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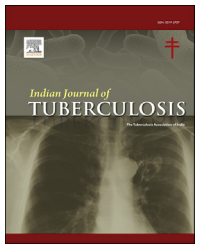

Editorial

Towards building a tuberculosis free world

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ABSTRACT

The Honourable Prime Minister of India set a target of year 2025 for elimination of TB from the country, 5 years ahead of the Sustainable Development Goal of 2030. Last few years, India has made significant improvements, towards elimination of tuberculosis from the country in the form of bold policies and unprecedented political commitment. While COVID-19 has resulted in setbacks for TB elimination efforts, it has also offered an opportunity to revisit and structurally redesign the public health infrastructure/system in our country. The dream of TB elimination is possible with active participation of all stakeholders and community at large coupled with accelerated development of new diagnostics, drugs, and development of a new TB vaccine. COVID-19 pandemic has shown that vaccines can be developed in a year, contrarily, the lack of a TB vaccine is deterrent in the efforts towards a TB free world. A progress towards TB elimination would require potential contribution of novel TB vaccine. Now, is the time for mobilization towards a TB vaccine to make an impact towards our end TB goal.

As per WHO Global Tuberculosis (TB) Report 2020, India ranks 39th globally in terms of the TB incidence rate i.e. the number of new TB cases per lakh population. In terms of the Multi drug resistant TB incidence rate, India also ranks 39th globally. However, owing to India's overall population, these rates translate to very large numbers. An estimated 26 lakh TB cases occurred in India in 2019 and 4.36 lakh people succumbed to the disease in the same year. Approximately 1200 people die of Tuberculosis every day. Considering the huge load of this deadly disease, Central TB Division (CTD) of Ministry of Health & Family Welfare, had developed National Strategic Plan (2017–2025) in line with UN's Sustainable Development Goal 3 of 2015 and on 13th March, 2018, at "Delhi End TB Summit", the Honourable Prime Minister of India set a target of year 2025 for elimination of TB from the country, 5 years ahead of global target of 2030.

In the last few years, the country has made significant strides in improving the public health system and health indicators across the country. These advances are unprecedented both in terms of their scale and reach and bear a rich testimony to the sincere commitment towards a healthy and more productive India. The Ministry of Health & Family Welfare has also taken definitive steps towards elimination of tuberculosis from the country with tailwinds in the form of conducive plans, policies, and commensurate resources to match unprecedented political commitment.

The National Tuberculosis Elimination Programme has significantly scaled up access to free rapid molecular diagnostics, treatment and nutritional support to patients. Relentless efforts have led to a record increase in TB notifications and considerable improvements in time-to-diagnosis, adherence, and treatment outcomes. This reflects that the Programme now has better access to TB patients and the ability to provide free treatment to patients from both public and private sectors. The programme has also advanced leaps and bounds in the services being provided for TB, with substantial ramping up of diagnostic capacities for TB with rapid molecular diagnostic facilities available in all districts of the country. Going forward, they will be further decentralized to block levels. High quality anti TB drugs, use of digital technology, multisectoral and community engagements, and integration of TB services within all levels of the health system, are all aligned to detect cases early and prevent the emergence of new cases of TB by expanding TB care and services to rapidly decline TB incidence and mortality in the country.

Between January and February 2020, the National Tuberculosis Elimination Programme was on an up-hill trajectory notifying more than 4,11,000 patients (~6% more than

in the corresponding months of 2019). And then COVID-19 hit the world full force and unleashed a devastation unprecedented in modern times. The national lockdown imposed in March and April resulted in tumbling notifications by almost 38%, and NTEP worked hard to mitigate the impact of this new threat to regain the lost momentum. Central TB Division had sent a number of advisories which included TB and COVID-19 bi-directional screening among ILI/SARI patients and COVID-19 patients, at various health facilities of the country to detect TB cases among the COVID-19 affected persons and vice-versa. This practice of bi-directional screening yielded better diagnosis in both TB and COVID-19 patients which helped to reduce the transmission of both the diseases.

The National TB Prevalence Survey resources were also repurposed and optimally utilized to conduct three rounds of national SARS-cov2 sero-surveillance by ICMR in India. A large capacity of Nikshay Sampark (National TB Call Centre) was also re-purposed to serve as COVID-19 helpline. By December 2020, the Programme had almost closed the gap on TB treatment enrolment with a total of 18,05,670 patients notified, 11% more than the estimated projections made in April. The private sector too contributed significantly by notifying ~5.49 lakh patients (31% of total notifications), 3% more than in 2019. More than 95% of total patients notified were put on treatment.

At the community level, large scale active TB case finding campaigns with massive screening and testing in campaign mode, engaging health outreach workers and community volunteers to facilitate surveillance of symptoms within households, doorstep delivery of drugs and doorstep collection of sputum samples, tele-consultations over phone and video, and ensuring uninterrupted supply of anti TB drugs are being undertaken. We have also supported the States with periodically updated advisories, directives, and guidance documents.

The sweeping global COVID-19 pandemic has reversed years of progress made in the fight against Tuberculosis. Currently India is in the midst of the second wave of the pandemic. This huge surge in cases has posed unprecedented challenges for the health system in general and particularly for the National Tuberculosis Elimination Programme. But we are committed to keep the TB Programme on the track and focus on continued TB services. Even in these trying times, the Central TB Division is implementing TB Mukt Bharat Abhiyan, a People's Movement for TB launched by the erstwhile Honourable Union Health Minister Dr Harsh Vardhan. The campaign aims to build awareness about TB, address the deep-seated stigma around the disease in the community, raise awareness about the available TB services under the program, and generate demand for TB services in the community by building strong political and administrative commitment at the grassroots level and through inter sectoral coordination and synergy with other public health programmes in the country. Earlier, "TB Harega Desh Jeetega" campaign was launched by the erstwhile Honourable Union Health Minister Dr Harsh Vardhan on 25th September 2019 to make every citizen of India aware about TB and kick-start an awareness campaign for the TB services throughout the country. This should effectively lead to better reach of TB services in remote areas of India.

The programme has identified key thematic areas to cover across the year with exhaustive activities at State/District and Facility levels to be undertaken. These are over and above the regular calendarized plans followed by States/UTs. A State Guidance Document has been developed to provide much needed direction for State TB Offices to implement the activities in line with the TB Mukt Bharat calendar together with the processes that need to be followed for implementation and their expected outputs and outcomes. Several development partners too have come forward in support of the Abhiyan with commitments in the areas of Advocacy, Communication, Sectoral engagements including the communities, treatment and management of TB, diagnostics, logistics and supply chains, Surveillance and Monitoring, use of Artificial Intelligence for improving patient care, Research etc. Many have started interventions across geographies, expanding activities under various aspects of the TB care cascade for a more patient-centric delivery of health services.

While COVID-19 has resulted in setbacks for TB elimination efforts, but as a silver lining to every dark cloud, it has also provided the chance to revisit and structurally redesign the public health infrastructure/system in our country. Dedicated Infectious Disease Hospitals being established as part of the pandemic preparedness and response, would contribute significantly to TB care and management. The measures undertaken by various states to control COVID-19 infection will alter the transmission dynamics of infectious diseases in the health facilities. The behavioural change acquired by the common citizens during this COVID-19 pandemic will further contribute to reducing transmission of all respiratory illnesses including TB. Adoption of technology and use of online platforms for trainings, meetings and reviews will allow staff getting better adapted to the use of these modalities. The scale-up of telemedicine and teleconsultations during the pandemic will provide additional channels of consultation on TB.

But, despite everything, an uphill task remains. The dream of TB eradication will not bear fruit without the active participation of all stakeholders and community at large. To end TB, we need to accelerate development of new diagnostics, vaccines, drugs etc. Today, the world lacks an effective TB vaccine. The COVID pandemic has shown us that united efforts can lead to vaccines in less than a year. We need a similar effort for a TB vaccine too, and soon. We must leverage all available opportunities to foster awareness and advance indigenous technologies along with learning and sharing of innovative research and development activities for TB drug discovery, diagnostics, and vaccine development. It is only through continued efforts by all of us in the scientific advancement of knowledge, that persisting challenges facing TB will find a resolution.

The National Tuberculosis Elimination Programme remains committed to the deadline of 2025 that India has set for itself.

Conflicts of interest

The authors have none to declare.

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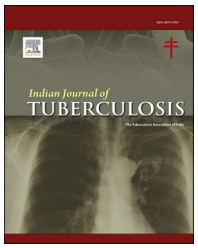

Viewpoints

Chronic obstructive pulmonary disease in Latin America and the Caribbean: Mapping the research by bibliometric analysis

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Dear Editor,

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of mortality worldwide and is projected to be the third leading cause of death by 2030.[1] Underdiagnosis and a lack of precise population-based studies from developing countries likely contribute to the underestimates of the actual COPD burden globally.[2] Respiratory medicine research in Latin America and the Caribbean is still limited. No analysis of COPD-related research in this region has been performed. Given the paucity of data, we conducted a bibliometric analysis of scientific literature contributions on COPD in the aforementioned region.

A cross-sectional bibliometric analysis of COPD scientific production in Latin America and the Caribbean was carried out. Information was retrieved from four databases: MEDLINE/PubMed (1945–2019), Scopus (1982–2019), LILACS (1982–2019), and SciELO (1982–2019). (1998–2019). For the search pipeline we used the following combination of keywords (MeSH, DeCS) in English, Spanish and Portuguese: "COPD" AND "Latin America", and this strategy was maintained including the name of each country as a keyword. We searched for the 33 countries included in the United Nations list. All article types were included. Data was exported to Microsoft Excel® and then transferred to SPSS Version 15 program for analysis.

In PubMed/Medline, 85,172 COPD-related documents were found, 2724 (3.19%) from Latin American countries. The top five countries were: Brazil (48.31%), followed by Mexico (18.46%), Argentina (8.62%), Chile (7.37%) and Colombia (3.78%). The number of publications increased significantly over the years ($r2 = 0.7364$, $p < 0.001$), with the major output observed in 2018.

In Scopus, 54,812 global articles were found, 1846 (3.36%) from this region. Top five countries were: Brazil (51.14%), followed by Mexico (13.34%), Chile (8.72%), Argentina (7.83%) and Colombia (5.08%). The number of papers has progressively increased over the years ($r2 = 0.816$, $p < 0.001$).

In ScIELO, 303 articles were found. 23.1% from Argentina, 17.8% from Colombia, 17.16% from Chile, 15.18% from Brazil

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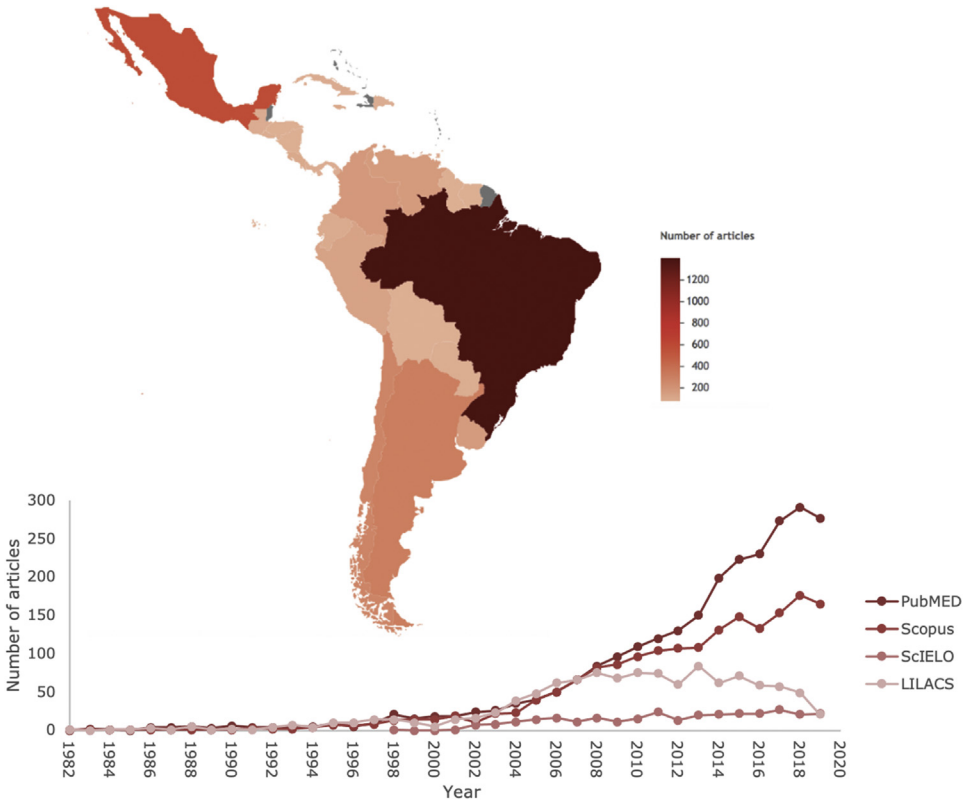

Fig. 1 – Distribution of COPD-related research in Latin America and trends in scientific production time in four medical databases (1982–2019).

and 10.23% from Mexico. In LILACS, the search showed 860 articles, 50.93% from Brazil, 12.09% from Chile, 11.16% from Argentina, 10.58% from Colombia and 4.30% from Cuba, with an annual production of 30.18 articles. The findings are distributed geographically in Fig. 1.

The data of this study suggest that COPD-related scientific output in the region is very poor (between 3.19 and 3.36% of global publications), which is consistent with low productivity (1.09%) of the region on research in the area of respiratory medicine.[3] However, there has been a strong upward trend in the number of articles published in recent years, especially over the last decade. As in previous bibliometric articles, the top contributors in this research were Brazil, Mexico, Argentina, and Chile.[4,5]

According to the findings, COPD research in Latin America and the Caribbean is low, but there has been an increase in scientific output in recent years. As a result of the significant impact COPD has on the area, more research, a better understanding of the epidemiology, and better intervention designs are needed to reduce COPD-related morbidity, mortality, and costs. More international and national collaborative research projects are recommended.

Conflicts of interest

The authors have none to declare.

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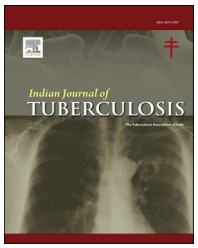

Viewpoint

Recent updates in diagnosis and management of drug-resistant tuberculosis in India: A paradigm shift and the way ahead during the COVID-19 crisis

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ABSTRACT

The recent guidelines on the Programmatic Management of Drug-Resistant Tuberculosis (DR-TB) in India (PMDT) have been released in March 2021 on World TB Day. The new guidelines have considered emerging diagnostic trends including TrueNat, Xpert Mtb/XDR, Next generation sequencing and evaluation for resistance to newer drugs including Bedaquiline (Bdq) and Delamanid. The emerging therapeutic trends include focus on oral shorter Bdq based regimen with phasing out injectables use. The replacement sequence of drugs for DR-TB have also been updated. Updated definitions for pre-XDR, XDR, culture conversion and default have also been added. These guidelines are a paradigm shift which will make treating DR-TB easier and more efficient especially during the ongoing COVID-19 pandemic crisis.

1. Introduction

India has the highest burden of tuberculosis (TB) in the world, having an estimated incidence of 2.7 million cases in 2019.[1] This crisis evokes a deeper concern considering the increasing proportion of drug-resistant TB (DR-TB) cases. India had an estimated 130,000 DR-TB cases in 2018, while the estimated percentages of new cases and previously treated cases with multidrug-resistant (MDR) or rifampicin-resistant (RR) TB in 2017 were 2.8% and 12% respectively. As per the National Drug Resistance Survey (NDRS) released in 2018, only 44% of the estimated MDR-TB cases were diagnosed and almost 65% of the cases remained untreated.[1] The increasing burden of DR-TB has led to the rapid expansion of Programmatic Management of Drug Resistant Tuberculosis (PMDT) services in recent times. Endorsed by the World Health Organization (WHO) in 2002, India adopted the PMDT services in 2007 and complete geographic coverage was achieved in 2013. Since then, PMDT, a comprehensive document on diagnosis and treatment of DR-TB is updated regularly anticipating the current needs in providing for the DR-TB services.

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The latest guidelines on management of DR-TB have been released on 24th March 2021, the World TB Day. The new document incorporates emerging diagnostic and therapeutic trends, while focussing on the elimination of TB under the National TB elimination program (NTEP).[1] In order to achieve the sustainable development targets by 2025 (i.e., five years before 2030, the global target year), the national strategic plan (NSP) 2017-25 emphasizes on the 'prevent-detect-treat-build' strategy. The PMDT-2021 guidelines are a welcome step, with the updated recommendations backed by the evolving evidence and are in coherence with the WHO technical report.[2] The update spans from definitions and diagnostics; to treatment and logistics. The main driving force behind majority of the suggested changes in these guidelines is the current evidence regarding the promising efficacy of the two newest members of the antitubercular treatment (ATT), i.e., Bedaquiline (Bdq) and Delamanid (Dlm).

2. Changes in diagnosis of DR-TB

The WHO recently revised the definition of extensively drug-resistant tuberculosis (XDR-TB), who have also defined pre-XDR-TB for the first time, highlighting the seriousness of these forms of TB. XDR and Pre-XDR TB are defined on the basis of resistance to certain key drugs. The WHO had issued an updated interim guidance in 2019 which re-classified anti DR-TB drugs into three categories (A, B, and C).[2] The update was based on evidence of the high effectiveness of levofloxacin/moxifloxacin, Bdq, and linezolid in DR-TB. The PMDT- 2021 India update has now officially adopted this new classification. The drugs which form Group-A, are the key pillars on which the new PMDT regimens stand. Since these key pillars have evolved, and the second-line injectable drugs (SLIDs) are given less importance now, these definitions have also been changed appropriately. New definitions for pre-XDR and XDR-TB aim to define more precisely the groups of TB patients who require complex treatment regimens. The (SLID) resistance-based criteria for the diagnosis of Pre-XDR/XDR TB have been removed.[1] Pre-XDR TB is now defined as Multidrug-resistant/rifampicin-resistant (MDR/RR) TB with FQ resistance. XDR-TB is now defined as Pre-XDR TB with additional resistance to Bdq and/or linezolid. These new definitions are not only expected to lead to better reporting, surveillance and monitoring of DR-TB, but also, stimulate the development of better treatment regimens for these dangerous forms of TB.

PMDT-2021 also focuses on the challenges with the available diagnostic modalities for DR-TB in India. CBNAAT (Xpert-Mtb) is a cartridge-based nucleic acid amplification test with a short turn-around time for diagnosing TB and at the same time, identifying rifampicin (R) resistance. This PCR-based test has managed to change the landscape of DR-TB diagnosis and treatment in our country. However, it requires an air-conditioner and uninterrupted power supply (UPS) for its functioning. These become a hindrance in many of the TB centres in remote areas of the country. The newer diagnostic modality, TrueNat is a chip-based test and is devoid of the above two liabilities, while maintaining the advantages offered by CBNAAT. The adoption of TrueNat in TB management may eventually prove to be a crucial step towards eliminating TB from the country. All samples that test positive in TrueNat, are further going to be subjected to TrueNat-MTB-Rif-Dx, to rule out rifampicin-resistance. Additionally, ruling out isoniazid- or fluoroquinolone-resistance by second line–line probe assay (SL-LPA) currently requires a turn-around time of at least 2 days. An advanced version of CBNAAT, the Xpert-Mtb/XDR can detect resistance against isoniazid (H), fluoroquinolones (FQs), SLIDs and ethionamide (Eto). The PMDT-2021 aims to progressively introduce this modality throughout the country. Xpert-Mtb/XDR will not only reduce turn-around times, but also lessen the excessive burden faced by the various centres which perform SL-LPA on samples from a large geographical area.

Currently, the Phenotypic Drug Susceptibility Testing (pDST) in India is available for R, H, Pyrazinamide (Z), FQs and SLIDs. The programme aims to commence Clofazimine (Cfz) pDST this year (2021).[1] Bedaquiline (Bdq) and Delaminid (Dlm) DST are currently available only at National Institute for Research in Tuberculosis (NIRT) and National Institute of Tuberculosis and Respiratory Diseases (NITRD). As the coverage of these drugs is expected to become incrementally more frequent, it is prudent to provide DST for these drugs to avoid a clinical or epidemiological crisis. These will soon be made available at other laboratories, providing greater access. Infrequent as it maybe, discordance of drug resistance results between genotypic and phenotypic DSTs is a potentially catastrophic clinical problem. In its technical report-2021, the WHO advised lowering the critical concentration in Mycobacteria Growth Indicator Tube (MGIT) based DST from 1 μg/ml to 0.5 μg/ml to reduce discordance between genotypic and phenotypic DSTs.[2]

Furthermore, the PMDT-2021 now explicitly recommends resolving discordance in RR between NAAT & first line–Line probe assay (FL-LPA) with a successively repeated NAAT.[3] Moreover, the pDST performed in MGIT can miss RR-TB associated mutations. Another new update has added for next generation sequencing (NGS) which can help in detection of genomic sequence variants[3] to predict TB drug-resistance phenotypes.

3. Changes in treatment of DR-TB

The treatment of DRTB rests on using all three key drugs (Group A), supplemented by one or both Group B drugs, to form a regimen of four or five total drugs. In the 2020 guidance, the WHO review group found similar success rates on using four, five or six drugs. The change in PMDT-2021 aligns with this. The focus has now clearly shifted away from SLID containing regimens. Citing the meta-analysis and trial data, the WHO re-classified anti DR-TB drugs. In view of the poorer outcomes with kanamycin and capreomycin, the WHO recommends avoiding these drugs. Amikacin, however, can be used as a Group-C drug, provided susceptibility to it has been confirmed. The focus now rests on an all-oral shorter regimen containing Bdq, in place of kanamycin containing (modified Bangladesh) regimen. The new Bdq-containing shorter oral regimen is recommended for all DRTB patients, provided there is no history of prior exposure to these drugs for 1 month or more, no resistance to FQ, and no extensive-TB defining

presentation such as bilateral lung cavities, extensive parenchymal damage, miliary/meningeal/central nervous system TB, and/or any extrapulmonary TB in children except lymphadenopathy, children less than 5 years of age, pregnancy less than 32 weeks and lactation unless mother willing for formula feed. If any of the exclusion criterion is present, Bdq-containing longer oral regimens are used. The updated regimens are summarised in Table 1.

The evidence on shorter oral Bdq-regimen is primarily based on data from South Africa, which was reviewed by the WHO. The analysis of this standardized shorter regimen containing Bdq place of SLID, in combination with FQ, Cfz, High dose H, Ethambutol (E), Z and ethionamide (Eto) revealed a 13% higher treatment success rate of Bdq over SLID-regimen, with a treatment success rate similar to the conventional longer oral MDR-TB regimen. The change, which essentially replaces SLID with Bdq reduces the need for trained personnel for injection-administration and makes the all-oral regimen more accessible in remote and difficult-to-reach areas of the country. This along with the reduced pill burden, and consequently the adverse event durations associated with long term drug use, while achieving a treatment success rate comparable to the longer conventional regimen is likely to improve adherence and compliance to therapy. The new regimen has the potential of being a paradigm shifting change.

Regarding Bdq, the other important updates include the conditional approval for its use in ages 5–18 years, provided the body weight is above 15 kg and clearance by pediatrician has been obtained. Pregnant or lactating women, and stable-arrhythmia patients can also be prescribed Bdq based treatments. This is extrapolated from a recent study from South Africa which reported non-inferior treatment outcomes with the use of Bdq in pregnant or nursing mothers, and infants.[4] However, the decision to give Bdq must always be a concurrent decision of the obstetrician and physician weighing-in the risk-benefit ratio. Further, as ethionamide is contraindicated in pregnancy up to 32 weeks of gestation, longer oral Bdq containing regimen should be used if the patient wants to continue with her pregnancy. However, if period of gestation is more than 32 weeks, the Bdq-containing shorter oral regimen is preferred. Lastly but equally importantly, Bdq can now also be used in Extra-pulmonary TB (EP-TB).

Delamanid (Dlm) can now be used in ages 6 years and above, in doses of 50 mg twice daily (6–11 years) and 100 mg twice daily (12 years and above). Recently, WHO has also stated that no extra safety concerns could be found for the concurrent use of Bdq and Dlm. These can be used together and even extended beyond 6 months if only 2 drugs are useable from groups A and B, and adequate group C drugs are not available or are contraindicated. Dlm requires no dose adjustments when used with antiretroviral therapy in patients of TB with human immunodeficiency virus infection (HIV).

The replacement sequence of drugs has also been updated in PMDT-2021 based on efficacy, resistance, adverse drug events, prior use, and background level of resistance. For H mono-/poly-resistance, FQs are to be used. If there is resistance to levofloxacin, then high dose moxifloxacin can be used, provided the susceptibility to the latter has been found. Else, the replacement order is linezolid (Lzd) followed by a combination of Cfz and cycloserine. Rifampicin is a key component in H-resistant TB regimens. However, it is decreases drug levels of Bdq. This precludes Bdq from being used in H-resistant TB. Dlm, Amikacin, Z, Eto is the recommended order, replacing the previous order (Z, A, Eto in 2019). The BPaL regimen (Bdq, pretomanid and Lzd) can also be used as a last resort in individualized settings since the evidence for this combination is still evolving.[5]

4. Other updates

The requirement of a DR-TB centre at every medical college has been stressed. The patient turn-around time has been updated and shortened. A digital e-Nikshay system for monitoring has been advocated. It has been shown that delay in referral to DR-TB centre is an important cause of delayed treatment initiation.[6] To improve the quality of care, a difficult-to-treat TB clinic (DT3C) is to be established at various state levels (Bihar and Maharashtra already have started). Benchmarks for turnaround time for CBNAAT (5–10 days),

Table 1 – Updated regimens for treatment of Drug-resistant Tuberculosis.

Clinical scenarios	Treatment duration and regimen
H mono/poly DR-TB regimen (R resistance not detected & H resistance detected)	(6 or 9 months) Lfx-R-E-Z
All MDR/RR-TB patients with no history of prior exposure to these drugs for 1 month or more, no resistance to FQ, and no extensive-TB defining presentation such as bilateral lung cavities, extensive parenchymal damage, miliary/meningeal/central nervous system TB, and/or any extrapulmonary TB in children except lymphadenopathy, children less than 5 years of age, pregnancy less than 32 weeks and lactation unless mother willing for formula feed. If any of the exclusion criterion is present, Bdq-containing longer oral regimens are used	(4–6 months) Bdq-Lfx-Cfz-Z-E-H-Eto followed by (5 months) Lfx-Cfz-Z-E
MDR/RR-TB patients who are excluded from shorter oral bedaquiline-containing MDR/RR-TB regimen including the XDR-TB patients.	(18–20 months) Lfx-Bdq-Lzd-Cfz-Cs (Bdq for six months or longer)

H: isoniazid; DR-TB: Drug-resistant tuberculosis; R: Rifampicin; Lfx: Levofloxacin; E: Ethambutol; Z: Pyrazinamide; MDR:Multidrug-resistant; RR: Rifampicin resistant; FQ: Fluoroquinolone; Bdq:Bedaquiline; Cfz:Clofazimine; Eto: Ethionamide; XDR:extensively drug-resistant; Lzd: Linezolid; Cs: Cycloserine.

LPA (8–12 days) and culture-based DST (29–58 days) have been tabulated with an emphasis to achieve them as they serve as a quality indicator for care. Bacteriological conversion is now defined when 2 cultures, 7-days apart (originally 1 month) are negative. Lost-to-follow-up is now defined as interruption in treatment for more than 2 months (originally 1 month). Guidelines for prophylaxis of house-hold contacts in DR-TB have been defined- 6 months of levofloxacin for MDR-TB with FQ susceptibility, 4 months of R in H resistance and 6 months of H in RR-TB with H and FQ susceptibility, with monitoring up to 2 years for any symptoms.

5. Future directives

The diagnosis and treatment guidelines of DR-TB is still evolving, and India is updating the same in concurrence with the WHO recommendations. However, at the national level, there are various gaps including logistics, equipment, training, soci0-economic and cultural factors, which also need to be tackled. Integration of the private sector with public health services can definitely help to increase and improve the coverage and treatment of DR-TB in India. Linking the TB control programme with the Ayushman Bharat-Pradhan Mantri Jan Arogya Yojana (PM-JAY) can alleviate the economic constraints, as it can help provide free indoor care to patients. National level centres of excellence, State level difficult-to-treat TB clinics and regular training of the doctors and other supportive health care workers can help in capacity building and improving patient care and outcomes. Diagnostics must adhere to expected turn-around times. Whole genome sequencing platforms are to be made available in the national and state laboratories, to detect known and novel mutations responsible for the drug resistance patterns. Eventually, the treatment algorithms based on such platforms will need to be developed. COVID-19 pandemic has created big hurdles in DR-TB program at all levels, but intensive implementation of the updated PMDT guidelines must be ensured and continued.[7] Fearing a risk of increased morbidity and mortality,[8] COVID-19 and TB coinfection also needs to be timely evaluated[9] in all suspected cases for effective therapy and best outcomes.

Contributions

All the 3 authors contributed to –Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; & Drafting the work or revising it critically for important intellectual content; & Final approval of the version to be published; & Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interest

The authors have none to declare.

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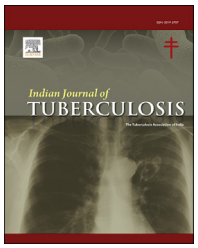

Review article

Rectal tuberculosis: A systematic review

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ABSTRACT

Rectal tuberculosis is an uncommon entity. It has unique epidemiological features, specific medical treatment and surgery is rarely indicated. The first case of rectal tuberculosis was reported in 1957. Delayed diagnosis is common. Patients who develop rectal tuberculosis have been reported to have some risk factors or associated comorbid conditions or pathologies with some form of abnormal host-defence mechanism such as acquired immunodeficiency syndrome, complement deficiency. Rectal tuberculosis has been reported to be more common in females as compared to males. Haematochezia is the most common presenting symptom. The definite diagnosis requires demonstration of *Mycobacterium tuberculosis* bacillus on histopathologic examination. Once a correct diagnosis has been made, rectal tuberculosis is curable with antituberculous treatment. Surgery is indicated for diagnostic dilemmas, non-responsive disease and complications. The authors encountered 3 cases in the last 10 years. The aim of this study is to provide our data on this rare disease and to review the reported literature comprehensively so as to provide guidelines for diagnosis and management.

1. Introduction

Rectal tuberculosis (TB) is an uncommon entity with vague clinical presentation. It shares clinical characteristics of rectal malignancy. Rectal TB has unique epidemiological features, specific medical treatment and surgery is rarely indicated. Davis et al reported first case of rectal TB in 1957.[1] Misdiagnosis was common earlier; because of improvement in imaging and endoscopic techniques, rate of misdiagnosis has reduced. Morbidity has also reduced because of improvement in antituberculous chemotherapy. There is no detailed literature on this rare pathology. Till date, only 28 cases have been reported. The authors encountered 3 cases in last 10 years. The aim of this study is to provide our data on this rare disease and to review the reported literature comprehensively so as to provide guidelines for diagnosis and management.

2. Methods

The systematic search of the literature was performed on Pubmed and Medline according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (Fig. 1). Articles from 1950 till now, using MeSH terms "tuberculosis" and "rectum" in combination with each other and with "epidemiology", "clinical presentation", "imaging", endoscopy", "antituberculous therapy", "surgical management" and "prognosis".

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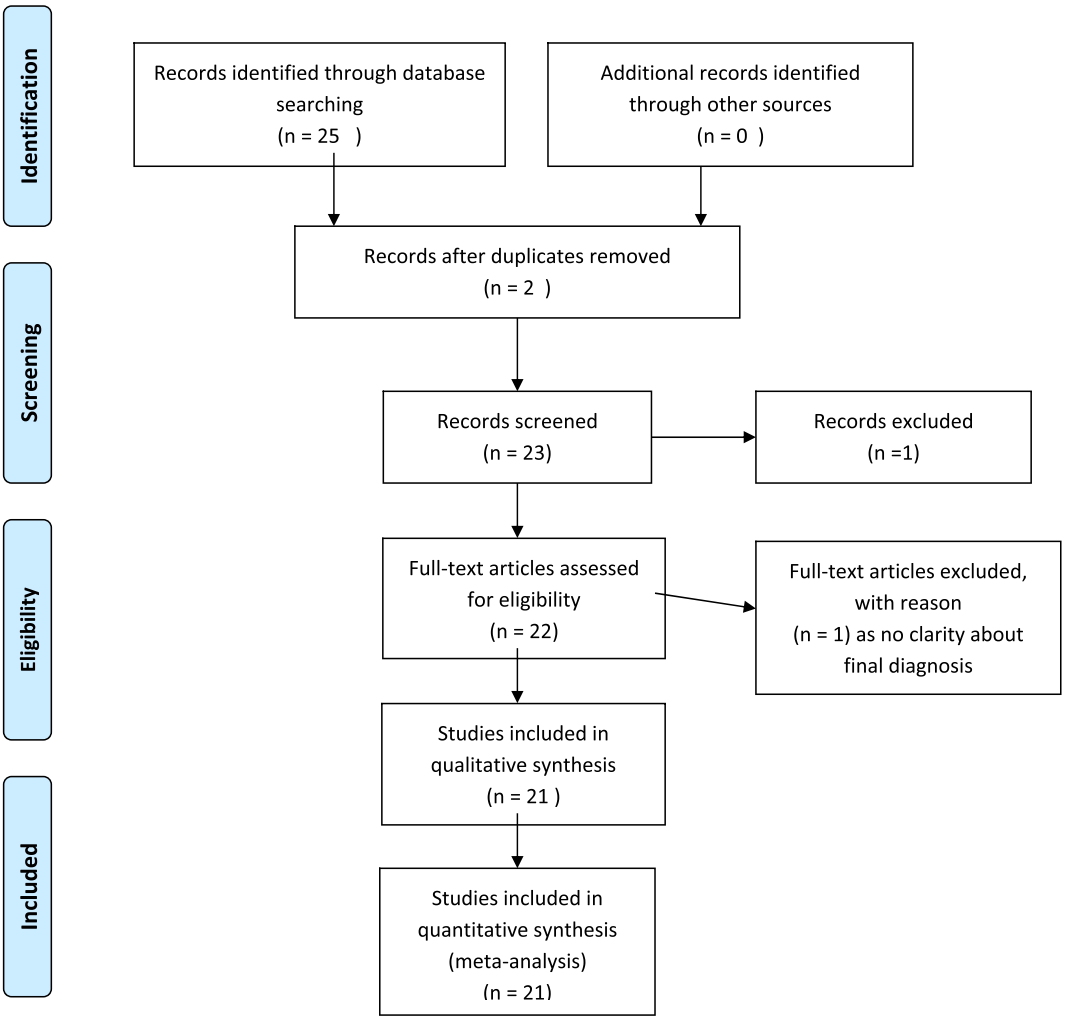

Fig. 1 – PRISMA flow chart.

Data were extracted by one author independently and then compared. The study author provided additional data if incomplete data were noted. Titles/abstracts considered potentially relevant were retrieved for review of the full manuscript. The list of full manuscript meeting inclusion criteria were compared, and any disagreements were resolved by discussion and consensus.

3. Results

One case series and 20 case reports have been identified. All the resulting titles, abstract and full text, when available were read, analysed and kept for reference.

4. Discussion

4.1. *Epidemiological features*

TB is a worldwide health problem. It is a disease of great public health importance in India; India accounts for the highest TB burden (20%) in the world.[2] In patients with gastrointestinal TB, ileocaecal region is the most commonly involved site followed by jejunum and colon.[3] The incidence of rectal TB is 4.5% among all gastrointestinal TB patients.[4] Majority of the reported cases of rectal TB are of Asian origin. There is a rise in the incidence of rectal TB cases. Only 3 cases of rectal TB were reported during the period 1950 to 1990, 11 cases were reported from 1991 to 2000; while, the number of cases reported during 21st century are 14. The reasons for this rise are-increased frequency of abdominal TB among immunocompromised patients, resurge in TB cases in developed nations due to HIV infection and the incidence has not decreased in developing nations, reporting has increased because of improvement in imaging studies and other diagnostic modalities, and there is an increase in emigration from TB endemic countries.

4.2. *Etiopathogenesis*

Patients who develop rectal TB have been reported to have some risk factors or associated comorbid conditions or pathologies with some form of abnormal host-defense mechanism such as acquired immunodeficiency syndrome (AIDS), complement deficiency.[5] Patients with malignancy such as

leukemia or patients on antineoplastic therapy, corticosteroid therapy, TNF-α inhibitors[6] are also at an increased risk of developing tuberculosis at rare sites including rectum. Singh et al suggested that TNF-α inhibitors are associated with a definite risk of reactivation of TB.[6] An adjusted risk ratio of 11.7 has been reported in patients exposed to TNF-α inhibitors, with 20% developing extrapulmonary TB.[7] Occurrence of rectal TB in renal transplant patient supports the role of immunosuppressive states and immunosuppressive therapy.[8] For all types of rectal/perirectal abscesses including tuberculous abscess, immunosuppressed states are the predisposing factors.[9] Rectal trauma resulting from blunt or stab or unrecognized iatrogenic injury, and traumatic rectovaginal fistula with foreign body retention in rectum with superadded infection also have been associated with rectal tuberculosis. Rectal TB has also been reported in association with rectal carcinoma.[10] Immunocompromised state associated with rectal cancer acts as a predisposing factor. Immunocompromised states are also responsible for reactivation of a dormant bacillus. The possible routes of tuberculous infection of rectum are:

(1) Haematogenous spread is considered to be the most common route of spread as tuberculous lesions.[4,10]
(2) Lymphatic dissemination from perirectal lymph nodes.
(3) Direct spread through tuberculous lesions in contiguous structure such as urinary bladder, spine and psoas muscle.
(4) Superinfection of a malignant lesion.

Rectal tuberculosis can be due to primary or secondary infection. In primary cases, there is no evidence of TB elsewhere. Primary and isolated rectal TB is a rare occurrence. Rectal TB is usually secondary to pulmonary TB, or occurs as a part of multifocal gastrointestinal TB, or miliary TB. Based on the mode of involvement, rectal tuberculosis can be classified into the following:

(1) Local, with isolated involvement of rectum in the form of primary complex with caseation of the associated perirectal lymph nodes.
(2) Rectal TB developing secondary to pulmonary TB.
(3) As a part of multifocal involvement of gastrointestinal tract, rectum being accidental rare involvement.
(4) Miliary TB, as a part of generalized TB resulting in involvement of pancreas as well. This is most commonly associated with immunosuppressed states.

The most common site of rectal TB is lower third of rectum, followed by middle third and upper third is least commonly affected by TB. Diffuse involvement of rectum has not been reported.

4.3. *Clinical features*

Rectal TB has been reported to be more common in females as compared to males. Youngest reported patient is 6 years old[3] while the oldest is 67 years old.[8] Majority of the patients were in 3rd and 4th decade of life.

Clinical features of rectal TB are vague and non-specific; presentation depends upon manifestations of TB. Various manifestations of rectal TB are[11,12]:

1. Ulcer formation.
2. Fistula formation
3. Stricture formation
4. Multiple aphthous ulcers
5. Submucosal nodule
6. Nodule with ulceration
7. Verrucous form with warty excrescences
8. Non-specific diffuse edema with stricture formation

Lower abdominal pain, fever, anorexia, weight loss and change in bowel habits have been reported in more than half of the patients. Haematochezia is the most common presenting symptom. Constitution features of TB are seen in up to 75% of patients.[13] Patients may also present with, intestinal obstruction, constipation and diarrhoea.[8,12–14] Massive haematochezia is rarely seen in patients with rectal TB as TB causes end arteritis; however, mucosal trauma caused by the hard stools traversing the strictured segment can cause massive haematochezia in rectal TB patients.[15] Intestinal TB accounts for 3–4% cases of gastrointestinal bleeding leading to haematochezia.[16] In a study by Puri et al, haematochezia was the most common symptom (88%), followed by constitutional symptoms of TB (75%) and constipation (37%).[13]

Constipation and features of intestinal obstruction are most commonly due to stricture formation. TB is responsible for up to 2% of cases of rectal stricture.[13,17] Other rare presentations are passage of mucoid stools and rectal prolapse.[17] Table 1 shows clinical features and other parameters of previously reported cases.

4.4. *Pathology*

Rectal tuberculous lesions are nodular and/or ulcerative or rarely an abscess. Such lesions are usually solitary. Ulcerative lesions are usually superficial and multiple while nodular lesions are solitary and large. Miliary lesions are multiple. 62% of lesions were found to be 2–3 cm in diameter, 44% were 3–4 cm and 12% were 4.5 cm or larger. Majority (85%) of the lesions are seen in the lower third of rectum.

The affected part of rectum is mild to moderately edematous in most cases. Diffuse massive involvement of rectum is rare. The most common gross pathological finding is presence of multiple white caseating nodules in the lower third of rectum present up to 8–10 cm from anal verge, coalescing to form a large yellowish mass of solid consistency (Figs. 2 and 3). Pathologically, rectal TB may have following types[8,18]-

(1) Hyperplastic or hypertrophic lesions: 70% of the patients with primary TB have hyperplastic or hypertrophic lesions. Overall, hypertrophic lesions constitute only 10% of tuberculous lesions. Nodular hypertrophic lesions may mimic carcinoma.[13,18]
(2) Ulcerative type: secondary lesions are mainly ulcerative types. As secondary tuberculous lesions are more common, ulcerative lesions constitute 60% of tuberculous

Table 1 – Demographic, clinical, diagnostic parameters and prognosis of previously reported cases, TB: tuberculosi, HIV: human immunodeficiency virus, -: Not available, PCR: polymerase chain reaction.

