 year old Boy.

affliction by migration of either active/latent TB individuals.[9,10] With the strain of the current (Coronavirus disease – 19) COVID-19 pandemic on the healthcare systems of low- and middle-income countries, a 20% increase in the incidence of TB is projected due to delayed diagnosis and treatment.[11] Nevertheless we opine that the widespread use of masks, maintaining social distance and avoiding large gatherings could lead to a decline in the incidence of TB.

Studies have shown that 50–80% of patients with smear-positive TB will succumb to their disease if undiagnosed and left untreated.[12] The similarities in the symptomatic presentation of SARS COVID and TB have a masking effect on the diagnosis of TB.[13] Although, oral lesions are uncommon, they are crucial for diagnosis and interception of systemic TB.

Diagnosis of oral TB is most often by exclusion because: lesions are rare; have multiple forms of presentation; do not have pathognomonic signs and most importantly Primary Oral TB could present without any respiratory symptoms.[3,4,14]

The three cases presented in this paper had no preexisting history, symptoms, characteristic signs and radiographic features of any form of TB. All the patients underwent oral surgical procedures; subsequently, refractory lesions developed in the same region of surgery which mimicked various oral lesions as mentioned earlier. These lesions were refractory to local measures and were eventually diagnosed as oral TB.

The route of Tuberculous Bacilli to the oral cavity is unsettled; owing to the resistance of oral mucous membrane and inhibitory action of saliva against the bacilli. Direct endogenous/exogenous inoculation and hematogenous/lymphatic spread are believed to cause oral TB; however, a break in oral epithelium or trauma is alleged to cause oral TB.[6,7,15]

The pattern noticed in our patients is very similar to existing reports of patients that underwent a recent invasive oral surgical procedures pursuant to which, the refractory lesions which developed were diagnosed as Oral TB.[16–21] Chaudary et al have shown that the spread of infection may be through an extraction socket or mucosal opening associated with an erupting tooth.[22] In a case series published by Wang et al, 5 out of 7 individuals with cervical TB lymphadenitis have had a previous history of Tooth extraction.[21] Meng et al state that the mandible may be involved from extension of a tubercular lesion of the mucous membrane or from infected gingivae.[23]

Case reports of exogenous inoculation following oral surgical procedures have been documented. Smith et al reported an outbreak of oral TB among patients that underwent an invasive dental procedure by a dentist with preexisting pulmonary TB.[16] Remarkably, those who were only examined did not develop Oral TB. Petti states that Dental Health care workers (DHCW) with active pulmonary TB who do not conform to Infection control protocols could be responsible for Primary Oral TB of the surgical site and occasionally active pulmonary TB.[24]

Although case reports to corroborate trauma as one of the precursor conditions for Oral Tb are existing, Shengold and Shengold state the contrary due to the following reasons: all sputum positive patients who undergo oral surgical procedures don't develop oral TB and patients with negative sputum nevertheless develop oral lesions.[15] Therefore, the association of Oral TB to a preexisting extraoral TB is non-conclusive; however, any chronic, refractory postoperative oral lesion could be Tuberculous.

Most studies published on this subject state that Oral TB should be suspected for any chronic oral lesion but, we opine that the chronicity of the lesion combined with a history of an oral surgical procedure should particularly warrant a differential diagnosis of Oral TB. This pattern described in our paper could be a significant clinical clue to enable a quicker diagnosis of Oral TB. It is essential for Dental Healthcare workers in TB endemic countries to be able to identify Oral TB despite its varied presentation. Our patients completely recovered following surgery combined with ATT. More studies are required to determine the pattern and predisposing conditions for Oral TB.

6. Conclusion

Although the occurrence of Oral TB is rare, there is a strong likelihood of its presentation in longstanding, non-responsive post-operative lesions. Early detection of the disease results in complete cure of the lesion and reduces morbidity.

Conflicts of interest

The authors have none to declare.

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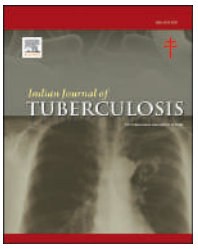

Case report

Molecular detection of *Mycobacterium tuberculosis* complex in a captive aguará popé (*Procyon cancrivorus*) with macroscopic tuberculosis like-lesions

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ABSTRACT

Tuberculosis is a chronic and contagious infectious disease caused by multi-host species of the genus *Mycobacterium* grouped within the *Mycobacterium tuberculosis* complex. These pathogenic bacteria mainly affect mammals, including humans. The most recognized species is *Mycobacterium bovis*, the causative agent of bovine tuberculosis in livestock. Although livestock is the main host of *M. bovis*, this species is frequently isolated from wild animals. Wild native mammals from Central and South America, as the crab-eating raccoon or "aguará popé" (*Procyon cancrivorus*), may act as a source of tuberculosis and may represent a human health risk, especially in captive scenarios, due to closer animal-human interaction. However, the only presence of infection in wild animals is not enough to determine their epidemiological role in the disease. Here we identify tuberculosis in a captive aguará popé with clinical signs and lung macroscopic tuberculosis-like lesions during necropsy. We detected tuberculosis by polymerase chain reaction assay. DNA was extracted directly from lung tissue and the amplification target was the insertion sequence 6110. This study contributes to investigate the presence of the disease in wild native animals of Argentina and supports the knowledge that wild mammals may act as a source of TB for humans and domestic animals.

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1. Introduction

Tuberculosis (TB) is an endemic and reemerging infectious disease that generates hundreds of deaths and a significant economic cost around the world each year.[1] Globally, about 10 million people fell ill whit TB in 2019.[2] TB is caused by species of the genus *Mycobacterium* grouped within the *Mycobacterium tuberculosis* complex (MTBC). MTBC species are multi-host pathogenic mycobacteria with a high genetic similarity.[3] The most recognized members of MTBC are *M. tuberculosis*, a human-host adapted species, and *Mycobacterium bovis*, an animal-host adapted species.[3] In livestock, bovine tuberculosis (BTB) is a zoonotic disease cause by the latter *M. bovis*. Although livestock is the main host of *M. bovis*, this species is also frequently isolated from wild animals. The infection with *M. bovis* has already been reported in more than 85 animal species around the world.[1] In the Americas, TB caused by *M. bovis* in wild animals has been reported as well.[4] Moreover, the prevalence of this endemic disease is known in livestock but is unknown in wild animals.[5] However, the only presence of infection MTBC in wild animals is not enough to determine if they play an epidemiological role within the disease.[7] In this sense, more studies are needed to establish the role of wild animals in BTB in Argentinean ecosystems.[8]

The aguará popé (*Procyon cancrivorus*), also called "crab-eating raccoon", is a wild carnivorous mammal native to Central and South America.[9] The population of aguará popé is likely to remain stable throughout South America, thus its species conservation category is "least concern" (LC).[10,11] In the environments in which it is distributed it has not been established yet whether this species plays any epidemiological role in BTB or other zoonotic diseases.

Animals and humans affected by TB may develop a characteristic macroscopic lesion with a nodular and irregular appearance, a firm consistency and a caseous center. Although these lesions are quite suggestive of TB, its etiology must be confirmed.[12] In wild animals, TB diagnostic tests commonly used are bacteriological culture (as the gold standard method), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).[13–15] Our aim was to confirm TB in a captive aguará popé from Argentina with tuberculosis-like lesions (TBL), applying molecular and microbiological diagnostic tests.

2. Materials and methods

This wild specimen of aguará popé was captured in Misiones (Argentina) when it was a few months old, and it has been kept as a pet in Buenos Aires (Argentina) since then. The animal lived apparently healthy for 15 years until it showed signs of health deterioration. Veterinary clinical evaluation showed loss of teeth, shaggy hair, poor body condition and respiratory signs (mucus and labored breathing). The veterinarian used amoxicillin-clavulanic (for 15 days), cephalexin (during the next 20 days) and azithromycin (for 10 days after that) as an empirical antibiotic therapy. Those antibiotics were alternated because the patient did not show clinical improvement. The animal died after 45 days of treatment. At necropsy, multiple macroscopic TBL were observed in both lungs (Fig. 1).

TBL samples were sent to the Mycobacteria Veterinary Diagnostic Laboratory of the School of Veterinary Sciences, University of Buenos Aires. For bacteriological culture, TBL samples were homogenized and decontaminated by Petroff's method using 4% sodium hydroxide. After decontamination process, the samples were cultured in specific solid media: Stonebrink medium (in duplicate), containing pyruvate for the specific isolation of *M. bovis*, and Löwenstein Jensen medium, containing glycerol as carbon source for the isolation of *M. tuberculosis*. All cultured media were incubated at 37 °C and observed for 12 weeks (Jorge et al., 2005). In parallel, DNA extraction from TBL was performed using the commercial DNA Puriprep T-kit (InBio Highway, Argentina). We used sterile distilled water as a negative control on this step. From extracted DNA, PCR was performed to amplify the insertion sequence *6110* (Hermans et al., 1990), a target sequence conserved within the MTBC. In PCR, we used the reference strain of *M. bovis* AN5 and sterile distilled water as a positive and negative control, respectively.

3. Results and discussion

MTBC was molecularly confirmed by PCR IS*6110* performed on DNA extracted directly from the TBL samples. Although the isolation of the pathogenic mycobacteria could not be achieved, the detection of *Mycobacterium* DNA belonging to the MTBC, together with TB clinical signs and TBL, are quite suggestive of a case of tuberculosis in this aguará popé.[16,17]

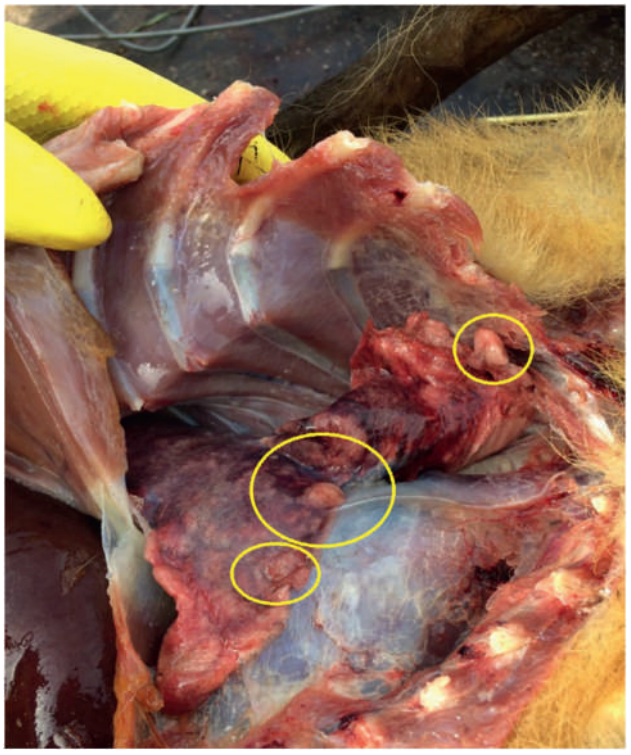

Fig. 1 – TBL in the right lung of an aguará popé (*Procyon cancrivorus*) observed during necropsy. TBL are shown inside the yellow circles.

Bacteriological culture is the gold standard test for TB diagnosis. Nevertheless, its sensibility is variable (69–78%).[18] The result of the test depends on mycobacteria viability, and that is its main limitation. This result also depends on the initial load of viable mycobacteria present in the sample, and the percentage of surviving mycobacteria after the decontamination process (Jorge et al., 2005). Even though we cultured three consecutive times, none of them resulted in positive isolation after 12 weeks of observation. In this sense, the mycobacteria viability in the sample may have been affected by an incorrect conservation method during its transportation and also by the antibiotic therapy administered to the aguará popé. Antibiotics used in this therapy were reported to be bactericidal for MTBC members (Cynamon and Palmer, 1983) and potential enhancers of antibiotic therapy used in the treatment of TB caused by multidrug-resistant strains (Ramón -Garciá et al., 2016).[27]

In contrast, PCR can sensitively detect the genetic material of the primary pathogen regardless of the viability of the mycobacteria.[17] Therefore, molecular diagnosis offers an advantageous alternative as a screening test for TB diagnosis in mammals[19] and its results can be obtained faster (2/3 days) than mycobacteria culture (12 weeks).[20] The need to obtain faster results becomes more relevant if we need to diagnose animals that maintain close contact with humans.[21]

In this work, we suspected TB infection in an aguará popé based on clinical sings and macroscopic TBL found during necropsy and confirm the disease by molecular diagnosis. However, MTBC species probably involved as the causative agent of TB in this aguará popé could be either *M. bovis* or *M. tuberculosis*, regarding to the epidemiology of this case. First, *M. bovis* infection may have been acquired in the wild or by feeding contaminated raw meat or milk in captivity.[22] On the other hand, *M. tuberculosis* may have been transmitted by its owners. It is known that *M. tuberculosis* have humans as primary hosts. The exposure of wild animals to *M. tuberculosis* becomes higher when the animal-human interaction is closed due to mascotism or captivity life.[23,24] However, neither of those scenarios was confirmed.

Finally, we should improve community education to prevent TB and other zoonotic diseases, emphasizing the risks that arise from maintaining a close contact between wild animals and humans. Moreover, captivity generates negative experiences in wild animals that impacts on their welfare[25] and also could impact the health of both animals and humans.[23]

In conclusion, we detected MTBC by PCR from an aguará popé with TBLL for the first time through the amplification of IS*6110*. This study supports the knowledge that wild mammals may act as a source of MTBC, especially when MTBC members are involved in scenarios that generate close contact between wild animals and humans[24,26] and contributes to investigate the presence of the disease in wild native animals of Argentina.

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Dra. Marcela Martínez Vivot is in charge of direction of the UBACyT Project.

Conflicts of interest

The authors have none to declare.

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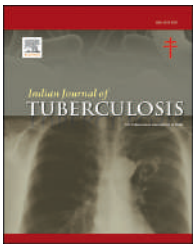

Case report

Odd presentations of skeletal tuberculosis: A case series

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ABSTRACT

Tuberculosis has been afflicting mankind since times immemorial and yet can still present itself in such a disguised manner that even the bests of experts may be duped. Any site from head to toe can be affected but certain sites are far less common than the others. We came across three inconspicuous manifestations at atypical sites-parapharyngeal abscess, wrist joint and foot ulcer. No other primary site could be identified in any case. Two cases were diagnosed microbiologically and one with radiological evidence. All the three cases were medically managed and depicted positive response.

1. Introduction

Tuberculosis is a chronic infectious disease caused by the resilient bacillus mycobacterium tuberculosis (M.tb). The disease is endemic to developing and underdeveloped countries and is responsible for chronic morbidity and associated adverse socioeconomic and psychological implications.[1] Although, tuberculosis can affect any organ of the body but most commonly the lungs are involved while extra-pulmonary tuberculosis (EPTB) accounts for only about 15–20% of all cases.[2] The extra-pulmonary sites are often secondarily involved via hematogenous or lymphatic spread from lungs or abdomen. It is rare for isolated involvement of a different site as a primary site.[3] These sites are notorious for atypical presentation and consequent deleterious delayed diagnosis that often leads to development of residual changes. Another challenge is availability of tissue for microbiological confirmation of diagnosis as often the representative sample is inadequate and not available for repeat access.[3,4]

Skeletal TB cases comprise of only about 10% of EPTB and roughly 1–3% of total TB cases.[2,5] Lower thoracic and upper lumbar spine are the most commonly affected sites in Pott's spine, which itself is the commonest site involved in skeletal TB. We present here three cases with unusual rare sites of involvement and conspicuous presentation- TB of upper dorsal vertebrae manifesting as parapharyngeal abscess; isolated wrist TB and tubercular foot ulcer. We had to rely on

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radiological evidence for wrist TB, while the other two cases were microbiologically confirmed. The patients were given the standard regimen for drug-sensitive TB under National Tuberculosis Elimination Programme (NTEP)[6] and showed improvement in follow-up.