Author (year) [reference]	No. of cases	Clinical presentation	Age/sex	History of TB/TB contact	Associated comorbidity	HIV status	Site of lesion	Endoscopy with biopsy/Surgical biopsy
Davis et al (1957)[1]	1	Tenesmus, weight loss	52/M	No	No	_	Lower third rectum	Endoscopic Biopsy
Gupta et al (1970)[4]	1	Pain abdomen, tenesmus, fever, weight loss	40/M	No	No	_	Lower third	Endoscopic biopsy
Chaudhary et al (1986)[27]	1	_	_	_	_	_	_	_
Josh et al (1992)[28]	1	_	_	_	_	_	_	_
Rai et al (1993)[11]	1	Pain abdomen, tenesmus, fever, weight loss	40/M	No	No	_	Lower third	Endoscopic biopsy
Puri et al (1996)[13]	8	Haematochezia, constitutional symptoms, constipation	_	_	_	Negative	Lower third in 7 patients	Endoscopic biopsy in 7, surgical resection and biopsy in 1 case
Das et al (1996)[29]	1	Constipation, intermittent diarrhoea, weight loss	12/F	No	No	_	Lower third	Resection of segment and Swensen pull through surgery, biopsy of resected specimen
Rege et al (2002)[14]	1	Haematochezia, constipation	40/M	No	No	Negative	Lower ectum	Laparotomy, sigmoid loop colostomy, biopsy resected rectum
Nagi et al (2003)[30]	1	_	_	_	_	_	_	_
Sahoo et al (2004)[31]	1	Haematochezia, mucoid stool	35/F	No	No	Negative		Anterior resection and biopsy
Fernandez-Rivero et al (2007)[5]	1	Atypical anal ulcers	50/F	No	No	Negative		Endoscopic biopsy
Sotoudehmanesh et al (2007)[8]	1	Haematochezia, painful defaecation, weight loss	67/M	No	h/o renal transplant	Negative	Lower third	Endoscopic biopsy
Samarasekara et al (2008)[9]	1	Per rectal discharge, weight loss	41/M	No	h/o recurrent intra-rectal abscess	Negative	Lower third	Endoscopic biopsy positive, PCR and culture negative
Sebastian et al (2008)[32]	1	Low backache, haematochezia, weakness, weight loss, fever	44/M	No	No	Positive	Lower third	Endoscopic biopsy, developed drug induced hepatitis, died
Subnis et al (2008)[12]	1	Haematochezia, painful defaecation, altered bowel habits	31/F	No	No	Negative	Lower ectum	Endoscopic biopsy and PCR positive

(*continued on next page*)

Table 1 – (continued)

Author (year) [reference]	No. of cases	Clinical presentation	Age/sex	History of TB/TB contact	Associated comorbidity	HIV status	Site of lesion	Endoscopy with biopsy/Surgical biopsy
Khaniya et al (2009)[10]	1	Haematochezia, altered bowel habits, weight loss	35/F	No	Carcinoma rectum	Negative	Lower rectum	Endoscopic biopsy s/o TB + carcinoma, APR done
Patil et al (2013)[17]	1	Constipation, weight loss, Rectal prolapse	45/M	No	No	Negative	Lower third	Endoscopic biopsy, Laparotomy done for prolapse, ileostomy made
Santra et al (2013)[33]	1	Haematochezia, fever, diarrhoea	17/F	No	No	–	–	Endoscopic biopsy
Singh et al (2016)[6]	1	Diarrhoea	38/M	Disseminated TB, colon normal	Ulcerative colitis on TNF-α inhibitors	Negative	Middle and power third	Endoscopic biopsy
Liu et al (2017)[34]	1	Pain abdomen, bloody purulent stool	29/F	No	Hyperthyroism, history of insertion of chopsticks into anus by herself	Negative	Negative	Diagnosed by Empirical ATT
Pandit et al (2018)[3]	1	Pain abdomen, weight loss, vomiting	6/M	No	No	Negative	Anorectal involvement	Endoscopic biopsy

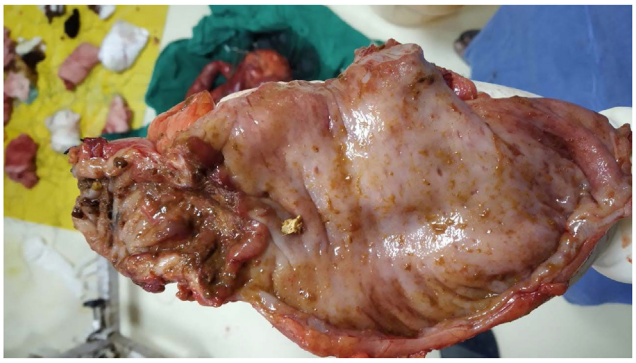

Fig. 2 – Growth with white cheesy material in lower rectum.

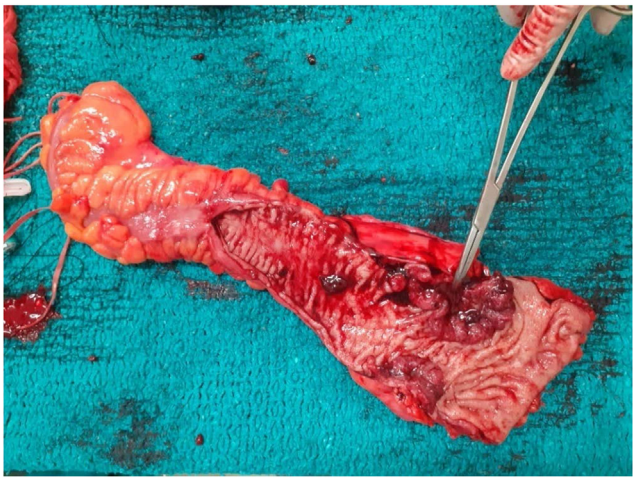

Fig. 3 – Ulcerative growth in lower rectum.

lesions.[8,18] Ulcerative lesions are superficial and irregular with a necrotic base.

(3) Ulcerohypertrophic type: these lesions constitute 30% of tuberculous lesions.[8,18]

4.5. *Microscopy*

The hallmark of tuberculosis caused by Mycobacterium tuberculous bacillus are granulomatous inflammatory lesions and typically shows central caseation with aggregates of macrophages, epitheloid cells and langhans giant cells. The lesions vary in size from 1mm to >2cm in diameter. Fibrosis may develop in relation to granulomas. Epitheloid granulomas may be non-caseating also; in such granulomas, presence of acid fast bacilli is diagnostic.

4.6. *Diagnosis*

Diagnosis of rectal TB is difficult and often gets delayed because of its rarity, vague presentation, and overlapping of clinical features with the more common pathology of rectum i.e., adenocarcinoma of rectum. The definite diagnosis of rectal TB requires demonstration of *Mycobacterium*

tuberculosis bacillus on histopathologic examination. Demonstration of acid and alcohol fast bacilli is also pathognomonic; though not commonly seen. The criteria/definition for diagnosis of rectal TB is given in Table 2.

4.7. *Imaging studies*

X-ray chest: X-ray should be done as it detects the presence of past or active tuberculous focus.

Conventional Ultrasonography (USG): It is the imaging modality of choice for initial evaluation of patients presenting with symptoms related to gastrointestinal system. USG is an excellent modality for preliminary screening to confirm the presence of any extraluminal lesion including presence of space occupying lesion suggestive of tuberculous in solid organs, mesenteric, omental nodules, lymph node enlargement and ascites. It is difficult to pick up rectal lesions on conventional USG.

Endorectal ultrasound (ERUS): ERUS is easy to perform, causes very less discomfort and reconstruction of 3D images is also possible.[19] It is the most accurate method for evaluation of rectal cancer, perianal sepsis and also of value in evaluating other anatomical structures in the pelvis.[20] The presence of slight mucosal invasion in early rectal cancer may be imaged in greater detail with ERUS.[21] Unfortunately, there is no study on assessment of rectal involvement in tuberculous pathology with ERUS; however, ERUS might become an excellent and safe alternative to expensive computed tomography and magnetic resonance imaging study.[22]

4.8. *Contrast enhanced computed tomography (CECT)*

USG has resolution limitations. CT scan is the most effective study to demonstrate changes in the peritoneum, mesentery, bowel, lymph nodes and other organs. Though, rectal involvement with tuberculosis was associated with disseminated disease only in 1 case,[6] it is important to rule out or confirm any tuberculous lesion in the abdomen other than rectum as management plan changes accordingly. It is not easy to differentiate rectal adenocarcinoma from tuberculous lesions of rectum as both the pathologies may have associated lymphadenitis and ascites. Sinan et al reported that CT evaluates both mucosal and extramucosal components; barium enema is better in demonstrating mucosal lesions but it does not provide direct evidence of extramucosal pathology.[23] On CECT, the findings suggestive of TB of rectum are circumferential enhancing mural thickening of rectum with luminal narrowing, longitudinal stricture with mucosal irregularity, widened presacral space due to perirectal and perianal fibrosis associated with stricture, diffuse nature of lymphadenopathy.[3,15] Now, CT colonography has gained popularity as a new diagnostic tool in early detection of colorectal pathologies by acquiring volumetric CT data sets of the abdomen.[24]

Table 2 – Criteria for diagnosis of rectal tuberculosis, TB-tuberculosis, PCR-polymerase chain reaction, ZN- Zeihl Nelson stain.

1. Relatively young patient, especially female
2. Patient from areas having high incidence of active TB
3. Past history of TB and/or exposure to TB patient
4. Patients with active tuberculosis at other site
5. Presence of associated congenital or acquired immunodeficiency state
6. PCR/ZN stain/culture obtained from pus/ascetic fluid/tissue demonstrating acid fast bacilli
7. CECT showing mass/ulcerative lesion in rectum with associated perirectal lymphadenitis with/without associated tuberculous lesions in the abdomen
8. Endoscopic biopsy demonstrating caseating granulomas

4.9. *Bacteriology and polymerase chain reaction (PCR)*

Acid fast bacilli (AFB) staining and culture and PCR may be obtained from ascitic fluid, pus from rectal ulcers, rectal ulcer/growth biopsy. The success rate of these modalities for rectal TB has not been reported. In reported cases, AFB staining, culture and PCR failed to diagnose rectal TB. Rai et al reported that cultures obtained from biopsy specimen are positive in 36% of cases.[11]

PCR assay is helpful in diagnosing tuberculous lesions present at rare sites including rectum. However, out of 29 reported cases, PCR assay was done on tissue in 2 cases but failed to diagnose TB. Out of 3 cases of cases, PCR assay was done in 1 case and the result was negative.

4.10. *Endoscopic biopsy*

Endoscopic biopsy is the best modality to diagnose rectal TB as it provides tissue for direct histopathological examination. The success rate is high; of 28 reported cases, 17 were diagnosed on endoscopic biopsy. If the suspicion of TB is high based on clinical and epidemiological features, and endoscopic biopsy comes as unfavourable; in such a situation, endoscopic biopsy should be repeated as it saves the trauma of laparotomy. Major surgical intervention is indicated for diagnostic purposes only when repeat endoscopic biopsy fails to confirm the diagnosis and associated rectal malignancy cannot be ruled out. Laparotomy was done in 1 case out of 29 reported cases for diagnostic purposes. Out 3 of our cases, laparotomy and abdominoperineal resection were done in 1 case, while 2 cases were diagnosed on endoscopic biopsy (Table 3).

4.11. *Other investigations*

HIV testing should always be done in all the patients diagnosed to have rectal TB as occurrence of TB at rare sites is more common in patients who are HIV positive. Tumor marker such as carcinoembryonic antigen (CEA) is helpful in patients with coexisting carcinoma and tuberculous lesion.

4.12. *Differential diagnosis*

The differential diagnosis of rectal TB includes rectal adenocarcinoma, crohn's disease, amebic colitis, pseudomembranous colitis.

4.13. *Treatment*

The management of rectal TB depends on the clinical manifestations, pathological type, presence medical comorbidities

Table 3 – Demographic, clinical, diagnostic parameters and prognosis of three cases, TB- tuberculosis, CECT-contrast enhanced computed tomography, ATT-antituberculous therapy, APR-abdominoperineal resection.

Case	Age/ sex	Clinical presentation/ duration of symptoms	History of TB/TB contact	Associated comrbidity	HIV status	CECT	Site of lesion	Endoscopic biopsy	Follow up and prognosis
1	48/F	Haematochezia, constitutional symptoms/14 weeks	No	No	Negative	Circumferential thickening of lower rectum	Lower third	Positive	Received ATT for 12 months, recovered
2	54/F	Haematochezia, altered bowel habits/ 12 weeks	History of pulmonary TB at the age of 19 years, family history positive	No	Negative	Growth in lower third rectum	Lower third	Negative on two occasion, laparotomy with APR done, post-operative biopsy showed TB	Received ATT for 12 months, recovered
3	39/M	Mucoid stools mixed with blood, pain during defaecation, constitutional symptoms/12 weeks	No past history of TB, family history of pulmonary TB	No	Positive	Ulcerated growth in middle and lower third rectum	Distal two-third	positive	Received ATT for 12 months, on ART and follow up

and carcinoma, HIV status, and its occurrence in isolation or as a part of diffuse gastrointestinal TB or disseminated disease. The principles of treatment are timely diagnosis with fewer diagnostic modalities, treatment with antituberculous drugs for optimum duration, timely surgical intervention whenever indicated, pre- and post-operative ATT should be given, identification, and management of associated medical illness.

There are no specific guidelines for management of rectal TB because of the rarity of disease. Once a correct diagnosis has been made, rectal TB is curable with antituberculous treatment (ATT).[12,13,25] Treatment regimen consists of intensive phase with four drugs: isoniazid (5 mg/kg BW/day), rifampicin (10 mg/kg BW/day), pyrazinamide (30 mg/kg BW/day), ethambutol (20 mg/kg BW/day) for 2–4 months followed by four months continuation phase with two drugs: isoniazid and rifampicin.[25] Isolated extrapulmonary tuberculosis (EPTB) is associated with lower bacillary burden than pulmonary disease. According to WHO guidelines, isolated tuberculous lesion with no history of TB should be considered as a new case of EPTB, and ATT should be given for six months.[26] Therefore, isolated EPTB, such as rectal tuberculosis can be treated with standard short-course regimens (6 months) that are effective for pulmonary disease. However, disseminated disease with tuberculous rectal involvement and rectal involvement as a part of diffuse gastrointestinal TB needs to be treated with 12 months of chemotherapy.

Colonoscopy is the investigation of choice for follow up and also in guiding the duration of ATT for patients of isolated rectal TB. CT imaging is preferred for patients with rectal TB with associated rectal carcinoma and also for patients with diffuse gastrointestinal TB for confirmation of complete resolution of the pathology and also in patients requiring any form of surgical intervention.

4.14. *Surgical intervention*

Indications of surgical intervention are[14]:

(1) Tuberculous abscess
(2) Coexistence of malignancy and TB
(3) When surgery is indicated for involvement of other parts of gastrointestinal system with malignancy and/ or TB.
(4) Imaging studies and endoscopic biopsy fails to diagnose rectal TB and malignancies such as rectal malignancy cannot be ruled out.
(5) The disease fails to show complete clinical response with ATT and stenosis/stricture persists for more than three months.
(6) No endoscopic/radiological improvement in the lesion even after standard ATT for 3–6 months, the mass needs to be resected for histological confirmation of diagnosis. For resection, oncological principles should be followed.
(7) In the presence of complications such as rectovaginal fistula, rectal prolapse, and rupture of an abscess cavity into the adjacent hollow viscera or pelvis.

4.15. *Role of surgical intervention*

Tuberculous rectal abscess: the management of tuberculosis abscess is prompt surgical drainage and initiation of antituberculous chemotherapy. It can be drained rectally, laparoscopically, and by open exploratory laparotomy method with extensive surgical drainage.

Endoscopic balloon dilatation: there is proven role of endoscopic balloon dilatation of gastric and ileal lesions; however, there is no successful report of endoscopic balloon dilatation of strictures due to rectal TB.

Exploratory laparotomy with resection of diseased segment with anastomosis with or without proximal stoma creation: there are reports of anterior resection, abdominoperineal resection, rectosigmoidectomy with or without proximal stoma formation.[10,14]

4.16. *Prognosis and complications*

Complications are rare, and delayed diagnosis is responsible for their occurrence. Intestinal obstruction is a common complication of rectal TB; occurs due to stricture formation. Massive haematochezia can occur, and treatment is emergency surgical resection of diseased segment. Rectal prolapse has also been reported.[17] Of 29 reported cases, one patient died due to sepsis.

5. Conclusion and recommendations

Tuberculous involvement of rectum is a rare manifestation of a common infective pathology caused by *M. tuberculosis*. Diagnosis often gets delayed. Rectal TB patients respond well to standard ATT. The authors recommend-

1. Rectal TB often gets confused with rectal adenocarcinoma. Rectal TB should be suspected when symptoms are of prolonged duration, patient is relatively young, in the 3rd or 4th decade of life, presence of constitutional symptoms, past and/or family history of TB, or in a patient who is from a region with high prevalence of TB.
2. There are no specific features on imaging studies. However, CECT should always be done to rule out disseminated nature of TB and also to rule out any other intra-abdominal pathology.
3. Endoscopy and histology is the best and direct way to diagnose. In patients with high level of suspicion with negative biopsy for TB, endoscopy should be repeated as it is essential to diagnose this pathology without major surgical resection.
4. Surgical intervention is indicated for non-responsive disease and complications.
5. When it is not possible to differentiate between TB and malignancy, intra-operative frozen section helps.

Conflicts of interest

The authors have none to declare.

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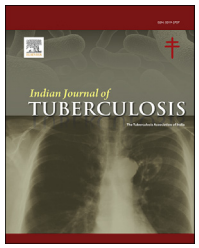

Review article

Review on public private mix TB control strategy in India

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ABSTRACT

In India, around 70% of health care services are offered by the private sector. National strategic plan (NSP) has emphasized private sector engagement to TB program. Public private mix strategy along with web based mandatory notification of TB cases were established in 2002. However, feasibility of consulting an informal provider first was seen to be associated with significant increases in total delay (absolute increase 22.8 days, 95%CI 6.2–39.5) and in the risk of prolonged delay >90 days.

Study design: A mixed method literature review, descriptive information and evaluative outcomes data extracted and analysed.

Objective: This review aimed to systematically review public private mix strategy in TB control in Indian tuberculosis disease burden and efforts towards elimination.

Methods: Available published literatures were searched with key words, articles related with objectives were selected, analysed and systematically synthesized. Overall 30 studies were reviewed.

Result: Available literatures were selected based on study objective and analysed. The modes of PPM strategy its success and problems of implementation and shortcomings were synthesized.

Discussion: After implementing PPM from 2002, case detection is seen to have significantly increased for smear positive cases and high detection rate and better treatment outcomes achieved. However, implementation of PPM has been challenged to fully deliver the intended services. Interestingly, seeking initial care from PPs is significant risk factor for diagnostic delay.

Conclusion: PPM is a proven and tested strategy to achieve End TB goal globally and even in India. However, studies indicated there is the need to strengthen and motivate public sector to engage private practitioners in specific districts and sync their activities into the mainstream programme. Conflict of interest and mistrust between private practitioners and public sector has to be well addressed to build sustainable relationship among the

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sectors. Routine and institutionalized systematic monitoring and evaluation of the system is required to meet the End TB goal by 2025.

1. Introduction

Worldwide, Tuberculosis (TB) is the cause of millions of deaths annually, in 2019 there were an estimated 1.2 million TB deaths among HIV-negative people and an additional 208,000 deaths among HIV-positive people globally. Geographical TB distribution were WHO regions of South-East Asia (44%), Africa (25%) and the Western Pacific (18%), Eastern Mediterranean (8.2%).[1] India reported incidence of TB cases (includes HIV + TB), 2.690 incidence (HIV + TB only) 71,000. Incidence (MDR/RR-TB) was 124,000, mortality (excludes HIV + TB) 436,000, mortality (deaths) (HIV + TB only) is 9500 (3%).[2]

Utilization of Private Practitioners has raised to fivefold worldwide in programmatic service delivery of TB control programme. Public Private Mix strategy is recognized globally and brings tremendous impact on TB control by improving access to patients. Although RNTCP has insured DOTS in 2006 coverage, delay in initiation of TB treatment was reported as in smear-positive patients mean duration between TB diagnosis and treatment initiation was 21 days with a range of 8–207 days (median = 14 days).[1] Substantial delay was identified from previous studies on public private mix TB care. Care given by private sector is often substandard and inconsistent.[3] Similarly, baseline study conducted in the urban slums of Mumbai showed that patients experienced delays beyond two months from the onset of symptoms to first care seeking and reasons for delay were provider shopping for confirmation of diagnosis in approaching the next provider after leaving one provider.[4] Patients visit up to four health care facility before initiation of anti-tuberculosis treatment.[5]

RNTCP has insured universal access to TB in 2006 in India. Public private mix strategy was designed and implemented as a way to ensure access to TB care and control. However, substantial delay in diagnosis, initiation of TB treatment and delay in seeking care is common. Studies identified longer health system delay among symptomatics who approached a private health provider at first point of care patients; higher delay being associated with private providers than government. Recent study conducted in Kerala identified delay in diagnosing tuberculosis especially with private health providers. The study reported the mean time taken by the patient for consultation after onset was 36 days and the mean time for diagnosis was 42 days and total time until diagnosis was 78 days. 72.8% patients consult within 6 weeks of onset and 74.7% are diagnosed within 6 weeks of consultation.[6] Similarly study in Southern India identified among the study participants 73.7% delayed seeking care (cough duration of ≥4 weeks more delay occurred)higher delay was observed among risky alcohol users and patients with higher income which is significant to risk adjustment analysis to delay in seeking care.[7]

Public Private Mix Strategies in developing countries have faced conceptual imprecision. There are also claims of gaps in understanding how PPM concept has been applied at the ground level, the role of the partner organizations in relation to the state, the programme and the health system in general among scholars and implementers. Partnership modes differ in different TB programs identifying successful partnership helps to scale up and implement effective strategies. Documented successful PPM strategies, dynamics and constraints of implementation on the ground are few.

1.1. *Objective*

This review aimed to review literatures on public private mix strategy in TB control in Indian TB to get insight on recent PPM strategy and inform bigger study.

2. Methods

Published literatures available on Scopus, PUBMED and Google Scholar were searched with key words, articles related with objectives were selected, analysed and systematically synthesized. Overall we reviewed over 20 studies. PRISMA method was used to synthesise the literatures.

3. Result

Studies on Pubic private mix reported on the success of the strategy, the challenges faced during implementation and the reasons stated by patient to prefer private sector are discussed.

Private health sector is a key health service provider which is considered as more accessible responsive and individualized for patients. The private sector is highly diversified ranging from practitioners of western medicine MBBS doctors, those trained in Indian systems termed as AYUSH an acronym (Ayurveda, Yoga,Unani, Siddha and Homeopathy) to those without formal training.[8]

Private practitioners can be involved in the programme depending on their capacity interest and programe requirements in a multiple or single activity. Private sectors detect suspected cases and then either refer cases to NTP affiliated facilities for treatment or provide the TB treatment and notify NTP through laboratory register.

3.1. *Mechanisms of RNTCP private sector engagement*

3.1.1. *Public private mix performance in TB programme*

Findings from previous studies reported PPM programme achieved successful improvement in case detection, treatment outcomes and case management. DOTS utilization was

highly improved. PPM has created favourable environment to practise standard TB care. Knowledge of private practitioners improved through NTP advocacy. Increased use of x-ray and sputum testing and prescription of only x-ray has decreased. Case detection was significantly increased for smear positive cases and high detection rate achieved on all TB cases. Treatment outcome success rates also increased.[9]

A qualitative study conducted in Southern Indian State reported improved accessibility of quality TB care with greater geographic coverage.[3] A policy analysis done on Private Providers of health care India reported private sector enjoys the advantages of more convenient timings and locations, shorter waiting periods, closer identity with the communities they serve, and greater trust enjoyed. In addition, it will accord a sense of joint ownership and accountability to TB control activities in India.[10]

Better treatment outcomes were reported in an armed controlled trial in the PPM areas. PPM DOTS setting achieved higher treatment success rates than purely public service settings. Lesser default rates, improved case management, better case registration, patient tracing, follow up and rate of case referral significantly increased. Confirmed diagnosed cases were also significantly increased.[11]

3.2. *Problems of PPM implementation*

Perceived good quality of care was one of the reasons for clients to seek care from private sector while another mostly mentioned reason was convenience of distance and flexible opening times and confidentiality across the Indian health system. However, private sectors have not well positioned to the patient expectation of timely diagnosis and treatment provisions. Seeking initial care from PPs is significant risk factor for diagnostic delay. Study from Mumbai found pathway from the onset of symptoms suggestive of TB to initiation of TB treatment the mean duration for the total pathway was 65 days. Importantly the mean duration of first care seeking was similar in new (24 days) and retreatment patients (25 days). Diagnostic duration contributed to 55% of the total pathway the finding shows higher health system delay.[4] Shift of patients from the private to public sector and non-allopaths to allopaths was observed, particularly for treatment initiation. Failure to direct the patient to RNTCP forced patients to concurrently seek care from Public and Private providers which caused delay leading long care seeking pathway.[11] Consulting an informal provider first was associated with significant increases in total delay (absolute increase 22.8 days, 95%CI 6.2–39.5) and in the risk of prolonged delay >90 days was reported from study in Chennai.[12]

3.2.1. *Knowledge and attitude of private practioners towards RNTCP*

Study on, Enhancing the role of private practitioners in tuberculosis prevention and care activities in India finds, Private doctors generally have inadequate training and lack of information about DOTS. Second, they lack confidence in treatment regimens and diagnostic methods of the national TB Control program. Private practitioners also claim on the public dominance in the overall implementation of DOTS strategy and not fully participated as partner with the policy and program.[13]

Lack of knowledge of private practitioners (PPs) about the best protocols for the diagnosis and treatment of TB was reported by study in Uttarakhand a State of North India reported that out of 71 PPs, almost 83% knew that in RNTCP sputum acid fast bacillus (AFB) examination was the most important diagnostic test for pulmonary tuberculosis, 66.2% knew that intermittent regimens under direct observation are practiced in RNTCP and 50.7% PPs showed readiness to support the programme with government support.[10] In another study conducted in Pune, use of sputum microscopy for TB diagnosis was reported 63%.[14]

3.2.2. *Perception towards PPs and monitoring gaps by RNTCP/the public sector*

The preconceived notions and prejudices against PPs perception of the public that private sectors have money motives only was identified as constrain to collaborate.[13] Another study conducted on Private Practitioners' Perspectives on their involvement With the Tuberculosis Control Programme in Southern India reported low perception of private practitioners partnership with the TB programme.[15]

Lack of strong regulatory mechanisms by the public sector, resources and coordination was another factor to hinder the inter collaboration. Private sectors reported to have in adequate training and information about DOTS.[12] Private practitioners claimed limited involvement of private sectors in planning stage of the programme as factor which challenge the effective collaboration.

3.2.3. *Adherance to guidelines and protocols*

Adherance to RNTCP diagnostic and treatment protocol is vital in control and prevention of TB. Smear microscopy is the recommended method of diagnosis for newly suspected symptomatic. However, studies have reported sticking to other methods like serological tests by private practitioners. Study from Pune India reported 63% (158/249) of provider responses were consistent with ISTC diagnostic practices, and 34% (84/249) of responses were consistent with ISTC treatment practices. However, 48% (120/249) PP also reported use of serological tests for TB diagnosis.[16]

4. Discussion

The second pillar of End TB strategy is Engagement of communities, civil society organizations and public and private care provider. Establishment and sustained PPM strategy implementation is a way to achieve universal access to TB care, prevent the emergence and spread of multidrug resistant TB. In India where more than 50% of TB symptomatic sought care from private sector as first choice, private sector engagement is critical to provide quality TB care.[17]

Better access and equity is aimed to be insured through PPM programs. Free diagnosis and anti TB medicines are supplied by NTPS which increases affordability of TB care to a

large extent. Restriction put on private sale of TB medicine which insures affordability and patients treated under standard treatment guideline. Studies reported patient attitudes and care seeking behaviour has changed through PPM and a high degree of satisfaction with TB treatment was expressed.[11,13,15]

Patients switch between public and private health sector mainly due to felt poor user experience, perceived greater protection and confidentiality when receiving services from private providers and claim more user friendly and patient centred care in allowance of flexibility in DOTS. Patients feel alienated and ignored due to less time with doctors in overburdened public sector.[12,16]However, public practitioners reported to be more technically competent compared to PPS with regard to detecting and treating TB.[12,13,16]

The private sector enjoys the advantages of convenient timings and locations, shorter waiting periods, closer identity with the communities they serve, and greater trust enjoyed. Further, involving PPs to co-deliver DOTS would rapidly enhance the case finding and treatment outcomes.[13] Another benefit of PPM is its cost effectiveness in treating TB patients shifting from non-DOTS to DOTS has subsequently reduced the cost per patient successfully treated following the initiation of PPM.[18]

PPM can be established in different models related to the private practitioners capacity and nature. Overall there are four types of PPM models. Model 1.The referral of presumptive TB patients identified by the private providers to NTP for further diagnosis and treatment. Model 2 encompasses the diagnosis of TB and referral to the NTP. Model 3 Provision of directly-observed treatment. Model 4 provides both diagnostic and treatment services similar to a TBMU.[17]

Missing of TB patients due to selective TB referral was observed in study in Southern India found among the referred TB symptomatics, smear positivity were 24% which was higher than the expected 10% implies selective referrals and missing of cases among ther referred cases 50% were grade three sputum positive.[19] This finding has similarity with finding from Vietnam within the generic symptomatic referral about 30% of presumptive TB patients referred to NTP for sputum microscopy did not reach the diagnostic facility and nearly 60% of the patients diagnosed were lost to follow-up before starting treatment. Of those started on treatment, only 60% successfully completed it.[20]

Although more than half present of TB symptomatic prefer as first point to seek care from the private sector significant health system delay was reported through studies conducted on delay to TB diagnosis. Findings revealed that private sectors are not still as equipped as public sector to provide timely diagnosis fort TB patients thus RNTCP program has to provide trainings and further awareness for private providers. Strong linkages between public and private sectors, referral system strengthening and patient awareness creation on TB symptomatic and seek immediate accessible care could reduce the delay associated within public private mix system. Local NGOs serve as intermediaries in Indian PPM program however research in Southern India suggested enhancing the capacity of national TB program and general health system staff has better communication and efficiency after randomized controlled trial.[21]

5. Conclusion and recommendations

PPM is a proven strategy for DOTS coverage and would help achieve End TB goal in India. However, studies indicated there is the need to strengthen and motivate public sector to engage private practitioners. Private sectors perceive their inclusion in the system is not sufficient. Conflict of interest and mistrust between private practitioners and public sector has to be well addressed to build sustainable relationship among the sectors.

Some recommendations that emerge from the review of literature is that there should be an *inclusive list of private sector service providers* inbuilt into the programme and the listing process should be dynamic), depending on new additions of health care providers in the area, every six months. This list may be available with the frontline workers who are regularly in touch with the community. During home visits, vaccination or adolescent camps these lists should be displayed and discussed with participants, and they may be informed that these private providers are trained in TB programme and may encourage the community to seek their advice for sake of convenience. The private providers may be sent updates on the programmatic front and would feel encouraged by the involvement in the programme, as GOI gives small monetary support for any referred case from this sector. A list of referrals per month may be displayed at a prominent side of the clinic of the private provider and their contributions acknowledged in all programmatic forums. The linkage of the private and the public provider, needs to be streamlined and any referrals from private sector should be honoured and the private providers may also be taken up for DOTs services and trainings, whenever planned and their time and participation reimbursed. Routine and institutionalized systematic monitoring and evaluation of the system should be planned and the gaps be further identified. Such a cohesive and inclusive strategy which is already being done in many parts of the country is the need of the hour to achieve the End TB goal by 2025.

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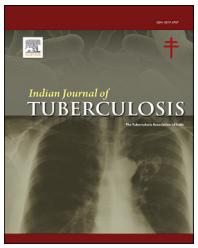

Review article

Recent updates in natural terpenoids as potential anti-mycobacterial agents

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ABSTRACT

Tuberculosis is considered as a leading health issue globally. Even though, the todays first line anti-mycobacterial treatments used in the hospital have low deaths, multidrug-resistance forms of the ailment have now spread globally and become a major issue. The wide-ranging biodiversity of medicinal plants, ocean animals have gained considerable attention for drug discovery in previous spans, and the emergence of TB drug resistance has inspired interest in judging natural products (NPs) to cure this disease. Till now, several compounds have been isolated from natural sources with anti-mycobacterial activity, few of which demonstrate significant activity and have the potential for further development. Worldwide huge natural flora and fauna are existing, this flora and fauna must be investigated for new potent lead against infectious TB. This review systematically surveys various classes of terpenoid molecules obtained from different medicinal plants, fungi, sponges, and sea plumes with anti-TB activity, which could be useful for further optimization and development in this field.

1. Introduction

Tuberculosis is considered as dangerous, lethal, communicable, contagious, epidemic and pandemic disease caused by various forms of TB bacteria like *Mycobacterium tuberculosis, Mycobacterium bovis, M. africanum, M. avium, M. laprae, and M. microti.*[1–9] It is known to be one of the top 10 causes of death worldwide and the leading cause of death of a single infectious agent beyond HIV/AIDS.[10–13] All TB bacteria's mainly attack the lungs alveoli and respiratory system of human body. Most of the tuberculosis cases are from *Mycobacterium tuberculosis* H37Rv strain discovered by Robert Koch in 1882.[4,5,14,15] Almost all the treatment options are failing to cure tuberculosis completely. Worst thing is *Mycobacterium tuberculosis* shows more or less resistance to all first and second line anti-TB drugs.[16] Hence, treating MDR-TB is the major challenge in front of today's scientist.[2] Drug-resistant TB is still a rising public health threat. Approximately half a million people worldwide developed rifampicin-resistant TB in 2019, of which 78% were found to be multidrug-resistant TB cases. Currently 2 novel anti-TB drugs namely Bedaquiline and Delamanid were approved by US FDA and EMA for treatment of MDR-TB patients.[17,18] Bedaquiline is found to show certain adverse effects like hepatotoxicity and cardiotoxicity. Hence,

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to treat MDR, XDR, TDR-TB cases there is a need of safe, efficacious, small duration and cost effective drug treatment.[10] According to the WHO Global Survey of 2019, 10.0 million people had TB disease in 2019. 1.2 million deaths have been reported among HIV negative people and an additional 2,08,000 deaths among HIV positive people have been reported. All age group people (56% of men aged more or equal to 15 years, 32% women, 12% children aged below 15 years) have developed TB in 2019 and from these people, 8.2% people were living with HIV disease (Fig. 1). In 2019, 44 per cent of citizens from South-East Asia, 25 per cent from Africa, 18 per cent from Western Pacific, 8.20 per cent from Eastern Mediterranean, 2.90 per cent from America and 2.50 per cent from Europe developed TB (Fig. 2).[15]

Nature always supplied complete medication to cure all maladies of mankind.[19,20] There are many mega-diversity hot spots on the planet with a rich history in traditional medicine knowledge. Almost 60–80% of current medicines in practice are from herbal origin and most of the people rely on them.[21,22] Approximately 4.5 million plant species available on the earth, among which only 250,000–500,000 plant species have been scientifically studied for therapeutic operation. A significant number of higher plants are still to be toured as a source for new lead molecules.[23] Medicinal plants contain polychemicals like alkaloids, glycosides, tannins, flavonoids, lignans, resins, volatile oils, terpenoids, saponins *etc.* in them (Fig. 3).[8] These polychemicals shows multiple actions against different types of diseases and disorders.[8] Considering this wide application of medicinal plant chemicals in treating ailments and in continuation of our research on finding new anti-mycobacterial agents[24–28] here, in this review we have compiled sixty terpenoids isolated from various natural sources with their anti-mycobacterial potential. This review will help researchers to know anti-mycobacterial potentials of these isolated natural terpenoids and their further development.

2. Data sources, and data extraction process

Literature search is conducted using various search engines such as Scifinder, PubMed, PubChem, Science Direct and Google Scholar. Espacenet, and the world intellectual property organisation (WIPO) employed for patent hunt. From the above search engines, Scifinder, Science Direct, PubMed and Google scholars and WIPO were considered useful for the compilation of related literature and patents. Not a single patent found on topic of our interest. Search has been carried out by using keywords like phytochemicals in TB, natural products TB, anti TB phytocompounds, anti-TB secondary metabolites, terpenoids as anti-mycobacterial agents, herbal compounds TB, terpenoids, terpenoids tuberculosis, terpenoid MTB *etc* (Fig. 4).

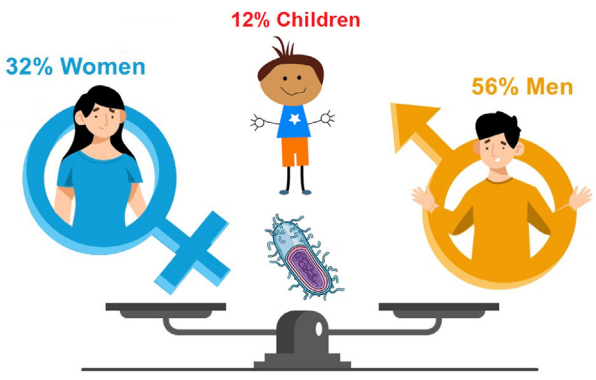


Fig. 1 – Percentage TB cases developed in different age groups as per WHO global TB report 2020.[15]

3. Anti-mycobacterial potential of terpenoids

Oladosu *et al.*, isolated the two lupane type isoprenoid constituents namely betulinic acid methylenediol ester (**1**) and betulinic acid (**2**) from stem bark of *Syzygium guineense* through anti-mycobacterial activity directed solvent fractionation and chromatographic separation. They extracted the plant powder with the help of chloroform menstrum using cold maceration technique. Resultant extract was concentrated in vacuum evaporator. It was defatted later with n-hexane and partitioned with acetone to get both the n-hexane and acetone fractions. With the isolation technique of column chromatography, both the lupane isoprenoids (white solid compounds) were separated from acetone fraction successfully.

The anti-mycobacterial activity was performed using the *Mycobacterium* Growth Indicator Tube (MGIT) process. Both lupane isoprenoids (**1** and **2**) had corresponding MIC values of 150 μg/mL and 600 μg/mL (Fig. 5). Higher lipid solubility of betulinic acid methylenediol ester could be the reason for its higher anti-mycobacterial activity as compared to the betulinic acid.[29]

Madikane *et al.*, reported isolation of newer drimane sesquiterpenoid lactone, 11α-hydroxycinnamosmolide (**3**) from stem bark of the *Warburgia ugandensis* medicinal herb.

The bark was dried in air away from sunlight for 7 days before it was ground into a fine powder. A dichloromethane extract was prepared from the ground powder. The dichloromethane was removed by rotary evaporation at 40 °C to get dried crude extract. The crude dichloromethane extract of *Warburgia salutaris* bark was dissolved in 100 mL of 100% acetonitrile before it was filtered through a Whatman filter paper to exclude insoluble waxy material. Double distilled water was added to yield a milky solution. Fractionation of 50 mL of this solution was carried out on pre-conditioned C-18 reversed phase columns (70 mL, 10 mg silica bed embedded). The solution was allowed to drain through the column at a flow rate of 1 mL/min to release unbound material. Bound material was eluted at the same flow rate with increasing concentrations of HPLC grade acetonitrile in water, in the ratios of 30:70, 60:40 and 100:0 yielding 3 fractions Fr 30, Fr 60 and Fr 100, respectively. Fr 30 was subjected to further purification using HPLC. A range of individual peaks were identified in Fr 30 based on their absorbance at 220 nm. compounds **3** was successfully isolated using preparative HPLC.