2. Case 1. Parapharyngeal abscess as a presenting manifestation of Pott's spine

A-41-year old Indian male presented with complain of sensation of swelling in the floor of mouth and difficulty in deglutition since 4 months. He also felt mild pain in upper back in midline. There was mild fever in the evening and loss of appetite. There was no history of trauma or tuberculosis. On examination, he was thin built, anemic, afebrile, anicteric and had no clubbing, cyanosis or peripheral lymphadenopathy. Vitals were within normal limits. On oral examination there was edema in the floor of the mouth and pharynx more on the left side and tenderness in the D1 and D2 vertebrae. Neurological examination was within normal limit and there was no deficit. Auscultation revealed bilateral vesicular breath sounds.

Baseline investigations revealed the hemoglobin to be12.4 g/dL, total leukocyte count (TLC) of 11,700/mm3, 60% polymorphs, 37% lymphocytes and 3% monocytes on differential count. Inflammatory markers were raised with C-reactive protein (CRP) of 9.4 mg/L and erythrocyte sedimentation rate (ESR) 35 mm/hour. On mantoux test there was 15 mm induration. Chest radiograph showed a reduced space between the first two thoracic vertebrae, which was further confirmed on antero-posterior and lateral radiograph of the cervico-thoracic spine. There was a large left sided parapharygeal abscess extending from the floor of the mouth to the D2 vertebra with osteolytic and spondyloarthropathic changes in D1 and D2 vertebrae on magnetic resonance imaging of the spine (Fig. 1). The pus was aspirated intra-orally and submitted for microbiological studies. The cartridge based nucleic acid amplification test (CBNAAT) was positive wherein Rifampicin sensitive M.tb was detected. The patient was registered under NTEP and was initiated on Rifampicin (R), Isonizid (H), Pyrazinamide (Z) and Ethambutol (E) as per the guideline.

3. Case 2. Isolated wrist swelling

A young adult Indian male (aged 20 years) had mild pain and a gradually increasing swelling in the right wrist joint since last 18 months. He had started to experience difficulty in movements of the wrist since last 6 months. He had visited several local physicians during this time and was prescribed varying durations of non-steroidal anti-inflammatory drugs with minimal effect. The patient felt generalized weakness but denied any history of trauma or tuberculosis. Vitals were within normal range. On local examination, the wrist was warm with mild diffuse swelling. Tenderness was present; there was no fluctuation or local or distal lymphadenopathy. Routine blood investigations showed normal hemoglobin (13 g/dL) with TLC of 9800/mm^3 with 62% neutrophils, 33% lymphocytes and 5% monocytes. Platelet count was 1.67 lac/mm^3 with an elevated ESR of 27 mm/hour and CRP of 8.2 mg/L. The mantoux test was positive with 13 mm induration. With the differential of tubercular etiology in mind, a chest radiograph was done to rule out pulmonary TB along with gadolinium enhanced MRI right wrist. There was no parenchymal or pleural abnormality on chest x-ray while the MRI revealed post contrast enhancement in the distal ends of right radius, ulna and all the bones of proximal row of carpals (Fig. 2). A collection was also noted in the wrist space, which was aspirated and sent for smear examination, CBNAAT and liquid culture and sensitivity testing. The smear and CBNAAT for M.tb were negative and while awaiting the culture (which later turned up negative), the four-drug regimen (HRZE) was initiated on clinical and radiological basis after discussion with the orthopedic surgeon. The patient reported relief in pain and weakness on follow-up visit 4 weeks after initiation of therapy.

4. Case 3. Foot sinus

A 32-year-old Indian male had pain in left foot since six months for which he initially resorted to over the counter analgesics with little benefit. The pain persisted and gradually the movements of the foot became restricted. Four months back he developed an ulcer with oozing of non-foul smelling yellowish pus that did not grow any organism on pyogenic culture. The sinus did not heal despite receiving courses of broad-spectrum antibiotics from local doctors. However, the discharge from the ulcer confirmed presence of M.tb without the rpo-B mutation on CBNAAT and the patient was initiated on a private regimen of HRZE and referred to our center for registration under the NTEP program. He reported to us after a month of receiving the anti-tubercular drugs.

On examination, the ulcer was bone deep with inverted healing bluish-grey margins and necrotic base (Fig. 3). He had gained 3 kg weight in the past one month. A chest x-ray was done which was normal. He was registered under the national program, provided with the drugs according to his weight band and counseled for regular follow-up.

5. Discussion

Spinal TB is the most common type of skeletal TB with potential to spread along the different fascial planes causing extensive morbidity and likelihood of mortality if left untreated.[2,5] While TB can affect any live tissue in the human body, certain sites are very rarely involved.[7–11] Parapharyngeal space is one such potential space in the pharynx bounded medially by the superior constrictor muscle and pretracheal fascia and laterally by pterygoid muscles, anteriorly by ramus of mandible and posteriorly by the vertebral column; containing important neurovascular structures.[12] Most of the parapharyngeal abscesses are pyogenic in origin and are potentially fatal if not managed timely.[13] Patient presents with fever, dysphagia, odynophagia and swelling.[14] High-grade fever associated with oral symptoms point towards pyogenic cause; the chronic course of the disease associated with evening rise of temperature, fatigue, night sweats, weight loss and lack of response to broad spectrum antibiotics are indicative of

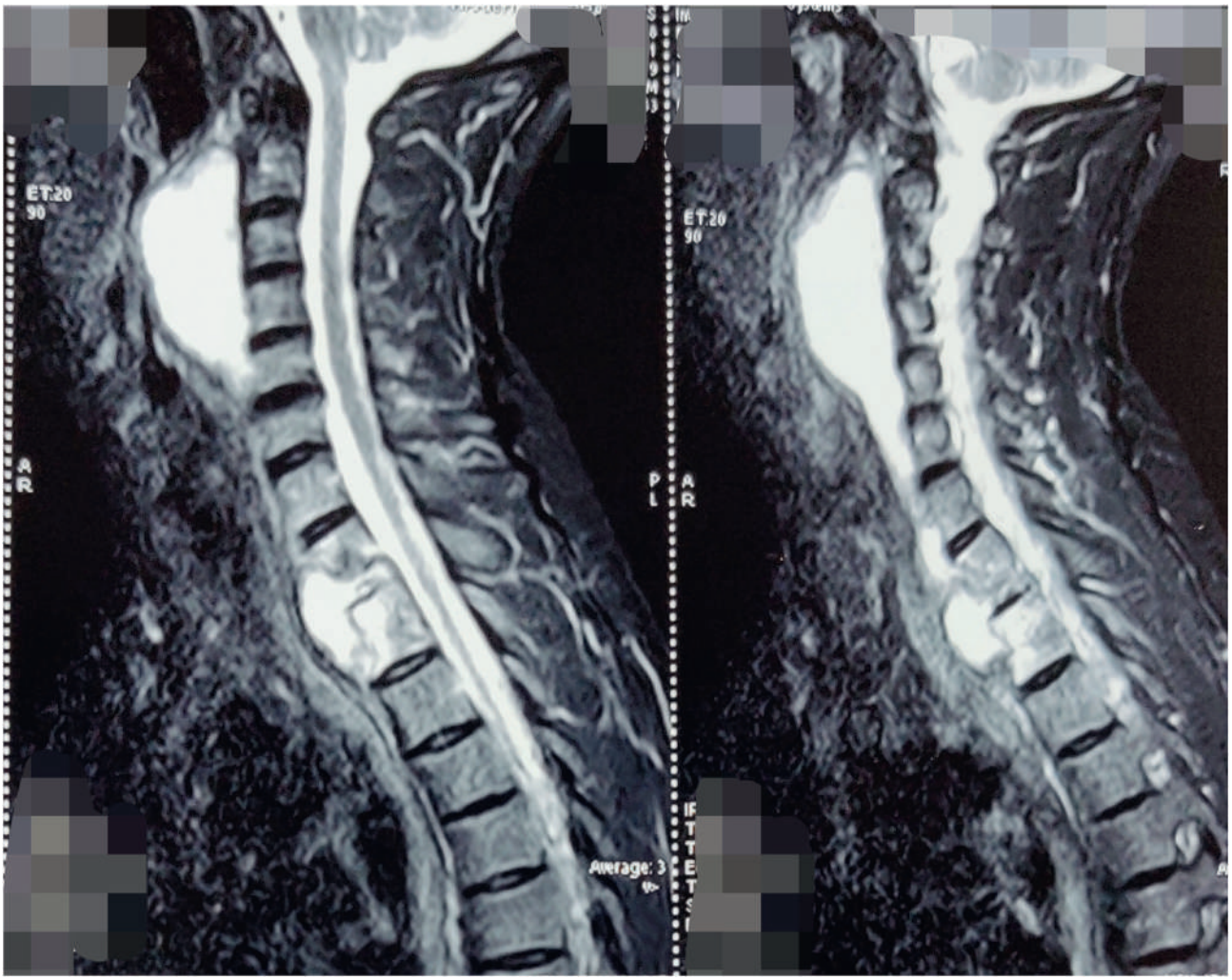

Fig. 1 – Sagittal section of magnetic resonance imaging cervicothoracic spine shows a large pocket of left sided parapharyngeal abscess extending from floor of the mouth upto D2 prevertebral level, ? Stress fracture of D1 vertebrae with ? osteolytic lesion of D1 and D2 vertebrae with bone marrow edema with associated spondyloarthropathic changes cause ? Pott's Spine.