The separated 11α-hydroxycinnamosmolide (**3**) demonstrated anti-mycobacterial action against *M. bovis* BCG in dose dependent manner. Isolated Compound **3**, inhibited recombinant arylamine *N*-acetyltransferase (NAT) by 35% at 0.5 mg/

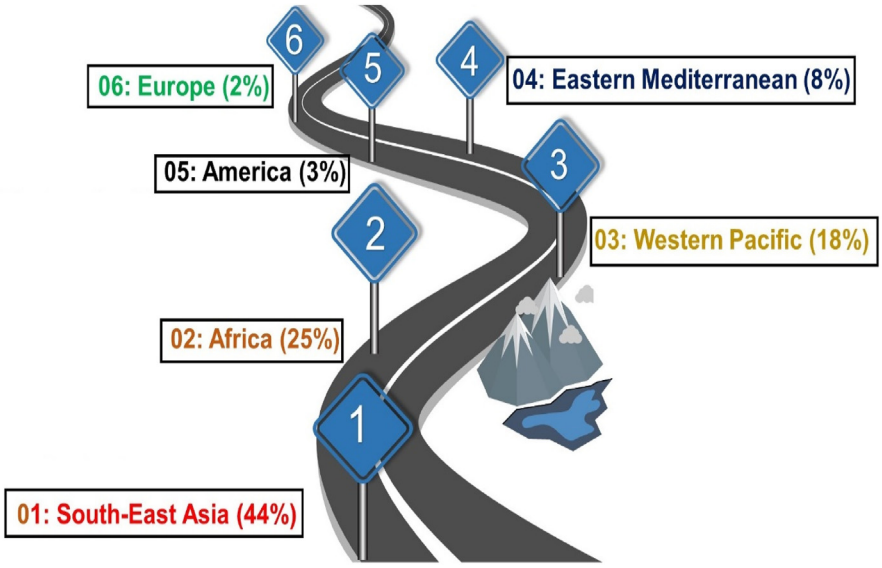


Fig. 2 – Continent wise estimated percentage of TB-deaths as per WHO global TB report 2020.[15]

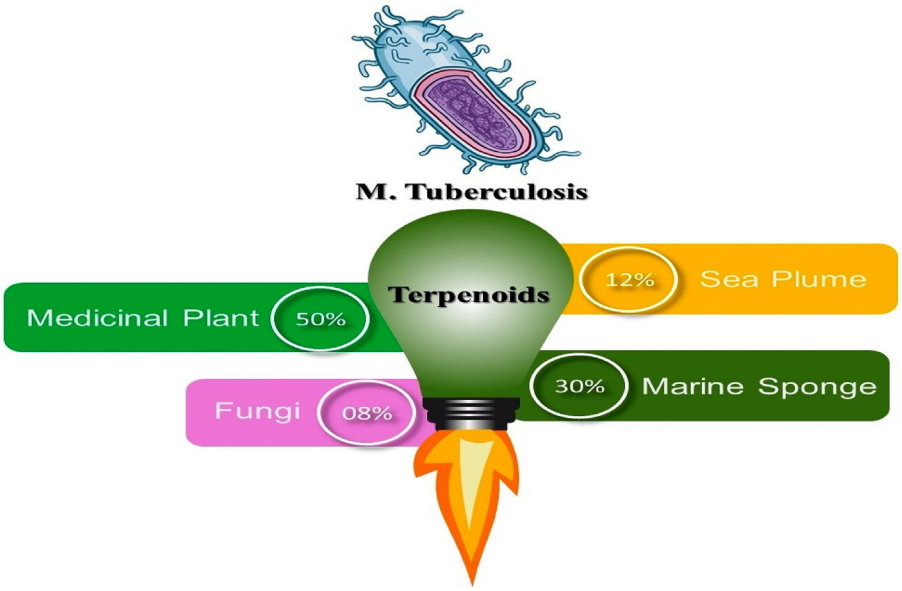


Fig. 3 – Percentage of potential terpenoid molecules found in various natural sources.

mL (Fig. 6). NAT is an enzyme that plays a key role in mycobacterial cell wall phospholipid production. Exocyclic α-methylene-γ-lactone ring is essential for the anti-mycobacterial activity.[30]

Prabu *et al.*, demonstrated potent anti-mycobacterial activity of the phyto-compound Andrographolide (**4**) separated from herb *Andrographis paniculata*. The dry powdered crude drug of *A. paniculata* was extracted in hexane and methanol solvent using soxhlet extractor. The resultant extract is processed to get dry residue and stored at − 20 °C until its use. They have screened the six fractions of *A. paniculata* against the *M. tuberculosis* eluted from column chromatography.

Among them methanolic extract of *A. paniculata* showed maximum *anti-mycobacterial* activity against all the strains of mycobacterium at 250 μg/mL concentration. From methanolic extract of *A. paniculata* they have further isolated Andrographolide (**4**) and docked against the 22 drug targets to find out the affinity of the compounds and result indicated that aminoglycoside 2-*N*-acetyltransferase AAC (RV0262C) had the highest gold score of 68.01. Andrographolide (**4**) was found to interact with most of the key residues Leu 16, Phe 32, Phe 36, Asp 40, Thr 44, Val 84, Glu 82, Ser 117, Trp 181, Val 81, and Tyr 126 of AAC protein (Fig. 7).[31]

Kanokmedhakul *et al.*, isolated the triterpenoid β-acetyllolean-12-en-28-olic acid (**5**) from the roots and stems of the medicinal plant *Prismatomeris fragrans*. The powder obtained from dried root and stem part of the plant extracted by using hexane and dichloromethane. The solvent is removed from resultant extract by vacuum evaporator to get crude extract residue. This residue is further loaded on column for isolating

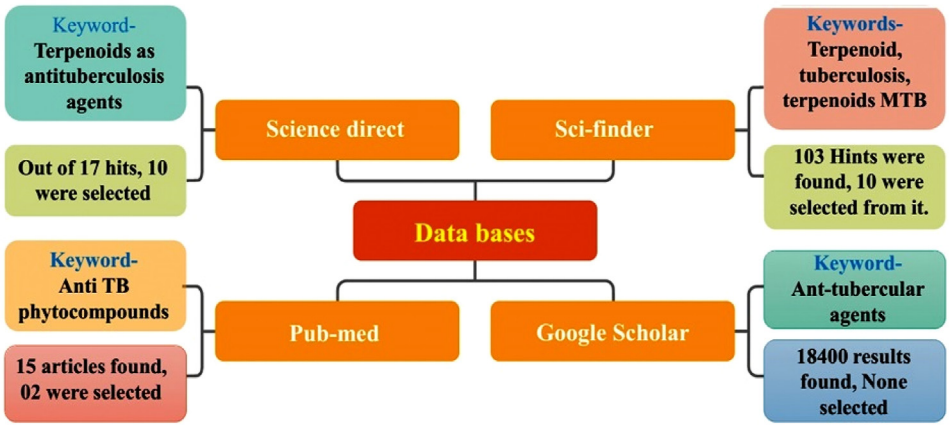


Fig. 4 – Methodology adopted for information collection at the time of literature review.

desired phytomarker. On eluting extract with hexane–EtOAc, EtOAc–MeOH mobile phase, 18.5 mg of colourless needles of β-acetylolean-12-en-28-olic acid (**5**) found.

The isolated compound was having significant anti-mycobacterial activity with MIC value of 50 μg/mL (Fig. 8). They also isolated the Anthraquinone compound like Nor-damnacanthal, Damnacanthal, Rubiadin and 1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone, which showed significant anti-mycobacterial activity.[32]

Tiwari *et al.*, have isolated the two novel labdane diterpenoids like 6α, 7α-diacetoxy-13-hydroxy-8(9),14-labdadien, 9-hydroxy-13(14)-labden-15,16-olide (**6**) and another known diterpenoid called isoambreinolide (**7**) from the leave part of *Vitex trifolia*. Cold methanol was used for extraction of powdered leaves of *V. trifolia*. Extraction was carried out at room temperature and resultant liquid extracts concentrated under reduced pressure. After that, obtained methanolic extract was suspended in sufficient quantity of water and partitioned with n-hexane. Partitioned n-hexane fraction was loaded on column and eluted with n-hexane initially and later with ethyl acetate to isolate 6α,7α-diacetoxy-13-hydroxy-8(9),14-labdadien,9-hydroxy-13(14)-labden-15,16-olide (**6**) and isoambreinolide (**7**).

All the isolated diterpenes were tested for anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain by using BACTEC-460 assay. 9-hydroxy-13(14)-labden-15,16-olide (**6**) and isoambreinolide diterpene (**7**) displayed significant anti-mycobacterial activity with MIC value of 100 and 25 μg/mL, respectively (Fig. 9).[33]

Fischer *et al.*, determined anti-mycobacterial activities of sesquiterpenes like Costunolide (**8**) and Parthenolide (**9**) against *M. tuberculosis* and *M. avium by* radiorespirometric bioassay.

Considerable anti-mycobacterial activity was exhibited by a number of extracts of plants from the south eastern United States including *Mangolia grandiflora* and *M. virginiana*. Therefore, their pure constituents Costunolide (**8**) and Parthenolide (**9**) as well as a series of related germacranolides were tested against *M. tuberculosis*. Costunolide (**8**) showed MIC values of 32 and 128 μg/mL against *M. tuberculosis and M. avium* respectively. Parthenolide (**9**) was found to be utmost effective and active germacrolide against both *M. tuberculosis and M. avium* strains with MIC values of 16 and 64 μg/mL (Fig. 10). Existence of exocyclic α-methylene-γ-lactone moiety and α, β-unsaturated carbonyls, epoxides or carbocationic intermediates are responsible for anti-mycobacterial activity of these compounds.[34]

Jimenez-Arellanes *et al.*, isolated three pentacyclic triterpenoids like 3-acetoxy-22-(2′-methyl-2Z-butenyloxy)-12-oleanen-28-oic acid (**10**), 3-hydroxy-22β-(2′-methyl-2Z-butenoyloxy)-12-oleanen-28-oic acid (**11**) and oleanolic acid (**12**) from hexane extract of *Lantana hispida* aerial parts.

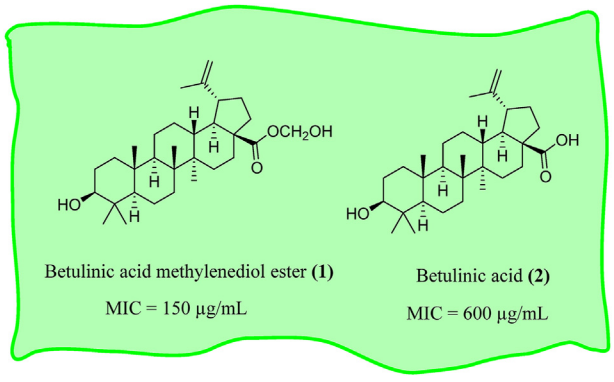


Fig. 5 – Structure and anti-mycobacterial spectrum of betulinic acid methylenediol ester (1) and betulinic acid (2).

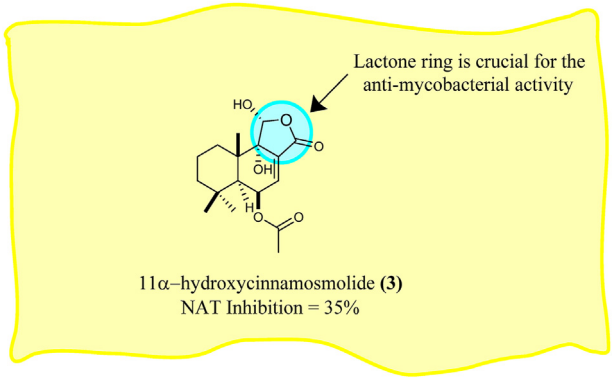


Fig. 6 – Structure, SAR and NAT inhibition of 11α-hydroxycinnamosmolide (3).

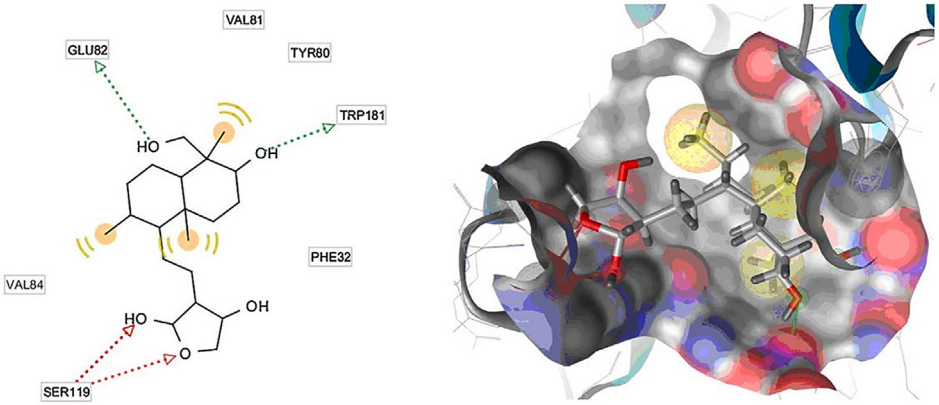


Fig. 7 – Binding interaction of the Andrographolide (4) with AAC protein after MD simulation (*Figure has been reproduced with permission*).

The dried crude drug material macerated with hexane for 24 hours at room temperature. After that, liquid extract is concentrated under reduced pressure. 2 gm of the extract is partitioned on silica gel column to yield eight fractions. Most active fractions again re-chromatographed to get 3 pentacyclic triterpenoids (**10**,**11** and **12**). These terpenoids were evaluated for *anti-mycobacterial* activity against *Mycobacterium tuberculosis* H37Rv strain by microdilution alamar blue assay method. Compounds (**10** and **11**) exhibited MIC value of 50 μg/mL and oleanolic acid (**12**) displayed significant MIC value of 25 μg/mL indicating ambient potential for further development (Fig. 11). In all three compounds (**10**,**11**, and **12**) presence of hydroxyl or keto groups in A or B rings and the carboxylic group in D/E rings play crucial role for the anti-mycobacterial activity.[35]

Joycharat *et al.*, reported isolation of triterpene 23,24,25-trihydroxycycloartan-3-one (**13**), two exceptional pregnane steroids 2β,3β-dihydroxy-5α-pregn-17(Z)-en-16-one (**14**) and 2β,3β-dihydroxy-5α-pregn-17(E)-en-16-one (**15**), as well as the bisamide pyramidatine, the sesquiterpene spathulenol and the common triterpenoids lupeol, lupenone, and a mixture of β-sitosterol and stigmasterol.

The dried powdered leaves of *A. Forbesii* extracted with methanol. Resultant liquid extract is evaporated in vacuum evaporator under reduced pressure to get residue. Later,

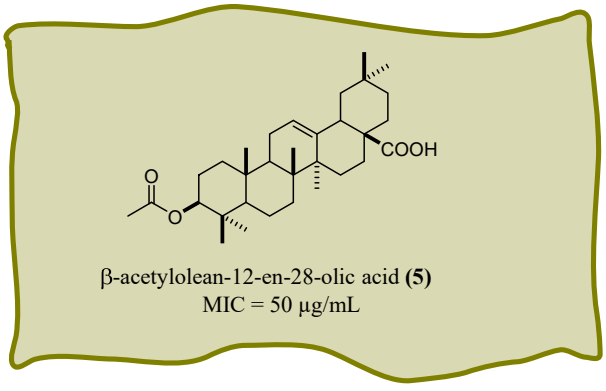


Fig. 8 – Structure, and anti-mycobacterial spectrum of β-acetylolean-12-en-28-olic acid (5).

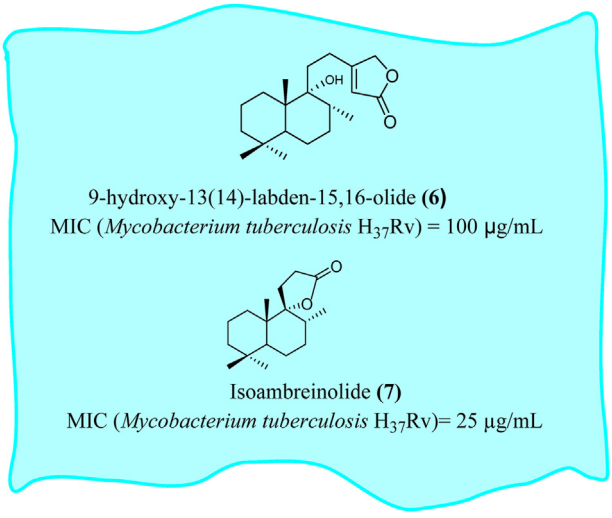


Fig. 9 – Structure, and anti-mycobacterial spectrum of 9-hydroxy-13(14)-labden-15,16-olide (6) and Isoambreinolide (7).

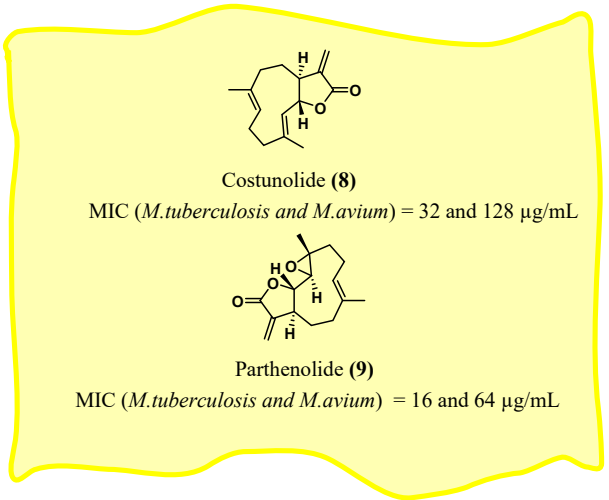


Fig. 10 – Structure, and anti-mycobacterial spectrum of Costunolide (8) and Parthenolide (9).

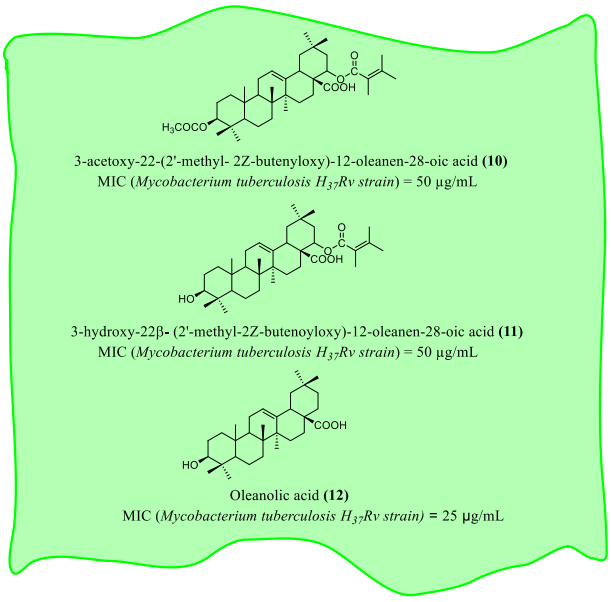


Fig. 11 – Structure, and anti-mycobacterial spectrum of 3-acetoxy-22-(2′-methyl-2Z-butenyloxy)-12-oleanen-28-oic acid (10), 3-hydroxy-22β-(2′-methyl-2Z-butenoyloxy)-12-oleanen-28-oic acid (11) and oleanolic acid (12).

residue mixed with silica and eluted with n-hexane, dichloromethane, ethyl acetate and methanol to get terpenoid macromolecules **13**, **14**, **15**.

Compound **13**, **14**, **15** were tested for the anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain. 23,24,25-trihydroxycycloartan-3-one (**13**) and 2β,3β-dihydroxy-5α-pregn-17(E)-en-16-one (**14**), found weakly active against *Mycobacteria* (MIC = ˂ 200 µg/mL). Pregnane steroid called 2β,3β-dihydroxy-5α-pregn-17(Z)-en-16-one (**15**) found inactive (MIC= > 200 µg/mL) (Fig. 12).[36]

Kanokmedhakul *et al.*, successfully isolated new meroterpenoids chevalones C (**16**), sequiterpene alkaloid eurochevalierine (**17**), terpenoid pyrrolobenzoxazine named CJ-12662 (**18**) from the fungus *Eurotium chevalieri*. Dried fungal biomass of *E. chevalieri* was ground into powder and then extracted successively with hexane, EtOAc and MeOH. Removal of solvents under reduced pressure gave crude hexane, EtOAc, and MeOH extracts, respectively. The hexane extract was subjected to silica gel column chromatography (CC) and eluted with a gradient system of hexane- EtOAc and EtOAc-MeOH, to give ten combined fractions from HF_1–HF_{10}. Fraction $HF_{6.4.3}$ was recrystallized from MeOH to yield yellow needles of **17**. Fraction $HF_{6.4.4}$ was separated by FCC, eluted with a gradient system of hexane-EtOAc to give an additional amount of 18. Fraction HF_8 was recrystallized from hexane-CH_2Cl_2 to yield a white solid of **16**.

Anti-mycobacterial activity of the separated biomarkers was assessed against *M. tuberculosis* H37Rv using the Microplate Alamar Blue Assay (MABA). Compound **16**, **17**, **18** showed significant anti-mycobacterial activity against *Mycobacterium* with MIC values of 6.3, 50.0, and 12.5 µg/mL respectively (Fig. 13).[37]

El Sayed *et al.*, have researched several structurally diverse marine-derived natural products for *in vitro* activity against *Mycobacterium tuberculosis*. Puupehenone (**19**), 15-cyanopuu pehenone (**20**), puupehedione (**22**), 15-oxopuupehenol (**23**), 15α-methylpuupehenol (**24**), 15α-cyanopuupehenol (**25**), compounds **21**, and **26**–**30** were natural sesquiterpene-shikimate derived metabolites or semisynthetic derivatives of puupehenone, which was isolated from sponges of the orders Verongida and Dictyoceratida. Puupehenone (**19**), 15-cyanopuupehenone (**20**), and 15α-cyanopuupehenol (**25**)

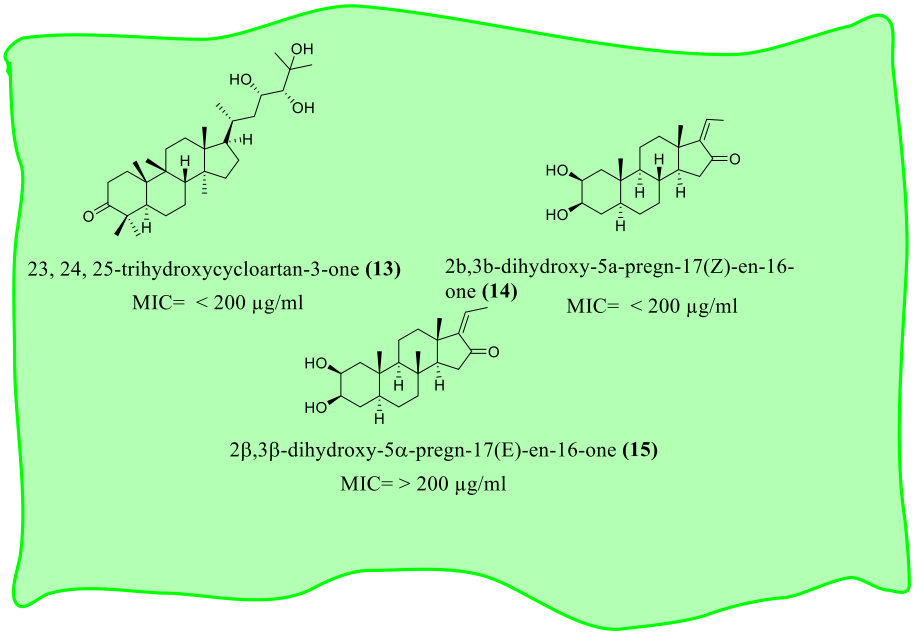


Fig. 12 – Structure, and anti-mycobacterial spectrum of 23, 24, 25-trihydroxycycloartan-3-one (13), 2β,3β-dihydroxy-5α-pregn-17(Z)-en-16-one (14) and 2β,3β-dihydroxy-5α-pregn-17(E)-en-16-one (15).

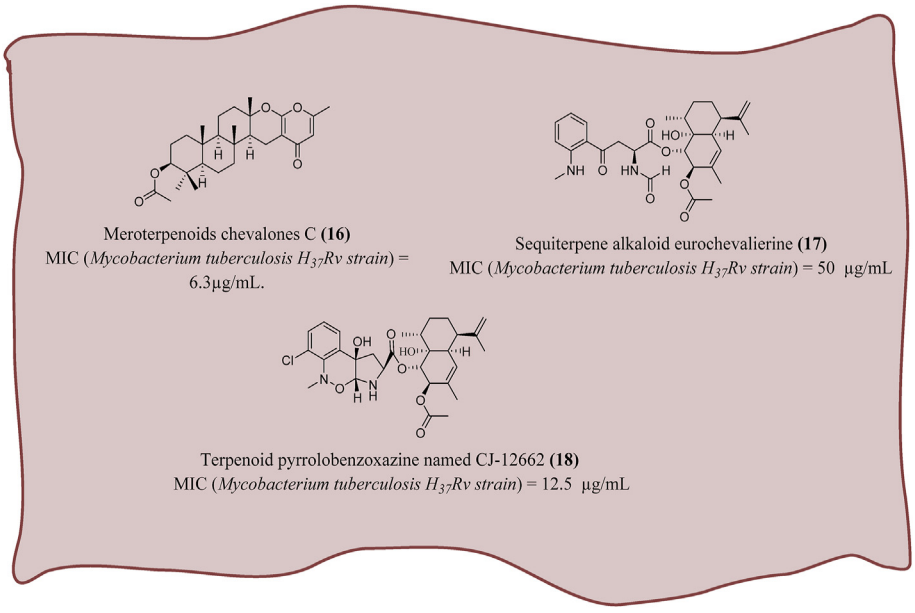


Fig. 13 – Structure, and anti-mycobacterial spectrum of meroterpenoids chevalones C (16), sequiterpene alkaloid eurochevalierine (17), and terpenoid pyrrolobenzoxazine named CJ-12662 (18).

induced 99, 90, and 96% inhibition of *M. tuberculosis* (H37Rv) growth, respectively. Puupehenone (**19**) showed MIC of 12.5 mg/mL and an IC_{50} of 2.0 mg/mL. SAR study indicates that the quinone-methide system in ring D of puupehenone is essential for activity as denoted by compounds **19** and **20**. Heteronemin (**31**) is a scalarin-type sesterterpene previously isolated from the sponge *Heteronema erecta* and recently isolated by Hamann *et al.*, from a Red Sea sponge. Heteronemin displayed a 99% inhibition of *M. tuberculosis* (H37Rv) with an MIC 6.25 mg/mL and IC_{50} 1.3 mg/mL (Figs. 14 and 15). The high cytotoxicity of these compounds prohibited further testing; however, microbial and/or chemical modifications of these compound may produce less toxic and more active derivatives.[38]

Torres-Romero *et al.*, isolated four different dihydro-β-agarofuran sesquiterpenes from the leaves of *Celastrus vulcanicola*. The dichloromethane extract of the leaves of *C. vulcanicola* was subjected to repeated chromatography on Sephadex LH-20 and silica gel to isolate the compound **32**.

These isolated compounds were tested on *M. tuberculosis* H37Rv. 1α-acetoxy-6β,9β-dibenzoyloxy-dihydro-β-agarofuran (**32**) have anti-mycobacterial activity against the MDR-TB strain with a MIC value of 6.2 µg/mL (Fig. 16).[39]

Isaka *et al.*, performed isolation of seven lanostane triterpenoids ganorbiformins A–G from fungus *Ganoderma orbiforme* BCC 22324. The fungus isolated from a dead oil palm trunk. The fungus BCC 22324 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 8 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth. After incubation at 25 °C for 4 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1000 mL Erlenmeyer flask containing 250 mL of the same liquid medium (PDB), and incubated at 25 °C for 4

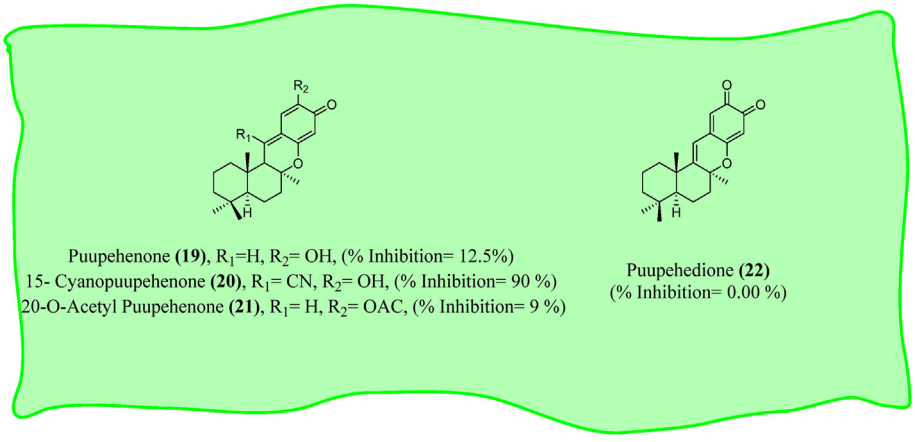


Fig. 14 – Structure, and anti-mycobacterial spectrum of Puupehenone (19) and its synthetic derivatives and puupehedione (22).

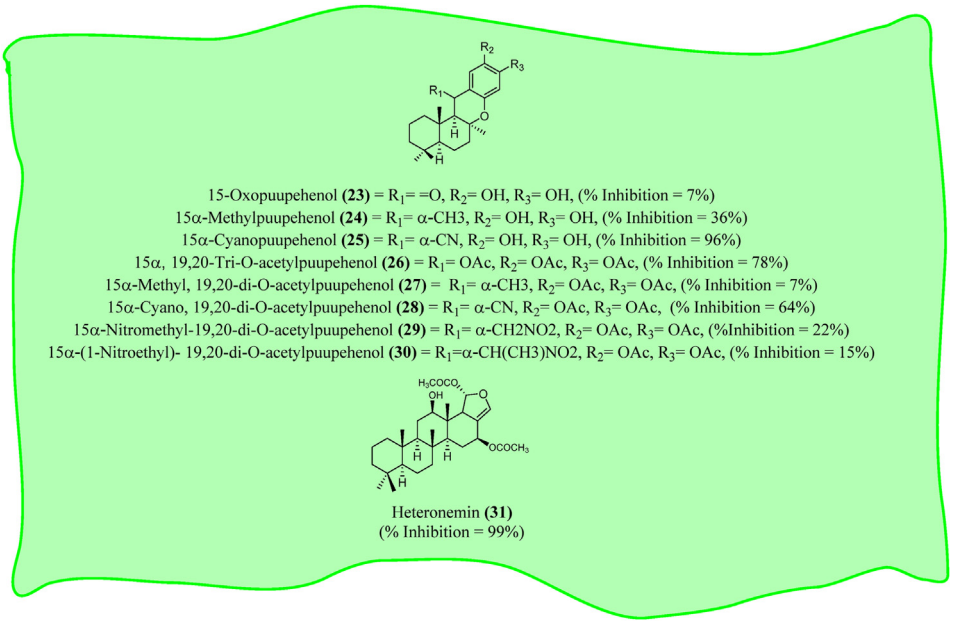


Fig. 15 – Structure, and anti-mycobacterial spectrum of Puupehenols (23–31).

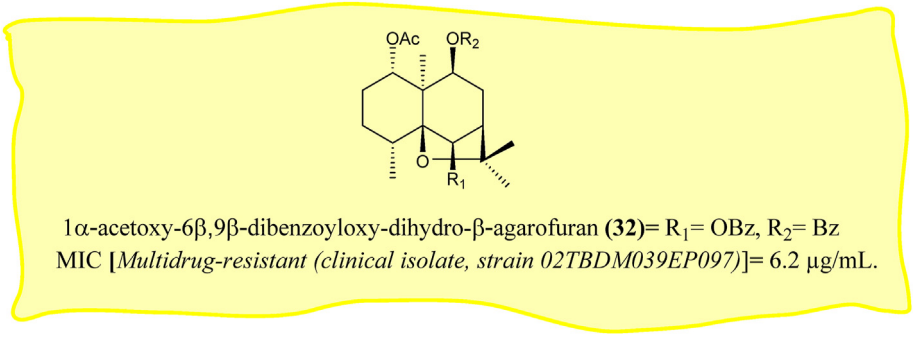


Fig. 16 – Structure, and anti-mycobacterial spectrum of 1α-acetoxy-6β,9β-dibenzoyloxy-dihydro-β-agarofuran (32).

days on a rotary shaker (200 rpm). The secondary cultures were pooled and each 25 mL portion was transferred into 80 × 1000 mL Erlenmeyer flasks containing 250 mL of malt extract broth, and the final fermentation was carried out at 25 °C for 20 days under static conditions. The cultures were filtered to separate broth and mycelia (residue). The broth was extracted with EtOAc and concentrated under reduced pressure to obtain a brown gum. The wet mycelia were macerated in MeOH and filtered. Hexanes and H_2O were added to the filtrate, and the layers were separated. The H_2O/MeOH layer was partially concentrated by evaporation, and the residue was extracted with EtOAc which was concentrated under reduced pressure to obtain a brown gum. The mycelial extract was passed through a column on Sephadex LH-20 and eluted with MeOH. The terpenoids-containing fractions were combined (3.45 g) and it was subjected to column chromatography (CC) on silica gel and the fractions were further fractionated and purified by silica gel CC (EtOAc/hexanes or MeOH/CH_2Cl_2) and preparative HPLC using a reversed phase column (Phenomenex Luna 10u C18(2) 100A, 21.2 × 250 mm, 10 μm; mobile phase MeCN/H_2O, proportions 50:50–85:15; detection UV 210 and 254 nm) to furnish pure compounds.

The C-3 epimer of ganoderic acid T (**34**) demonstrated noteworthy anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv with MIC 1.3 μM (Fig. 17).[40]

Pérez-González *et al.*, isolated two major terpenoids primarily moretenol (**35**) and moretenyl acetate (**36**) from *Cnidoscolu chayamansa* leaves.

Dry leaves were extracted successively by maceration at room temperature (25 °C) with $CHCl_3$: MeOH (1:1). The extract was concentrated at 40 °C in vacuum system (BuchiVac V-153), and maintained at room temperature under conditions of darkness until its use. Compounds **35** and **36** were isolated by preparative TLC using Hex:EtOAc 92:8 as elution system; the compounds showed an R_f = 0.56 and 0.23 by moretenyl acetate (**36**) and moretenol (**35**) respectively, and were identified by ^1H- and ^{13}C NMR for structural elucidation.

Moretenol and moretenyl acetate indicated good anti-mycobacterial activity with MIC value of 25 μg/mL against *Mycobacterium tuberculosis* H37Rv and also towards four mono

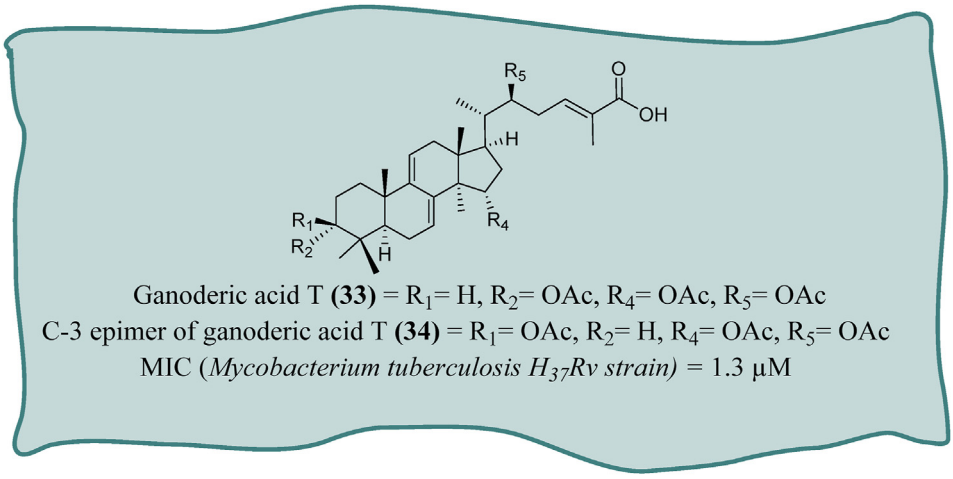


Fig. 17 – Structure, and anti-mycobacterial spectrum of Ganoderic acid (33) and C-3 epimer of Ganoderic acid T (34).

resistant strains of *M. tuberculosis* H37Rv (Fig. 18). The $CHCl_3$: MeOH extract of *C. chayamansa* leaves also exhibited anti-mycobacterial activity (MIC ˂ 50 µg/mL).[41]

Peng *et al.*, isolated twenty-seven diterpenes and cyanthiwigins A–AA from *Myrmekioderma styx* Jamaican sponge. Chemically cyanthiwigins are 5,6,7-tricarbocyclic diterpenes.

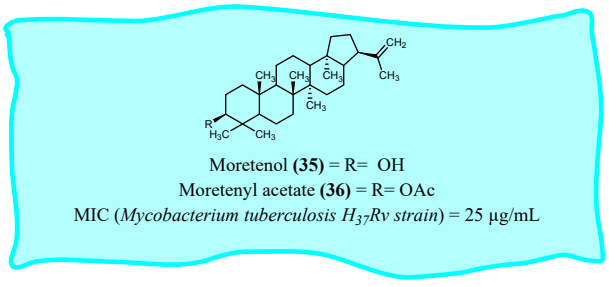


Fig. 18 – Structure, and anti-mycobacterial spectrum of moretenol (35) and moretenyl acetate (36).

Cyanthiwigins' isolation E–AA recorded for the first time. The sponge *M. styx* was collected and extracted with methanol. The extract was dissolved in acetone, and subjected to silica gel vacuum-liquid chromatography followed by column chromatography, preparative thin layer chromatography and reverse phase HPLC to yield Cyanthiwigins A (**37**), B (**38**), C (**39**) and D (**40**).

Cyanthiwigins A (**37**), B (**38**), C (**39**) and D (**40**) were investigated for *anti-mycobacterial activity tuberculosis* using a broth micro-dilution assay. Cyanthiwigins A, B, C and D demonstrated a significant anti-mycobacterial activity with a MIC value of 25, 9, 50, 30 µg/mL, respectively (Fig. 19). SAR study indicates that presence of the double bond at C-12 and 13 is required for anti-mycobacterial activity. Cyanthiwigin C (39) displayed the strong anti-mycobacterial activity due to mono hydroxyl group at C-1.[42]

Marrero *et al.*, isolated seven original diterpenoids of the pseudopterane class, namely, kallolide D (**41**), kallolide C acetate (**42**), kallolide E (**43**), kallolide F (**44**), kallolide G (**45**), kallolide H (**46**), and kallolide I (**47**) from the sea plumes *Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos*. The

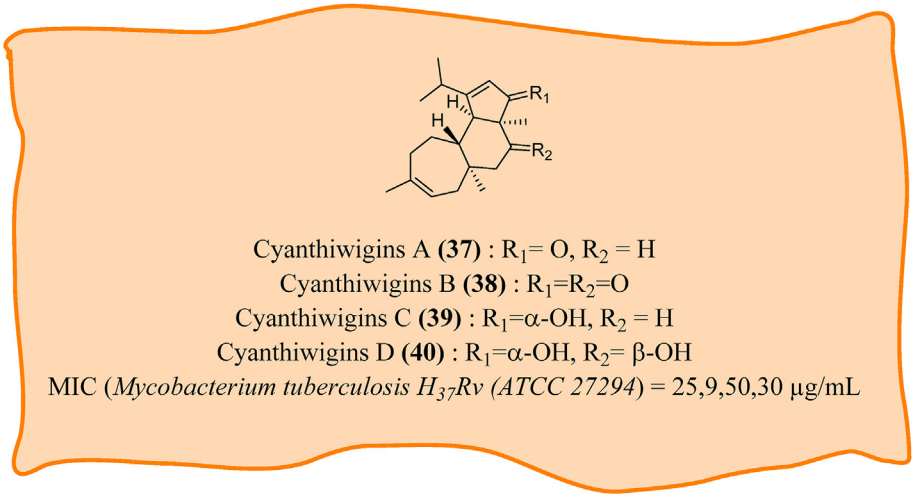


Fig. 19 – Structure, and anti-mycobacterial spectrum of Cyanthiwigins A (37), B (38), C (39) and D (40).

partially air-dried specimens of *P. bipinnata* were frozen, freeze-dried and cut in small pieces, and blended with 1:1 MeOH/$CHCl_3$. The combined organic extracts were filtered and concentrated to give a brown residue that was suspended in H_2O and extracted with hexane, CHCl3, and EtOAc. Rotoevaporation of the $CHCl_3$ extract of *P. bipinnata* produced a greenish oil that was loaded onto a column of silica gel and eluted with a 99:1 mixture of $CHCl_3$/MeOH. Several of the least polar fractions obtained were further purified by column chromatography over silica gel followed by normal-phase HPLC to afford known compound kallolide C and the new metabolites kallolide D, kallolide C acetate, kallolide E, kallolide F and kallolide G etc.

Isolated compounds were assessed *in-vitro* towards *M. tuberculosis* H37Rv strain. All of the seven novel pseudopterane diterpenes mentioned have been found to be moderately active against *M. tuberculosis* at two concentrations (128 and 64 μg/mL) with MIC value of less than 50 μg/mL (Fig. 20).[43]

de Araujo *et al.*, isolated triterpene β-amyrin (**48**) from the hexane fraction of *Passiflora mucronata*. Fresh leaves were triturated and extracted with 500 mL of hydroalcoholic solvent at room temperature by maceration for 24 h. The extract was lyophilized and then an aliquot of the total dried crude hydroalcoholic extract was re-suspended with methanol and partitioned with hexane to obtain PMH. An aliquot PMH was chromatographed on a silica column and eluted with hexane/ethyl acetate/methanol gradient solvent system, yielding 271 fractions. Fraction PMH-113 crystallized *via* the slow evaporation of hexane/ethyl acetate, affording compound **48** as white crystalline needles. Compound **48** was identified as triterpene β-amyrin based on MS data.

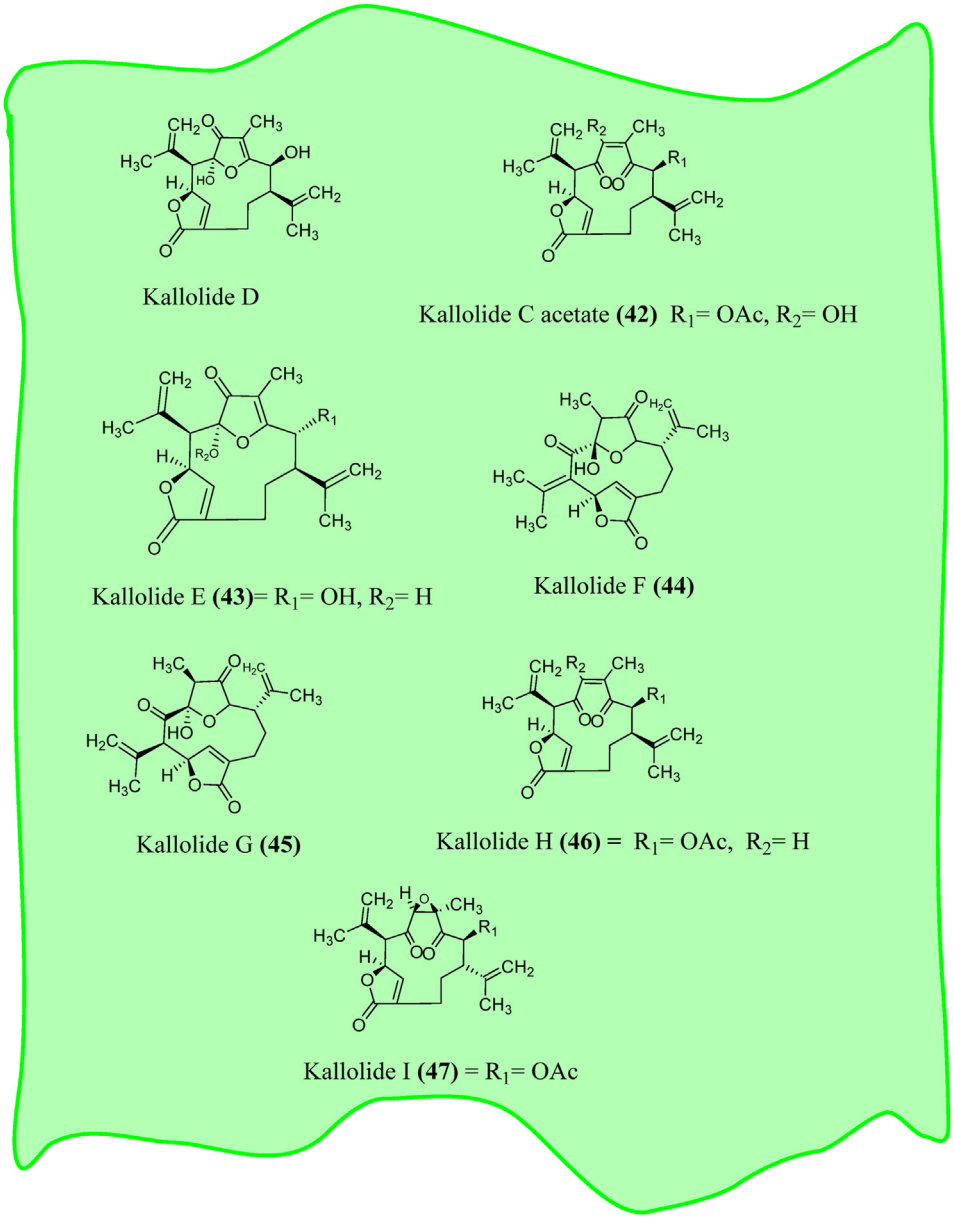


Fig. 20 – Structure, and anti-mycobacterial spectrum of kallolides (41–47).

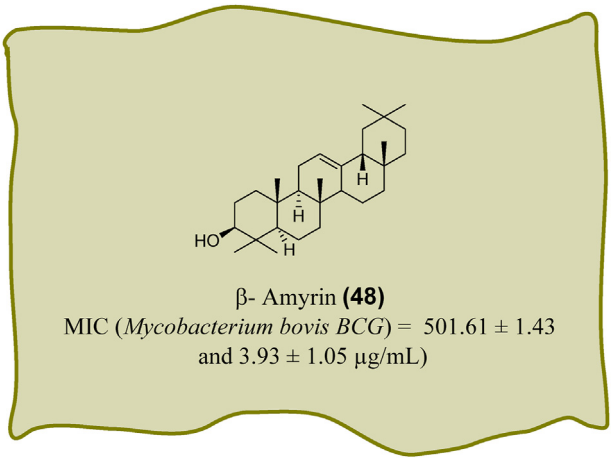


Fig. 21 – Structure, and anti-mycobacterial spectrum of β-amyrin (48).

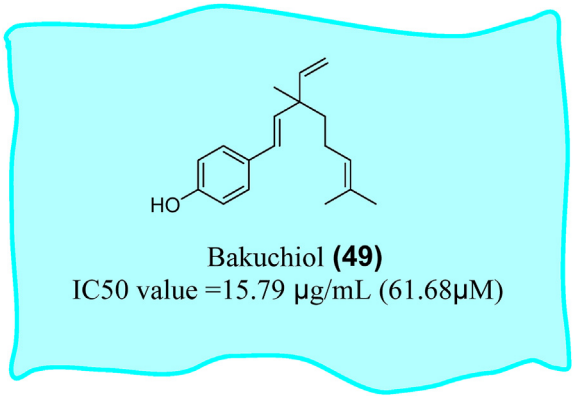


Fig. 22 – Structure, and anti-mycobacterial spectrum of Bakuchiol (49).

Triterpene β-amyrin (**48**) exhibited substantial growth inhibitory activity against *Mycobacterium bovis BCG* (MIC 501.61 ± 1.43 and 3.93 ± 1.05 µg/mL, respectively) (Fig. 21). For the first time, β-amyrin tritepene was isolated from the *Passiflora* species.[44]

Newton *et al.*, performed bioassay-guided fractionation, isolation of phenolic meroterpene bakuchiol (**49**) from *Psoralea corylifolia* seeds and demonstrated appreciable anti-mycobacterial activity. A sequential extraction at room temperature was performed on powdered seeds *of P. corylifolia*. The hexane extract was found to be the most active. Further bioassay guided fractionation of the hexane extract, using positive pressure column chromatography over silica gel, eluting with hexane and increasing amounts of ethyl acetate, lead to the isolation of the known phenolic meroterpene bakuchiol (**49**). The compound was characterised using spectroscopic techniques.

Crude methanolic plant extract *P. corylifolia* also demonstrated strong anti-mycobacterial activity against *Mycobacterium aurum* with MIC value of 62.5 µg/mL only (Fig. 22). *Mycobacterium bovis* BCG strain used as a screening organism to detect anti-mycobacterial activity. Bakuchiol (**49**) has been found to be active against *M. aurum* with IC_{50} value = 15.79 µg/mL (61.68 µM).[45]

Kaemchantuek *et al.*, isolated six innovative diterpenoids namely A-F trigonoreidons, together with eight famous diterpenoids from *Trigonostemon reidioide* roots. The pulverized, air-dried root of *Trigonostemon reidioides* was successively extracted by percolation with *n*-hexane, EtOAc and MeOH at room temperature. The extracts were evaporated to dryness under reduced pressure at temperature 40–45 °C to give the hexane extract, the EtOAc extract and the MeOH extract. The hexane extract was fractionated by column chromatography using a gradient solvent system of *n*-hexane, *n*-hexane-EtOAc and EtOAc with increasing amounts of the more polar solvent. The eluates were examined by TLC and 13 combined fractions

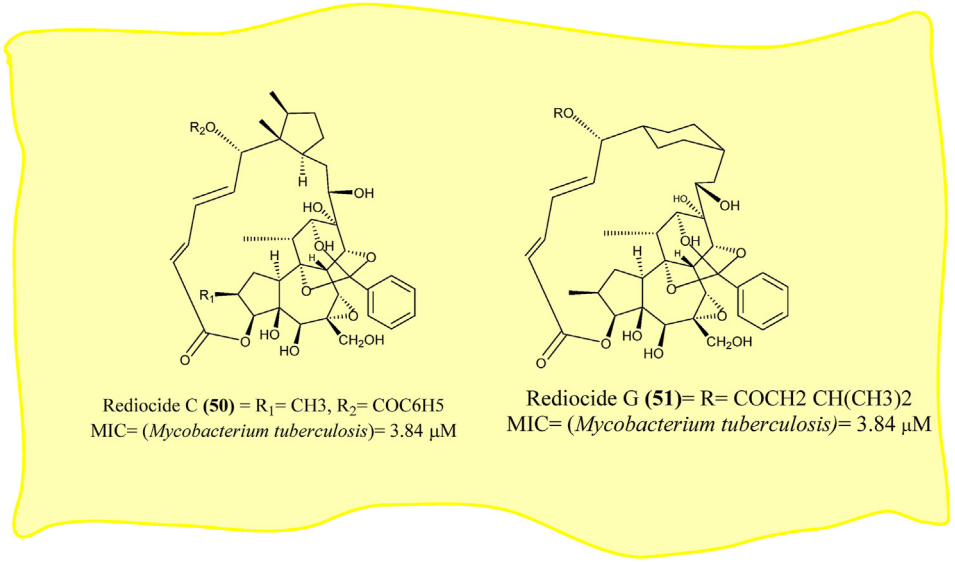


Fig. 23 – Structure, and anti-mycobacterial spectrum of Rediocide C (50) and Rediocide C (51).

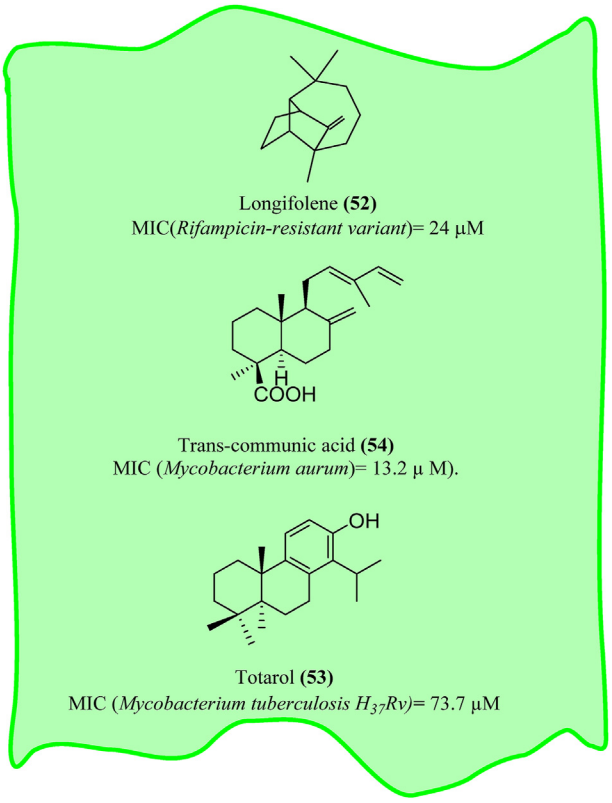


Fig. 24 – Structure, and anti-mycobacterial spectrum of longifolene (52), Totarol (53) and Trans-communic acid (54).

(E1-E13) were obtained. Fraction E8 was chromatographed over silica gel and eluted under isocratic condition (0.5% MeOH in CH_2Cl_2) to give 8 subfractions (Ef1-Ef8). retention time for rediocide G (**51**) and rediocide C (**50**) was found to be at 13.66 and 15.30 min respectively.

The isolated compounds were tested for anti-mycobacterial activity against *Mycobacterium tuberculosis*. Diterpenoids Rediocide C (**50**) and Rediocide G (**51**) were the most active compounds, with the MIC value of 3.84 μM. they showed more potent activity than kanamycin (MIC = 4.29 μM) and 10 times less than streptomycin positive control (Fig. 23).[46]

Gordien *et al*., isolated sesquiterpene longifolene (**52**) and two diterpenes namely totarol (**53**) and trans-communic acid (**54**) from roots and the aerial parts of *Juniperus communis* herb.

The dried powdered roots of *J. communis* were extracted using pressurised liquid extraction process. Extractions were performed under pressure (1500 psi) at 100 °C with a flush volume of 60% and 4 static cycles (static time of 8 min/cycle), sequentially using HPLC grade n-hexane, ethyl acetate and methanol to get the **52**,**53** and **54**. The behaviour of pure isolated compounds against drug-resistant *Mycobacterium tuberculosis* strains, non-replicating *Mycobacterium tuberculosis* and a number of non-tuberculosis mycobacteria was also evaluated.

Isolated components were tested against the non-replicating strain of *Mycobacterium tuberculosis H37Rv* using a low oxygen recovery assay and a broth microdilution process. Totarol (**53**) demonstrated the highest activity against *Mycobacterium tuberculosis* H37Rv (MIC of 73.7 μM) and also the most active against isoniazid, streptomycin and moxifloxacin-resistant variants (MIC of 38.4, 83.4 and 60 μM, respectively) (Fig. 24). Longifolene (**52**) and totarol (**53**) were highest active against the rifampicin-resistant variant. Totarol (**53**) demonstrated the best activity in the LORA assay (MIC of 81.3 μM) and MICs of 7–14 μM against all on-tuberculosis mycobacteria. Trans-communic acid (**54**) exhibited decent activity towards *M. aurum* (MIC of 13.2 μ M). This is the very first report on isolation and evaluation of longifolene, totarol and trans-communic acid as anti-mycobacterial agent. In the structure of trans-communic acid (**54**), a double bond between 13, 14 carbon and 3-O-acetylated position on the labdane skeleton necessary for activity.[47]

Lu *et al*., observed the anti-mycobacterial activity of sophoradiol (**55**), a vegetal derived triterpenoid against drug-resistant strains of *Mycobacterium tuberculosis*, and also in the murine tuberculosis model.

Results showed that sophoradiol (**55**) has remarkable activity (8.5 μg/mL) against the H37RV strain. The MIC of sophoradiol towards the drug resistant strains of *M. tuberculosis* was found in between 9 and 16 μg/mL (Fig. 25). Sophoradiol can be used as a lead molecule for the development anti-mycobacterial drugs in the future.[48]

Jyoti *et al*., assessed *in-vitro* anti-mycobacterial potency of triterpenoid ursolic acid (**56**) against *Mycobacterium tuberculosis* H37Rv strain in Resazurin assay and MGIT 960 assay with MIC

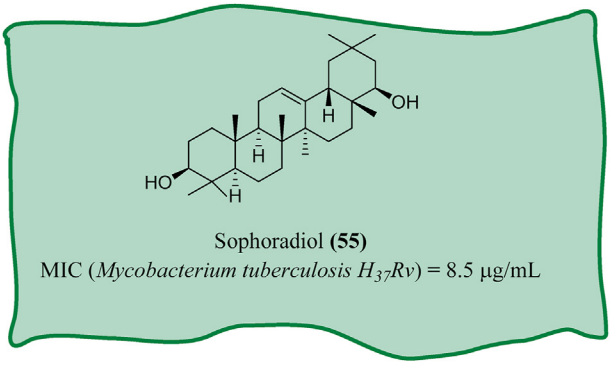


Fig. 25 – Structure, and anti-mycobacterial spectrum of Sophordiol (55).

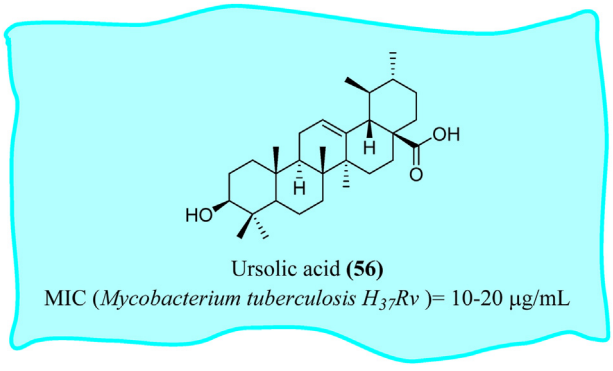


Fig. 26 – Structure, and anti-mycobacterial spectrum of Ursolic acid (56).

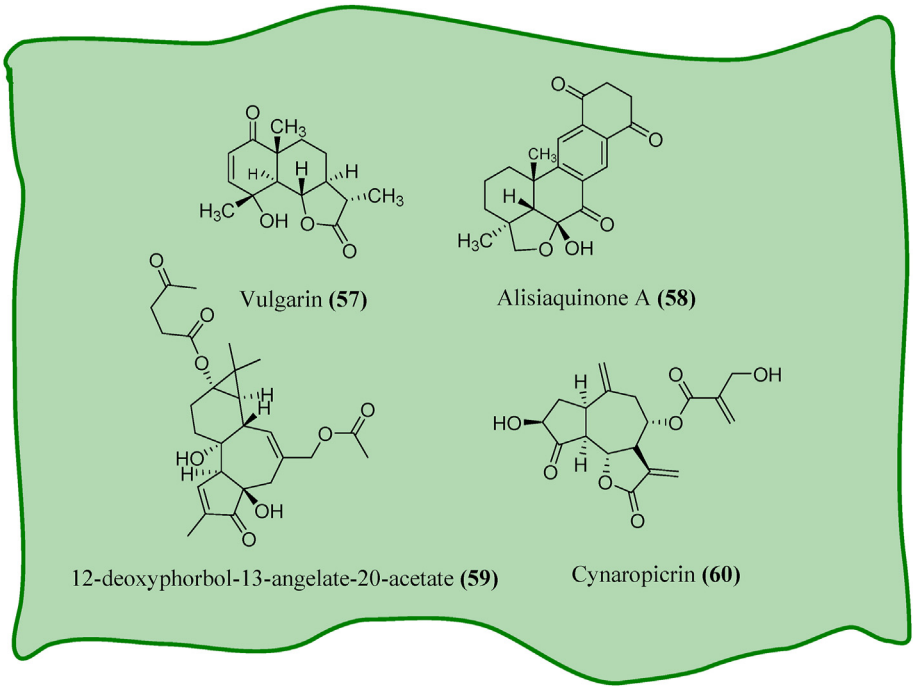


Fig. 27 – *In silico* screened hits against the PKS18.

value 10–20 μg/mL (Fig. 26). Experimental findings showed that Ursolic acid interferes with mycolic acid biosynthesis in *Mycobacterium tuberculosis*.[49]

Sharma *et al.*, identified potential drug candidates by *in silico* screening of the 672 terpenoid compound library against type III polyketide synthase18 (PKS18) as anti-mycobacterial agents. PKS18 is a mycobacterial enzymes crucial for its intracellular existence, pathogenicity and drug resistance. Pharmacokinetic analyses revealed that 18 plant-derived and 1 marine sponge-derived natural compounds satisfy all the ADMET criterion and also those of Lipinski's Rule of Five. Out of 672 terpenoid compounds, 19 compounds docked efficiently inside the active site of PKS18 and shown greater binding affinity and pharmacokinetics.[50] These natural compounds were docked successfully within the active site of type III polyketide synthase18 (PKS18) and exhibited low binding energy indicating high affinity (Fig. 27). Among them, vulgarin (**57**) showed the best docking score followed by alisiaquinone A (**58**), 12-deoxyphorbol-13-angelate-20-acetate (**59**) and cynaropicrin (**60**). Vulgarin (**57**) forms hydrogen bond interaction with the Arg45 residue of the PKS18.

4. Discussion and conclusion

TB remains the highest infectious killer in the world claiming nearby 4000 lives a day. Currently, the widespread occurrence of resistance toward *Mtb* strains is becoming a significant concern to public health. This scenario exaggerated the need for the discovery of new inhibitors. In August 2019, FDA approved novel treatment for drug-resistant *Mtb* (MDR-TB, XDR-TB) under the Limited Population Pathway for Antibacterial and Antifungal Drugs (LPAD pathway), as part of a three-drug regimen containing bedaquiline, pretomanid, and linezolid (collectively known as BPaL regimen) has an efficacy rate of 90%. The period of treatment is six months that significantly lower than the present treatment regimens for MDR-TB and XDR-TB, which last 18–24 months, and it has the potential to boost effectiveness against drug resistance *Mtb* substantively. However, lipophilicity of these drugs limited the complete exploration of its potential dose range. In this review article, we have concentrated on anti-mycobacterial potentials of the terpenoids obtained from natural sources. Almost sixty terpenoid molecules were reported in this review (Table 1).

The lanostane triterpenoids C-3 epimer of ganoderic acid T (**34**) demonstrated noteworthy anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv with MIC 1.3 μM. Meroterpenoids chevalones C (**16**) isolated from the fungus *E. chevalieri* have the remarkable MIC values of 6.3 against *M. tuberculosis* H37Rv. Cyanthiwigins A (**38**) isolated from the *M. styx* Jamaican sponge has a noteworthy MIC of 9 μg/mL against the *Mycobacterium tuberculosis*.

Among the studied terpenoids, 1α-acetoxy-6β,9β-dibenzoyloxy-dihydro-β-agarofuran (**32**), Longifolene (**52**) and Sophoradiol (**55**) have shown activity against the resistant strain of the *Mycobacterium tuberculosis*. Longifolene (**52**) was obtained from the aerial parts of *J. communis* having MIC of 24 μM against the Rifampicin resistant Mtb. The MIC of Sophoradiol (**55**) was in between 9 and 16 μg/mL towards the drug resistant strains of *M. tuberculosis*. Compound **32**, isolated from the leaves of *C. vulcanicola* have a MIC value of 6.2 μg/mL against the MDR-TB strain. Future studies could focus on the large number of unexplored species in this order, expecting many newer compounds to be discovered. Further optimization of these derivatives using an appropriate drug target may

Table 1 – Various terpenoid molecules obtained from different natural sources, shown with their reported MIC values against the *M. tuberculosis*.

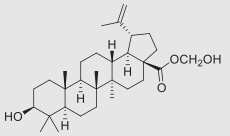
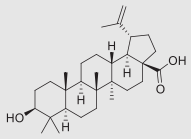
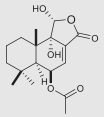
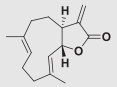
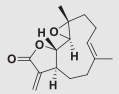
Class of terpenoid	Name of terpenoid compound	Molecular Weight	Source of compound	MIC Value	Reference
Lupane type isoprenoids	 Betulinic acid methylenediol ester	486.74	Stem bark of *S. guineense*	150 μg/mL	29
	 Betulinic acid	456.71	Stem bark of *S. guineense*	600 μg/mL	29
Drimane sesquiterpenoid lactone	 11α-hydroxycinnamosmolide	324.37	Stem bark of *Warburgia ugandensis*	–	30
Germacranolide-type sesquiterpene lactones	 Costunolide	232.32	*Magnelia grandiflora* and *M.virginiana*	32 and 128 μg/mL	34
	 Parthenolide	248.32	*Magnelia grandiflora* and *M.virginiana*	16 and 64 μg/mL	34

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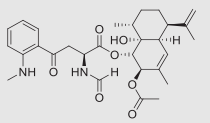
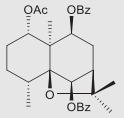
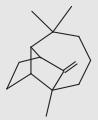
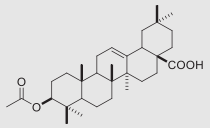
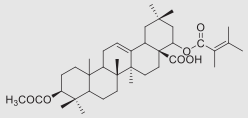
Table 1 – (*continued*)

Class of terpenoid	Name of terpenoid compound	Molecular Weight	Source of compound	MIC Value	Reference
Sequiterpene alkaloid	 Eurochevalierine	526.63	Fungus *Eurotium chevalieri*	50.0 μg/mL	37
Sesquiterpenes	 1α-Acetoxy-6β,9β-dibenzoyloxy-dihydro-β-agarofuran	521.25	Leaves of *Celastrus vulcanicola*	6.2 μg/mL (MDR-TB)	39
	 Longifolene	210.41	Roots and the aerial parts of *Juniperus communis*	50.50 μg/mL	47
Triterpenoids	 β-acetylolean-12-en-28-olic acid	498.75	Roots and stems of *Prismatomeris fragrans*	50 μg/mL	32
	 3-acetoxy-22-(2'-methyl-2*Z*-butenyloxy)-12-oleanen-28-oic acid	610.88	Aerial parts of *Lantana hispida*	50 μg/mL	35

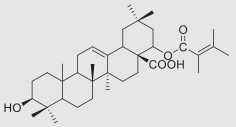
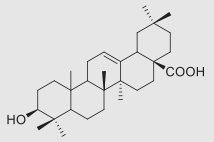
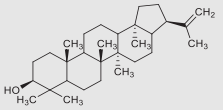
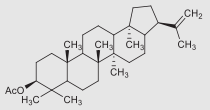
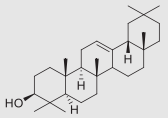
 3-hydroxy-22β- (2'-methyl-2*Z*-butenoyloxy)-12-oleanen-28-oic acid	568.84	Aerial parts of *Lantana hispida*	50 μg/mL	35
 Oleanolic acid	456.71	Aerial parts of *Lantana hispida*	25 μg/mL	35
 Moretenol	426.73	*Cnidoscolu chayamansa* leaves	25 μg/mL	41
 Moretenyl acetate	468.77	*Cnidoscolu chayamansa* leaves	25 μg/mL	41
 β-amyrin	412.70	*Passiflora mucronata* herb	501.61 ± 1.43 and 3.93 ± 1.05 μg/mL	44

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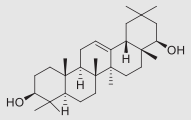
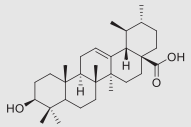
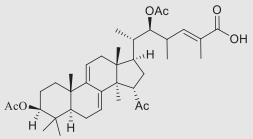
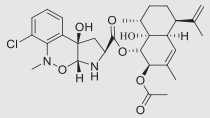
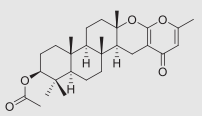
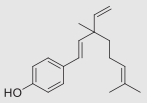
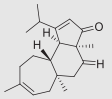
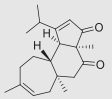
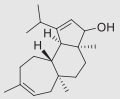
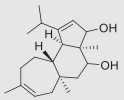
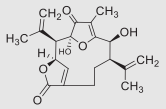
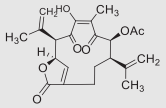
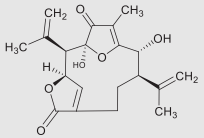
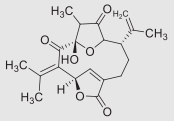
Table 1 – (*continued*)

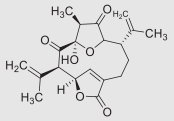
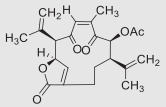
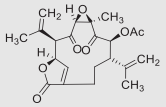
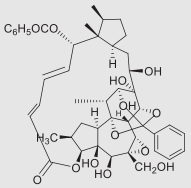
Class of terpenoid	Name of terpenoid compound	Molecular Weight	Source of compound	MIC Value	Reference
	 Sophoradiol	442.73	–	8.5 μg/mL	48
	 Ursolic acid	456.71	*Ocimum sanctum* and *Rosmarinus officinalis*	10 and 20 μg/mL	49
Lanostane triterpenoid	 C-3 epimer of ganoderic acid T	610.83	Mushroom fungus *Ganoderma orbiforme* BCC 22324	7.58 μg/mL	40
Terpenoid pyrrolobenzoxazine	 CJ-12662	561.07	Fungus *Eurotium chevalieri*	12.5 μg/mL	37

Meroterpenoid	 Chevalones C	456.62	Fungus *Eurotium chevalieri*	6.3 μg/mL	37
	 Bakuchiol	256.39	Seeds of *P. corylifolia*	15.79 μg/mL	45
Diterpenes	 Cyanthiwigins A	285.45	Jamaican sponge *Myrmekioderma styx*	25 μg/mL	42
	 Cyanthiwigins B	300.44	Jamaican sponge *Myrmekioderma styx*	9 μg/mL	42
	 Cyanthiwigins C	288.48	Jamaican sponge *Myrmekioderma styx*	50 μg/mL	42
	 Cyanthiwigins D	304.47	Jamaican sponge *Myrmekioderma styx*	30 μg/mL	42

(*continued on next page*)

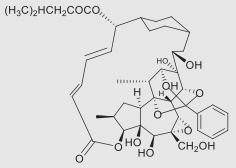
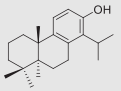
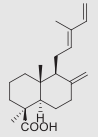
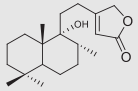
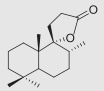
Table 1 – (*continued*)

Class of terpenoid	Name of terpenoid compound	Molecular Weight	Source of compound	MIC Value	Reference
Diterpenoids of the pseudopterane class	 Kallolide D	360.41	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
	 Kallolide C acetate	402.44	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
	 Kallolide E	360.41	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
	 Kallolide F	360.41	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43

Diterpenoids of the pseudopterane class	 Kallolide G	360.41	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
	 Kallolide H	386.44	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
	 Kallolide I	402.44	*Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* Sea plume	< 50 μg/mL	43
Diterpenoid	 Rediocide C	819. 97	Roots of *Trigonostemon reidioide*	31.49 μg/mL	46

(*continued on next page*)

Table 1 – (*continued*)

Class of terpenoid	Name of terpenoid compound	Molecular Weight	Source of compound	MIC Value	Reference
Diterpenoids	 Rediocide G	785.95	Roots of *Trigonostemon reidioide*	30.18 μg/mL	46
	 Totarol	300.49	Roots and the aerial parts of *Juniperus communis*	221.47 μg/mL	47
	 Trans-communic acid	302.22	Roots and the aerial parts of *Juniperus communis*	38.34 μg/mL	47
Labdane diterpenoids	 9-hydroxy-13(14)-labden-15,16-olide	320.47	*Vitex trifolia* leaves	100 μg/mL	33
	 Isoambreinolide	279.44	*Vitex trifolia* leaves	25 μg/mL	33

provide potential scope for anti-mycobacterial therapies. Lack of mechanistic study is one of the major concern in case of phyto-constituents. Therefore, target-based *in vitro* approaches may enable a broader and more sensitive screening approach for new anti-TB agents. These lead terpenoid molecules could be useful for further optimization and development in this field.

Author's contribution

Author Vilas Jagtap and Iqrar Ahmad were involved in the data collection and manuscript writing. Harun M. Patel contributed for the idea generation and finalising the manuscript.

Conflicts of interest

The authors have none to declare.

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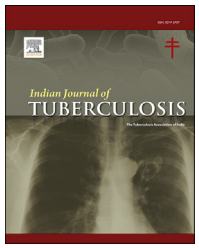

Original article

Obstetrics outcome in pulmonary tuberculosis

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ABSTRACT

Background: To evaluate the maternal and perinatal outcome in pulmonary tuberculosis cases as compared to low risk pregnancies in a tertiary referral hospital.
Methods: A total of 15 cases of pulmonary tuberculosis over a period of two years who delivered in our unit was studied in the retrospective study. The maternal and perinatal outcome in them was compared with 191 low risk pregnancies who delivered at the same time in the hospital after taking into account inclusion and exclusion criteria.
Results: The mean age and mean parity was 25.73 ± 2.85 and 28.75 ± 3.11, 2.1 and 1.9 in the 2 groups. Symptoms of pulmonary tuberculosis were cough (100%), chest pain (80%), expectoration (100%), hemoptysis (33.3%), fever (93.33%), anorexia (86.66%) and loss of weight (80%). Symptoms in study patients were significantly more common in study patients. The presence of associated medical problems was similar in the 2 groups. The prevalence of oligoamnios, gestational diabetes mellitus, antepartum hemorrhage and intrahepatic cholestasis was similar in the 2 groups. Prevalence of preterm labor was 53.33% in study group which was significantly higher than in controls (8.9%). Risk of premature rupture of membrane was also significantly higher in the study groups (53.33%) as compared to control groups (8.9%). Mean gestational age was also significantly lower (36.2 weeks) in study group as compared to 38.6 weeks in control group. The incidence of cesarean delivery was similar in the 2 groups (26.66% vs 28.79%). The mean birth weights was 2308.6 gm in the study group as compared to 2707.56 gm in control group. Fetal growth restrictions and Respiratory distress syndrome in babies was significantly higher in study group than in control group. Low APGAR score (<8) was also higher (33.3%) in study group as compared to control group (2.61%).
Conclusion: Pulmonary tuberculosis during pregnancy is associated with increased perinatal morbidity, low birth weight, poor APGAR and increased respiratory distress rates.

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1. Introduction

Tuberculosis is considered to be a major public health problem all over the world with an estimated 15 million TB cases annually all over the world, out of which 5 million are women.[1] Greatest burden of acquired TB in women is between 15 and 44 years of age, making them more vulnerable to contract TB during childbirth years and in pregnancy.[2,3] The disease particularly affect low and middle income countries with asia and Africa bearing the main brunt of disease.[1] Tuberculosis is the leading infectious cause of mortality in women and alongside HIV ranks as the leading cause of death all over the world.[1,4]

The exact prevalance of TB in pregnancy is not well known and varies from time to time and place to place.[5] Prevalence of TB is lowest in developed countries like USA being less than 10 cases per 100,000 population per year with foreign born pregnancies from endemic countries accounting for most cases in USA.[6] The prevalence was found to be 4.2 per 100,000 in UK in 2009 but was as high as 2010 per 1,00000 in HIV negative and 6880/100,000 in HIV positive women in Africa.[7,8]

TB in pregnancy can increase the risk of morbidity to mother and fetus both.[9] Risk of vertical transmission is very small in antenatal period as compared to risk of acquiring disease by newborn in postpartum period from open pulmonary TB in mother.[10]

Higher incidence of abortions, pre-eclampsia, postpartum hemorrhage, difficult labor and acute respiratory failure has been reported in women with TB as compared to controls.[11,12] The effect of TB on pregnancy mainly depends upon various factors like severity of disease, site of TB, HIV coinfection, gestation at diagnosis and treatment initiation and compliance.[11,13] TB in pregnancy has been associated with adverse perinatal outcome in the form of prematurity, lowbirth weight, small for gestation age with adverse outcome being more in presence of poor drug compliance, severe pulmonary involvement, HIV coinfection and extrapulmonary disease excluding lymph node TB.[15–17]

The aim of this study was to establish the maternal and perinatal outcome in pregnancies complicated by pulmonary tuberculosis.

2. Material and methods

It was a retrospective study on 15 women of pulmonary tuberculosis with pregnancy over 2 year period from June 2016 to June 2018 in a unit of obstetrics and gynecology of a tertiary referral centre. Inclusion criteria were Age <35yrs, primi and second gravida. Exclusion criteria is –elderly, multigravida (more than third gravida), obstetric comorbidity like placenta previa, medical comorbidity pre pregnancy like DM, CKD, Chronic hypertension, Autoimmune disorders and severe anemia (Hb < 7g%). The diagnosis of pulmonary TB was made on clinical, microbiological and radiological findings. Sputum examination was performed for acid fast bacilli, microbiology, culture, gene Xpert in all cases. To have more statistical power for the study and also to serve the purpose of better representation of control population, 191 controls were chosen who were low risk pregnancy and fit into our inclusion and exclusion criteria, these were the patients who delivered in the same time frame. The data of study and control patients was collected in relation to age, parity, gestation. Clinical features, method of diagnosis of TB, associated medical problems, obstetric complications like preterm labor, premature rupture of membranes, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, obstetric outcome (gestation at delivery, mode of delivery, cesarean section rate) and fetal outcomes (birthweight, APGAR scores any congenital malformation or any neonatal complications), antitubercular therapy received and any adverse effect of drugs.

2.1. *Statistical analysis*

Data were analysed using SPSS version 20 with Fischer 's exact test, student 't'test and ANOVA test. A two sided value of <0.05 being taken as significant. conditional logistic regression analysis conditioned for maternal age and years of delivery were carried out to investigate all the risk of fetal growth restrictions, preterm birth and other perinatal outcome in pulmonary TB versus non pulmonary TB cases.

3. Results

The baseline characteristics and associated medical problems in the two groups are shown in Table 1. The age ranged from 19 to 35 years with mean age 25.73 ± 2.85 years in group 1 (pulmonary tuberculosis) while in group 2 mean age was 28.75 ± 3.11 years. The parity ranged between 0 and 3 in group 1 with mean being 2.1, while it was between 0 and 3 with mean being 1.9 in group 2 ($p > 0.05$). there were 7 (46.6%) primigravida in group 1 as compared to 152 (79.5%) in group 2, while the number of multigravida was 8 (53.3%) in group 1 as compared to 39 (20.4%) in group 2. Those with parity more than three were excluded and elderly were also excluded from the study and control group. We have excluded multigravida (more than or equal to three) due to associated complications like gestational diabetes, pre-eclampsia, oligoamnios and fetal growth restriction associated with of multiparity that can bias our comparative study. Various associated medical problems are also shown in Table 1. Hypothyroidism, lower urinary tract infections, upper respiratory tract infections, seizure disorders, hepatitis, HIV, Bronchial asthma, beta thalassemia trait were seen in 1 (6.66%) and 15 (7.8%), 0 and 4 (2.09%), 0 and 4(2.09%), 1 (6.66%) and 0 and 2 (1.04%), 0 and 1 (0.52%),0 and 2 (1.04%) and 0 and 3 (1.5%) respectively in the two groups. Various symptoms seen in the 2 groups are also shown in Table 1. Thus cough, chest pain, expectorations, hemoptysis, fever, anorexia, loss of weight were seen in 15 (100%) and 4 (2.09%), 12 (80%) and 2 (1.04%), 15 (100%) and 3 (1.57%), 5 (33.3%) and 0, 14 (93.3%) and 6 (3.14%), 13 (86.6%) and 3 (1.57%), 12 (80%) and 4 (2.09%) respectively in the two groups. All the symptoms were statistically more common in pulmonary TB cases as compared to non tuberculosis cases. Diagnosis of pulmonary TB was made by sputum AFB IN 12 (80%) cases, culture in 15 (100%)and gene Xpert in 13 (86.68%) cases. Out of 15 patients of pulmonary tuberculosis who were diagnosed during pregnancy 6 were diagnosed between 10

Table 1 – Baseline characteristics, symptoms and associated medical problems in two groups.

S.NO	OUTCOME	Group 1 N = 15 (%) Pulmonary TB group	Group 2 (Low risk Pregnant patients) N = 191 (%)	P value and significance
1	Age range	19–36 years	18–40 years	
2	Mean age (yrs)	25.73	28.75	P > 0.05 NS
3.	**OBSTETRIC HISTORY:**			
	Primigravida	7 (46.66)	152 (79.58)	P > 0.05 NS
	Multigravida	8 (53.33)	39 (20.41)	P > 0.05 NS
	Previous abortions	5 (33.33)	26 (13.61)	P > 0.05 NS
4.	**Symptoms:**			
	Cough	15 (100%)	4 (2.09%)	p = 0.001 HS
	Chest pain	12 (80%)	2 (1.04%)	p = 0.01 SIG
	Expectoration	15 (100%)	3 (1.57%)	p = 0.001 HS
	Hemoptysis	5 (33.3%)	0	p = 0.02 SIG
	Fever	14 (93.3%)	6 (3.14%)	p = 0.03 SIG
	Anorexia	13 (86.6%)	3 (1.57%)	p = 0.01 SIG
	Loss of weight	12 (80%)	4 (2.09%)	p = 0.02 SIG
5.	**Associated Medical Problems:**			
	Hypothyroidism	1 (6.66)	15 (7.85)	P > 0.05 NS
	LRTI	0	4 (2.09)	P > 0.05 NS
	URTI	0	4 (2.09)	P > 0.05 NS
	Seizure disorder	1 (6.66)	0	P > 0.05 NS
	Hepatitis/HIV	0	2 (1.04)	P > 0.05 NS
	Bronchial asthma	0	2 (1.04)	P > 0.05 NS
	Beta thal trait	0	3 (1.57)	P > 0.05 NS
6.	**Diagnosis of pulmonary TB:**			
	Sputum positive by AFB	12 (80%)		
	Positive AFB culture	15 (100%)		
	Sputum positive by gene Xpert	13 (86.6%)		

LRTI: Lower respiratory tract infection; URTI: Upper respiratory tract infection.

and 14 weeks and 9 between 14 and 20 weeks. All patients took ATT deligently without miss. Various obstetric complications are shown in Table 2. Preeclampsia, oligohydraminos, gestational diabetes mellitus, antepartum hemorrhage, intrahepatic cholestasis, need of blood transfusion and postoperative complications were seen in 2 (13.3%)and 15(7.8%), 5(33.3%) and 17 (8.9%), 0 and 5(2.65%), 2(13.3%)and 8(4.18%), 2(13.3%)and 2 (1.04%) respectively in the 2 groups and were not different. However premature rupture of membrane and preterm labor were significantly higher in pulmonary TB cases as compared to control and were 8 (53.3%) and 17 (8.9%) and 8 (53.3%)and 4 (2.09%) respectively in 2 groups (p = 0.02 and p = 0.024).

The gestation age at delivery and mode of delivery in the two groups is shown in Table 3. The gestation age ranged from 26 to 39 weeks with mean being 36.2 weeks in group 1 which was significantly lower than mean gestation of 38.6 weeks in group 2 (p = 0.02).

In study group, there were 11 (73.33%) vaginal delivery with 5 (33.3%) being spontaneous and 6 (40%) being induced as compared to 134 (70.17%) in group 2 with 103 (53.9%) being spontaneous and 31 (16.2%) being induced (p = 0.03). Cesarean

Table 2 – Obstetric complications and mode of delivery in two groups.