tubercular etiology. Spinal involvement has more commonly been reported with retropharyngeal abscesses.[13,15] However, pharyngeal abscesses could also associated with pott's spine as in our case but there could be clinical latency.[16] Since last four cranial nerves pass through this space, a detailed nervous system examination should be done to assess the extent of involvement of the nerves. Cases of isolated tubercular parapharyngeal abscesses have been reported previously.[17–19] A tubercular abscess responds well to anti-tubercular therapy while a pyogenic abscess requires urgent incision and drainage. Only large tubercular abscess requires intra-oral or subcutaneous aspiration otherwise conservative management is sufficient as there is risk of formation of non-healing sinus with surgical management.[13,17–19]

Among skeletal TB cases, wrist is involved in only about 1% cases.[20–22] Capitate and radius are the most commonly involved bones. The involvement is either post-trauma or via hematogenous route.[20–22] Our patient denied the trauma history and had no abnormality on chest radiology. The subtle presentation of pain and swelling in the absence of respiratory involvement often delays the diagnosis leading to perpetual damage and residual deformity. Sinus formation may occur in complicated cases.[22,23] Fortunately, in our case, the anatomy was not disrupted yet so medical management alone sufficed in restoring complete range of movement. In advanced cases, surgical exploration and fixation may become a necessity along with anti-tubercular chemotherapy.[22,23]

TB of the foot or ankle is yet another rarity. There may be underlying tubercular osteomyelitis or arthritis that may present as swelling and pain or may burst forming an ulcer or a sinus. The delay in diagnosis ranges from 3 months to 5 years. Most cases reported are males due to more outdoor exposure.[24] Nearly half the cases have no pulmonary involvement as in our case as well. Like in other forms of skeletal TB, conservative management is adequate for uncomplicated cases, with surgical options reserved for refractory cases.[24–26]

Early diagnosis is the key to reduce morbidity in all forms of EPTB. Skeletal TB often gets confused with pyogenic,

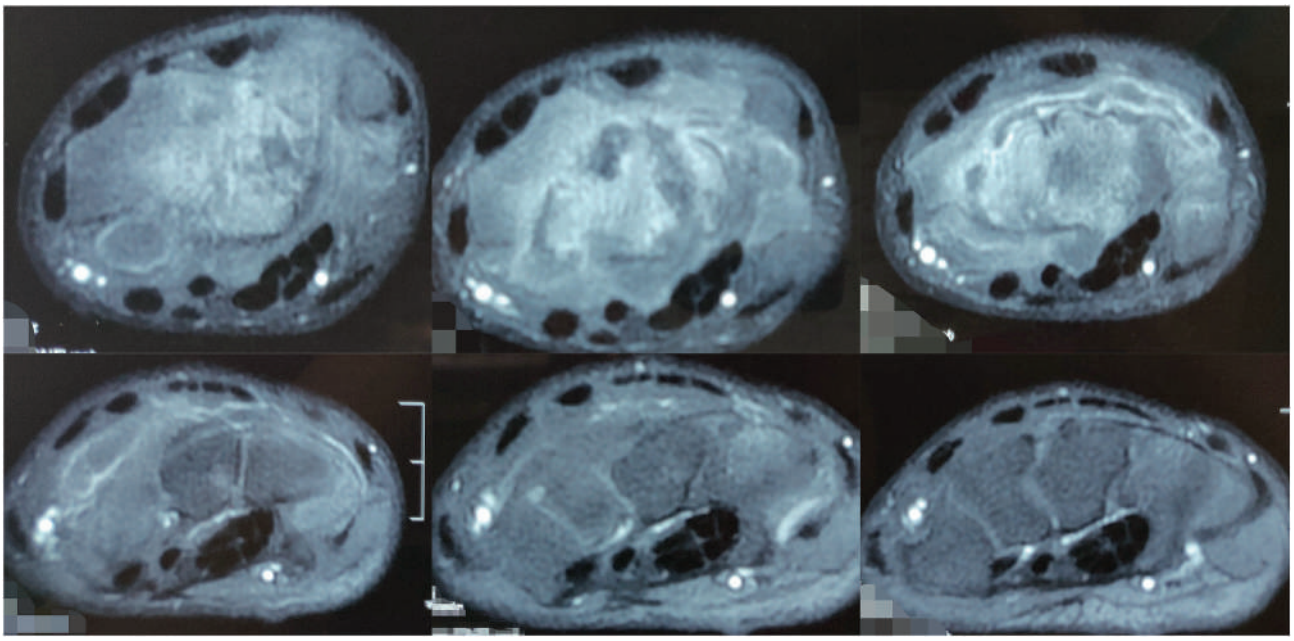

Fig. 2 – Magnetic resonance imaging right wrist shows patchy area of signal intensity alteration with post-contrast enhancement in distal ends of radius and ulna and scaphoid, lunate, triquetral and pisiform. Peripheral enhancing collection is noted in joint space extending into radio-ulnar joint and along inter-osseous membrane. The findings are suggestive of infective etiology ? Tubercular.

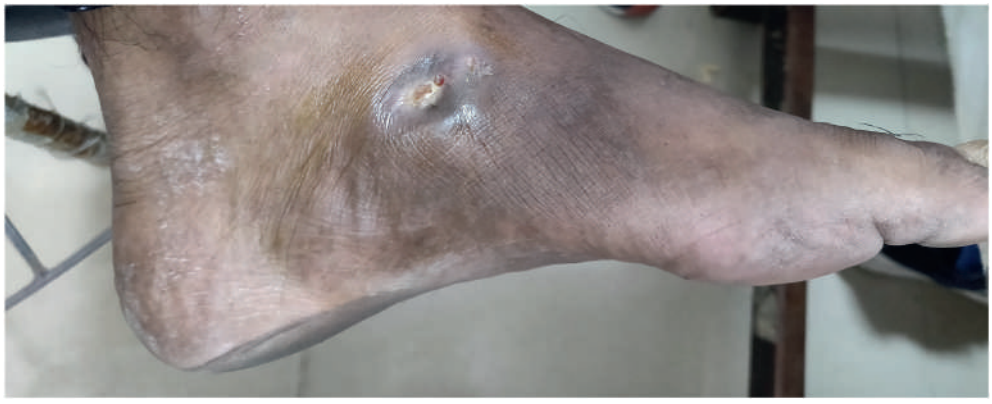

Fig. 3 – A 2.5 cm × 2 cm oval healing ulcer present medially on the dorsum of left foot showing bluish-grey indurated margins with sloping edge and part-scarred, part-granular, reddish-yellow base. Surrounding skin is thinned out and shiny.

rheumatoid or systemic inflammatory disorders.[24] Most cases reported have asserted the dominant role of clinical, laboratory and radiological features to obtain the diagnosis.[20–26] Microbiological evidence is scarcely reported. We were able to detect M.tb in two of our cases via CBNAAT. It was impressive that the microbiological diagnosis of the tubercular foot ulcer was made at a remote center in India. It is likely that strengthening of the NTEP and widespread availability of CBNAAT machines with ease of sample submission led to accurate diagnosis of drug sensitive TB which is a positive event. The treatment of skeletal TB is prolonged with respect to pulmonary TB and extends from 9 to 18 months depending on clinical and radiological follow-ups.[2]

None of our patients had any low-immunity state so as to make them more prone. Ergo, TB being endemic to our country with a rising number of drug-resistant cases even in extra-pulmonary cases, a high index of suspicion is required even in absence of immune-compromised status.[27,28] We assert repeated periodic sensitization of all specialties other than pulmonary is highly desirable so as to update them regarding the changing epidemiology and available diagnostic and therapeutic options.