S.NO	OUTCOME	Group 1 N = 15 (%) Pulmonary TB group	Group 2 (Low risk Pregnant patients) N = 191 (%)	P value and significance
1.	**OBSTETRIC EVENTS:**			
	PIH	2 (13.33)	15 (7.85)	P > 0.05 NS
	Oligohydramnios	0	3 (1.57)	P > 0.05 NS
	GDM	5 (33.33)	17 (8.9)	P > 0.05 NS
	PROM	8 (53.33)	4 (2.09)	P = 0.024 SIG
	ICP	2 (13.33)	8 (4.18)	P > 0.05 NS
	Preterm labor	8 (53.33)	17 (8.9)	P < 0.02 SIG
	Post partum complication	2 (13.33)	2 (1.04)	P > 0.05 NS
	Need for blood transfusion (antepartum or intrapartum)	0	1 (0.05)	P > 0.05 NS

GDM: Gestational diabetes mellitus.
APH: Antepartum hemorrhage.
PROM: Premature rupture of membrane.
ICP: Intrahepatic cholestasis of pregnancy.

Table 3 – Gestation age at delivery and mode of delivery in the 2 groups.

S.NO	OUTCOME	Group 1 N = 15 (%) Pulmonary TB group	Group 2 (Low risk Pregnant patients) N = 191 (%)	P value and significance
1.	GESTATIONAL AGE AT DELIVERY			
	Range	26–39 weeks	29–41 weeks	
	Mean number	36.2 weeks	38.26 weeks	P=0.02 SIG
	MODE OF DELIVERY: VAGINAL:	11 (73.33)	134 (70.15)	P > 0.05 NS
	SPONTANEOUS	5 (33.33)	103 (53.92)	P > 0.05 NS
	INDUCED	6 (40)	31 (16.23)	P > 0.05 NS
	LSCS	4 (26.66)	55 (28.79)	P > 0.05 NS
	ELECTIVE	0	40 (20.94)	P > 0.05 NS
	EMERGENCY	4 (26.66)	15 (7.85	P > 0.05 NS

section were needed for 4 (26.6%) patients with all being emergency in study patients as compared to 55 (28.79%) in group 2 with 40 (20.9%) being elective and 15 (7.85%) being emergency cesarean sections, but the difference was not statistically different in the 2 groups.

Fetal outcome in the 2 groups is shown in Table 4. The range of birth weight in study group was between 900 gm and 3085 gm with mean being 2308.6 gm as compared to 1280 to 3575 gm in group 2 with mean being 2707.56 gm in group 2. It was significantly lower in study patients. (p = 0.024), APGAR score of <8 was seen in significantly higher number of cases 4 (26.6%) in pulmonary TB cases as compared to 14 (7.32%) cases in control group (p = 0.034). Fetal growth restriction was seen in significantly higher number of cases 4 (26.6%)in study cases as compared to 14 (7.32%) in control group (p = 0.034). Large for date babies were seen in 0 and 5 (2.65%)respectively in the 2 groups, but was not significantly different (p > 0.05). Respiratory distress syndrome was seen in 4 (26.6%) cases in group 1 as compared to 3 (1.57%) cases in group 2 which was significantly higher in pulmonary TB cases (p = 0.026). However stillbirth rate was seen in 0 and 1 (0.057%) and congenital anomalies were seen in 1 (0.66%) and 0 cases respectively in the 2 groups and were not different (p > 0.05).

4. Discussion

Tuberculosis especially pulmonary TB remains a major public health problem all over the world but mainly so in developing nations like India.[1] It involves women in almost one third cases in reproductive age group and can thus complicate pregnancy.[2,3] pulmonary tuberculosis in pregnancy is associated with adverse fetal outcome as shown by previous studies.[9–14] the present study shows increased risk of fetal growth restriction (28.66%) in pulmonary TB cases in contrast to control pregnancies (7.32%). There was also increased risk of respiratory distress rate (26.66%) in them as compared to control (1.37%). Low APGAR score was also seen amongst them more commonly. Mean birth weight was significantly lower in them (2308 gm) as compared to controls (2707 gm). Risk of preterm labor was significantly higher (53.3%) amongst them as compared to controls (8.9%). Lin et al[18] also observed significantly higher low birth weight and small for gestation age newborns in TB patients in their study. However they did not observe increased preterm birth rate in their study.[18] however results are conflicting in literature. El-Messidi et al[4] did not find any difference in fetal growth, preterm birth and still birth in TB patients in contrast to controls in their study, but found higher rate of congenital malformations. While in the present study congenital malformation rate was not increased in pulmonary TB cases as compared to non TB cases. TB incidence in pregnancy in various countries varies being 12.8 per 100,000 pregnancies in UK,[19] but is up to 26.8 per 100,000 pregnancies in USA.[4] The burden of active TB cases in pregnant women is substantial in India.[20] Sugarman et al[21] estimated about 216,500 active TB cases amongst pregnant women globally in 2011 with 44,500 cases being in india, which thus contribute 20.8% of global burden of disease. Jana et al[20] also calculated 20,000–40,000 pregnant women with TB in India in 26 million births annually (around 100 cases per

Table 4 – Fetal outcome in two groups.

S.NO	FETAL OUTCOME	Group 1 N = 15 (%) Pulmonary TB group	Group 2 (Low risk Pregnant patients) N = 191 (%)	P value and significance
1.	**FETAL OUTCOME:**			
	Range of birth weight	900–3085 grams	1280–3575 grams	
	Mean Birth Weight (in gram)	2308.6	2707.56	P = 0.024 SIG
	FGR	4 (26.66)	14 (7.32)	P = 0.034 SIG
	LFD	0	5 (2.61)	P > 0.05 NS
	APGAR<8	5 (33.33)	5 (2.61)	P = 0.042 SIG
	Still birth	0	1 (0.05)	P > 0.05 NS
	Congenital anomaly	1 (6.66)	0	P > 0.05 NS
	Respiratory distress	4 (26.66)	3 (1.57)	P = 0.026 SIG

LFD: Large for dates.
FGR: Fetal growth restriction.

100,000 women). Jana et al[14] found two fold increased risk of preterm birth, low birth weight, intrauterine growth restriction and six fold increase in perinatal death in pulmonary TB cases. Recent systemic analysis which included studies from India and other countries clearly showed that active TB in pregnancy is associated with adverse maternal and fetal outcome.[22] Chopra et al[23] on their experience of over 10 years period from a tertiary referral centre observed five times higher risk of prematurity and three times higher risk of intrauterine growth restriction in TB patients as compared to controls. We also observed adverse maternal and perinatal outcome in our study on extra pulmonary TB cases.[24] TB has also been observed to be an important contributor to the maternal mortality in India in a recent postmortem analysis of maternal deaths in india.[25] TB can thus result in nearly 10 million cumulative orphans globally through parental deaths.[26]

Hence active TB risks grave maternal and perinatal risks necessitating early diagnosis and appropriate and adequate anti tubercular treatment of the mothers for successful pregnancy outcome.[27] Informed maternal care services can be utilized as a platform for TB case discussion in pregnancy.[26]

World health organization (WHO) has recommended systematic screening for active TB during antenatal period in populations where prevalence of TB is more than 100 per 100,000 population or higher.[28] India thus is suitable country for routine screening of all pregnant women for active TB for its early diagnosis and timely treatment for optimum maternal and perinatal outcome during pregnancy.

5. Conclusion

Tuberculosis especially pulmonary TB during pregnancy is associated with adverse maternal and perinatal outcome. However the present study was small. Larger multicentered studies are recommended to confirm the findings of the present study.

Conflict of interest

All authors have none to declare.

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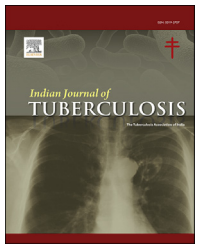

Original article

Plasma drug concentrations of 4-drug fixed-dose combination regimen and its efficacy for treatment of pulmonary tuberculosis under National Tuberculosis Elimination Programme: A prospective pilot study

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ABSTRACT

Background: The thrice weekly dosing regimen of DOTS has shown low rifampicin plasma concentrations as an independent risk factor for unfavourable tuberculosis (TB) outcome. With introduction of daily regimen using fixed dose combinations (FDC) under National Tuberculosis Elimination Programme (NTEP) the existence of suboptimal plasma levels of first-line antitubercular drugs and its clinical significance remain poorly understood.

Method: We included a prospective cohort of newly diagnosed pulmonary tuberculosis (PTB) patients receiving 4-FDC daily regimen under NTEP. Plasma concentration at 2 hours (C_{2h}) of each drug was determined after two weeks of treatment using liquid chromatography (LCMS/MS) developed by us. TB card and laboratory reports were reviewed for baseline characteristics and clinical status at 2, 4 and 6 months after the initiation of treatment. At a 1 year follow-up, therapy failure was defined as death or a relapse of tuberculosis.

Results: Among 40 PTB patients, the C_{2h} post dose plasma concentrations of H, R and E were suboptimal in 25%, 60% and 10% respectively. The C_{2h} of H, R, Z and E were respectively 4.2 ± 2.0, 7.3 ± 2.8, 39.2 ± 8.8 and 3.5 ± 1.2 μg/ml; 60% of the patients had suboptimal plasma concentrations and commonly it was observed with H and R. C_{2h} were lower than expected for at least two drugs i.e. H and R in 25% (10/40) of the patients. Plasma concentration of isoniazid and rifampicin has always been considered important for microbiological

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response and treatment outcome and low concentrations has been associated with poor treatment response. These patients may require a two year follow up and critical evaluation for prevention of MDR-TB. However, all the TB patients were cured and none of them had recurrence within one year follow up.

Conclusions: All the pulmonary TB patients administering 4-FDC daily regimen under programmatic settings were cured despite the suboptimal levels of isoniaizd and rifampicin. All the patients achieved pyrazinamide plasma levels and probably this could be the reason behind favourable outcome. Further study is required on large sample size with various subset of population to understand the need of therapeutic drug monitoring.

1. Introduction

Tuberculosis (TB) is the leading cause of morbidity and mortality in Asia and Africa, where India accounts for 1/5 of the global TB burden.[1] While the TB patients responds to antituberculosis therapy (ATT), recurrence and multidrug resistant (MDR)/RR-TB are high among previously treated patients.[2] A study showed 13% of recurrence of TB under programmatic settings within a year[3] and India bears the considerable burden of rifampicin resistant TB (RR-TB).[1]

India's Revised National TB Control Program (RNTCP) has been treating new pulmonary TB patients with thrice-weekly, directly observed treatment short course (DOTS), until a study reported poor outcomes due to low rifampicin and pyrazinamide levels.[4] Low plasma concentrations of isoniazid and rifampicin resulted into acquired drug resistance and few studies have reported about predictive nature of low drug exposures to clinical outcome, but data are limited and conflicting.[5–7]

The Area under the concentration-curve (AUC) is pharmacokinetic parameter that best describes the plasma drug exposure and its response, but expensive and impractical to conduct in programmatic settings. So, blood collected 2-hours post dosing are often used to estimate maximum plasma concentration (Cmax) of TB drugs, and generally considered to predict therapeutic targets and TB treatment outcomes.[8–10] Even the plasma concentrations at 6 hours (C_{6h}) of the first-line TB drugs are performed to identify patients with delayed/poor absorption.

Until recently in India, daily dosing of fixed dose combinations (FDC) under programmatic settings was introduced not only to improve adherence but to achieve lower relapse rate.[11] Plasma drug exposure data related to daily dosing FDC DOTS under Indian programmatic settings remain unavailable. Very few studies outside India correlated plasma drug exposure and TB outcome upon treatment with either FDC or non FDC (i.e single drug products) daily dosing ATT but the results were variable and conflicting.[12]

As low TB drug concentration is concerning, so we aimed not only to determine the variability in 2 hours post-dose concentrations (C_{2h}) of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) but also attempted to describe the relationship between drug plasma concentrations and treatment outcomes in a prospective cohort of newly diagnosed adult pulmonary TB patients treated under FDC daily regimen DOTS under the national program. The results may be beneficial in understanding the variability in plasma concentrations of first-line ATT drugs dispensed as 4-FDC and document clinical outcome.

2. Materials and methods

2.1. Study design and setting

This prospective observational pilot study was performed at the Bharati Hospital and Research Centre, a tertiary care teaching hospital in Pune, India. Our cohort comprised of 40 adult patients with newly diagnosed pulmonary tuberculosis (PTB) who were enrolled from January to June 2019.

Inclusion criteria were age ≥18 years, ATT for >14 days under DOT, not critically ill, willing to participate and give informed written consent, and agreeable to visiting the same DOTS center until study completion. Diagnosis and treatment was administered by RNTCP according to national guidelines.[11] Patients were administered the conventional daily 2-month intensive phase of the four-drug antituberculosis regimen (4FDCs: HRZE) and three drugs (3FDCs: HRE) for next 4 months as continuous phase. TB Patients received 150, 225, 300, 375 and 450 mg of isoniazid, 300, 450, 600, 750 and 900 mg of rifampicin, 800, 1200, 1600, 2000 and 2400 mg of pyrazinamide and 550, 825, 1100, 1375 and 1650 mg according to their body weight (the body weight bands were 25–34 kg, 35–49 kg, 50–64 kg, 65 to 75 and > 75 kg).[11] Patients with any of the following conditions HIV infection, chronic alcohol consumption, pregnancy, known drug resistance, end stage renal or hepatic disease and cystic fibrosis, and patients receiving drugs known to interact with HRZE (antifungal azoles, macrolids that may increase the concentration of rifampicin, antacids, and corticosteroids, which can reduce isoniazid values) were excluded from the study. Patients suspected of non-adherence or in whom blood sampling did not fulfill the study requirements were also excluded. This study was approved by institution ethics committee.

2.2. Study procedure

The TB card of patients were reviewed for baseline characteristics and clinical and paraclinical status at 2, 4 and 6

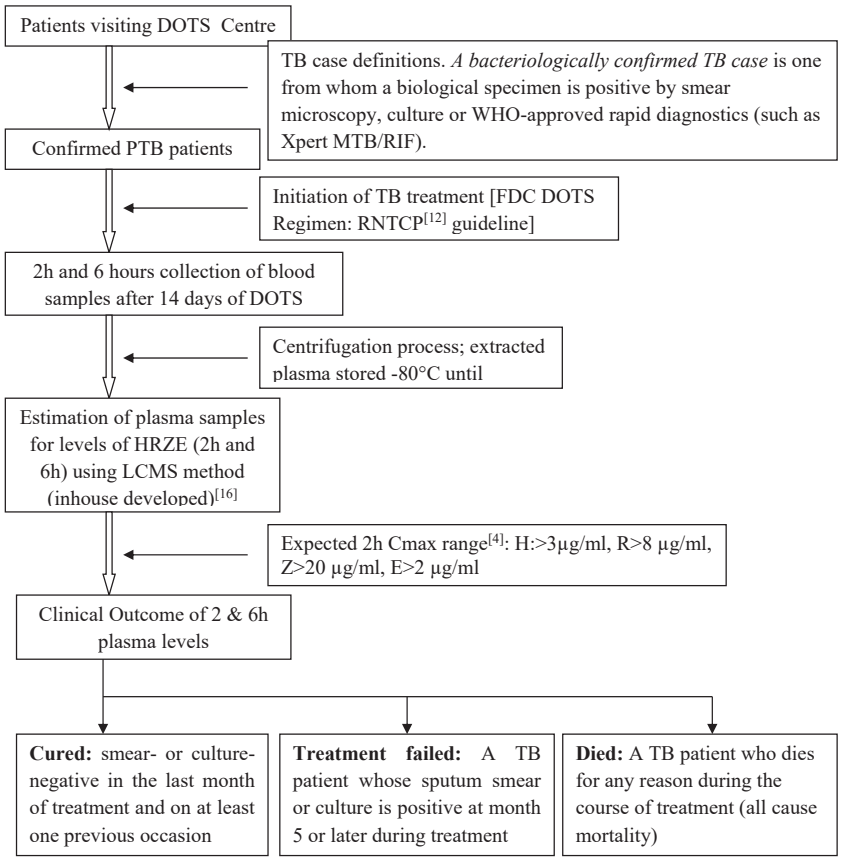

Fig. 1 – Schematic representation of the non randomized observational study.

months after the initiation of treatment. Outpatients visiting to the study DOTS Centre were instructed by the physician to take their daily dose of TB FDCs medication supplied under national program. The patients were instructed to take fixed dose combinations of first-line antitubercular agents on an empty stomach. They were contacted after two weeks of treatment (14 days) for blood samples because of the expected steady-state in the pharmacokinetics of rifampicin. Venous blood (3 ml) samples were drawn after 2 hours (h) of drug administration in empty stomach and another sample after 6 hours to identify patients with delayed absorption. Patients were instructed to take breakfast immediately after the 2 h blood sample was withdrawn. The duration of receiving treatment at the time of sampling ranged between 16 and 28 days. Separated plasma was centrifuged and immediately frozen at −80 °C. Samples were transported on dry ice to the nearest laboratory for analysis.

2.3. *ATT plasma concentration measurements*

The plasma concentrations of all the first-line ATT drugs (H, R, Z and E) were simultaneously quantified using a simple and validated liquid chromatography tandem mass spectrometry (LCMS/MS) developed in collaboration with Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Pune, Maharashtra, India.[13] The method adopted for simultaneous estimation of H, R, Z and E was selective, precise and accurate as per USFDA norms.[14] The method was validated over the concentration range 0.05–10 μg/ml for isoniazid (H), 0.1–20 μg/ml for rifampicin (R), 0.5–100 μg/ml for pyrazinamide (Z) and 0.05–10 μg/ml for ethambutol (E). The lower limit of quantification was 0.05 μg/ml for H and E, 0.1 μg/ml for R, and 0.5 μg/ml for Z. Intra-day and inter-day precision data in two patient samples were carried out and the imprecision estimated by the coefficient of variation was less than 15% and the inaccuracy estimated by the relative errors was within ±15% for all four drugs. Plasma concentrations below the lower limit of quantification were assumed to be zero.[13]

Plasma concentration reference ranges for H, R, Z and E were 3–5, 8–24, 20–50 and 2–6 μg/ml.[9,10] These ranges represent the expected (average) concentrations (Cmax in μg/ml) in adults with standard daily doses of first-line drugs used to treat tuberculosis.[9]

2.4. *TB treatment outcomes and definitions*

Participants were followed at 2 weeks, 8 weeks, 6, 12 and 18 months following ATT initiation to determine treatment outcome. The TB treatment outcome was defined according to RNTCP[11] guidelines (Fig. 1). Successful treatment (i.e. favourable outcome) was defined as treatment completion or cure (defined as the absence of symptoms suggestive of TB disease and absence of microbiological evidence of *Mycobacterium tuberculosis* by smear or cultures at the last month of treatment and on at least one previous occasion). Unfavourable outcome was defined as a composite outcome of death,

treatment failure (sputum smear or culture is positive at month 5 or later during treatment) and recurrence (presence of symptoms suggestive of TB disease and acid-fast bacilli (AFB) detected on smear microscopy after successful treatment completion). Death was defined as all-cause mortality. A year follow up was carried out telephonically (at 12 and 18th month after initiation of therapy) to assess their clinical status by enquiring the presence or absence of any sign and symptoms of TB as per RNTCP guideline.[11]

2.5. *Statistical analysis*

The demographic data of eligible patients were extracted from the patient treatment cards. The analysis included participants with drug concentrations available. Data were verified and normality checked by Shapiro–Wilk test. Categorical and continuous variables are summarized as proportions and means with standard deviation. Suboptimal drug concentrations were defined as: isoniazid < 3μg/mL; rifampicin < 8μg/mL; pyrazinamide <20μg/mL and ethambutol < 2μg/mL.[11] Single and multivariable linear regression analysis was performed to identify factors influencing drug concentrations, wherein the drug concentrations were transformed using log function, without adjusting for multiple testing. For group comparison the student's t-test was applied for normally distributed variables. Categorical variables were analysed using χ^2 test. $P < 0.05$ was considered statistically significant. Statistical analyses were done using Microsoft Excel 2010.

3. Results

During the study period 2 and 6 hour post dose blood samples from a total of 42 adult newly diagnosed pulmonary TB patients were collected. Two patients were excluded from the study because of resistance. Of 40 adult pulmonary TB patients (PTB) 22 (55%) were females. The mean age±SD of the study patients were found to be 32.2 ± 11.1 years and average weight±SD was found to be 47.8 ± 4.6 kgs. The pharmacokinetic time of sampling ranged between 15 and 30 days. Overall, mean ± SD for estimated plasma concentrations (C_{2h}) were 4.2 ± 2.0, 7.3 ± 2.8, 39.2 ± 8.8 and 3.5 ± 1.2 μg/ml for H, R, Z and E respectively. Sixty percent (24/40) of the patients had drug plasma concentrations lower than the target ranges. Of 40 patients, isoniazid in 25% (10/40), rifampicin in 60% (24/40) and ethambutol in 10% (4/40) were found to be suboptimal under programmatic settings. Pyrazinamide was found to be within the C_{2h} normal plasma target range in all the patients (range 21.6–57.1 μg/ml). The patients found to have atleast one first-line ATT drug in suboptimal range were 25% (10/40), whereas 35% (14/40) had two ATT drugs within suboptimal range, mostly rifampicin and isoniazid. Table 1 provides the baseline patient characteristics and average plasma concentrations of HRZE estimated in adult pulmonary tuberculosis (PTB) under programmatic settings. Inter-individual variability was observed in the 2 h plasma concentrations (C_{2h}) of TB drugs and low C_{2h} was seen in three TB agents (H,R and E).

The mean age of male and female was found to be 34.3 ± 11.3 and 30.6 ± 11.1 years with a mean weight of 45.2 ± 4.3 and 43.6 ± 4.9 kg respectively. Upon estimation of C_{2h} plasma samples, 77.7% (14/18) and 45.5% (10/22) PTB adult male and female patients had either one or more than one first line anti-tubercular drugs below the target normal concentration ranges. Isonaizid (H) C_{2h} plasma concentration was found to be low in 33% (6/18) and 18% (4/22) in males and females respectively, whereas, rifampicin (R) C_{2h} plasma concentration was found to be low in 66.6% (12/18) and 54.5% (12/22) in males and females respectively. Table 2 compares the baseline characteristics and C_{2h} plasma concentrations of HRZE in adult male and female PTB under programmatic settings.

Table 1 – Baseline patient characteristics and C_{2h} plasma concentrations of HRZE estimated in adult pulmonary tuberculosis (PTB) patients under programmatic settings.

Characteristics	Patients (n = 40)	P-value
Male n (%)	18 (45%)	0.65
Female n (%)	22 (55%)	
Age in years, mean (SD)	32.2 (11.1)	
Body weight in Kg, mean (SD)	47.8 (4.7)	
Prior TB History	2 (5%)	
C_{2h} plasma concentration of Analytes in μg/ml	**Mean (SD)**	**Range**
Isoniazid (H)	4.2 (2.0)	0.9–9.0
Rifampicin (R)	7.3 (2.8)	1.1–13.1
Pyrazinamide (Z)	39.2 (8.8)	23.9–57.1
Ethambutol (E)	3.5 (1.2)	0.9–5.8
C_{6h} plasma concentration of Analytes in μg/ml		
Isoniazid (H)	2.6 (1.3)	0.9–5.6
Rifampicin (R)	4.8 (1.6)	2.3–7.7
Pyrazinamide (Z)	29.0 (8.7)	16.1–48.1
Ethambutol (E)	1.9 (0.6)	0.9–3.2
Frequency of C_{2h} plasma concentration in μg/ml (below normal ranges)		
	n (%)	
Isoniazid (H)	10 (25%)	
Rifampicin (R)	24 (60%)	
Pyrazinamide (Z)	0	
Ethambutol (E)	4 (10%)	

Table 2 – Comparison of baseline characteristics and C_{2h} plasma concentrations of HRZE between male and female TB patients under programmatic settings.

	Male (n = 18)	Female (n = 22)	P-value[a]
Age in years, mean (SD)	34.3 (11.3)	30.6 (11.1)	0.23
Body weight in Kg, mean (SD)	45.2 (4.3)	43.6 (4.9)	
Prior TB History	1	1	
C_{2h} plasma concentration in μg/ml, mean (SD)			
Isoniazid (H)	4.1 (2.1)	4.4 (1.9)	0.36
Rifampicin (R)	7 7 (4.7)	7.5 (2.8)	0.46
Pyrazinamide (Z)	38.1 (13.3)	35.1 (9.2)	0.29
Ethambutol (E)	3.3 (1.4)	3 9 (1.9)	0.19
Frequency of C_{2h} plasma concentration (below normal ranges)	14 (77.7%)	10 (45.5%)	0.4
Isoniazid (H)	6 (33.3%)	4 (18%)	
Rifampicin (R)	14 (77.7%)	10 (45.5%)	
Pyrazinamide (Z)	0	0	
Ethambutol (E)	2 (11%)	2 (9%)	

[a] Student t test.

Table 3 – Frequencies of concurrent 2 h plasma concentration below normal range.[9]

	Isoniazid (n = 10)	Rifampicin (n = 24)	Ethambutol (n = 4)
Isoniazid	–	10/24 = 42%	0
Rifampicin	10/10 = 100%	–	4/4 = 100%
Ethambutol	0	4/24 = 17%	–

Table 3 shows the frequencies of concurrent suboptimal (low) plasma drug concentrations (C_{2h}) in patients. Out of 24 PTB patients having suboptimal rifampicin plasma levels, 10 (42%) patients concurrently had low isoniazid plasma levels. Plasma concentrations (C_{2h} post dose) were lower than expected for at least two drugs i.e. H and R in 25% (10/40) of the patients. Concurrent suboptimal plasma levels of R and E was seen only in 10% (4/40) patients. None of the TB patients had suboptimal plasma levels (C_{2h}) more than two ATT drugs at the same time.

We also estimated 6 hour post dose plasma concentrations of the first-line antitubercular drugs in PTB outpatients to confirm rate and completeness of drug absorption. Upon analysis, the mean plasma concentrations documented for H, R, Z and E were 2.6, 4.8, 29 and 1.9 μg/ml (Table 1). Delayed absorption of isoniazid (H) was documented in 7.5% (3 out of 40) of PTB outpatients and 5% (2 out of 40) each of rifampicin (R) and ethambutol (E). Rest others showed a complete absorption within 6 hours of ingestion of fixed dose combinations.

Fig. 2 Median concentrations of Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) at C_{2h} (n = 40). The error bars denote the ranges of concentrations and the boxes represent the 25% to 75% percentile ranges. In the present study the median plasma concentration of isoniazid, rifampicin, pyrazinamide and ethambutol was found to be 3.9 (2.9–5.1), 7.45 (5.8–8.7), 37.4 (34.9–40.8) and 3.5 (2.9–4.1) μg/ml respectively. Age, body weight, BMI and gender did not affect the C_{2h} plasma concentrations of ATT first-line drugs.

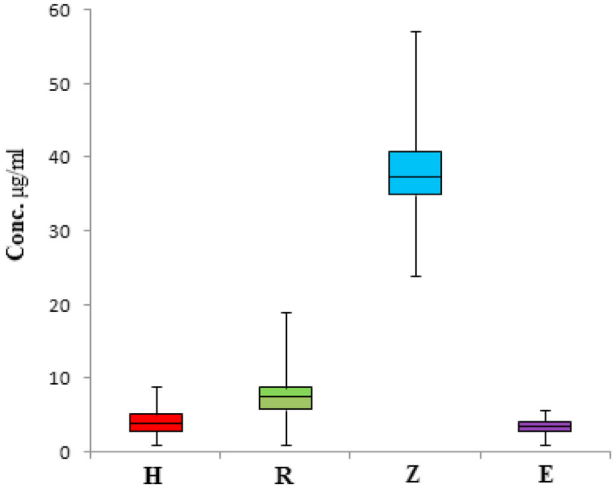

Fig. 2 – Median concentrations of Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) at C_{2h} (n = 40). The error bars denote the ranges of concentrations and the boxes represent the 25% to 75% percentile ranges.

3.1. *Effect of tuberculosis drug concentrations on treatment efficacy*

Upon assessment all the adult pulmonary TB patients had favourable outcome. All the patients completed the therapy successfully. At the end of the intensive phase and completion of therapy the sputum results were negative in all patients. No recurrence was observed within the patients when followed up for 1 year after completion of treatment.

A comparative study was done with other published reports on plasma concentrations of first-line antitubercular drugs administered as daily regimen with either fixed dose combinations or single drug products. Table 4 shows the various other studies that focused on measurement of first-line antitubercular drugs with clinical outcomes in TB patients. There were 29 studies which reported therapeutic outcomes pertaining to drug plasma concentrations[12] but only four studies[7,15–17] reported therapeutic outcomes pertaining to all four agents (isoniazid, rifampicin, pyrazinamide, and ethambutol). These studies were related to TB patients without HIV and were on either single ATT drugs or FDC given as daily regimen. All were prospective cohort study except one.[17]

4. Discussions

This is one of the rare study that examined plasma concentrations (C_{2h}) of all the four first-line ATT drugs in patients on daily regimen using FDC under programmatic settings in India. We observed inter-individual variability in plasma concentrations of these drugs like other studies[7,15–20] despite administration of daily regimen. When compared to a review[12] published on drug concentrations of first-line antitubercular agents in adults, we found low frequency (60%) of suboptimal plasma levels of first-line ATT drugs. The reason of higher frequency in reported the review[12] published could be different regimens, ethnicity and co-morbid conditions. Rifampicin was the most common amongst the first-line TB drugs for not achieving the target plasma concentration range followed by isoniazid. Similar observation was seen in our patient but at lower frequency. Comparing to the studies[7,16–19] where daily regimen was administered, the frequency of suboptimal levels of atleast one ATT drug was reported higher compared to our study.

A low isoniazid (C_{2h}: 25%) plasma levels was observed but frequency was low as compared to studies conducted by Burhan et al[15] (88%), Prahl et al[16] (71%) and Park JS et al[17] (60%). A low isoniazid levels was found to be common in patients with previous history of TB treatment ($P=0.026$) and low isoniazid groups had more drug resistant strains ($P=0.049$),[18] but its implication is unclear and might represent indirect evidence. It is a known fact that fast acetylators are more common in Asian than in Western population.[23] The high prevalence of low isoniazid levels is reported in these studies due to prevalence of fast acetylators. However, the slow and fast acetylation influence on isoniazid concentration is currently not a matter of concern as the DOTS regimen has been shifted from twice/thrice weekly to daily. N-

Table 4 – Comparison of study design, population, pharmacokinetic, predictive factors and clinical outcomes of plasma studies on first-line antitubercular drugs.

[Ref] Study type	No. of Patients enrolled	First line TB drugs/doses	Pharmacokinetic results (C_{2h})	Predictive factors	Clinical outcomes
[7] Prospective cohort study	142 TB patients	R: 600 mg/day if >50 kg or 450 mg/day If < 50 kg H: 300 mg/day Z: 20–35 mg/kg/day E: 15 mg/kg/day **(Single drug products+FDC) +2 years follow up** 29 (20%) fixed-dose combination and 109 (77%) single drug products	Low Z conc. was seen in 11% of the patients unable to achieve sputum conversion.	Z peak conc. was highest predictor of 2-month sputum conversion. 24-h AUCs of Z (<363 mgh/L), R (<13 mgh/L), and H (<52 mgh/L) most predictive of poor long-term outcome. Low R and H peak and AUC concentrations preceded all cases of acquired drug resistance.	15/142 (11%) did not achieve sputum conversion within 2 months. 25% had poor long-term outcomes (19 relapses, 15 deaths, 2 therapy failures)
[15] Prospective cohort study	181 TB patients	WHO weight based dosage recommendations **(FDC-DOTS) + 6 to 8 months follow up**	% patients below reference range: H 88% R 49% Z 39% 91% patientshad low H,R, or Z; 60% had at least two low C_{2h} conc.	Patients with low Z plasma C_{2h} levels and patients with large extensive lung lesions were at risk of at least one positive culture at 4, 8, or 24/32 weeks (SS).	82% were cured at the end of therapy.
[16] Prospective cohort study	35 TB patients	H: 5 mg/kg to maximum of 300 mg once daily, R: 10 mg/kg to maximum of 600 mg once daily, E: 20 mg/kg of ethambutol to maximum of 1200 mg once daily Z: 30 mg/kg of pyrazinamide to a maximum of 2000 mg once daily **(Single drug products and FDC)+ 1 year follow up**	86% (30/35) had plasma conc. of at least one drug below the normal range. % patients below reference range: H 71% (25/35), R 58% (19/33), E 46% (13/28) Z 10% (3/29). A total H and R 45% (15/33) (concurrent low plasma concentrations) of these E: 47% (7/15) and Z: 20% (3/15) had plasma concentrations below the normal ranges.	Plasma conc. of R decreased with increasing age (SS) and in anaemia (SS). Anemia may be a marker of disease severity and decreased absorption of rifampicin.	5 (14.3%) therapy failure (3 patients died during treatment, and 2 patients relapsed of TB within 1 year after the end of therapy). Low H and R plasma levels are frequently correlated with therapy failure.
[17] Retrospective Cohort study	413 TB patients	>50 kg: H 300–400 mg; R 600 mg; E 800 mg and Z 1500 mg. <50 kg: H 300 mg; R 450 mg; E 600 mg and Z 1000 mg. **(Single drug products) + 2 year follow up**	% patients below reference range: H 60% R 27.8% Z 8.7% E 12.8%	Low H group: greater % of patients with a history of TB treatment (SS) and H resistant (SS). Low H levels were associated with male sex. Recurrence rate was not different between patients with low and normal H levels (NS).	17 (4.1%) had recurrence rate.

Current study	40 TB patients	Standard weight based dosing as per RNTCP (**FDC DOTS**)+ **1 year follow up**	% patients below reference range: H 25% (10/40) R 60% (24/40) Z 0% E 10% (4/40)	Z concentrations were within range for all patients. It is plausible that this is contributing towards favourable outcome by attenuating synergism effect. Low H levels were associated with male sex.	All were cured at the end of therapy. No unfavourable outcome when followed for 1 year.

TB = Tuberculosis; PTB= Pulmonary tuberculosis; EPTB = Extrapulmonary tuberculosis; H= Isoniazid; R = Rifampicin; Z = Pyrazinamide.
E = Ethambutol; FDC = fixed dose combinations; conc. = concentrations; C_{2h} = plasma conc. At 2h levels post dose; SS = statistical significant; NS = non significant.

acetyltransferase 2 (*NAT2*) gene has several alleles responsible for fast and slow acetylation and *NAT2* guided therapy has not only reduced isoniazid induced liver injury but early treatment failure.[24–26] *NAT2* genotype assessment was not performed in our study and this is not a routinely performed under programmatic settings. Acetylation status for isoniazid is of no prognostic significance in daily dosing regimen, it may be of significance in twice or thrice weekly dosing.[20,27] Mah et al[21] and Sloan et al[22] found a likelihood association of C_{2h} and AUC of isoniazid with sputum culture conversion and failure or relapse rate which was completely contradicted with results published by Burhan et al[15] and Park JS et al.[17] Prahl et al also found an association between low plasma concentrations of isoniazid and rifampicin and therapy failure. However, our study had favourable treatment outcome despite suboptimal C_{2h} plasma levels of isoniazid.

Rifampicin level (C_{2h}) was comparatively low with respect to other three ATT drugs in our study patients but compared to other studies it was less frequently observed. Plasma concentration of isoniazid and rifampicin has always been considered important for microbiological response and treatment outcome and low concentrations has been associated with poor treatment response. But this assumption was not confirmed in our study though concurrent suboptimal plasma levels of H and R was seen in 25% of study population. The reason that could be explained is total drug exposure of drug (AUC) correlates well with treatment response, not C_{2h} or C_{max}. However, according to studies both C_{max} and AUC of rifampicin and isoniazid shows good correlation with response. Nevertheless, C_{2h} has been utilised as surrogate of C_{max} and was found to correlate well with C_{max} and AUC in this study and other studies.[9] But, in programmatic settings to withdraw blood at multiple time points is a challenge. However, it also reported that C_{max}/MIC or AUC/MIC[28] is more reliable in predicting the response but in our study MIC for various drugs was not determined.

Treatment response was good as all the enrolled patients had a favorable outcome. Our data analysis showed no relationship between C_{2h} of all the four ATT drugs and sputum smear at 8 weeks (2 months) and 24 weeks (6 months) which is performed routinely in programmatic settings. All the patients showed decrease in clinical symptoms and improvement in the weight. Other studies[9,15–17] have reported treatment response in the range of 82%–91%. Low H and R levels were considered for delayed culture conversion.[17] Therapy failure[16] was frequent in patients due to concurrent low levels of H and R in a study population[4,16] and low Z levels responsible for recurrence and poor treatment outcomes.[4,16] The reason could be different ethnicities or patients with comorbid conditions. Two studies[16,18] recommended dose adjustment or use standard higher doses of pyrazinamide for beneficial outcome as they experience a low Z levels in 5–39% of TB patients. Prahl[16] and Park et al[17] did not find any association of pyrazinamide with TB treatment response. But we had a favorable outcome with same standard doses of Z in FDC daily regimen, as C_{2h} levels of Z was in expected target range.

The other possible reason for favorable outcome was the median values of isoniazid (3.9 μg/ml) and rifampicin (7.45 μg/ml). A study estimated that a C_{2h} plasma concentration of

2.19 μg/ml of isoniazid is associated with 90% of the maximal killing (EBA_{90}) of metabolically active bacteria present in the sputum during the first 2 days of treatment with ATT.[29,30] With regard to rifampicin, a peak concentration threshold of 6.6 μg/ml is predictive of 2 month sputum conversion in a study of 142 TB patients.[7] The present study thus shows that the clinically relevant thresholds are considerably higher than predicted in literatures.

The study had several limitations like small sample size when including all 4 ATT drugs, thus arising the problem of comparison of multiple covariates. Limitations do exist in terms of patients with co-morbid conditions (diabetes, HIV, malnutrition, anemia) and geriatrics which may be a scope of future study. Ideally, liquid culture should have been a routine marker for testing disease activity, cure and relapse[29] but this is again a quite impractical in programmatic setting due to its cost.

5. Conclusion

Low plasma concentrations for isoniazid, rifampicin and ethambutol was observed in newly diagnosed pulmonary tuberculosis patients administering daily regimen with fixed dose combinations of first-line antitubercular drugs introduced in programmatic settings. Pyrazinamide levels were found to be within target range in all the patients. All the patients had a favourable outcome despite low drug concentrations. A study in large sample of patients and in a subset of the population is required to identify the effect of these suboptimal levels of first-line antitubercular drugs.

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Conflicts of interest

The authors have none to declare.

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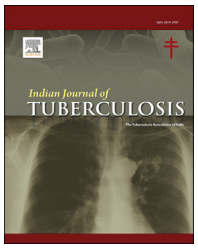

Original article

Implementation of revised national tuberculosis control program guidelines: Practitioner's perspective and awareness-a questionnaire based study

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ABSTRACT

Background: Strengthening public health services, setting up directly observed therapy short course Centres, introduction of Revised National tuberculosis Control Program (RNTCP) are aimed to eradicate tuberculosis by 2025. The aim of this study was to assess physician's awareness, and perspectives about the recent RNTCP guidelines.

Methods: This was a cross-sectional, opinion deriving, study through a tested, validated, standardized questionnaire that covered the role of physician in the diagnosis or treatment of tuberculosis, and tested their awareness about various aspects of RNTCP guidelines. Descriptive statistics was used.

Results: Of the 96 participants, 61.5% were involved in diagnosis and management, 15.6% in diagnosis only, three in treatment aspect of tuberculosis, 19 (19.8%) were not involved in any activity. Awareness regarding RNTCP guidelines was high (90.6%). Forty-five (46.9%) opined that revised Indian program was different from the World Health Organization End Tuberculosis Strategy. Understanding the definitions of diagnosis (DoD) (92.7%), guideline (92.7%), implementation of revised DoD (89.6%), guidelines ((82.3%) was considered simple. Awareness regarding the implementation of revised DoD (86.5%) and guidelines (78.1%) was below expectation.

Participants were less aware (80.2%) of reporting adverse drug reactions to the deputy drug controller; 41.7% each responded that the treating physician or any of the listed persons can report. Reporting ADR to the supervising committee was not clear as >50% did not answer. Awareness about Nikshay (86.5%), procedure for procuring Nikshay ID (46.9%), Institute's Nikshay ID (53.1%) was less.

Conclusions: Knowledge about RNTCP guidelines is satisfactory among participants. Extensive training, continued medical education programs are required to increase awareness.

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1. Introduction

National tuberculosis programme (NTP) launched in 1962 in India helped to reduce the disease burden, but not for eradication. Despite active implementation of NTP, challenges associated with the management of tuberculosis (TB) continues to haunt India. An estimation by the World Health Organization (WHO) indicates high mortality associated with tuberculosis; 480,000 Indians are succumbing every year and >1400 lives are lost every day. As India accounts for 25% global incident cases, associated mortality is expected to be high without medical intervention. With the emergence of multidrug resistant TB (6.19% in India), complexities in the treatment have increased.

Introduction and implementation of national strategic plan for the elimination of TB by the government of India is an important and significant step to achieve the mission. 'Stop tuberculosis' strategies have been developed as a road map to complete the mission. Strengthening the public health services, setting up of directly observed therapy short course Centres (DOTS) Centres, introduction of Revised National tuberculosis Control Program (RNTCP) are other measures aimed to achieve the target.

Achieving the goal of tuberculosis free India is not without challenges; apart from disease related issues (latent infection, reactivation of latent infection, malnourishment & undernourishment, greater risk factors for development of TB, inadequately treated TB), major challenge is low awareness among the general population to seek early medical intervention and physicians with respect to early detection, misdiagnosis, and proper and adequate treatment regimen to prescribe.[1] Studies have shown that delays in the diagnosis is a major hurdle, more due to patient related but a small proportion is health care related. These observations suggest that physicians need to be updated on the existing and changing treatment guidelines.

Poor knowledge on TB and national programs has proved to be a major hurdle in the management of TB, particularly in the private sector. In government sector, physicians are better aware of national programs and are better armoured to fight against the disease. There are not many studies on the awareness of these programs among the physicians in the teaching hospitals.

With this backdrop, we planned this study to identify factors, recognize gaps in the knowledge and awareness of the recent RNTCP guidelines 2017, which introduced fixed dose combinations and daily dose regimens to improve treatment compliance among TB patients in an attempt to reduce the disease burden in our country.[2] there is less awareness regarding RNTCP guidelines among the healthcare providers including medical interns and students.[3–6] It is necessary that our physicians are equipped with adequate awareness, knowledge and are ready to face the challenges. Hence, we assessed the awareness level regarding the changed regimen of tuberculosis treatment among physicians in our Institution.

2. Material and methods

This was a cross-sectional, opinion deriving, hospital based questionnaire study and was approved by the Institutional Ethics Committee. Written informed consent was obtained from the prospective participants. Physicians and pulmonologists in a tertiary care teaching hospital of a medical college, who were willing to participate were given a pre-structured questionnaire containing 20 questions. This study aimed to assess the awareness and perspectives about the recent guidelines in the management of TB among the medical practitioners.

This questionnaire covered the role of physician in the diagnosis or treatment of tuberculosis, tested their awareness about various aspects of revised guidelines on the mission, diagnosis, treatment, adverse effects of anti-tubercular drugs used and notifications, was validated and standardized prior to use.

3. Statistical analysis

3.1. *Sample size*

Minimum sample size for this study was 96 (assuming 50% of the clinicians are aware of the latest RNTCP guidelines), by the following formula –

$$n = (Z\_\alpha^2\ p(1 - p)) / e^2$$

where $z\_\alpha = 1.96$ at 95% C.I., e = allowable error, p = 50%

Data from the questionnaire was transferred to MS–Excel 2007 worksheets and analysed using SPSS V23.

Descriptive analysis was used. Chi-square test was used as appropriate. Results were expressed as mean, percentage (%), and interquartile range.

4. Results

Total of 96 physicians participated in this study. Forty-nine (61.5%) were involved in the diagnosis and management of tuberculosis, 15 (15.6%) in the diagnosis; only three were involved in the treatment aspect while 19 (19.8%) were not involved in any activity. Eighty-seven (90.6%) were aware of the revised TB control guidelines (2017) and the source of information were training (n = 37, 38.5%) and sensitization programmes (n = 36, 37.5%) (Table 1).

Forty-seven (49.0%) answered the year of elimination of TB from India as the year 2030, 37 (38.5%) as the year 2025. Forty-five (46.9%) opined that our revised program was different from the WHO End TB Strategy (Table 1).

Eighty-nine (92.7%) considered understanding the definitions provided in the guidelines to be simple. Regarding implementation of these definitions of diagnosis from this revised program, 86 (89.6%) considered it easy. Eighty-three

Table 1 – Physician's involvement, source of information, training programs attended.

Questions	Response	n	Percentage %
Activities Involved	Diagnosis	15	15.6%
	Treatment	3	3.1%
	Both	59	61.5%
	None	19	19.8%
	Total	96	100.0%
aware of any revision in the TB control guidelines in the recent past	Yes	87	90.6%
	No	9	9.4%
	Total	96	100.0%
If yes, source of information			
Online	Yes	23	24.0%
	No	73	76.0%
	Total	96	100.0%
Sensitization Programmes	Yes	36	37.5%
	No	60	62.5%
	Total	96	100.0%
Training	Yes	37	38.5%
	No	59	61.5%
	Total	96	100.0%
Newspapers and others	Yes	7	7.3%
	No	89	92.7%
	Total	96	100.0%
Year intended to eliminate TB as a public health programme in our country	2025	37	38.5%
	2030	47	49.0%
	2040	4	4.2%
	2050	5	5.2%
	No response	3	3.1%
	Total	96	100.0%
Is our elimination target of TB different from WHO End TB strategy?	Yes	45	46.9%
	No	43	44.8%
	No response	8	8.3%
	Total	96	100.0%
Have you attended any Training Programme/CME/Sensitization programme on TB	Yes	49	51.0%
	No	44	45.8%
	No response	3	3.1%
	Total	96	100.0%

(86.5%) were aware about the implementation of the program in our institution (Table 2).

Eighty-nine (92.7%) participants agreed that the guideline on the treatment of TB was simple to understand. Regarding the feasibility of implementation, 79 (82.3%) opined it was easy to implement; regarding implementation of treatment guidelines from the revised program in our institution, 75 (78.1%) were aware of the implementation (Table 2).

We included a question on their opinion if adherence of the patient to the drugs will be affected in the newer guidelines. Only 31 (32.3%) opined that it will affect the patient adherence to treatment in a positive way, while 60 (62.5%) opined it does not.

We asked our participants can there be presence of family DOT provider to supervise the therapy of a family member for which 69 (71.9%) agreed for the presence.

We assessed our participants awareness on the reporting of adverse drug reactions (ADRs). To the reporting of ADRs to the deputy drug controller, 77 (80.2%) agreed that it should be reported. About the reporting person, 40 (41.7%) each responded that the treating doctor or any of the listed persons can report (Table 2).

Table 2 – Responses on the diagnosis of tuberculosis.

TB Diagnosis			
Are the new guidelines simple to understand	Yes	89	92.7%
	No	4	4.2%
	No response	3	3.1%
	Total	96	100.0%
Are the new guidelines easy to implement	Yes	86	89.6%
	No	9	9.4%
	No response	1	1.0%
	Total	96	100.0%
Has it been implemented in your institution.	Yes	83	86.5%
	No	10	10.4%
	No response	3	3.1%
	Total	96	100.0%
Are the new guidelines simple to understand	Yes	89	92.7%
	No	5	5.2%
	No response	2	2.1%
	Total	96	100.0%
Are the new guidelines easy to implement	Yes	79	82.3%
	No	15	15.6%
	No response	2	2.1%
	Total	96	100.0%
Has it been implemented in your institution.	Yes	75	78.1%
	No	16	16.7%
	No response	5	5.2%
	Total	96	100.0%
Will newer guidelines affect adherence of the patient to the drugs.	Yes	31	32.3%
	No	60	62.5%
	No response	5	5.2%
	Total	96	100.0%
Can there be a Family DOT provider to supervise the therapy of a family member.	Yes	69	71.9%
	No	24	25.0%
	No response	03	3.1%
	Total	96	100.0%
Are the drug reactions to be reported to the Deputy drug controller.	Yes	77	80.2%
	No	12	12.5%
	Not sure	03	3.1%
	No response	04	4.2%
If yes,	Total	96	100.0%
Who should report	Patient	11	11.5%
	Treating Physician	40	41.7%
	Nurse	1	1.0%
	Any of the above	40	41.7%
	No response	4	4.2%
	Total	96	100.0%
Is there any number given to the patient to call in case of drug reaction	Yes	61	63.5%
	No	24	25.0%
	No response	11	11.5%
	Total	96	100.0%
Which committee supervises the reporting of drug reactions in your institution	ADR committee	13	13.5%
	Department Pharmacology	18	18.8%
	Pharmacovigilance committee	10	10.4%
	No response	55	57.3%
	Total	96	100.0%

Sixty-one (63.5%) participants were aware of the phone number to be contacted for the patient to inform.

We included a question about the committee that supervises the reporting of drug reactions in the institution. This question was unanswered by 57 (57.3%).

Eighty-three (86.5%) of our participants were aware of Nikshay, 51 (53.1%) were aware that our institution has Nikshay ID and 45 (46.9%) were aware of the procedure for the Nikshay registration (Table 3).

Table 3 – Responses on Notification of tuberculosis, and Nikshay.

Notification			
Is TB a notifiable disease.	Yes		100%
Have you heard of Nikshay.	Yes	83	86.5%
	No	13	13.5%
	Total	96	100.0%
If yes, is your institution having a NIKSHAY ID.	Provided ID	51	53.1%
	Wrong ID	42	43.8%
	No response	3	3.1%
	Total	96	100.0%
If No, how you or your institution should enroll for Nikshay.	Yes	45	46.9%
	No	15	15.6%
	No response	36	37.5%
	Total	96	100.0%

There was a significant statistical difference ($p < 0.05$) for all the parameters tested except for three (difference in TB strategy from that of WHO, attending any training program and awareness about Hospital's Nikshay ID).

5. Discussion

Availability and easy access to better diagnostic technologies, effective interventions and monitoring, trained physicians, establishment of DOTS Centres have made India confident in attaining the mission of eradicating TB by 2025 and making a TB free country. Failure to control the tubercular infection even after 5 decades of introduction of TB control programs demanded a change in the disease control strategy that led to revision of these programs; after DOTs, RNTCP was introduced, which was revised in 2016 and 2017. These revisions were made to ensure to achieve the goal of eradication of TB in India by 2025 and TB free India by 2030.

Constant revisions to the guidelines for the management and prevention of tuberculosis, have made it difficult for the medical practitioners to keep themselves abreast of all the revisions of RNTCP, resulting in an inadequate knowledge and poor adherence to the standard treatment regimens. Many studies have illustrated a mis-match between the physician's knowledge and their practices in the private sector, with <1/3rd of them knowing the standard regimens for the management of tuberculosis.[7,8] Awareness and knowledge among the government/public sector was better than those in the private sector.[8]

However, the awareness and knowledge about these programmes was inadequate and misunderstood by many practitioners.[2,9] Even in the most developed cities of India, knowledge regarding the disease is inadequate with misconception being very high.[8,10,11]

Most of the studies done were on the knowledge, awareness of Interns, medical students,[4,12–17] which were good to satisfactory. Ours is the study in which participants were pulmonologists and physicians, apart from from medical Interns, who were involved in the management of tuberculosis.

We noticed that awareness regarding the revised TB programme (2017) was satisfactory (90.6%) among our participants. Training (38.5%) and sensitization programmes (37.5%) have proved to be an efficient way of bringing awareness in our study; 45.8% did not receive any training nor attended any program indicating that the target remains large; though a few participants (24.0%) sought online information, incomplete and incorrect information can be misguiding; Mass communication media which is generally considered useful to increase the awareness for the general public was useful only in 7.3% of participants, which, if utilised can be a useful tool.

There was no clear consensus regarding the mission of the national strategic plan for elimination of TB, with 49.0% stating the year 2030, and 38.5% the year 2025. Though later years (2040 & 2050) were stated by smaller proportion of participants, it is suggestive that the goal of these revisions has not been understood clearly and has to be propagated more.

We wanted to know if our participants thought that the revised program is different from that of WHO End TB Strategy; we did not find much difference among those who were for (46.9%) and against this statement with 46.9% considering the revised program to be different and 44.8% considering not different.

The guidelines were found to be simple to understand and implement indicating its feasibility in daily clinical practice. We implemented this program in September 2017 in our hospital; 86.5% of our participants were aware about the implementation of the program but 10.4% were unaware indicating the presence of robust inhouse awareness programs.

Our participants did not believe that the patient adherence to the treatment can be affected positively (62.5%) with these revised guidelines but were supportive of family involvement, support, cooperation in the treatment and can play a significant role to play in the treatment compliance and outcome.

Our participants (80.2%) were aware that ADRs must be reported to the deputy drug controller but divided in their knowledge about the reporting person (patient, doctor, nursing staff). Only 63.5% participants were aware of the contact number to report.

Our participants were not clear about the committee that supervises the reporting of drug reactions in our institution. This question was unanswered by more than half of our participants (57.3%) and is clearly indicative of lack of awareness.

Our study revealed that there is a need for awareness programs regarding Nikshay (In Sanskrit, ni = End, Kshay = Tuberculosis) platform. With 86.5% of our participants being aware of Nikshay, we find that awareness is satisfactory but there is a need for others to be aware of the same.

Our hospital has a Nikshay ID (058265); unfortunately, many (43.8%) of our participants are unaware of it. Only 15.6% were unaware of the procedure for the Nikshay registration; but 37.5% did not provide any answer probably due to lack of awareness.

Our study proves that there is a dearth of awareness among the study participants who are actively involved in the management of TB, similar to previous studies.[12,18]

Better implementation of the revised national TB program is required for better treatment outcome and on the national front to achieve the mission 2025. Already the deadline being extended to 2030, there is a doubt if this is achievable

successfully. Though few express their concern on achieving the goal, it is neither an impossible task nor unachievable. Our study clearly documents the lack of awareness among the treating physicians who are the main contributors in completing this mission.

Training that includes in-house interdepartmental interactions, Regional training programs by the trained persons/mentors, on the RNTCP guidelines must be made mandatory for the staff, interns and mandatorily to be included in the teaching curriculum.

6. Conclusion

The knowledge about the RNTCP guideline is satisfactory among our participants but there exists a gap. Extensive training, and Continuing Medical Education are required to increase physician's awareness.

Contribution details

Kundapur Anurag, Design, Literature search, Clinical studies, Data acquisition, Data analysis, Manuscript preparation, Udaykumar Padmaja, Concepts, Design, Definition of intellectual content, Data analysis, Statistical analysis, Manuscript editing, Manuscript review, Guarantor

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Conflicts of interest

The authors have none to declare.

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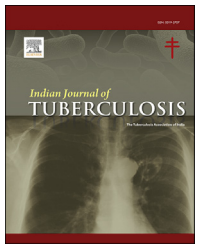

Original article

Cyclophosphamide therapy as an adjunct in refractory post-tubercular arachnoiditis

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ABSTRACT

Introduction: There is no satisfactory treatment for post tubercular arachnoiditis (TB arachnoiditis). We did this study to investigate the efficacy and safety of cyclophosphamide as adjuvant therapy for post TB arachnoiditis refractory to corticosteroids and anti-tubercular therapy (ATT).

Methods: This was a retrospective case series of patients of refractory post TB arachnoiditis leading to paraparesis and vision loss who received cyclophosphamide as an adjuvant therapy along with standard ATT and corticosteroids. These patients were treated with intravenous cyclophosphamide (dose 500 mg/m^2) once a month for 4 consecutive months after informed written consent and were assessed clinically and radiologically before and after cyclophosphamide therapy.

Results: We had 4 patients with refractory post TB arachnoiditis of whom three became independently ambulatory. There was significant clinical as well as radiological improvement in all the patients.

Conclusions: Cyclophosphamide therapy could be an effective therapy for patients with refractory post TB arachnoiditis. Well-designed randomized controlled studies are essential to study the safety and efficacy of cyclophosphamide in this condition.

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1. Introduction

Tubercular meningitis (TBM) accounts for around 1% of patients with tuberculosis and 10% of those with extrapulmonary tuberculosis.[1] Spinal involvement in TB may be due to tubercular myelitis, spinal radiculo-myelitis due to tubercular arachnoiditis (TB arachnoiditis), tuberculoma, syringomyelia, due to bony tuberculous spondylitis or tubercular abscess. Arachnoiditis can affect any part of the neuraxis surrounded by the meningeal layer, however, one of the important causes of morbidity is the sequelae of TBM due to arachnoiditis involving the spinal cord and optic nerve/chiasm(optico-chiasmatic Arachnoiditis).[2] TB arachnoiditis may develop as a paradoxical reaction anytime during the course of treatment or later. Whether this is an immune response related to organism, host or interaction between the two is currently unknown. One of the hypotheses is that it is an enhanced delayed-type hypersensitivity response.[3] Paradoxical reactions to anti-tubercular drugs can present with new deficits or worsening of pre-existing neurologic deficits in the central nervous system and have been traditionally treated with corticosteroids, infliximab or other immunesuppressants such as cyclophosphamide.[4,5] The treatment of post TB arachnoiditis is far from satisfactory. Intra-thecal hyaluronidase, thalidomide and other immunomodulators have been used as adjuvant therapy of TB spinal arachnoiditis, hydrocephalus and optico-chiasmatic arachnoiditis.[6,7] Since post TB arachnoiditis is a chronic inflammatory process, a trial of anti-inflammatory drugs sounds justified. We hypothesized that since cyclophosphamide has potent anti-inflammatory effects and is used in various inflammatory and vasculitic conditions, it may be effective in controlling the relentless progression of post TB arachnoiditis. It is also inexpensive and easily available as compared to other biologics such as infliximab and the onset of action is relatively quick as compared to other immunosuppressants such as azathioprine or methotrexate. Cyclophosphamide has also been used previously in two case reports for the treatment of CNS vasculitis secondary to tubercular meningitis.[8,9]

Here, we describe our experience in patients who received cyclophosphamide off label for refractory TB arachnoiditis, which was defined as arachnoiditis refractory to high doses of corticosteroids for atleast 4 weeks along with standard antitubercular therapy (ATT).

2. Methods

All records of patients with TB meningitis were screened and those who received cyclophosphamide therapy for post TB arachnoiditis (diagnosed based on clinical features of myeloradiculopathy or vision loss along with imaging features of proliferative arachnoiditis) were retrieved. The clinical data, including demography, the dosage of ATT and steroids, the number of hyaluronidase injections, duration of treatment, stage of disease when cyclophosphamide injections were started, disability and functional status at baseline and at last follow up were extracted in a proforma. Following parameters were analyzed: 1) Change in functional status (modified Rankin scale(mRS)) from baseline to last follow up, 2) Change in limb power, sensation and sphincter function, 3) Change in visual acuity from baseline to last follow up, 4)Degree of improvement in radiological findings and clinical correlation. The MR images of the patients were retrieved and imaging pre and post-cyclophosphamide therapy were compared.

3. Results

In patients with symptomatic post TB arachnoiditis, a trial of high dose methyl-prednisolone was given because the patients were developing significant functional decline due to blindness, paraparesis and sphincter dysfunction. Weekly intra-thecal hyaluronidase was also tried. Since the disease was relentlessly progressive in spite of these interventions, cyclophosphamide was administered off label in those who consented.

We found 4 patients who had received cyclophosphamide therapy for refractory post TB adhesive arachnoiditis. All the patients had been diagnosed to have TBM based on clinical, radiologic and CSF findings and categorized into various levels of diagnostic certainty according to Ahuja et al.[10] They had been treated with ATT and cortico-steroids and had had initial improvement in clinical features including resolution of fever, improvement in sensorium etc. Following this initial improvement, these patients deteriorated in terms of limb power, sensation, sphincter function and vision loss. Imaging showed dense adhesive basal arachnoiditis as well as spinal tubercular arachnoiditis (Figs. 1–4). Table 1 summarizes patients' clinical profile, neurological manifestations, radiology and therapy received.

3.1. *Patient 1*

A 28-year-old man presented with fever and headache of 5 months duration with recent-onset altered consciousness. Examination, MRI and CSF analysis were consistent with chronic meningitis. He was diagnosed with probable TBM as per the criteria validated by Ahuja et al.[10] and treated with ATT and steroids. With treatment, the patient's sensorium improved over 3–4 days. The clinical course of the patient and treatment offered are detailed in Supplementary illustration 1 and it is clear that the patient deteriorated after an initial period of improvement. Repeat CE-MRI brain showed multiple intracranial tuberculomas with optico-chiasmatic and spinal arachnoiditis [Fig. 1(a-d)]. Since there was no improvement at 12 months from onset of illness, after informed written consent, he was treated with intravenous cyclophosphamide. The patient had gradual improvement in bladder symptom, power and sensation in the lower limb with a complete improvement of vision over the next 12 months. Follow up imaging showed significant radiologic improvement [(Fig. 1(e-h)]. After one year of cyclophosphamide therapy, he was independently ambulatory with almost complete recovery in vision, bladder/bowel function, sensation and power in both upper and lower limb. Last follow up (5 years after cyclophosphamide therapy) at 6 years post-diagnosis, he was doing well.

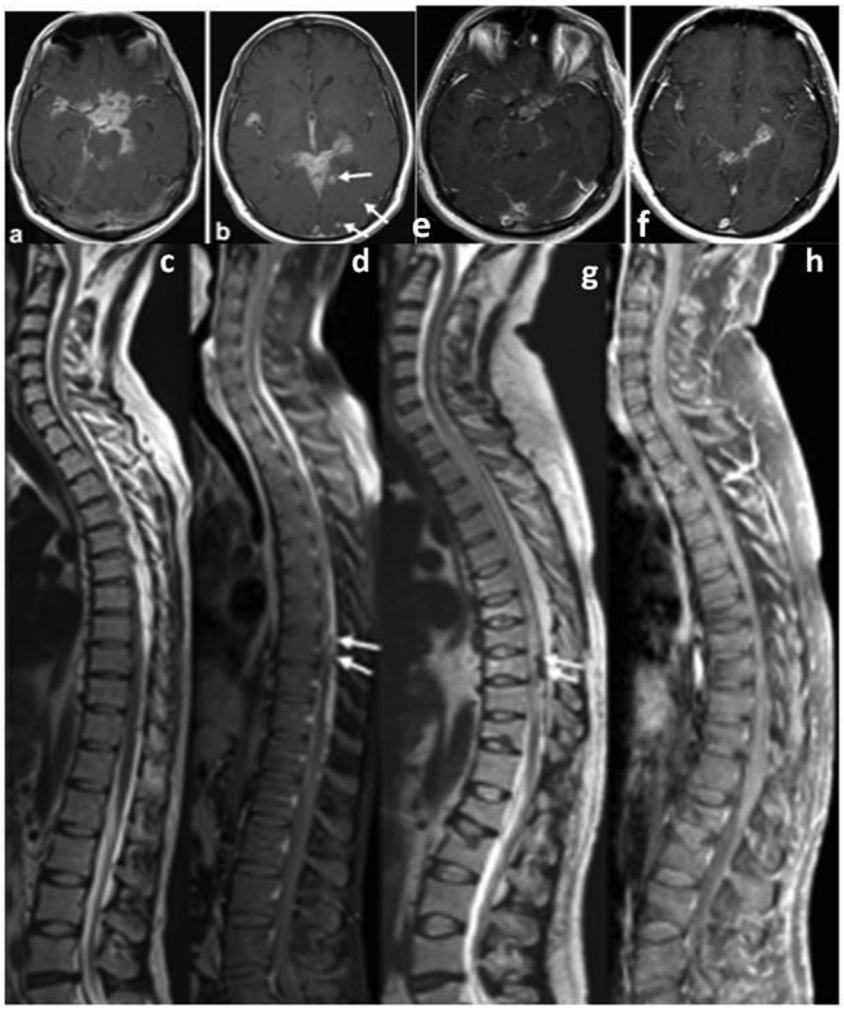

Fig. 1 – MRI brain post gadolinium axial T1 (a & b) show thick enhancing exudates in supra-sellar cistern, bilateral sylvian fissure, inter-peduncular cistern and bilateral peri-mesencephalic cisterns. Enhancing granulomas (arrows) are present in left occipital and temporal lobes. Whole spine sagittal T2-WI (c) and post-gadolinium T1-WI (d) show thick leptomeningeal enhancement and sub-pial peripherally enhancing granulomas in lower dorsal region (arrows in d) suggesting arachnoiditis. Follow-up post-gadolinium MRI brain T1-WI (e & f) shows) image significant reduction in enhancing exudates and resolution of enhancing granulomas. Follow-up MRI of whole spine-sagittal T2-WI (g) shows mild cord hyperintensity in dorsal region and hypointense granulomas (arrows in g). Leptomeningeal enhancement and enhancement of subpial granulomas has reduced in postgadolinium sagittal T1-WI (h).

3.2. *Patient 2*

This 23-year-old lady was treated with ATT (HRZE) for pulmonary tuberculosis for two months following which she developed TBM. Due to no clinical improvement after 2 months of ATT, she was switched on 2nd line ATT (Table). Further course of disease and treatments offered are summarized in the Table and Supplementary illustration 2. CEMRI was done [Fig. 2(a-d)]. At 20 months of illness, (18 months after hyaluronidase therapy), due to non-satisfactory improvement in vision and weakness, she was treated with Cyclophosphamide after consent. At follow-up after 3 months of cyclophosphamide therapy, she had significant improvement in vision, limb power, sensation and complete recovery of bladder and bowel functions. At the last follow-up at 6 years of illness, the patient reported improvement in lower limb function to the extent that she could walk independently within her house. Repeat imaging showed radiological improvement [Fig. 2(e-h)].

3.3. *Patient 3*

A 22-year young man was diagnosed with TBM and started on HRZE with corticosteroids. He improved initially. At the 15th month of illness, he developed gradually progressive paraparesis, acute urinary retention and vision loss. CE-MRI brain and spine showed significant arachnoiditis with cord signal changes from the cervico-medullary junction to the conus. (Fig. 3(a-d)]. Further course and treatment are summarized in the Table. At 31 months of illness, he was treated with cyclophosphamide after consent and his lower limb power improved with residual spasticity. At the last follow-up (42

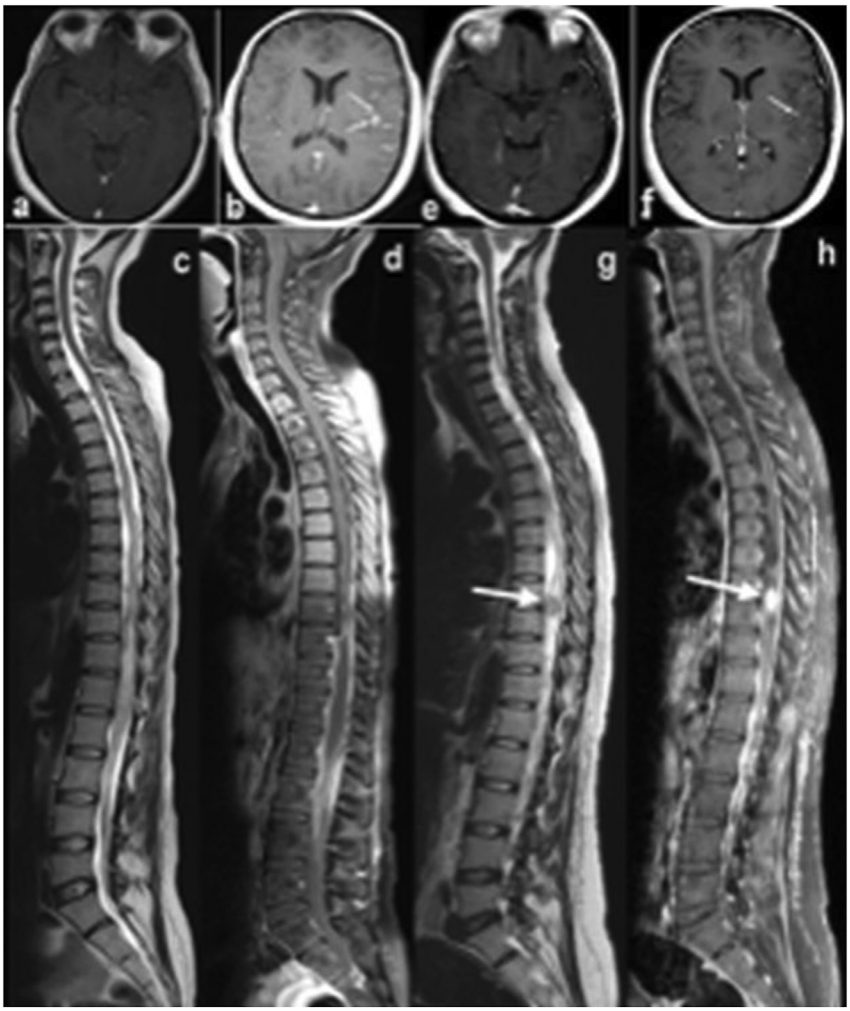

Fig. 2 – Post gadolinium MRI brain axial T1 (a & b) show leptomeningeal enhancement in left sylvian fissure (arrows in b). Whole spine sagittal T2-WI (c) show T2 -hyperintensity extending from D2 to conus. The spinal cord has undulating outline with thecal sac dilations suggesting CSF loculations. Post-gadolinium T1-WI (d) show leptomeningeal enhancement suggesting arachnoiditis with CSF loculations. Follow-up post-gadolinium MRI brain T1-WI (e & f) shows) image show decrease leptomeningeal enhancement in left sylvian fissure. A ring-enhancing lesion in left sylvian fissure is a new finding (arrow in f) suggest organized granuloma. Follow-up MRI of whole spine-sagittal T2-WI (g) shows increase in CSF loculations, however post-gadolinium T1-WI (h) shows decrease in leptomeningeal enhancement esp. in cervical and upper dorsal region. T2 hypointense and enhancing intramedullary/subpial granuloma in thoracic region is a new finding (arrow in g & h).

months after the onset of illness) there was a complete improvement in bowel and bladder function, improvement in vision and he was able to walk with support. Follow up MRI brain and spine [Fig. 3(e-h)] at last follow up showed reduction intracranial tuberculoma, leptomeningeal enhancement and arachnoiditis (Table).

3.4. *Patient 4*

A 13-year-old girl had fever, headache, vomiting for 4 months and her CSF was consistent with chronic meningitis. She was started with ATT and steroids. At that time, she noticed weakness in both lower limbs with complete loss of sensation below D6 level. There was acute retention of urine with constipation. CE-MRI brain and spine suggested myelitis with syrinx formation and arachnoiditis [Fig. 4(a-d)] with multiple granulomatous lesions in the brain. The treatment she received is summarized in the Table. At 45 months, after discussion and consent with the patient and her caregivers, she was treated with cyclophosphamide. At 51 months of follow up, there was an improvement in vision (6/6 bilaterally) without any cranial nerve palsy. Upper limb power improved completely but power in lower limbs did not improve and she continued to be on bladder self-catheterization. Repeat CE-MRI brain & spine [Fig. 4(e-h)] showed partial improvement (Table 1).

4. Discussion

In a recently published study on TBM, (1) one of the most common complications was paraparesis or hemiparesis 48/

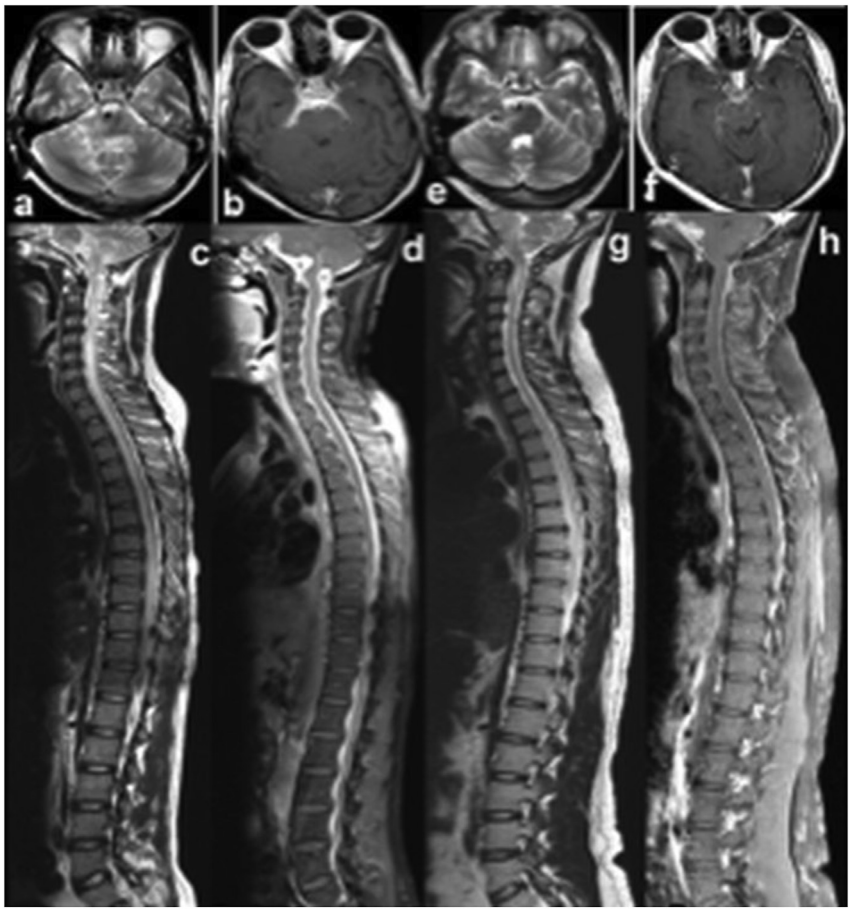

Fig. 3 – MRI brain axial T2 (a) shows T2 hyperintensity in the right cerebellopontine angle cistern, right middle cerebellar peduncle and dorsal pons. Post-gadolinium T1-WI (b) shows enhancement in the interpeduncular cistern and suprasellar cistern. Whole spine sagittal T2-WI (a) and post-gadolinium T1-WI (b) show holocord T2 -hyperintensity with thick leptomeningeal enhancement suggesting arachnoiditis with cord edema. Follow-up MRI brain axial T2 (e) image show decrease in signal change in right middle cerebellar peduncle and dorsal pons. Multiple T2 hypointense lesions are seen in right cerebellopontine angle cistern suggesting organized granulomas. Post-gadolinium T1-WI (f) show decrease in enhancement in interpeduncular and suprasellar cistern. Follow-up MRI of whole spine-sagittal T2-WI (g) and post-gadolinium T1-WI (h) shows decrease cord T2-hyperintensity (g) and leptomeningeal enhancement (h).

244 (19.6%) and visual involvement (22/244, 9.0%). Symptomatic weakness of both lower limbs (n = 33, 16.7%) was due to myelo-radiculitis or intradural lesions and in 29 patients (14.6%) MRI documented arachnoiditis.

There are only two case reports describing the use of cyclophosphamide in CNS tuberculosis: One case report where 2 doses, (750 mg/m^2 BSA, 1gm) of cyclophosphamide, 5 weeks apart was used in the treatment of CNS vasculitis secondary to tuberculous meningitis and the patient remained free of further strokes. This was based on the hypothesis that this measure was effective in primary CNS vasculitis. The patient also had a significant decrease in the CSF protein value, decreased erythrocyte sedimentation rate as well as improvement in TCD velocity. The response was stable over months after follow up.[8] Celotti et al. reported a patient with tubercular meningitis on ATT with steroids. During the second attempt of steroid tapering, there was a worsening of clinical condition in the form of reduced level of consciousness, lethargy and obtundation. Along with it there was worsening of biochemical and radiological profile like elevated CSF protein and hydrocephalus. Cyclophosphamide at 750mg/m2 BSA every 3 weeks was given. The patient showed rapid and progressive improvement with mild residual memory impairment.[9] In one of the studies, authors used cyclophosphamide in idiopathic hypertrophic pachymeningitis which is a chronic inflammatory process causing fibrous thickening similar to TB arachnoiditis but without any recognized etiology. After recurrence on tapering of steroids, they administered cyclophosphamide and there was clinical as well as radiological improvement.[11] With these hypotheses and after a detailed discussion with patients and their caregivers about the risks and possible benefits we administered cyclophosphamide in 4 different patients in which other modalities had failed (steroids, hyaluronidase, thalidomide, VP shunt).

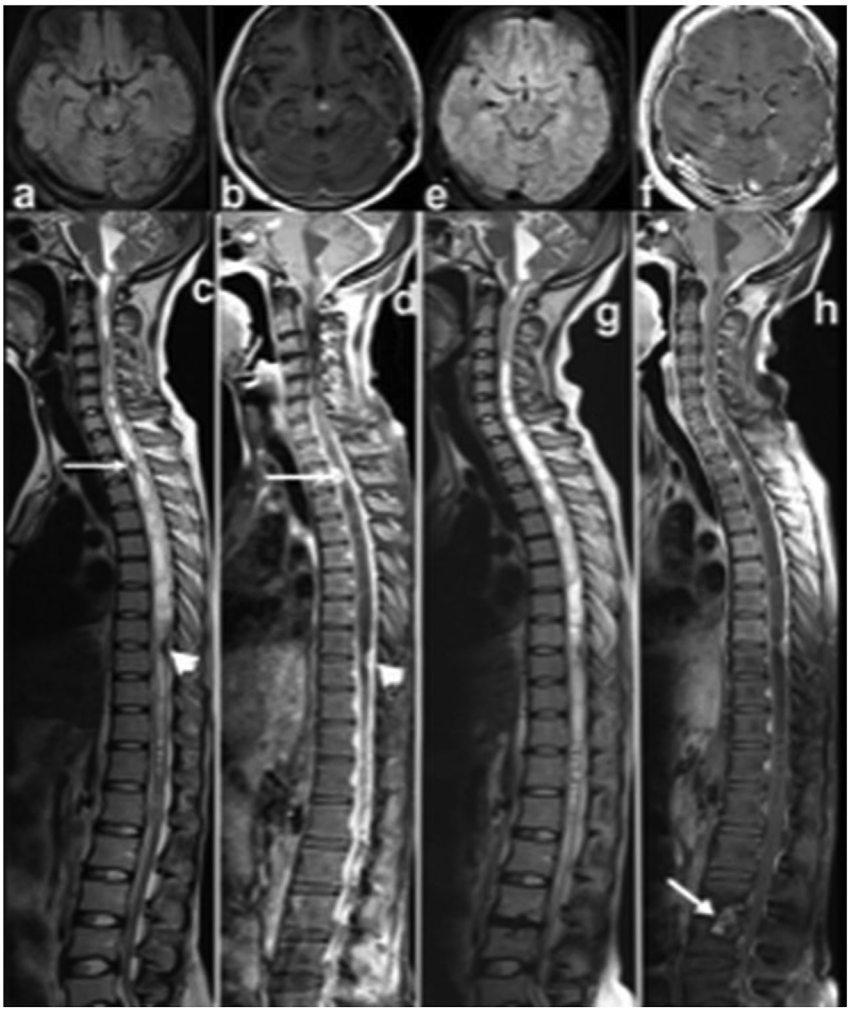

Fig. 4 – MRI brain axial FLAIR (a) and post-gadolinium T1-WI (b) shows enhancement in the interpeduncular cistern and diffuse pachymeningeal thickening and enhancement. Whole spine sagittal T2-WI (c) and post-gadolinium T1-WI (d) show holocord multiseptated CSF-like T2 -hyperintensity with thick leptomeningeal enhancement suggesting arachnoiditis with syrinx. T2-hypointense and peripherally enhancing subpial granulomas are present on anterior aspect of spinal cord at D2 (arrow) and posterior aspect of spinal cord at D8 (arrowhead) (c & d). Follow-up MRI brain axial FLAIR (e) and Post-gadolinium T1-WI (f) show significant decrease in enhancement. Follow-up MRI of whole spine-sagittal T2-WI (g) shows decrease in syrinx in upper cervical region and increase in syrinx in dorsal region. Leptomeningeal enhancement and enhancement of subpial granulomas has reduced in postgadolinium sagittal T1-WI (h). The enhancement of inferior endplate of L3 and superior endplate on L4 with intervening disc suggests spondylodiscitis and is a new finding (arrow in h).

We had four patients with post TB arachnoiditis and quadriparesis (spinal arachnoiditis) with or without visual loss (optico-chiasmatic arachnoiditis) who were treated with cyclophosphamide therapy (Table 1). These 4 patients had had an initial improvement with ATT followed by gradually progressive paraparesis and some of them had partial/transient improvement either spontaneously or with hyaluronidase. We hypothesized that in these patients; clinically and radiologically progressive arachnoiditis was due to an autoimmune inflammatory phase triggered by the initial tubercular infection. This could be due to enhanced delayed-type hypersensitivity reaction leading to activation and aggregation of lymphocytes, macrophages and other immune mechanisms at the site of bacterial deposition and site where bacteria died and produced toxin.[8] Because of the chronic inflammatory process in tuberculous meningitis, patients have very high levels of tumor necrosis factor and one study reported no change in levels when corticosteroids were used with ATT.[3] Based on this hypothesis various other immunomodulatory drugs like thalidomide have been used as adjuvant therapy in TBM.[7,12] Cyclophosphamide being an anti-metabolite with strong immunosuppressive property is an already established treatment modality in various autoimmune disease conditions like systemic lupus erythematosus,[13] multiple sclerosis,[14] vasculitis including CNS vasculitis[15] is usually contraindicated in active systemic infections including tuberculosis. However, our patients had improved with ATT following which they developed progressive arachnoiditis

Table 1 – Clinical and radiological features of patients before and after Cyclophosphamide.