6. Conclusion

Despite housing nearly one-fourth of the world's TB cases, sometimes the diagnosis is delayed even in an endemic country like India owing to uncommon presentation. A thorough history with an index of suspicion is required to timely diagnose and prevent irreversible complications.

Conflicts of interest

The author have none to declare.

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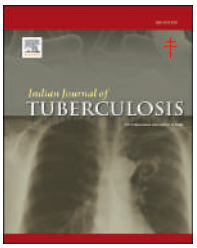

Case report

Disseminated multidrug-resistant tuberculosis and SARS-CoV-2 co-infection in a child with IL-12Rβ1 deficiency☆

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ABSTRACT

Mendelian Susceptibility to Mycobacterial Disease describes a spectrum of inherited defects, of which complete deficiency of the interleukin-12 receptor β subunit 1 (IL-12Rβ1) is the most common cause. This condition results in a predisposition to severe disease caused by mycobacteria. We report a case of disseminated multidrug-resistant tuberculosis with extensive central nervous system affection with SARS-CoV-2 co-infection, in a 4-year-old child with IL-12Rβ1 complete deficiency.

☆ All authors contributed to the writing of the final manuscript and acquisition of data.

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1. Introduction

Complete deficiency of the interleukin-12 receptor β subunit 1 (IL-12Rβ1 or MIM:614891) is the most common genetic etiology of Mendelian Susceptibility to Mycobacterial Disease (MSMD).[1] To present, only 213 patients from 164 families worldwide have been reported to have complete IL-12Rβ1 deficiency and possibly many others may have been diagnosed.[2] Mycobacterial disease is the most prevalent infection in IL-12Rβ1 deficiency due to an increased susceptibility to the manifestation of severe type infection in these patients and has been reported in 80% of them.[2,3] Complete IL12Rβ1 deficiency has incomplete clinical penetrance; In other words, some patients are asymptomatic.[4]

Each year, 1 million children contract tuberculosis (TB), of whom 210,000 die because of the disease. Approximately 10% of all TB cases have been reported to occur in children, and in this age group deaths are usually caused by Central Nervous System (CNS) TB or disseminated disease.[5]

To our knowledge, only three patients with IL-12Rβ1 deficiency and disseminated multidrug-resistant tuberculosis with CNS TB have been reported.[3,6,7]

We reported a child with disseminated multidrug-resistant tuberculosis and SARS-CoV-2 co-infection due to IL-12Rβ1 deficiency.

2. Case report

A 4-years-old child, born in March 2021, the second son of a non-consanguineous marriage, from Tenango del Valle, México. He was premature (32 weeks). Neonatal screening test of congenital hypothyroidism, galactosemia, adrenal hyperplasia, phenylketonuria, and biotinidase deficiency, was normal and his sibling is healthy. Neurodevelopment was normal. History of non-specified severe infections in her family and any other relevant medical information.

He received BCG vaccine at birth, and 3 months later a local edema appeared, which self-resolved one month later without treatment. Seven months later, showed 2 retro auricular masses that got resolved without treatment. In June 2019 (at 2 years), a regional left armpit lymphadenitis due to BCGitis was diagnosed, and received rifampicin for unknown time without improvement.

On April 2020, the patient was remitted for the first time to our hospital and a ganglionic tuberculosis was diagnosed by a lymph node biopsy. Blood cell count analysis showed leukocytosis, neutrophilia, and lymphocytosis and was treated for nine months with isoniazid (6 mg/kg/day), rifampicin (12mg/kg/day), pyrazinamide (32mg/kg/day), and ethambutol (24mg/kg/day). On January 3rd 2021, at the age of 3 years, presented non-productive cough, rhinorrhea and odynophagia, treated with symptomatic drugs. Ten days later, arrived to our hospital presenting tachypnea, tachycardia, fever, and oxygen desaturation (84%) and then SARS-CoV-2 infection was detected by a quantitative polymerase chain reaction on January 19th. The patient had a favorable evolution and did not require invasive mechanical ventilation nor aminergic support.

After recovering from SARS-CoV-2 infection, the patient had persistent fever and paroxysmal hemoptysis requiring blood transfusion on January 21st; and ceftriaxone IV 75mg/kg/12hrs was initiated. Simple and contrasted computed tomography (CT) scans found bilateral pleural effusions and brain abscesses, so thoracentesis, gastric liquid sampling and drainage of brain abscesses were done on January 22nd. Bacilloscopy and GenExpert analysis were positive to *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid in the brain abscess and pleural effusions. Serology for HIV, HBV, HCV and adenosine deaminase in pleural liquid, were normal. Levofloxacin (20mg/kg/day), amoxicillin with clavulanic acid (75mg/kg/day), linezolid (20mg/kg/day), propionamid (18mg/kg/day), cicloserin (18mg/kg/day) and fluconazole (6mg/kg/day) were given.

Additional 15ml of drainage of a brain abscess was done on February 5th 2021. Another head CT done on February 11th found a new brain abscess nearby the ventricular wall (15.7 × 22.25mm). On February 15th, Chronic granulomatous disease was ruled out after negative results of the reduction of nitroblue tetrazolium and dihydroergotamine techniques. A flow cytometry showed a low count of CD4 and CD3 lymphocytes.

On March 13th, the patient developed intracranial hypertension by hydrocephalus; and a third ventricle ventriculostomy was performed the next day without improvement, therefore a ventriculoperitoneal shunt was performed the same day. On March 24th another microsurgical resection of brain abscesses was done without any incident.

On April 29th the expression of IL-12R and Interferon gamma receptor 1 (IFN-γR1) was assessed in the patient and in his family members using flow cytometry. Expression of IFN-γR1 and IL-12Rβ2 were normal. However, no expression of IL-12Rβ1 was found in the patient's CD3+ T lymphocytes, and was minor in his family compared to control.

Functionality of the IL-12R was assessed measuring IFN-γ production in response to recombinant human interleukin 12 (rhIL12) and phytohemagglutinin (PHA) (Fig. 1). The patient and his parents showed decreased production of IFN-γ in response to any of the given stimuli compared to control, especially with the combination of rhIL12 and PHA. Interestingly, his sibling showed increased basal production of IFN-γ and higher response to PHA and rhIL12 given alone compared to control.

Functionality of the IFN-γR was assessed measuring tumoral necrosis factor alpha (TNF-α) production in response to lipopolysaccharide and IFN-γ at different concentrations (Fig. 2). All the subjects showed adequate response to any of the given stimuli.

In summary, an IL-12Rβ1 receptor complete deficiency was diagnosed in our patient.

Subsequently, right tempo-parieto-occpital tuberculomas were found and resected on May 11th and June 12th 2021. On July 1st, the patient was re-admitted to our hospital and a magnetic resonance imaging (MRI) of the head showed multiple brain abscesses (Fig. 3). On July 7th, the patient went through a non-successful resection of a fourth ventricle tuberculoma (Fig. 3).