Clinical/radiological features	Case 1	Case 2	Case 3	Case 4
Age (years)/Gender	28/M	23/F	22/M	19/F
Diagnosis of TBM	Probable TBM	- Definite TBM - BACTEC +, (HRZ resistance) - Disseminated (military pulmonary TB)	Probable TBM	Probable TBM
Symptoms before ATT, months	5	2	7	4
ATT regime	- 3 months: HRZE: - 3 months: HRZS: - 26 months: HRZL+ PTM	- 4 months: HRZ Ox - 2 months: HRZ Am - 30 months: HR Lvx+ETM+ Cyc	- 3 months: HRZES - 12 months: HRZE - 27 Months: HRZ	- 2 months: HRZE - 8 months: HRZ - 3 months: HRZSLvx - 23 months: HRZLvx
Duration of ATT, months	32	36	37	36
Dose & duration of Steroid, months	Prednisolone 40mg/day, 24m	Dexamethasone 12mg/day, 20 m	Dexamethasone 12mg/day, 28 m	Dexamethasone 12mg/day, 24 m
Previous Thalidomide	100mg, BD, 2 month	No	No	No
Intrathecal Hyaluronidase: 1500 IU/week	15 doses	10 doses	15 doses	7 doses
Duration of illness before Cyclophosphamide (months)	12	20	31	45
Cyclophosphamide dose (500 mg/m^2)	- 750mg/month - 4 doses	- 650mg/month - 4 doses	- 750mg/month - 4 doses	- 750mg/month - 5 doses
Improvement in power:	Yes	Yes	Yes	Yes
- UL	- 3/5 to 5/5	- 4/5 to 5/5	- 3/5 to 4+/5	- 4/5 to 5/5
- LL	- 3/5 to 5/5	- 0/5 to 3/5	- 1/5 to 3/5	- 0/5 to 0/5
Improvement in Vision	Yes	Yes	Yes	Yes
- Right	- R: 6/6 to 6/6	- R: PL Negative to 6/18	- R: 6/36 to 6/18	- R: 6/12 to 6/6
- Left	- L: PL+ to 6/6	- L: PL Negative to 6/24	- L: 6/36 to 6/9	- L: 6/9 to 6/6
- Field	- R eye constricted field to normal		- NA	- NA
Improvement in sensory loss	Yes 100%	Yes 70–80%	Yes 60–70%	Yes Sensory loss receded from D6 to D12
Improvement in bladder/ bowel symptoms	Yes	Yes	Yes	Yes Patient was shifted to CISC
mRS before cyclophosphamide	4	5	5	5
mRS at last FU	1	3	3	4
Independent in walking at last FU	Y	Y	No, walking with support	No, cannot walk

(*continued on next page*)

Table 1 – (continued)

Clinical/radiological features	Case 1	Case 2	Case 3	Case 4
MRI changes after cyclophosphamide	- Decreased in cord enhancement with reduction in number of granuloma	- Reduced leptomeningeal enhancement with decreased cord signal changes with formation of CSF loculations	- Reduction of extent of Right CP angle tuberculoma and perilesional oedema, reduced leptomeningeal enhancement & arachnoiditis	- Reduced tuberculoma - Reduced cranial and cervical meningeal enhancement

Am: Amikacin, CyC: Cycloserine, ETM: Ethionamide, Lvx: Levofloxacin, mRS: Modified Rankin Scale, Ox: Ofloxacin, PTM: Prothionamide.

which suggested triggering of persistent inflammation by TB. And we found significantly promising results in 3 out of 4 patients in terms of clinical benefit as well as radiologic improvement.

5. Limitations

It is important to mention that these patients also received corticosteroids, intrathecal hyaluronidase and also antitubercular drugs. So it is likely that the improvement seen was a result of combined action of all these drugs. The standalone effect of cyclophosphamide cannot be commented on based on this study. It seemed unlikely spontaneous improvement due to the natural history of the disease given that the patients showed no signs of clinical or radiologic improvement in arachnoiditis prior to administration of cyclophosphamide. A controlled trial is essential to confirm the efficacy and safety of the drug.

6. Conclusion

In selected cases of post TB arachnoiditis refractory to treatment with steroids and hyaluronidase, cyclophosphamide may provide a beneficial effect.

Before recommending this as standard of care, a well-designed randomized controlled study is required to confirm the safety and efficacy of cyclophosphamide in tuberculous arachnoiditis.

Ethics approval

Not required.

Patient consent

Obtained.

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.2021.05.002.

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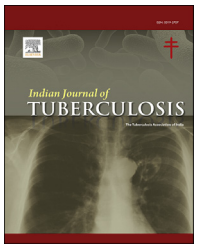

Original article

Treatment adherence status of the TB patients notified from private sector and its associated factors: Findings of a secondary data analysis from West Bengal, India

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ABSTRACT

Introduction: In India, each year, estimated one million TB cases are missing from notification, most of them being diagnosed treated in private sector. The large number of patients in private sector has raised concerns about suboptimal quality of care; lack of systems for treatment adherence thus raising the risk of drug resistance. The current analysis was conducted to find out the status of TB treatment adherence in private sector & to identify the factors associated with poor TB treatment adherence.

Methods: Analysis of secondary data obtained through adherence monitoring house visit by THALI (an USAID funded project) field workers during July 2018–June 2019, was done.

Results: Default rate among the private patients was 5%. Among the private TB patients 81.6% & among the defaulter 87.3% were in the age bracket of 15–59 years. Reasons stated for being a defaulter were 'Medicine is not working' (30%), 'Travel' (28.6%), 'Cost involved in the treatment' (21.8%), 'Side effects of ATD' (11.6%), 'Anxiety or Depression' (7.2%) & 'Feeling of completely cured' (0.8%). Despite best of efforts only 36.9% defaulter could be retrieved. Factors associated with increased risk of lost to follow-up were 15–59 years age, male sex, earning member of the family,tobacco user, alcohol user, DR-TB, continuation phase of treatment, previous history of TB, presence of symptoms & inability to walk.

Conclusion: Privately treated TB patients are vulnerable for non-adherence. Once defaulted, it is difficult to retrieve them. Economically productive age group is at higher risk of being defaulter. Commonest reason for lost to follow up is wrong impression about TB medicine. Program should think of extensive engagement & sensitization drive for the private

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providers; Strict adherence monitoring of private TB patients, extensive advocacy communication & social mobilization program in the community & workplaces/ institutions.

1. Introduction

Tuberculosis (TB) is one of the world's most neglected health crisis. In spite of its alarming danger, surprisingly little action has been taken to address the TB Epidemic. The World Health Organization (WHO) declared TB a global public health emergency in 1993, and also recommends what specific steps should be taken to address the epidemic.[1] TB is a communicable disease requiring prolonged treatment, poor adherence to a prescribed treatment increases the risk of morbidity, mortality spread of disease in the community. The therapeutic regimens given under direct observation as recommended by WHO have been shown to be highly effective for both preventing treating TB but poor adherence to anti-TB drugs (ATD) is a major barrier to global control.[2]

India notified 1.9 million TB cases in 2016 of which, 1.6 million were from public sector 0.3 million from private sector. A staggering one million TB cases are missing from notification, most of them being diagnosed treated in private sector. That is what makes the engagement with the private sector so very vital. The large number of patients in private sector has raised concerns about: delayed diagnosis; suboptimal quality of care; incorrect diagnostic treatment protocols; lack of systems for treatment adherence patient support; a high drop-out rate, thus raising the risk of drug resistance to first-line/or second-line drugs.[3]

The United States Agency for International Development (USAID) funded Tuberculosis Health Action Learning Initiative (THALI) project launched its activity in 6 revenue districts of West Bengal viz Kolkata, Howrah, Hooghly, North 24 Parganas, South 24 Parganas & Purba Medinipur with an aim to improve the quality of TB care by introducing innovative solutions.[4–8] With the help of THALI project these districts were able to notify nearly 8000 10,000 privately treated TB patients respectively during 2017 2018.[9,10] After notification, taking public health action especially monitoring treatment adherence was a real challenge for the TB patients notified from the private sector. The project devised a plan to monitor the treatment adherence of private TB patients though house visit by the trained field workers of THALI.

There are numerous studies to assess the factors associated with poor TB treatment adherence, but most of them analysed data from public sector.[2,11–16] There is a dearth of scientific literature reporting the treatment adherence status for private sector. There is no study on treatment adherence of private TB patients in West Bengal.The current study was conducted to find out the status of TB treatment adherence in private sector & to identify the factors associated with poor TB treatment adherence. Now as India is moving towards TB elimination, strict treatment adherence, especially for those patients who have been seeking care from private sector, is a need of hour. Therefore, identifying the major thrust areas, bears policy relevance.

2. Methods

2.1. *Study design, settings & study population*

A secondary data analysis was performed on the data that was obtained through adherence monitoring house visit by THALI field workers during the period of July 2018–June 2019. Data was collected by visiting household of the notified TB patients from six districts of West Bengal namely Kolkata, Howrah, Hooghly, North 24 Parganas, South 24 Parganas & Purba Medinipur who were notified during January 2018 to June 2019. Along with the private patients few of the public patients, who were residing at hard to reach area or whose required details had not been collected by the system yet. The line-list with complete address, contact details of all private patients & selected public patients has been shared to THALI by the respective District TB Officers (DTO). THALI field worker targeted to visit all privately notified TB patients who were on treatment from any peripheral health institute (PHI) of these six districts. During the visit period from July 2018 to June 2019 twenty field workers visited & collected adherence information of more than 15,000 TB patients. After excluding the details of public patients incomplete details of private patients we had 7505 patients' details for the secondary analysis for the study.

2.2. *Data collection, definitions & analysis*

Data collected by reviewing the TB treatment card, medical records available with the patients & interview with the patients or the care giver. Collected data was entered into the CommCare HQ (https://www.commcarehq.org/accounts/login/) application based structured proforma. For quality assurance 10% patients house whose house had been visited by the field workers, was re-visited by supervisors & senior managers of the project (district coordinator, medical consultant & deputy project director & director). Information from the CommCare was extracted into Microsoft® Excel® (2016) was analyzed after cleaning validation. Stata 14.2 (StataCorp LP, College Station, TX, USA) was used for statistical analysis. Key analytic outputs were the number proportion of TB patients with difference socio-economic & clinical background.

A Private TB patient was considered as one who has been diagnosed & treated by a Private (non-government) health Care facility. However, a private health care facility may use

Government diagnostic facility Government supply ATD for that Private TB patients. A TB patient was considered as Defaulter if s/he was not taking ATD for 28 days or more, consecutively after starting treatment. This outcome is called Lost to Follow Up (LTFU). To define regular use of alcohol, one who takes alcohol at least twice a week, was considered as alcohol user. On the other h, who used any forms of tobacco at least once per day was considered as a tobacco user. Reasons for missing of drugs is tabulated dividing into two categories namely number of proportions of defaulter retrieval done & number & proportion who remain defaulted. Nonrom association of the reasons measured using Fisher's exact test. Association with socio-demographic & clinical factors for being a defaulter was measured through the Odds ratio (OR) using Generalized linear model (Poisson regression with robust stard errors) 95% Confidence Interval (95% CI) for each OR was obtained from the models. A P-value of <0.05 was taken as statistically significant.

3. Results

3.1. *Socio Demographic profile of the private treated TB patients*

Table 1 is showing the socio-demographic & clinical profile of the privately treated TB patients as well as the treatment defaulters. Default rate among the Private Patients was 5%.

The median age of the Privately notified TB patients was 40 yrs (25–53). Among them 81.6% were in the age bracket of 15–59 years (Economically productive age). 68.6% were males, 20.7% were illiterate, 45.1% were earning family members, 3.8% belongs to a migratory family, 22.3% tobacco user, 10.4% alcohol user, 98.1% non-veg eater, 4.8% were Drug resistant TB (DR-TB), 66.3% were in their Intensive Phase of therapy (IP), 16.2% had history of TB before, 15.1% were known diabetic, 0.7% were PLHIV (People Living with HIV/AIDS), 63% patients had some symptoms 7% were unable to walk.

The median age of the defaulter TB patients (subset of the private TB patients) was 38 yrs (25–50). Among them 87.3% were in the age bracket of 15–59 years, 72.9% were males, 22.6% were illiterate, 53.3% were earning family members, 4.2% belongs to a migratory family, 36.3% tobacco user, 19.9% alcohol user, 98.7% non-veg eater, 11.1% were DR-TB, 54.1% were in their Continuation Phase of therapy (CP), 22.3% had history of TB, 16.5% were diabetic, 0.8% PLHIV, 73.5% patients had some symptoms 9.5% were non-ambulatory.

3.2. *Reasons for lost to follow-up*

Table 2 is showing different reasons (as stated by the patients &/primary care giver) leading to discontinuation of ATD & becoming a treatment defaulter. The table is also showing the number & proportion of each category who has been reinstated to the treatment with counseling & motivation by THALI field workers.

Commonest reason for being a defaulter was the impression of 'Medicine is not working' (30%). Other reasons were 'Travel' (28.6%), 'Cost involved in the treatment' (21.8%), 'Side

Table 1 – Socio Demographic & Clinical profile of the Private TB Patients (N) & among those who missed dose of 28 days or more (n) as identified by the THALI project, West Bengal, during Jul'18-June'19. N = 7505; n = 377.

Characteristics	Total Number of TB patients (%)	Number of TB patients who missed dose (%)
Total	7505 (100)	377 (5.0%)
Age in years		
0-14	193 (2.6)	2 (0.5)
15-29	2253 (30.0)	119 (31.6)
30-44	1948 (26.0)	109 (28.9)
45-59	1918 (25.6)	101 (26.8)
60 and above	1193 (15.8)	46 (12.2)
Gender		
Male	5145 (68.6)	275 (72.9)
Female	2359 (31.4)	102 (27.1)
Transgender	1 (0.0)	0 (0.00)
Education		
Illiterate	1556 (20.7)	85 (22.6)
Primary School	3955 (52.7)	217 (57.6)
High School	1548 (20.6)	59 (15.6)
Graduate & Above	446 (6.0)	16 (4.2)
Occupation		
Student	704 (9.4)	21 (5.6)
Dependent Family Members	3414 (45.5)	155 (41.1)
Earning Family Members	3387 (45.1)	201 (53.3)
Migratory Family		
No	7218 (96.2)	361 (95.8)
Yes	287 (3.8)	16 (4.2)
Tobacco usage		
No	5831 (77.7)	240 (63.7)
Yes	1674 (22.3)	137 (36.3)
Alcohol usage		
No	6723 (89.6)	302 (80.1)
Yes	782 (10.4)	75 (19.9)
Type of Diet		
Veg	145 (1.9)	5 (1.3)
Non-veg	7360 (98.1)	372 (98.7)
Type of TB		
DS-TB	7147 (95.2)	335 (88.9)
DR-TB	358 (4.8)	42 (11.1)
Phase of Treatment		
IP	4974 (66.3)	173 (45.9)
CP	2531 (33.7)	204 (54.1)
History of TB		
No	6287 (83.8)	293 (77.7)
Yes	1218 (16.2)	84 (22.3)
Diabetes status		
Non-diabetic	5446 (72.6)	279 (74.0)
Diabetic	1133 (15.1)	62 (16.5)
Unknown	926 (12.3)	36 (9.5)
HIV status		
Negative	6324 (84.3)	320 (84.9)
Positive	53 (0.7)	3 (0.8)
Unknown	1128 (15.0)	54 (14.3)
Current Symptoms		
No_Symptoms	2774 (37.0)	100 (26.5)
Have some symptoms	4731 (63.0)	277 (73.5)
Ambulatory		
No	522 (7.0)	36 (9.5)
Yes	6983 (93.0)	341 (90.5)

Numbers within the parentheses indicate column percentages for each category.

effects of ATD' (11.6%), 'Anxiety or Depression' (7.2%) & 'Feeling of completely cured' (0.8%). Proportion of default retrieved was 36.9%.

3.3. Risk factors associated with lost to follow-up

Table 3 is showing the association of Socio Demographic & clinical factors of the TB Patients for 'being a defaulter'.

Economically productive age group (15–59 yrs) were associated with more than 5 times risk of being defaulter when compared with below 15 years age group. Other factors associated with increased risk were male sex {OR 1.3 (1.0–1.6)}, being earning member of the family {OR 1.3 (1.1–1.6)}, being tobacco user {OR 2.1 (1.7–2.6)}, Alcohol use {OR 2.3 (1.7–2.9)}, DR-TB {OR 2.7 (1.9–3.8)}, being in the CP {OR 2.4 (2.0–3.0)}, previous history of TB {OR 1.5 (1.2–2.0)}, presence of any symptoms {OR 1.7 (1.3–2.1)} & inability to walk {OR 1.4 (1.0–2.1)}.

4. Discussion

4.1. Key findings in light of relevant literatures

The current article is unequivocally the first article which described the treatment adherence status of the TB patients notified from private sector of West Bengal. Our study has few key findings which may have significant implication in National TB Programs (NTP).

Default rate among the Private Patients was 5%. Which was same as the aggregate default rate of privately treated TB patients of whole country during the year 2020. The private sector default rate is slightly higher than public sector TB patients (4%).[17] The default rate was better than the default rates among private TB patients elsewhere.[18] Among the private TB patients 81.6% & among the defaulter 87.3% were in the age bracket of 15–59 years (Economically productive age). As per census 2011 the age group 15–59 contributes 62.5 percent of total population.[19] Over representation of the age group for contracting TB might be due to more outdoor activity exposure, addictions, carrier, or work-space related stress. Our study also found that the economically productive age group were associated with more than 5 times risk of being defaulter when compared with below 15 years age group earning family members were associated with 30% increased risk of being defaulter. In most of the families, males are earning members, naturally study showing 30% increased risk of being defaulter among males. These findings might partially explain how TB can put economic burden to the affected family the society.

Commonest reason for being a defaulter was the impression of 'Medicine is not working' (30%). Other two most stated reasons were 'Travel' (28.6%), 'Cost involved in the treatment' (21.8%). A similar study which was conducted for public patients reported that three most common reason for being defaulter is side effects of ATD (42.2%), a feeling of early improvement (33.3%) Travel for work (9.6%).[2] The study also found that once defaulted, retrieval is tough among the private TB patients. Despite best of efforts only 36.9% defaulter could have been retrieved. Tobacco & Alcohol use was associated with increased risk of being a defaulter. Several other studies also reported similar association.[12,20–24] There was increased risk of being defaulter among the DR-TB patients & who have previous history of TB. Studies also reported similar findings.[20,25] The study also found that 'persistence of symptom & inability to walk was also associated with higher risk of getting defaulted.

The study found that among private TB patients who were on Continuation Phase of their treatment were at more than 2 times higher risk of getting defaulted. Though Most of the studies on Public sector patients of India & abroad[12,26,27] reported that Intensive Phase is associated with higher risk of getting defaulted, there is a study at Tajikistan which reported similar findings.[25] This is particularly important because it has a significant public health implication. Non-adherence in the presence of high bacillary loads typically seen in the intensive phase is likely to have greater impact than the same degree of non-adherence later during the continuation phase, when bacillary loads are generally several logs lower.[28]

4.2. Strengths limitations

The study was conducted under programmatic settings reflects the field realities. The study had a relatively large sample size for a secondary data analysis possibility of selection bias was minimized by virtue of the systematic selection criteria used in the program. Quality of the source data (treatment card) had been controlled & assured through cross-checking & validation by the field supervisor. Entry errors were also checked by inbuilt logical checking & consistency checking system in the CommCare Apps. However, despite the best efforts, the study had a few limitations. Potential

Table 2 – Reasons for "being a defaulter", as stated by the private TB patients or care giver *during patient's house visit by the volunteer of THALI project, West Bengal during Jul'18-June'19. N = 377.*

Reasons for missing dose of anti-TB drugs	Number patients who remained defaulter (%)[a]	Number of default patients who were retrieved (%)[a]	Total Number (%)[a]	p-value
Medicine is notworking	75 (31.51)	38 (27.34)	113 (30.0)	0.794
Travel	67 (28.15)	41 (29.50)	108 (28.6)	
Cost involved in the treatment	49 (20.59)	33 (23.74)	82 (21.8)	
Side effect of drugs	28 (11.76)	16 (11.51)	44 (11.6)	
Anxiety/ Depression	16 (6.72)	11 (7.91)	27 (7.2)	
Feeling of completely cured	3 (1.26)	0 (0.00)	3 (0.8)	
Total (%)[b]	238 (63.1)	139 (36.9)	377	

[a] Numbers within the parentheses indicate column percentages.
[b] Numbers within the parentheses indicate row percentages; LTFU: Lost to follow-up (Missed dose for more than 1 month); P-value obtained by Fisher's exact test.

Table 3 – Association of Socio Demographic & Clinical factors of the Private TB Patients for "being a defaulter" as identified by the THALI project, West Bengal, during Jul'18-June'19. N = 7505; n = 377.

Characteristics	Total Number of TB patients (N)	Number of defaulters (n, %[a])	OR (95% CI)
Age in years			
0-14	193	2 (1.0)	(base)
15-29	2253	119 (5.3)	**5.3 (1.3–21.7)**
30-44	1948	109 (5.6)	**5.7 (1.4–23.1)**
45-59	1918	101 (5.2)	**5.3 (1.3–21.7)**
60 and above	1193	46 (3.9)	3.8 (0.9–15.9)
Gender			
Male	5145	275 (5.3)	**1.3 (1.0–1.6)**
Feale	2359	102 (4.3)	(base)
Transgender	1	0 (0.0)	(empty)
Education			
Illiterate	1556	85 (5.5)	1.6 (0.9–2.7)
Primary School	3955	217 (5.5)	1.6 (0.9–2.6)
High School	1548	59 (3.8)	1.1 (0.6–1.9)
Graduate & Above	446	16 (3.6)	(base)
Occupation			
Student	704	21 (3.0)	0.7 (0.4–1.0)
Dependent Family Members	3414	155 (4.5)	(base)
Earning Family Members	3387	201 (5.9)	**1.3 (1.1–1.6)**
Migratory Family			
No	7218	361 (5.0)	(base)
Yes	287	16 (5.6)	1.1 (0.7–1.9)
Tobacco usage			
No	5831	240 (4.1)	(base)
Yes	1674	137 (8.2)	**2.1 (1.7–2.6)**
Alcohol usage			
No	6723	302 (4.5)	(base)
Yes	782	75 (9.6)	**2.3 (1.7–2.9)**
Type of Diet			
Veg	145	5 (3.5)	(base)
Non-veg	7360	372 (5.1)	1.5 (0.6–3.7)
Type of TB			
DS-TB	7147	335 (4.7)	(base)
DR-TB	358	42 (11.7)	**2.7 (1.9–3.8)**
Phase of Treatment			
IP	4974	173 (3.5)	(base)
CP	2531	204 (8.1)	**2.4 (2.0–3.0)**
History of TB			
No	6287	361 (5.7)	(base)
Yes	1218	16 (1.3)	**1.5 (1.2–2.0)**
Diabetes status			
Non-diabetic	5446	279 (5.1)	(base)
Diabetic	1133	62 (5.5)	1.1 (0.8–1.4)
Unknown	926	36 (3.9)	0.8 (0.5–1.1)
HIV status			
Negative	6324	320 (5.1)	(base)
Positive	53	3 (5.7)	1.1 (0.4–3.6)
Unknown	1128	36 (3.2)	0.9 (0.7–1.3)
Current Symptoms			
No Symptoms	2774	100 (3.6)	(base)
Presence of Symptoms	4731	277 (5.9)	**1.7 (1.3–2.1)**
Ambulatory			
No	522	16 (3.1)	**1.4 (1.0–2.1)**
Yes	6983	361 (5.2)	(base)

Stylistically significant findings are highlighted with bold font.
[a] Numbers within the parentheses indicate row percentages. (base): reference categories.

confounders like distance from referred health facility, socio economic status, fees structure of treating private health facility, marital status, were not adjusted for during regression analysis. Thus, the factors associated with "being a defaulter" should be interpreted with caution. It can be argued that to explain the outcomes of the quantitative analysis, qualitative interviews could have been of help, but the current article limited its reach within the bounds of quantitative analysis of the secondary data.

4.3. *Recommendations based on the findings*

Based on the study's findings, there are few implications recommendations for adherence monitoring of privately treated TB patients. Privately treated patients are most vulnerable to become defaulter, so more attention is needed, unfortunately which is, till now, missing in the program. It is also evident that once defaulted it is very difficult to back them into the track. So early counseling & regular follow-up is a must for all TB patients especially for the privately treated TB patients.

Economically productive & most active age group is more vulnerable of having TB. This group are at higher risk of being a defaulter. This may be due to stigma associated with TB in workspace &/due to work pressure. De-stigmatization activity including awareness program, periodic TB screening, IEC may be carried out in the offices, schools, other private & public sector enterprises. Paid-leave until end of the Intensive Phase of TB treatment can be a way forward.

Commonest reason for lost to follow up was wrong impression about TB medicine. Burden of treatment cost was the reason for more than one fifth of the defaulter, whereas TB diagnosis & treatment is freely available at Government Hospitals. Both the reason indicates for extensive awareness program, IEC & repeated counseling of the private TB patients. To implement these, sensitization program for the private providers is required in order to build capacity of these private sector providers.

5. Conclusions

Privately treated TB patients are highly vulnerable for becoming a defaulter. Once defaulted, it seems to be difficult to retrieve them. Economically productive & most active age group is at higher risk of being defaulter. Commonest reason for lost to follow up is wrong impression about TB medicine. NTP should think of three things-extensive engagement & sensitization drive for the private providers; Strict adherence monitoring of the privately treated TB patients by repeated house visit & counseling; Implementation of extensive advocacy communication & social mobilization program in community as well as work places/institutions.

Ethics approval consent to participate

The study has been approved & actively supported by the Project Director, THALI. As the analysis involved review of patient records (secondary program-data), so issue of clearance from institutional ethics committee was waived.

Consent for publication

Not applicable.

Availability of data materials

The datasets used/or analysed during the current study are available from the corresponding author on reasonable request. Also, the datasets are included in the supplementary information files of the article.

Authors' contributions

All authors have contributed to data curation, manuscript writing, proof reading &all have given approval for publication. Below is the thematic area wise contribution-

Conceptualization – AD & AL.

Data collection – VS, PS & AD.

Data entry- VS, AD & SSJ.

Data curation- AD, AL, SSJ, VS, PS &AC.

Formal analysis- AD, AL, AC & VS; Software-VS,AD; Supervision-AC & PS; Validation-AC, PS & AD.

Visualization-AD & AL.

Writing, review & editing-AD, AL, SSJ, VS, PS & AC.

Consented for publication: AD, AL, SSJ, VS, PS & AC.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2021.06.001.

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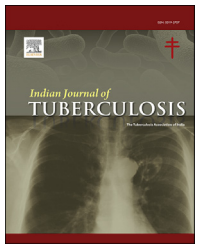

Original article

A prospective observational study to evaluate Glutathione S-transferase gene polymorphism and its association with Antitubercular drugs induced liver injury in tertiary hospital

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ABSTRACT

Background: Anti-TB drugs are most common cause of idiosyncratic hepatotoxicity worldwide. Reactive metabolite formed during drug metabolism has been involved in a clinical toxicity are described as 'idiosyncratic' drug induce liver injury (DILI). We have observed the distribution of glutathione S -transferase (GST) gene polymorphism & its association with drug-induced liver injury in patients taking anti-tubercular treatment.

Methods: A prospective observational study including 96 patients receiving anti-tubercular treatment. Blood sample was collected for LFT and gene extraction after ruling out other cause of liver injury. DNA extraction for GST gene was done follow by polymerase chain reaction to identify homozygous null mutation at GSTM1 and GSTT1 loci. Association of GSTM1 and GSTT1 gene with DILI was seen.

Results: Out of 96 tubercular patients under treatment, drug induced liver injury was found in 21 (21.9%) patients and 75 does not develop DILI, GST M1 gene null mutation was observed in 14 (66.7%), GST T1 gene null mutation was observed in 9 (42.9%), Both GST gene null mutation was observed in 8 (38.1%) in DILI group.

Conclusion: The GSTM1 gene null mutation and both GSTM1 and T1 gene null mutation were a risk factor for the development of DILI. But there is no significant association between GSTT1 gene null mutation and DILI in TB patients.

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1. Introduction

Tuberculosis (TB) is one of the most infectious diseases in India caused by bacteria *Mycobacterium tuberculosis*. Diagnosis of TB relies upon Radiographic Procedures, AFB Microscopy, Culture, Nucleic Acid Amplification, and Drug Susceptibility Testing for MDR TB. The most widely used drugs for the treatment of TB are Isoniazid (H), Rifampin (R), Pyrazinamide (Z), and Ethambutol (E) and considered as the first-line agents for the treatment of drug-sensitive TB (DS-TB). Isoniazid, rifampicin, and pyrazinamide are the most basic drugs used for the control of tuberculosis embraced by the World Health Organization (WHO) and all the three medications have been seen to have hepatotoxic potential.

The burden of tuberculosis is very high in India and looking at the enormous number of patients taking antitubercular treatment, drug-induced liver injury (DILI) is a noteworthy and most frequent adverse effect associated with antitubercular treatment.[1]

The pathogenesis of DILI induced by antitubercular drugs is not well-understood.[2] Alteration in antioxidant enzymes activity and an increase in lipid peroxidation showed that isoniazid and rifampicin-induced liver injury appears to interfere through oxidative stress.[3]

Glutathione S-transferase (GST) is well-known conventional detoxifying enzymes, which play an important role in protection as they catalyze the conjugation of different reactive drug metabolites which can cause cellular damage with glutathione, thus reduces the chance of drug-induced liver injury.[4–6] Glutathione-S-Transferase (GST) is a Phase II enzyme and the null activity of GST enzymes increases the risk of various diseases as they inactivate the metabolites of Phase I enzymes. Out of different GST isoforms, GST mu encoded by the gene GSTM1, GST theta encoded by the gene GSTT1 and are highly polymorphic. Absence of its activity because of homozygous null mutations at GSTM1, and GSTT1 loci make individual susceptible to drug-induced liver injury.[7]

In the general population, the GST enzymes are separated into various classes out of which five principle classes are: alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT) and zeta (GSTZ).[8] Of the different GST isoforms, GSTμ encoded by the gene GSTM1 located on chromosome 1p13.3, GSTT1 gene is encoded for GST θ who is located at chromosome 22q11.2. They are significantly polymorphic and thought to function in xenobiotic metabolism.[9] Homozygous null mutation of GSTM1 and GSTT1 are related with the diminution of glutathione content and bring about complete loss of enzymatic activity.[10]

The data on oxidative stress and the antioxidant profile in tuberculosis patients particularly concerning glutathione and GST polymorphism appears to be constrained. So the genetic association of liver injury in patients receiving an anti-tubercular drug will be evaluated as no study has been done among the Eastern UP population. Therefore, work on the genetics aspect in anti-TB drug-induced liver injury would be of clinical significance and may be an asset enlightenment to the medical community before starting ATT.

2. Aim & Objectives

1. To explore the distribution of *GST* gene polymorphism in TB patients.
2. To evaluate the association between the *GST* gene polymorphism with antitubercular drug induced liver injury.

3. Material and methods

The study was conducted in the Department of Pharmacology and Therapeutics, Department of Respiratory Medicine & Department of Clinical Hematology. Study was started after taking ethical clearance (**Ref. code: 93rd ECM 11B-Thesis/P30; No.-152/Ethics/19)** from Institutional ethical committee of King George's Medical University (KGMU), Lucknow and Patients were enrolled after taking the written informed consent.

3.1. *Study subjects*

A prospective observational study done on 96 DS-TB patients receiving antitubercular treatment from DOTS center KGMU, Lucknow. Different demographics (age, gender, smokers & intake of alcohol) and clinical variables (weight, height & BMI) was recorded and all patients were screened according to our inclusion and exclusion criteria.

3.1.1. *Inclusion criteria*

- Aged 15 years or above
- Bodyweight not less than 30 kg
- Patient on DS-TB treatment
- Not very sick or moribund

- Agreeing to come to the same DOT center until completion of the study

3.1.2. *Exclusion criteria*

- Confirmed chronic liver disease either clinically or laboratory.
- Hepatitis B and or hepatitis C positive cases, HIV and other serious problems.
- Habitual alcohol drinking
- Patients on other potentially hepatotoxic drugs.
- Refusal to participate in the study.

Baseline investigation was done for Liver Function Test (LFT) and Prothrombin Time (PT) and further samples were stored for genomic DNA extraction. Sample were repeated for LFT and PT after 21–28 days or when there were symptoms of liver injury like appetite loss, fever, nausea, vomiting, and jaundice, whichever of the two appeared first and difference from baseline was noted and patients were categorized into DILI group and non-DILI group. Patients were diagnosed as ATT induced DILI if any one of the following criteria is present:

- Five-times or more than five times increase in Alanine Transaminase (ALT) level above the upper limit of normal
- Twice or more than twice increase in Alkaline phosphatase (ALP) level
- More than or equal to threefold rises in ALT concentration and along with an increase in bilirubin by 2 times of the Upper limit of normal (ULN).

3.2. *Sample collection*

The peripheral blood sample was collected into ethylene diamine tetraacetic acid (EDTA) vials for extraction of genomic DNA and stored at −80 °C until further use.

For liver function test plain vials and for prothrombin time sample was collected in sodium citrate vials.

3.3. *Statistical tools employed*

SPSS (Statistical Package for Social Sciences) Version 21.0 statistical Analysis Software was used for statistical analysis. The values were represented in Number, percentage and Mean ± SD.

3.4. *Extraction of genomic DNA from blood*

Qiagen Blood DNA Mini Kit, Hilden, Germany (Qiagen, Germany) was used for the genomic DNA was extracted from the blood specimens as per the manufacturer's guidelines.

3.5. *Concentration and purity of DNA*

The Concentration and Purity of DNA were estimated through spectrophotometer as well as it was run on 0.8% gel.

3.6. *Purity of DNA*

The purity of DNA was dictated by measuring the OD (optical density) of the sample at 280nm for concentration of protein and at 260nm for concentration of DNA. The ratio OD260/OD280 was determine and ratio 1.7 or above was considered to be good. If the ratio was less than 1.2, DNA was re-extracted.

3.7. *Integrity*

The integrity of DNA is an important factor, which ought to be considered during extraction steps. Integrity was checked by

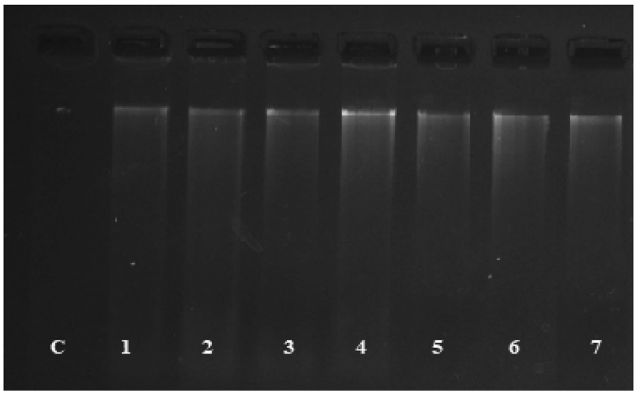

Fig. 1 – Lane C is Control, Lane 1–7 is Isolated DNA.

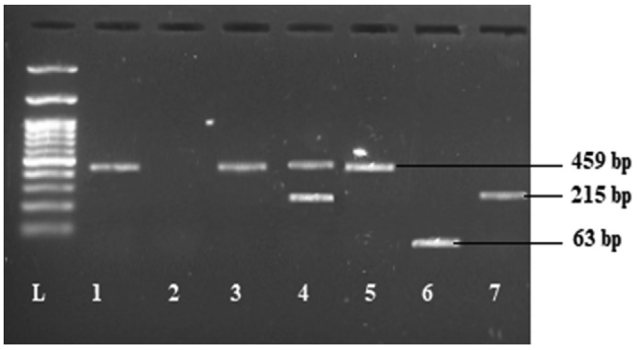

Fig. 2 – Lane L is Ladder, Lane 1,3 & 5 is wild type GSTT1 and Lane 2 is null, Lane 4 contain both GSTT1 & GSTM1, Lane 7 is wild type GSTM1 and Lane 6 is β-actin.

electrophoresis on 0.8% agarose prepared in 1X TBE buffer, containing Ethidium bromide (3μl of 10mg/ml stock for every 50ml of 0.8% agarose). A single band appear near the well for high molecular weight genomic DNA (Figs. 1–3).

3.8. *Primer sequence and method*

The genetic polymorphisms of GSTM1 and GSTT1 were assessed simultaneously using multiplex polymerase chain reaction (PCR) techniques. The primers used were GSTM1 F: 5′GAACTCCCTGAAAAGCTAAAGC -3′ and R: 5′-GTTGGGCTCAAATATACGGTGG −3'and primer used for GSTT1 gene are F: 5′- TCACCGGATCATGGCCAGCA -3'and R: 5′-TTCCTTACTGGTCCTCACATCTC3′ and actin was used as an internal standard. The PCR products were examined on 2% agarose gel pre-stained with ethidium bromide and visualized in transilluminator. The GSTM1 gene was recognized by presence of bands at 215bp whereas the GSTT1 gene by 459 and 63bp for actin product marked the presence of the GSST1 and GSTM1 null genotype.

3.9. *PCR amplification*

PCR amplification was performed in a final volume of 20μl (3μl DNA, 10μl Top Taq PCR Master Mix, 1μl primer; each forward and reverse, and 5μl HPLC water (Nuclease free water). The condition for PCR was in that sequence: The genomic DNA 100 ng was extracted, an initial denaturation at 94 °C for 5 min was done, then 30 cycles were performed consisting of denaturation at 94 °C for 1 min, annealing at 52.5 °C for 1 min, and an elongation at 72 °C for 1 min, which ended up with a final cycle of elongation at 72 °C for 10 min and store sample at 4 °C.

4. Observation and results

Out of 96 tubercular patients under treatment, drug induced liver injury was found in 21 (21.9%) patients only(Tables 1–4).

Drug-induced liver injury (DILI) among patients on anti-tubercular drugs. DILI had been defined as per World Health Organization criteria. As baseline above biochemical parameters of DILI and non-DILI cases were found to be comparable.

Table 1 – Prevalence of Drug Induced Liver Injury among patients on anti-tubercular treatment.

SN		No. of patients	Percentage
1-	Drug induced liver injury (DILI) present	21	21.9
2-	Drug induced liver injury (DILI) absent	75	78.1
		96	100.0

Table 2 – Association of baseline biochemical parameters and DILI.

SN	Parameters	DILI + nt (n = 21)		DILI −nt (n = 75)		Student 't' test	
		Mean	SD	Mean	SD	't'	'p'
1-	Bilirubin	0.53	0.13	0.57	0.13	−1.284	0.202
2-	SGOT/AST	28.62	6.10	29.53	6.27	−0.594	0.554
3-	SGPT/ALT	27.62	5.35	28.77	4.71	−0.963	0.338
4-	ALP	101.05	8.59	97.68	8.73	1.568	0.120
5-	Platelet	1.00	0.03	1.00	0.04	−0.148	0.883

Table 3 – Association of biochemical parameters at 3–4 weeks (21–28 days) and DILI.

SN	Parameters	DILI + nt (n = 21)		DILI −nt (n = 75)		Student 't' test	
		Mean	SD	Mean	SD	't'	'p'
1-	Bilirubin	1.53	0.58	0.59	0.13	13.039	<0.001
2-	SGOT/AST	170.24	57.53	34.28	5.63	20.393	<0.001
3-	SGPT/ALT	192.29	68.74	34.21	6.23	19.893	<0.001
4-	ALP	169.76	78.36	102.55	8.05	7.389	<0.001
5-	Platelet	1.21	0.13	1.02	0.04	11.684	<0.001

Table 4 – Association between the GST gene null mutation with antitubercular drug induced liver injury.

SN	GST gene null mutation	Total (N = 96)	DILI + nt (n = 21)		DILI −nt (n = 75)		Significance of differences	
			No.	%	No.	%	χ^2	'p'
1-	GST M1 gene	43	14	66.7	29	38.7	5.201	0.023
2-	GST T1 gene	43	9	42.9	34	45.3	0.041	0.840
3-	Both GST gene	19	8	38.1	11	14.7	5.673	0.017

At follow up at (21–28 days) all the above biochemical parameters were found to be significantly raised among DILI cases as compared to non-DILI cases.

4.1. *Distribution of GST gene polymorphism in tubercular patients*

Out of 96 tubercular patients enrolled in the study, frequency of GSTM1 gene and GSTT1 gene null mutation were found to be similar (n = 43; 44.8%) while the frequency of both GST (M1 & T1) null mutation was 19.8%.

Out of 21 patients of DILI, GST M1 gene null mutation was observed in 14 (66.7%). The proportion of GST M1 gene null mutation among DILI cases was significantly higher as compared to non-DILI cases but GST T1 gene null mutation difference was not significant. Both GST gene null mutation was observed in 8 (38.1%) and 11 (14.7%) cases among DILI and non-DILI patients respectively (Fig. 3). Both GST gene null mutation among DILI cases was found to be significantly higher as compared to non-DILI cases.

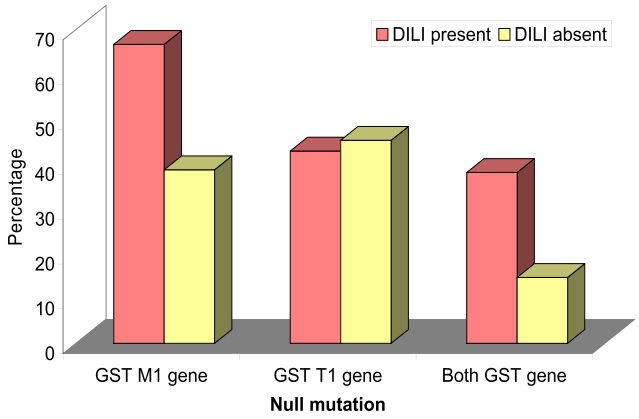

Fig. 3 – Association of GST Gene null mutation and DILI.