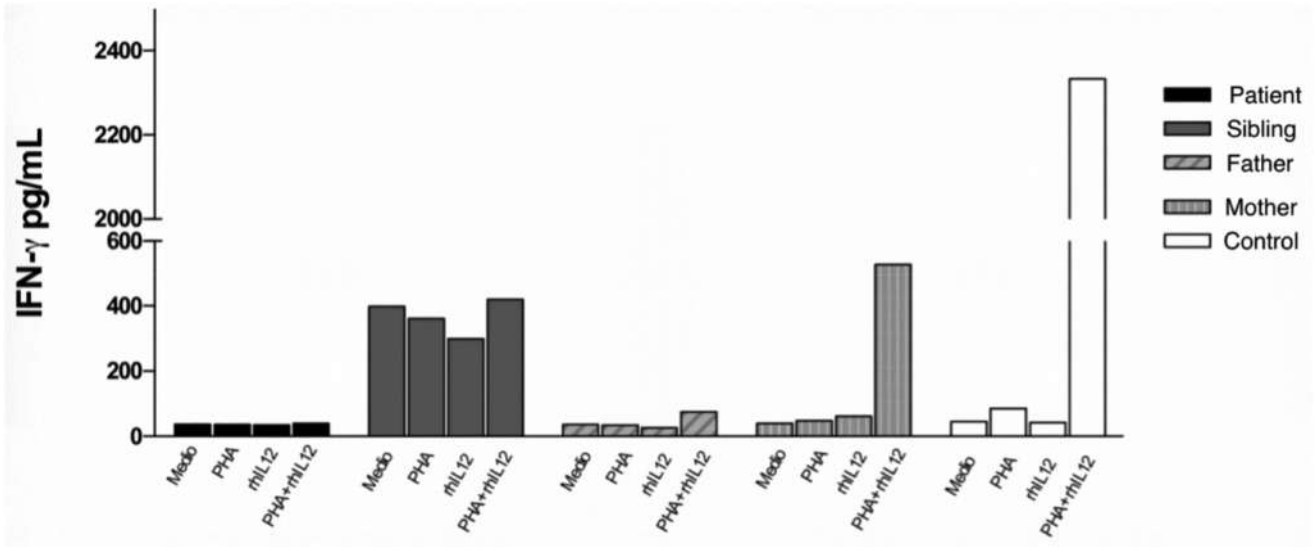

Fig. 1 – Evaluation of the functionality of IL-12R by IFN-γ production. The patient showed decreased production of IFN-γ demonstrating defects in the function of the IL-12R.

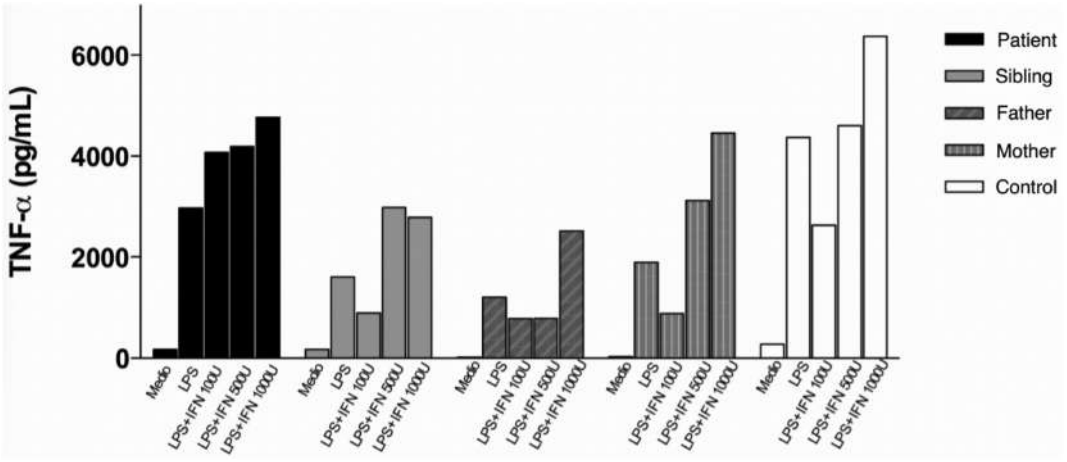

Fig. 2 – Evaluation of the functionality of IFN-γR by TNF-α production. The patient showed adequate production of TNF-α demonstrating correct function of the IFN-γR.

3. Discussion

We present the diagnosis and clinical evolution of a child with complete IL-12 Rβ1 deficiency. CNS TB is one of the most devastating clinical manifestations of TB and is associated with higher mortality.[8] Tuberculomas, together with leptomeningitis, are the most frequent tuberculous lesions. They are responsible for 10–30% of intracranial expansive processes in endemic countries, and a higher risk of CNS TB is described in children younger than 5 years and patients under immunosuppression, such as HIV-positive patients or any other cause of immunodeficiency such as MSMD.[6]

Previously, a six-year-old child from a town nearby our patient's residence presenting defects in the IL12/IL-23/IFN-γ axis, as well as disseminated TB was reported.[4] It is important to detect if there is a founder effect in the immunological deficiency between both patients. Similarly, there are three cases reported in India, Iran and Turkey with similar neurological pictures due to TB infection because of MSMD.[3,6,7]

However, unlike previously published cases, our patient showed substantially more severe neurological manifestations which have led him to be surgically intervened by the neurosurgery service on multiple occasions. All the patients reported above were vaccinated with BCG and had developed BCGitis.[3,4,6,7]

The basic model is that macrophages are infected by mycobacteria, leading to the elaboration of IL-12 by the infected cell. IL-12 acts on the IL-12 receptor on T and NK cells to elaborate interferon-gamma (IFNγ). IFN-γ, in turn, acts on

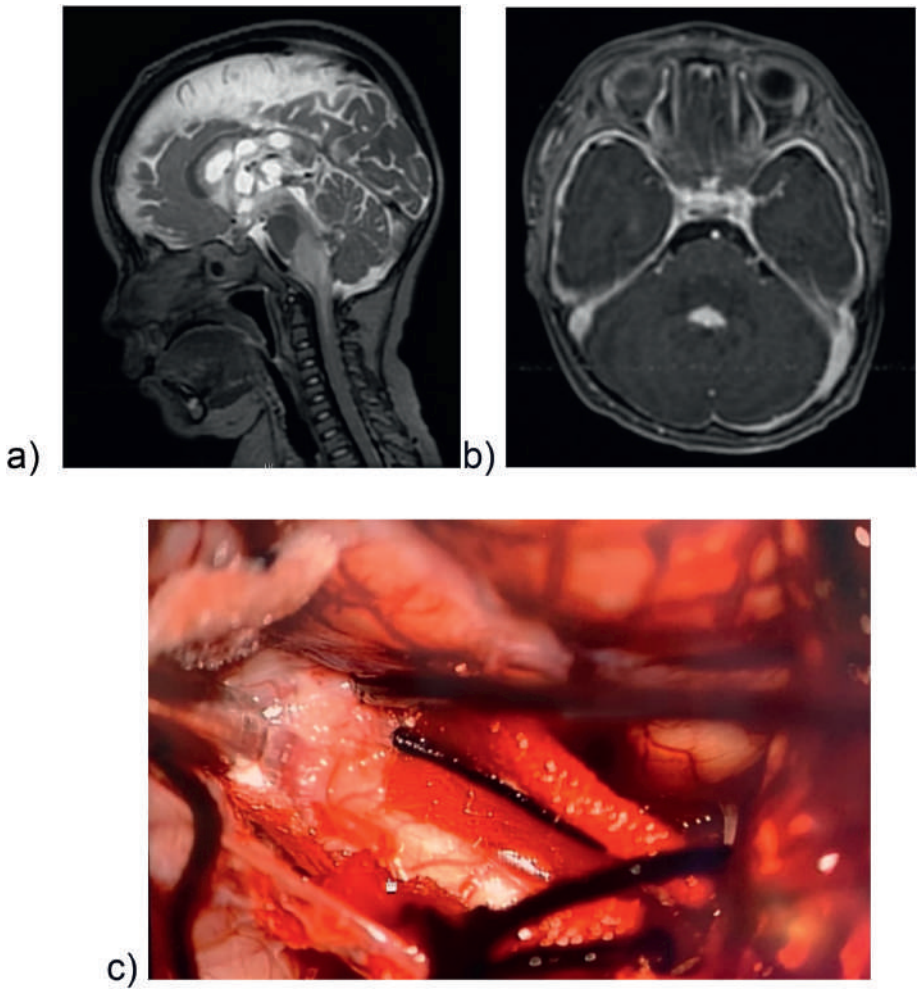

Fig. 3 – Brain abscesses and fourth ventricle tuberculoma. a) Sagittal T2 MRI showing multiple brain abscesses b) Transverse MRI enhanced with Gd showing a tuberculoma located in the fourth ventricle. c) intraoperative visualization of the tuberculoma.

the initiating macrophage through its interferon-gamma receptor phosphorylating the signal transducer and activator of transcription 1 (STAT1) and upregulating IFNγ responsive genes Co-expression of both β1 and β2 subunits is required for IL-12 binding and high-affinity signaling.[9] IL-12Rβ1 also combines with IL-23R to transmit the IL-23 signal, and therefore, mutations in IL-12p40 and IL-12Rβ1 affect IL-23 signaling as well.[3,4]

There are multiple ways in which diagnosis of complete deficiency IL-12R can be made. Among the options is the evaluation of the expression of IL-12Rβ1 by Real-time PCR. Another option is a whole-exome sequencing revealing where the molecular alteration lies.[2]

There is a standardized procedure for the evaluation of the IL-12Rβ1 expression by fluorescence-activated cell sorting and stimulation of T cells by rhIL12 and PHA to assess the production of IFN-γ.[10] These are the methods whereby diagnosis was achieved in our patient.

Treatment remains in debate because no evidence demonstrates the superiority of any treatment against others. In some cases, it has been reported the use of IFN-γ, prophylactic antimicrobials, and hematopoietic progenitor cell transplantation.[11]

However, due to the lack of solid evidence, none of the treatments mentioned above was implemented in our patient.

4. Limitations

Unfortunately, due to limitations in processing samples in our institution, the precise genetic mutation in our patient could not been identified. However, IL-12Rβ1 deficiency could be addressed by assessing the expression and function of the IL-12R and IFN-γR1 in the patient's CD3+ T lymphocytes.

Conflicts of interest

All authors have none to declare.

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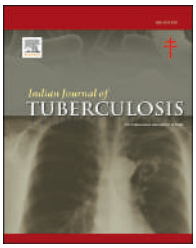

Case report

Eliminate TB by 2025? A case report of MDR TB to reaffirm the need of follow UP!

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1. Introduction

The World Health Organization (WHO) estimated that around 1.7 billion people to be infected with *Mycobacterium tuberculosis* (TB) in 2019, with 1.4 million annual deaths from TB.[1] A 95 percent of these TB cases occur in resource-limited countries with poverty, HIV and drug resistance being the major contributing factors.[2] The greatest obstacle to reduce the incidence and deaths due to TB is the significant proportion of patients with Tuberculosis that are never diagnosed and thus never treated. WHO estimation is that the undiagnosed cases of TB roughly approximate 30 percent. Detection and treatment of MDR-TB are also inadequate. According to WHO, only 44 percent of patients with MDR-TB were diagnosed in 2019 and, of those, only 86 percent were given effective second-line treatment.[2]

Drug resistant TB poses considerable risk to TB control programs worldwide. The proper therapy of MDR-TB and XDR-TB imposes huge financial burden, especially in low-income countries.[2] There is ever increasing incidence of multidrug-resistant tuberculosis (MDR-TB) and the emergence of extensively drug-resistant tuberculosis (XDR-TB) potentially creates lot more challenges. Hence, the rapid diagnosis of drug resistant-TB has received much attention especially in the last decade. Controlling resistance, reducing transmission and improving treatment outcomes in MDR/XDR-TB patients highly depends on early detection of drug resistance. The phenotypic methods used for susceptibility testing require more human resources and is time-consuming. At the same time, molecular methods for drug susceptibility testing are easier to perform and have a rapid turn-around time.[3,4]

In December 2010, WHO recommended the use of Xpert MTB/RIF assay for the detection of *M. tuberculosis* as well as to detect rifampicin resistance. The advantage of Xpert MTB/RIF assay is that it provides results directly from sample in less than 2 h.[5]

The World Health Organization (WHO) has approved the use of line probe assays (LPAs) for first and second line diagnostic screening of MDR/XDR-TB. In 2008, the World Health Organization (WHO) endorsed the use of the first line (FL) line probe assay (LPA), the GenoType MTBDRplus (referred to as GenoType MTBDRplus V1), which facilitated hasty diagnosis of multidrug-resistant TB (MDR-TB) as well

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as helped in concurrent detection of resistance to rifampicin (Rif) and isoniazid (H).[6] WHO endorsed second line LPA in 2016 to detect the resistance to flouroquinolones and second line injectables.[7] The LPA reports are usually available within 72 hours.

Next-generation sequencing (NGS) represents the futuristic method for TB diagnosis and detection of drug resistance characterization, and many NGS platform options now exist for DR-TB diagnosis. Unlike other genotypic methods used for DR-TB, which depend on the indirect identification of MTB and a limited set of resistance mutations through the hybridization of probes to specific genetic sequences, Next-generation sequencing give comprehensive information on the sequence of multiple gene regions or whole genomes of interest.[8]

Previous treatment regimens for MDR TB required intravenous treatments of long duration and high cost. However, the 2020 and 2021 recommendations from the WHO for the management of drug-susceptible TB and MDR TB have included oral treatment regimens and reduced treatment duration. The WHO recommends a shorter treatment regimen of between 9 and 11 months for patients with MDR TB not resistant to fluoroquinolones, including oral bedaquiline.[2] For patients with MDR-TB who also have fluoroquinolone resistance, a regimen composed of bedaquiline, pretomanid, and linezolid may be used for between 6 and 9 months.[2]

Despite these advancements in the diagnosis of TB, certain flaws still exist in the implementation of TB programmes especially in low income countries which affects treatment outcome. TB treatment failure can be sometimes due to insufficient supervision of field activities and laboratory services. So, we are presenting the case report of an MDR patient whose treatment outcome could have been improved by proper supervision and implementation of services as suggested by NTEP and WHO.

2. Case report

A 30 year old male patient, resident of Hathras District, Uttar Pradesh, a Northern State of India was referred from a peripheral health center to our hospital, which is a tertiary care center in Aligarh,Uttar Pradesh as a case of Multi drug resistant tuberculosis Treatment Failure. The presenting complaints of the patient were new onset cough with expectoration and fever of 1 month duration starting from November 2021. The patient had already taken MDR TB treatment with effect from January 2020, for a period of 9 months without any default before being declared as treatment completed on October 2020 from the same peripheral health center.

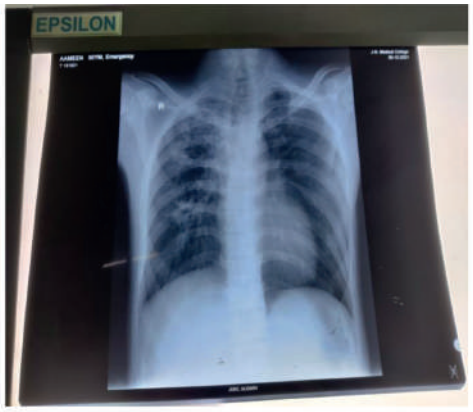
The patient was referred to us on 31st December 2021, when his sputum CBNAAT done on 15th December detected Rifampicin Resistance. Since this patient was referred as a case of MDR TB treatment failure, it was pertinent from our side to take proper history regarding his previous ATT history.

He took treatment for his primary MDR TB under NTEP (National Tuberculosis Elimination Programme), earlier known as RNTCP, with effect from January 4 2020, when his sputum smear detected Mtb and sputum CBNAAT confirmed Rifampicin Resistance. He was given medication under DOTS Category IV Shorter regimen as per his weight 51kg. It included 4 months Intensive phase of Inj. Kanamycin 750mg, Tab. Moxifloxacin 800mg, Tab. Ethionamide 750mg, Tab. Clofazimine 100mg, Tab. Isoniazid 900mg, Tab. Ethambutol 1200mg and Tab. Pyrazinamide 1750mg followed by 5 months Continuation phase comprising Moxifloxacin, Clofazimine, Pyrazinamide and Ethambutol in same doses. Soon after the initiation of this treatment, his sputum first line Line Probe Assay and Second line Line Probe Assay were sent on 7th January 2020. He was highly motivated to take his medications and was regular in taking his medication without any default.

The patient was on regular follow up till March 2020, when the nation-wide lock down was imposed in India owing to Covid-19 pandemic. During those periods, many health care facilities in India were converted to dedicated Covid care centers and as a result of which, the follow up of our patient was also affected. However, he was regularly getting medications from DOTS center. At the end of Intensive phase, his sputum smear was negative for AFB and sputum culture was also negative. Despite being unemployed due to lockdown, the patient completed 9 months of his medication. At the end of 9 months, he was completely free of symptoms and was declared as MDR TB treatment completed from the peripheral health center. Sputum culture done at the end of treatment reported contaminated specimen after 1 month of submission and no efforts were taken to submit new sample.

In January 2021, 4 months after he was declared as treatment completed, he got a call from peripheral DOTS center informing him that his treatment needed modification. Since he was having no symptoms, he refused to take any medications again. He was absolutely fine till November 2021, when he developed new onset cough with expectoration and low grade fever. He visited the same peripheral DOTS center now and his sputum smear was positive for AFB and sputum CBNAAT detected Rifampicin Resistance. Hence, the patient was referred to our center as a case of MDR TB treatment failure.

His past history was nothing significant except for previous MDR TB treatment. Two of his brothers had contracted TB and taken treatment for MDR TB 4 years and 3 years back respectively, out of which 1 brother died due to TB last year. He lives in a joint family consisting of 11 members and works as a delivery agent for a local company. His vitals were stable at the time of examination; Pulse rate-76/min, BP – 100/70 mm Hg in the right upper limb, Respiratory rate – 16/min, Saturation - 95% and Temp – 98.6 F. Respiratory system examination was normal except of bilateral coarse crepitations. Other system examinations were within normal limits. Chest X-ray revealed non homogenous opacities in the right upper and mid zone with cavities on the right side.



Chest X-ray taken on 30/12/2021 show non homogenous opacities on the right upper and mid zone

The first question that rose in our mind was whether this a true case of treatment failure. We checked the details of the patient in Nikshay web portal. It was realized that the Sputum F-LPA and S-LPA which was sent on January 4th 2020 was updated in Nikshay portal on 23rd March 2020. To our surprise, the F-LPA report was showing Rifampicin Resistance along with high level Isoniazid Resistance while S-LPA detected high level Flouroquinolone Resistance. The patient was not informed about this report then and was not adviced any change in treatment regimen. We enquired regarding the same with the concerned DOTS center and it was told that they got information regarding the reports on January 2021 and updated the patient about it promptly. Shortly, the patient was informed about his F-LPA and S-LPA reports, 1 year after he had submitted his sputum samples and by this time, he had already completed his MDR TB shorter regimen and was free of any symptoms.

3. Discussion

Tuberculosis caused by Mtb that is resistant to at least isoniazid and rifampin and an additional chemotherapeutic agent is known as MDR TB.[9] Infection with *M. tuberculosis* resistant to isoniazid, rifampin, and fluoroquinolones (levofloxacin or moxifloxacin) is now known as 'pre-extensively drug-resistant TB' (pre-XDR TB).[9] Infection with *M. tuberculosis* resistant to at least isoniazid, rifampin, and any fluoroquinolone, and at least one additional group A drug, which currently includes bedaquiline and linezolid, is now known as 'extensively drug-resistant TB' (XDR TB).[9]

Nikshay ID	Patient's Full Name (Address / Contact Details)	FL-LPA Valid (Y/N)	MTB (Y/N)	RIF (rpoB; RD/RI/S/I)	Mutation pattern	INH (katG; RD/RI/S/I)	Mutation pattern	INH (inhA; RD/RI/S/I)	Mutation pattern	SL-LPA-Valid (Y/N)	MTB (Y/N)	GYRA (RD/RI/S/I)	Mutation pattern	GYR Bi (h)(RD-Y/N)	Mutation pattern	SLID (rrs; RD/RI/S/I)	Mutation pattern	SLID (eis; RD/RI/S/I)	Mutation pattern
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MUTATION REPORT OF FL-LPA & SL-LPA sent on 4th JANUARY 2020

So this was actually a case of Pre-XDR Tb in January 2020 which required treatment with all oral longer MDR Tb regimen. However, due to the long delay in disposing the LPA reports to the patient, he was not given proper anti-tuberculosis medication as per his resistance pattern. We registered this patient under DOTS Cat IV all oral longer Bedaquiline containing regimen with effect from January 1st 2022.

Currently, the WHO collects MDR TB data and reports from more than 170 countries. The latest WHO Global TB Report estimated that in 2019, there were more than 500,000 patients of MDR-TB worldwide,[2] out of which only 186,772 patients

were confirmed to be of MDR-TB by laboratory and molecular investigations, and only 57% had positive treatment outcomes.[2] The need for a more rapid and accurate diagnosis of MDR TB, screening for MDR TB, and improving treatment and patient follow-up has been highlighted in the most recent WHO report.[2] The implementation of infection surveillance along with molecular testing has improved data collection. Hence, the prevalence of MDR TB can now be continuously monitored, even in countries with a high infection rate.[2]

Patient turnaround time (P-TAT) is defined as the time elapsed in days between identifying the eligible patient to initiating the patient on treatment, based on the laboratory result. The patient TAT has been divided into pre-lab, lab and post-lab TAT. Pre-lab TAT includes time taken for patient identification, referral for testing and specimen collection, packaging and transport. Lab TAT includes elapsed time in days for specimen receipt at the laboratory, testing and reporting results. Post-lab TAT is the time taken for receipt of laboratory results, pretreatment evaluation and treatment initiation/modification. TAT serves as a quality indicator to assess efficiency of field services as well as the diagnostic cascade in the patient pathway.[10]

The latest PMDT guidelines by NTEP have published the benchmark for total patient turnaround time. It has been set as 5–10 days for Nucleic Acid Amplification Test (NAAT), 8–12 days for line probe assay, 29–58 days for LC-DST and 11–45 days for follow up liquid culture.[10] Now, as far as our patient is concerned, he was informed about his LPA reports, 1 year after submission of sputum sample. His reports were made available in the Nikshay portal, 2 months after submission of specimen. So, it suggests that pre-lab, lab and post-lab TAT were significantly prolonged for this patient. The post-lab TAT of more than10 months is not affordable by any standards. This indicates that despite the introduction of rapid molecular tests, the overall patient turnaround time has not reduced significantly mainly due to inadequate monitoring of field level activities and laboratory services.

This case report is just a cross section of what actually happens at the peripheral levels of TB care. The author doesn't think it to be an isolated incident happening only in the country. In fact, this must be scenario in all high TB burden countries. Huge patient load, lack of sufficient laboratory facilitiess, inadequate supply of diagnostic logistics like specimen containers, catridges, lack of care providers and financial constraints are the common issues in low income countries that is affecting the TB treatment outcome.

Also, since early 2020, the COVID-19 pandemic has had a direct and indirect detrimental impact on health services in all areas, including infectious diseases and TB services.[11] Prioritized health services, clinical studies, and vaccine and drug development to treat SARS-CoV-2 infection have diverted these applications and resources from other infectious diseases.[11] These effects of the COVID-19 pandemic have come when TB cases, combined infections with TB, and MDR TB have all been increasing globally.[11] It has recently been estimated that MDR-TB cases will continue to rise in 2021 and 2022, further affecting already poor treatment outcomes.[11]

The previous ATT intake of the patient correlates with the periods of nationwide lockdown in India. It was a period of complete chaos in the public health sector and many of the peripheral primary health care centers in India were converted to dedicated Covid care centers. Also, the services of health care workers including laboratory technicians were shifted for Covid related services. This could be another reason for the delayed disposal of the reports.

Delayed diagnosis and treatment are detrimental to TB prognosis and sustain TB transmission in the community, making TB elimination a great challenge, especially in high burden countries. TB programs should strive to test and treat TB by adopting WHO recommendations for same-day TB diagnosis[12] to further reduce TB transmission and mortality.[13] Higher-level policies and interventions such as health system strengthening, universal health coverage, and the provision of sustainable social welfare schemes are important to reduce delays, improve access to TB care, and ultimately achieve the global TB targets.[14]

4. Conclusion

Moving forward, it is going to be challenging times for all TB care workers. What has happened in the past has happened and it is important to learn from the mistakes. It is important to consistently achieve benchmark TAT for all TB laboratory services and to periodically review process indicators for achieving quality improvement. To move in tandem with WHO End TB strategy, NTEP needs more political commitment and capacity building. More than implementation of the newer guidelines, monitoring and evaluation of TB services at the periphery is the need of the hour.

Conflicts of interest

The authors have none to declare

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