5. Discussion

Hepatotoxicity, ocular toxicity, and skin hypersensitivity reactions are important side effects of anti-TB drugs that have clinical implications. Liver injury is the most significant among all adverse drug reaction (ADR) of anti-TB drugs, which is mainly due to synergistic effect of first-line TB, that is given concurrently.[11]

There are different types of drug-induced liver damage observed which include idiosyncratic damage, dose-dependent toxicity, induction of liver enzymes, and allergic reactions among others.[12–14] As there is no relation observe between serum level of drug and liver injury a direct toxic effect is less likely.[15] There is a delayed onset of DILI, and as there are no symptoms of hypersensitivity like rashes, itching, and eosinophilia chances of hypersensitivity are less likely.[16]

Alteration in antioxidant activity with increased lipid peroxidation indicated that isoniazid- and rifampicin-induced hepatotoxicity seem to be mediated by oxidative stress.[17] Being a potent inducer of CYP450, rifampicin enhances the generation of the metabolites acetylhydrazine and hydrazine, which increases the hepatotoxicity of INH.[18] PZA is assumed as a genuine contributor to liver injury, its metabolites like Pyrazinoic acid (PA) and 5-Hydroxy Pyrazinoic acid (5-OH-PA) are mainly responsible for PZA-induced liver injury, pyrazionic acid as the principal offender of liver injury.[19] PZA exhibits both dose-dependent and idiosyncratic hepatotoxicity. Free radical generation may lead to liver injury but the exact mechanism of toxicity is unknown; it is unclear what enzymes are involved and who is responsible for toxicity, pyrazinamide, or its metabolites.[20]

Glutathione S-transferase is a super-family enzyme of drug-metabolizing enzymes that exists in various isoforms. It is a phase II enzyme which plays a crucial part as an intracellular free radical scavenger, that combines glutathione with toxic metabolites which are developed during the phase I metabolism of drug with enzyme like N-acetyltransferase 2 (NAT-2) and Cytochrome P450 2E1 (CYP2E1). The null genotype of GSTM1 or GSTT1 has been found to lack this defensive enzymatic action. It is hypothesized that GSTM1 or GSTT1 null genotype in an individual, couldn't remove the harmful metabolites efficiently and consequently have more risk of drug-induced liver injury.

This study reveals that GSTM1 null mutation alone and double null mutation of GSTM1 and GSTT1 were significantly more in DILI patients as compared to non-DILI patients whereas GSTT1 null mutation alone was not statistically significant.

Our results of the GSTMI gene mutation association with ATT induce liver injury were steady with results obtained by **Roy et al**[21] and **S.V Rana et al**[22] study in the Indian population. **Sotsuka et al**[23] in the Japanese population and **Huang et al**[24] study on Chinese population found the comparable outcome.

Our results of both GSTMI/T1 gene null mutation association with ATT induce liver injury were consistent with results obtained by **Gupta et al**[25] and **Singla et al.**[26]

However, opposite to the findings our study, **Chatterjee et al 2010**[27], **Sharma *et al*, 2014**[28] shows that GSTM1 or GSTT1 or both null genotypes do not appear to be associated with ATT induce liver injury in Indian population whereas **Teixeira *et al*2011**[29] and **Monteiro TP *et al* 2012**[30] also did not find any relation between *GSTM1* and *GSTT1* null genotypes with ATT-induced liver injury.

In a developing country like India which has a huge burden of tuberculosis (TB) cases globally. The strategy for TB treatment is undergoing massive expansion as we are in a way to eliminate TB by 2025, and this will be achieved by further increasing the treatment success rates and decreasing the failure associated with a regimen. Despite such a huge nationwide program for TB treatment, we still face numerous difficulties like the development of multi-drug resistance, immunodeficiency as well as anti-tuberculosis drug-induced liver injury (ATT induce DILI). Compliance with TB treatment is critical for a cure in active TB patients, as this is a long duration treatment. We need to concentrate on each part which may lead to its failure directly or indirectly as in the case of India where ATT therapy is one of the commonest reason of drug-induced liver injury.

It has now been recognized that differences in genetic polymorphism are noteworthy; so we should be more cautious about these genetic polymorphisms and exposure to these potential hepatotoxins that are derived during the metabolism of antitubercular drugs. Consequently, the risk assessment of GST polymorphisms or antitubercular drug-induced liver injury could be explicit for each population.[31] Therefore standard monitoring of patients for this genetic polymorphism could help in targeting the population who are at greater risk at the initial stage and, help in better TB management tailoring the antitubercular regimen should be considered to prevent liver injury and maintain therapeutic efficacy.

So to conclude, our study demonstrates that GSTMI null mutation alone and both GSTM1/T1 null mutation were associated with DILI. A detailed investigation of the association among significant enzymatic polymorphism and environmental influences can provide us with a superior comprehension of the event of such hepatotoxicity.

Conflicts of interest

The authors have none to declare.

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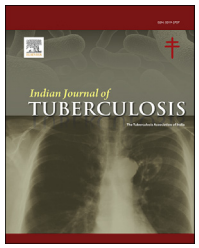

Original article

Health-related quality of life of multidrug-resistant tuberculosis patients: A study of eastern Uttar Pradesh, India

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ABSTRACT

Introduction: Much attention has been given to the microbiological aspect, drug treatment, and clinical indicators of MDR-TB, but patients' QOL has remained a neglected area. In this study, we aimed to find the quality of MDRTB on various quality of life domains during the initiation of the MDR Treatment regimen.

Materials & methods: A cross-sectional study was conducted over a period of 6 months at the Drug-Resistance Tuberculosis Management Centre (DR-TB Centre), of a tertiary care centre in the eastern Uttar pradesh, India. Patients with age >18 years diagnosed with MDR-TB (Multidrug resistance TB) were included in the study. The WHO QOL-BREF scale was used to assess the health-related quality of life of patients. Data were analyzed using SPSS version 21. The institutional ethical review committee approved the study, and consent was taken before the participation of patients.

Results: A total of 157 patients were included in the study & 45.85% were dissatisfied with their condition. Social domain of WHO QOL-BREF is having the lowest mean score (28.51 ± 15.4) while psychological has high mean values (39.92 ± 6.91). There was a significant difference in the physical health domain with respect to age (p-value 0.001). Similar differences have been seen in the psychological domain regarding patient sex (p-value 0.001), smoking and alcohol within the social domain, and loss of income in the environmental domain.

Conclusion: The mean value of different domains of WHO QOL-BREF is low in MDR-TB patients, with social relation domain being the most affected.

Abbreviations: MDR-TB, Multidrug Resistance Tuberculosis; WHO QOL BREF, World Health Organization Quality of Life Instruments; DOTS, Directly Observed Treatment, Short-course; RNTCP, Revised National Tuberculosis Control Programme; CDST, Culture and Drug Susceptibility Testing; CBNAAT, cartridge-based nucleic acid amplification test.

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1. Introduction

Tuberculosis (TB) is known to be one of the most transmissible diseases, causing around 2 million deaths per year.[1] A strain of *Mycobacterium tuberculosis* (MTB), which is resistant to both isoniazid and rifampicin, is responsible for multidrug-resistant TB (MDR-TB). MDR-TB is a chronic, debilitating disease requiring extended chemotherapy (about 20 months) with a potentially toxic regimen of second-line anti-TB therapy.[2] In addition to clinical symptoms, tuberculosis patients have to suffer from several psychological, physiological, and financial problems during their lifetime. Symptoms and burdens of chronic illness often continue beyond the span of treatment. In addition, treatment itself can be linked to several side-effects. All these aspects of the disease and its treatment have a major effect on the general well-being of the patient, and the burden of these causes can be equal to and even greater than the physical impact of the disease. Quality of Life (QOL) is defined as "the extent to which patient's subjective perception of physical, mental and social well-being is affected on a day to day basis by a disease and its treatment(s)".[3] Patients suffering from chronic diseases are considered to place high priority on their mental health and social well-being.[4] Regrettably, it is unlikely that patients with poor mental wellbeing would stick to treatment regimens, and this limits the effectiveness of treatment.[5]

As a result, the QOL assessment has become an important health outcome and an area of interest for policy makers, health practitioners and researchers. Much attention has been given to the microbiological aspect, drug treatment, and clinical indicators of MDR-TB, but patients' QOL has remained a neglected area. In this study, we aimed to find the quality of MDRTB on various quality of life domains during the initiation of the MDR Treatment regimen.

2. Material and methods

2.1. Study setting & population

The cross-sectional study was carried out in Gorakhpur, BRD Medical College, from 1 January 2016 to 30 June 2016 at the Drug Resistance TB Management Center (DR-TB Centre) in the Gorakhpur. BRDMC DR TB Centre, established as part of the RNTCP programme, covers four eastern part districts of the Uttar Pradesh namely Gorakhpur, Maharajganj, Deoria, and Kushinagar). Suspected MDR-TB cases are referred for further treatment and management from these four districts to this centre. After the drug sensitivity, the samples are sent to the nearest RNTCP accredited laboratories ie IMS, Banaras Health University, and Varanasi. When a patient is diagnosed with DR-TB, they have enrolled under the DOTS (Directly Observed Treatment, Short-course Plus Treatment) regimen. During their initiation of treatment, all registered patients were kept under observation for seven days in the DR-TB Ward. Subsequently, the closest DOTS provider to the patient location was contacted, and the patients were moved to the patient for further care.

2.2. Sample size and statistical analysis

Epi-info program version 7.2.0.1 was used to estimate the sample size. In the previously treated tuberculosis, the prevalence of MDR-TB was as high as 16%. The calculated sample size was 143, with a 95% confidence interval and an acceptable 6% error margin. The minimum sample size required for the study was 143. Patients ≥18 years of age were admitted to the DR-TB Center of BRD Medical College and diagnosed with MDR-TB from the RNTCP approved CDST (culture and drug susceptibility testing) or the CBNAAT (cartridge-based nucleic acid amplification test) laboratory was included in the study. Severely ill patients unable to interview and who did not give consent for participation were excluded. Consecutive sampling (all patients who meet inclusion & exclusion criteria during the study) was used to enrol the study participants.

Data obtained via the questionnaire were entered and analyzed using SPSS Version 26.[6] Means and standard deviation of the score were used and differences in mean scores were compared using independent T-test samples. The approval of the study was received from the College Research Council and the Institutional Ethical Review Committee of BRD Medical College, Gorakhpur.

2.3. Study tool

We used Hindi version of WHO QOL BREF scale. Quality of life describes what people feel about the various aspects of their lives. It is a subjective well-being assessment. The WHOQOL instrument was developed by the World Health Organization and has been widely tested. It has four domains: physical, psychological, social and environmental health, with 26 items. The response is available on a 5-point scale with description (not at all, through to completely). The assessment conceptually fits with the WHO definition of QOL. The time needed to complete the 26 items is around 10–15 minutes. The intra-rater reliability and subscale of the entire WHOQOL-BREF is high (ICC range: 0.84–0.93). For WHOQOL-BREF and its subscale inter-rater reliability is moderate to high (ICC range: 0.56–0.95). WHOQOL-BREF association with Well-Being Index satisfaction is moderate to high (Physical – Pearson's r = 0.63, Psychological – Pearson's r = 0.75, Financial/environment – Pearson's r = 0.59, Family/social – Pearson's r = 0.45).[7–9]

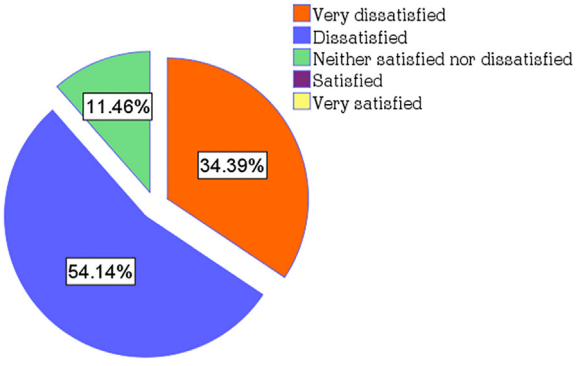

Fig. 1 – Distribution of patient feeling on their health status (How satisfied are you with your health?).

Table 1 – Descriptive Statistics of WHO QOL-BREF domains among study participants.

Domain	N	Mean (SD)	Min – Max
Physical	157	31.05 ± 6.9	17.14–51.43
Psychological	157	39.92 ± 6.91	23.33–53.33
Social Relations	87	28.51 ± 15.4	6.67–53.33
Environment	157	31.09 ± 7.40	17.50–51.43

Before collecting the data from the study subjects, written consent was taken in Hindi from all those approached for the study after explaining the study's procedure & purpose clearly. Patients were interviewed using our study questionnaire; privacy of the patients was taken care of during assessment of quality of life (QoL), and recommended precaution for prevention of transmission of MDR TB bacilli from patients was taken during the study. At the end of the interview, patients were sensitized about the cough etiquette, hand hygiene & importance of regular medication along with Nutrition & hygiene.

3. Results

Assessment of quality of life was done using WHO QOL BREF. Scores obtained from each item was added to obtained a raw score, and it is converted to a scale of 0–100 scale using the formula shown below –

$$\text{Transformed Scale} = \left[\frac{(\text{Actual raw score} - \text{lowest possible raw score})}{\text{Possible raw score range}}\right] \times 100$$

The questionnaire inquiries how you feel regarding the quality of your life, health, or other areas in the last two weeks.

Fig. 1 showing the distribution of responses in Item no. 2 of WHO QOL BREF scale, i.e., overall satisfaction level with their health. 54.14% were dissatisfied with their current health status, and 34.39% were very dissatisfied. 11.46% of patients claimed that they were neither satisfied nor dissatisfied with their health condition.

The mean score was highest for the psychological domain (Mean ± SD = 39.92 ± 6.91) while it was lowest for the social relation domain (Mean ± SD = 28.51 ± 15.4), but this domain has a higher value range than other domain (social relations 46.66, environment 33.93, physical 34.29, psychological 30) (Table 1).

The mean score of all four domains are compared with the demographic profile, and determinants of MDR-TB were presented in Tables 2–5. There was a significant difference in mean score in the physical domain between individual below

Table 2 – Comparison of Physical health domain Mean scores with characteristics of MDR-TB patients (N = 157).

Characteristic	Mean (SD)	Independent t-test
Sex		
Male	30.87 (7.1)	0.64
Female	31.43 (6.5)	
Age Category		
<40	33.73 (6.7)	0.001
40 & More	30.10 (6.8)	
Area of residence		
Urban	33.71 (7.2)	0.06
Rural	30.66 (6.8)	
Education Status		
Illiterate	33.16 (7)	0.03
Literate	30.37 (6.8)	
Loss in Income		
Yes	30.32 (7)	0.26
Dependent	31.59 (6.9)	
Food habits		
Vegetarian	31.61 (6.6)	0.10
Non-vegetarian	29.61 (7.6)	
Interval between TB and MDR-TB diagnosis		
< 1yr	31.55 (6.8)	0.87
> 1 yr	29.37 (7.3)	
Body Mass Index		
<16	30.41 (7.1)	0.12
16 & above	32.19 (6.5)	
Alcohol habits		
Habitual/Social	31.34 (7.2)	0.65
Never	30.84 (6.8)	
Smoking		
Past	30.71 (6.8)	0.60
Never	31.30 (7)	
Presence of BCG scar		
Yes	32.21 (6.4)	0.19
No	30.59 (7.1)	

Table 3 – Comparison of Psychological domain Mean scores with characteristics of MDR-TB patients (N = 157).

Characteristic	Mean (SD)	Independent t-test
Sex		0.001
Male	41.17 (6.8)	
Female	37.14 (6.2)	
Age Category		0.66
<40	40.06 (7.0)	
40 & More	39.51 (6.6)	
Area of residence		
Urban	39.33 (7.5)	0.68
Rural	40.00 (6.8)	
Education Status		
Illiterate	38.95 (6.7)	0.64
Literate	40.22 (6.9)	
Loss in Income		
Yes	40.00 (7.2)	0.89
Dependent	39.85 (6.7)	
Food habits		
Vegetarian	40.47 (6.9)	0.10
Non-vegetarian	38.48 (6.80)	
Interval between TB and MDR-TB diagnosis		
< 1yr	40.11 (7.1)	0.51
> 1 yr	39.26 (5.90)	
Body Mass Index		
<16	40.53 (6.4)	0.13
16 & above	38.81 (7.5)	
Alcohol habits		0.91
Habitual/Social	39.79 (6.60	
Never	40.00 (7.1)	
Smoking		0.32
Past	40.54 (7.1)	
Never	39.44 (6.7)	
Presence of BCG scar		0.12
Yes	41.29 (6.5)	
No	39.38 (6.9)	

40 (33.73 ± 6.70) than that of age >40 (30.1 ± 6.8) with a p-value of 0.001, while no such difference was found in other demographic properties of individuals (Fig. 2). Male (41.17 ± 6.8) has a better psychological score that than female (37.14 ± 6.2, p-value 0.001) (Fig. 2). The mean of married and unmarried psychological domains was compared using the independent t-test and found insignificant (p- 0.34). Similarly, other combinations, i.e., unmarried vs. separated and married vs. separated domain scores, were compared using an independent t-test and found an insignificant p-value of 0.45 and 0.91, respectively (Fig. 2). We found that smoking (25.28 ± 15.9) vs. never (33.53 ± 13.4, p-value 0.014) and alcohol (25.36 ± 15.1) vs. never (32.96 ± 15.0, p-value 0.02) are associated with lower mean scores in the social relations domain (Fig. 2). Loss of income has resulted in a significant change in the environmental domain. It was found that loss of income (29.57 ± 7.7 vs. dependent 32.22 ± 7.1, p-value 0.026) results in lower means score (Fig. 2).

4. Discussion

Quality of life in MDR-TB patients was assessed by using the WHO QOL BREF 26 item scale. Physical health domain scores were higher in males and those who were less than 40 years of age. However, the mean score difference in gender was insignificant (p = 0.64) while it was highly significant (0.001) between age category. The mean scores in the age category of <40 were 33.73 and 30.10 in >40 years of age. Similarly, a significant difference (p = 0.03) was found with the education status of patients. Mean scores of illiterate were higher than literate people, and these findings are rare in literature. Patients who had a BMI of more than 16 have recorded higher score as compared to those who are less than 16. The mean score of the BMI category of 16 & above was 32.19, and it was 30.41 among <16 categories. However, these differences were statistically insignificant (p = 0.10). A study from Pakistan assessed QOL using SF-36, they found that the physical component difference was related to the length of sickness before MDR-TB diagnosis.[7] In a study in Baltimore, patients were adversely affected in their financial well-being via loss of income and the treatment expenses of chronic disease; also, our study strongly support this issue as most MDRTB patients suffer in managing their own treatment expenses and in the developing countries, they are less likely to get any support from their families because of the chronic nature of the disease.[8]

The psychological health domain score was higher in males than females, and this difference was found highly significant (p = 0.001). The mean scores in the age <40 years of age category were found slightly higher than those who were more than 40 years of age; however, the difference was insignificant. Similarly,

Table 4 – Comparison of Social relations domain Mean scores with characteristics of MDR-TB patients (N = 87).

Characteristic	Mean (SD)	Independent t-test
Sex		
Male	27.96 (15.5)	0.60
Female	29.87 (15.5)	
Age Category		
<40	27.24 (15.8)	0.28
40 & More	31.03 (14.7)	
Area of residence		
Urban	30.26 (14.8)	0.66
Rural	28.20 (15.6)	
Education Status		
Illiterate	31.19 (15.4)	0.26
Literate	27.23 (15.4)	
Loss in Income		
Yes	29.33 (15.9)	0.60
Dependent	27.62 (15.0)	
Food habits		
Vegetarian	27.29 (16.3)	0.22
Non-vegetarian	31.88 (15.5)	
Interval between TB and MDR-TB diagnosis		
< 1yr	28.23 (15.2)	0.78
> 1yr	29.28 (16.3)	
Body Mass Index		
<16	29.15 (15.1)	0.64
16 & above	27.59 (16.1)	
Alcohol habits		0.02
Habitual/Social	25.36 (15.1)	
Never	32.96 (15.0)	
Smoking		0.014
Past	25.28 (15.9)	
Never	33.53 (13.4)	
Presence of BCG scar		
Yes	30.16 (15.1)	0.57
No	27.98 (15.6)	

Table 5 – Comparison of Environmental domain Mean scores with characteristics of MDR-TB patients (N = 157).

Characteristic	Mean (SD)	Independent t-test
Sex		
Male	30.70 (7.9)	0.32
Female	31.95 (6.1)	
Age Category		
<40	30.45 (6.9)	0.74
40 & More	32.90 (8.3)	
Area of residence		
Urban	34.43 (7.0)	0.34
Rural	30.60 (7.3)	
Education Status		
Illiterate	33.02 (7.9)	0.06
Literate	30.48 (7.1)	
Loss in Income		
Yes	29.57 (7.7)	0.026
Dependent	32.22 (6.9)	
Food habits		0.59
Vegetarian	31.29 (7.2)	
Non-vegetarian	30.59 (7.8)	
Interval between TB and MDR-TB diagnosis		
< 1yr	31.46 (7.4)	0.55
> 1yr	29.86 (7.1)	
Body Mass Index		
<16	30.36 (7.5)	0.09
16 & above	32.42 (7.0)	
Alcohol habits		
Habitual/Social	31.32 (7.4)	0.06
Never	30.93 (7.4)	
Smoking		0.14
Past	30.09 (7.6)	
Never	31.85 (7.1)	
Presence of BCG scar		
Yes	32.70 (6.5)	0.08
No	30.46 (7.6)	

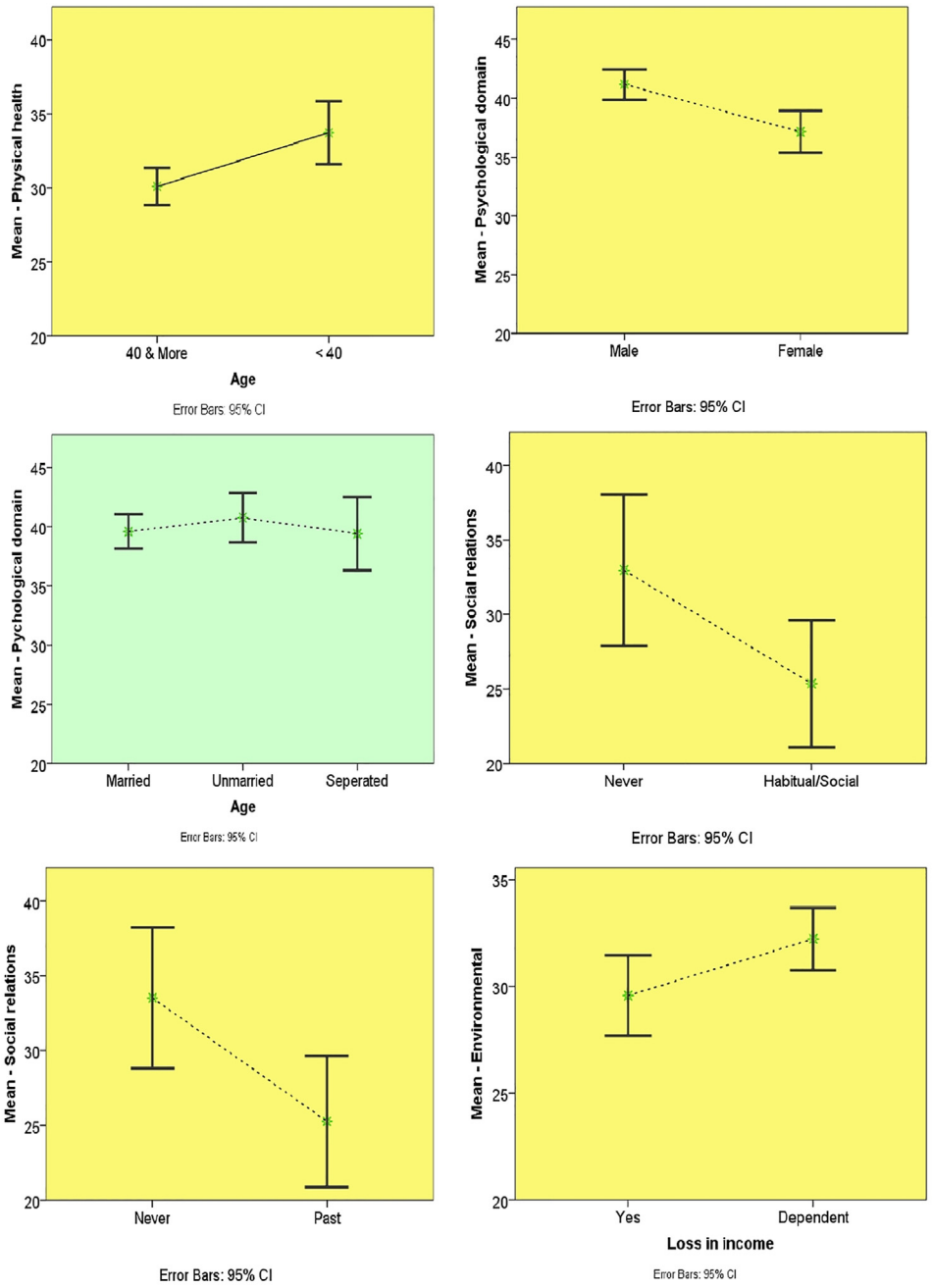

Fig. 2 – Mean values of various domain of WHO-QOL-BREF with respect of various demographic characteristics of patients.

the mean score was higher in literate than illiterate and vegetarian compared to non-vegetarian; the difference was statistically insignificant. Other determinants were also found insignificant. Ahmed et al assessed QOL using SF-36 and found that length of sickness and gender significantly affected the MDR-TB patients' mental scores.[7]

Out of 157, 87 responses are analyzed for the social domain as one item of the domain was missing in 70 patients, domain score for those respondents would not be calculated as per the instruction of the WHO QOL BREF Scale.[9] Missing item among 70 patients is item no. 21; the question was related to patients' sex lives, i.e., how satisfied are you with your sex life. Social relations were adversely affected, with the lowest scores in all 4 domains. The mean scores of social relation were compared with MDR-TB determinants and found a significant association with alcohol ($p = 0.02$) and smoking habits ($p = 0.01$). Those who had habits of alcohol and smoking were found lower scores in the social relation's domain.

The presence of loss of income was the only variable that was found significant ($p = 0.02$) in the environmental domain. The mean domain score in those who claimed loss of income was 29.57 ± 7.7 and 32.22 ± 7.8 in non-earning/dependent. Other variables were found insignificant differences in the environmental domain.

Another report from Ethiopia also concluded that TB and MDR-TB therapies had an effect on patients' self-perceived

health status.[10] Basit et al found that a large fraction of patients had achieved culture conversion at 2 months. The article stated that factors that adversely affected the conversion of culture are easily detected before and after the diagnosis of MDR-TB. This may help to enhance patient care through early detection and comprehensive treatment of individual patients.[11] A cross-sectional study, which was also conducted in the northern parts of India, identifies that QOL of MDR tuberculosis patients was worse than PTB (pulmonary tuberculosis) counterparts. They also calculated the mean scores of various 4 domains in MDRTB and PTB patients. While the difference in scores in physical and social domains less (MDRTB vs. PTB 19.03 vs. 20.05 and 7.88 vs. 9.61), the gap increases more in the psychological and environmental domains (17.46 vs. 15.23 and 22.00 vs. 18.91) domain, respectively.[12] While both MDRTB and PTB are affected socially due to the stigma of the disease but financially, MDRTB patients are amongst the worst sufferers than PTB as the former were not included in any program. By keeping the above points in mind, there is a need to develop a reliable and applicable measure to improve MDR-TB patients' quality of life. It will also enable health care and administration practitioners to implement effective steps to enhance patient safety and the Programme.[12-15]

The generalizability of the findings of this study, however, may be limited. The study was limited to MDR-TB patients who belonged to four eastern Uttar Pradesh districts and therefore was not representative of the state's total MDR pool. Another limitation is the patient taking treatment from Private sectors are not included. The study is a part of the MD thesis suffers from the limitation of time & resources. Assessment of Quality of life after completion of treatment has not been done in the present study & may be pursued in the future. Patients taking treatment from Private sectors were not included.

5. Conclusion

Among all four domains, the lowest score was recorded in social relations. The mean scores of social relation were compared with MDR-TB determinants and found a significant association with alcohol ($p = 0.02$) and smoking habits ($p = 0.01$). Those who had habits of alcohol and smoking were found lower scores in the social relation's domain. Physical health domain scores were higher in males and those who were less than 40 years of age. However, the mean score difference in gender was insignificant ($p = 0.64$) while it was highly significant (0.001) between age category. The psychological health domain score was higher in males than females, and this difference was found highly significant ($p = 0.001$). The presence of loss of income is the only variable that was found significant ($p = 0.02$) in the environmental domain.

Authors contribution

Conceptualization: U Venkatesh.
Methodology: U Venkatesh, D K Srivastava.
Formal analysis: U Venkatesh, Akash Sharma.
Data curation: U Venkatesh, Akash Sharma, R Durga.
Software: U Venkatesh.
Validation: U Venkatesh, Akash Sharma.
Investigation: U Venkatesh, R Durga.
Writing - original draft preparation: U Venkatesh, Akash Sharma, D K Srivastava, R Durga.
Writing - review and editing: U Venkatesh, Akash Sharma, D K Srivastava, R Durga.
Approval of final manuscript: all authors.

Conflicts of interest

The authors have none to declare.

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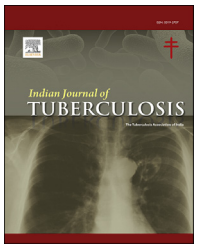

Case report

Ruxolitinib and tuberculosis: A case report with brief review

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ABSTRACT

JAK 2 inhibitors are widely used for the treatment of primary myelofibrosis. Ruxolitinib is the most commonly used JAK inhibitor in clinical practice. We report two cases of Primary Myelofibrosis who developed tuberculosis on active treatment with ruxolitinib. Our first case was a 48 year male who developed disseminated tuberculosis during fourth month of treatment and second case was a 50 year male developing tubercular lymphadenitis during second month of treatment respectively. These case reports indicate reactivation of underling tubercular infection as a very dreaded complication of this treatment. The prevalence of tuberculosis is much higher in India compared to the west. A thorough pretreatment evaluation should ideally be done using Mantoux test or interferon gamma release assay (IGRA) to rule out latent tuberculosis. Furthermore, the patients should be counselled regarding the possibility of reactivation of infections including tuberculosis. Also, proper follow up is the need of hour in all patients on any kind of immunomodulators.

1. Introduction

Primary myelofibrosis (PMF) is one of the classical myeloproliferative neoplasms (MPNs) and carries the worst prognosis.[1] Primary myelofibrosis (PMF) accounts for 3–15 per 1 million cases annually in the west. Indian data regarding incidence and prevalence of primary myelofibrosis is not available. PMF is characterized by ineffective erythropoiesis, extramedullary hematopoiesis, cytokine-mediated stromal changes including fibrosis and troublesome constitutional symptoms. It has the tendency for thrombo-hemorrhagic complications and risk of transformation to acute leukemia.[2,3] Most common mutations in Primary myelofibrosis are JAK2, CALR and MPL and these are present in 50%–60%, 20%–25% and 6%–7% cases respectively.[3] Mutually exclusive nature of these mutations is the hall mark of these Philadelphia negative MPNs. Triple negative MPNs constitute 10%–15% of all PMF cases and carry relatively poor prognosis.[4]

Ruxolitinib is a non-selective inhibitor of JAK-STAT (Janus Kinase-signal inducer and activator of transcription) pathway which is essential for host immunity and defense. Ruxolitinib is widely used for its excellent efficacy in decreasing the constitutional symptoms and splenomegaly in patients'

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primary myelofibrosis (MF). Ruxolitinib is a non-selective oral inhibitor of JAK1 and JAK2, is associated with reduction in the levels of inflammatory markers: IL-6, TNF-alpha, and C-reactive protein (CRP). Ruxolitinib has shown survival advantage in recent trial.[5–7]

Clinical trials of ruxolitinib have shown very few cases of infectious complications.[8,9] Tuberculosis is one of the major complications of Ruxolitinib treatment and few cases have been published on unmasking of latent tuberculosis and opportunistic infections in these patients.[10–18] Latent tuberculosis is characterized as a state of persistence of immune response to stimulation by *Mycobacterium tuberculosis* antigens. A direct measurement tool for *M. tuberculosis* infection in humans is currently unavailable. The vast majority of infected persons have no signs or symptoms of TB but are at risk for developing active tuberculosis (TB) disease which can be averted by preventive treatment.[19]

We report two case of PMF who developed tuberculosis on treatment. We will review briefly the association of JAK-2 inhibitor use and occurrence of active tuberculosis. This becomes very relevant in developing countries as they have high incidence of active and latent tuberculosis compared to the developed world.

2. Case report

2.1. *Case-1*

We report a case of 48 year old man who presented to the hematology OPD with complaints of off and on mild grade for the past 6 months. Fever was associated with generalized body weakness, loss of appetite and occasional nausea. Patient did not have any significant co-morbidity and he was a chronic smoker for the past 25 years. On examination the patient had severe pallor and moderate hepatosplenomegaly.

His baseline hemogram revealed a hemoglobin of 6.3 gm/dl, total leucocyte count of 7.1 $\times$ 10^9/L, differential counts showing 48% neutrophils and 44% lymphocytes and 2% monocytes. His peripheral smear revealed normocytic normochromic RBCs with moderate anisopoikilocytosis, tear drop cells and occasional nucleated RBC'S. Reticulocyte count was 1.5% and platelet count was 251 $\times$ 10^9/L. His renal and liver function tests were normal. His HIV, HBV, HCV tests were negative by ELISA. His Iron profile and serum ferritin were normal. His Vitamin B12 and folate levels were 2025 ng/ml and 131 ng/ml respectively.

Bone marrow aspiration and biopsy showed a hyper cellular bone marrow with increased megakaryocytes, clustering, nuclear atypia, cloud like nucleus and bare hyperchromatic nucleus. His reticulin grade was II [modified Bauermiester scale], BCR-ABL status was negative and JAK2 exon 14 was positive. He was diagnosed as a case of Primary Myelofibrosis (PMF) with Grade II fibrosis and was started on Ruxolitinib 20 mg twice a day. He was in regular follow up and doing well with improvement in baseline symptoms. In the fourth month of follow up he developed persistent fever and abdominal distension. His CT abdomen and thorax was suggestive of gross hepato-splenomegaly with moderate ascitis and effusion with military nodules in bilateral lung fields suggestive of tuberculosis. Ascitic fluid was exudative in nature. His peripheral smear did not show any increase in blasts. His mantoux test was negative and fluid TB-PCR was positive. Ruxolitinib was stopped after gradual tapering in 2 weeks. He was started on anti-tubercular drugs under Drug susceptible tuberculosis (DSTB) category including 2 months of isoniazid, rifampicin, pyrazinamide and ethambutol (2HRZE) followed by 6 months of isoniazid and rifampicin (6 HR). He responded to anti-tubercular treatment (ATT) with subsidence of fever at the end of 2 weeks. He took ATT for 8 months and Ruxolitinib was started after 1 month of ATT initiation. His clinical condition and hematological parameters remain stable and he is on monthly follow-up.

2.2. *Case: 2*

This 50 year male was diagnosed as PMF with WHO Grade II myelofibrosis and started on Ruxolitinib 20 mg twice a day. In the second month of follow up he developed enlargement of left submandibular lymph nodes without any fever or other B symptoms. FNAC of the lymph node was suggestive of granulomatous lymphadenitis. CXR PA View, USG Abdomen and Bone marrow was normal without any evidence of tubercular involvement. CBNAT was positive in lymph node aspiration sample. Ruxolitinib was stopped and he was started on ATT under drug susceptible TB category including HRZE. Patient lost to follow up after first OPD visit.

3. Discussion

Ruxolitinib is approved for the treatment of intermediate and high-risk disease and also for treatment of splenomegaly or constitutional symptoms. In addition, this drug is not specific and inhibits both the wild-type and mutated JAK2V617F. This efficacy of this molecule was tested in two randomized control phase III studies namely COMFORT-I and COMFORT-II.[9,20]

JAK STAT inhibition by ruxolitinib can lead to various immunosuppressive effects like depressed T helper cell type 1 response and reduction of cytokine production including IFN-γ and TNF-α. FN-γ and TNF-α have a critical and proven role in prevention of reactivation and control of TB infection.[6,7] Abnormalities in T cell function, macrophage activation, and granuloma formation occurs due to TNF-α reduction. This results in a threat for reactivation or dissemination of infections, particularly viral infections, tuberculosis, atypical bacterial and fungal infections.[8]

According to the WHO latent tuberculosis or asymptomatic tuberculosis affects almost one third of the population. Reactivation of latent tuberculosis occurs in almost 10% of patients accounting for 80% of all active tuberculosis cases.[21] Although the overall incidence of tuberculosis is decreasing worldwide it remains a The overall incidence of tuberculosis is decreasing worldwide, but it remains a concern in patients receiving biologics such as TNF-α inhibitors, interleukin antagonists, and JAK inhibitors especially in developing countries.[19]

In India, the incidence and prevalence of tuberculosis is still high and despite various government programs being run for its prevention, control and cure, most prominent being

Table 1 – Showing the case reports and case studies of Ruxolitinib and occurrence of tuberculosis in patients with Primary myelofibrosis.

Author	Number of patients	Site of TB	Outcome
Abidi MZ et al 2016[10]	1	Cervical nodes CT- bilateral lung nodules, left sided pleural effusion, and lower cervical and mediastinal conglomerate adenopathy	ATT for 6 months f/b ruxolitinib complete resolution and Alive
Branco et al 2016[11]	1		Alive
Chen Y.-H et al 2015[12]	1	Pulmonary nodules in in B/l upper lobes and mediastinal ln (Reactivation)	Alive
Colomba C et al 2012[13]	1	Inguinal lymph node, consolidation in the left middle lung field	Alive
Hopman R. K. et al 2014[14]	1	Retroperitoneal and gastrohepatic lymphadenopathy. Right supraclavicular, mediastinal, portacaval and retroperitoneal lymphadenopathy and lungs	Alive
Keizer S et al 2015[15]	2	Disseminated tuberculosis	Dead
Shamil E et al 015[16]	1	Right-sided cervical lymphadenopathy, lungs and perilymphatic nodes	Alive
Pepeler M S et al 2018[17]	1	Cervical lymphadenopathy, PPD 12 mm positive	Stopped Ruxolitinib for 6 months, Received ATT Alive
Lescuyer S et al 2019[18]	2 (out of total 65 patients)	1. Multiple pulmonary and cerebral micronodules 2. Excavated pulmonary lesions	1. Ruxolitinib stopped and ATT given, Patient died dur to cerebral hemorrhage 2. Ruxolitinib stopped, ATT started, patient died on D11 of ATT
Our report	2 (out of 23 patients)	1. Disseminated tuberculosis 2. Tubercular lymphadenopathy (submandibular gland)	Stopped JAKAVI in both cases. 1st patient recovered and second patient lost to follow up

National Tuberculosis Elimination Programme (NTEP), India still needs to work hard in achieving complete control over incidence of new cases, development of MDR/XDR and proper follow up of patients on treatment.[22]

Table 1 below shows various case reports and studies of Ruxolitinib and the occurrence of tuberculosis.

We could found 9 case reports including 11 patients of MPNs receiving Ruxolitinib and developing active tuberculosis. Dissemination of TB was reported in five of these cases while the remaining case reported were of reactivation of pulmonary TB. Four patients (37%) died due to complications of tuberculosis and 7 could be salvaged. In all these case reports it is obvious that ruxolitinib was withheld immediately after diagnosis or tapered and stopped. Standard four drug ATT was given in all eight cases except one reported by Branco et al.[11] Due to a relapse of MF related constitutional symptoms, ruxolitinib therapy was reinitiated with success in some cases. Duration of ATT treatment varied from 6 months to 12 months in cases with disseminated TB. Branco et al recently described a case of disseminated TB, occurring in a ruxolitinib treated patient, where ruxolitinib therapy was maintained while patient received rifampin.[11]

In our observation, 2 out of 23 (9%) PMF patients on ruxolitinib developed tuberculosis. Considering the previous data raising the concern of tubercular activation/development of new tubercular pathology, our cases also add to this significant problem and raise the issues to discuss need of screening for tuberculosis before initiation of JAK inhibitor and close follow up for new onset B symptoms like fever, weight loss in a patients already receiving JAK inhibitor. As far as concomitant Jakavi and ATT therapy is concerned few studies state that ruxolitinib is metabolized by CYP3A4 more easily as compared to CYP2C9. Rifampicin is a CYP3A4 inducer. Hence administering rifampicin with ruxolitinib reduces its half life by approximately 32%.

This is obvious from above case reports and literature review that the problem of developing tuberculosis on treatment is significant. This infection can be a new acquired infection or activation of latent tuberculosis.[21] Latent tuberculosis should be considered while planning to start ruxolitinib in these patients. Latent tuberculosis infection (LTBI) is defined as a state of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinically manifested active TB.[19] The reactivation of tuberculosis can be averted by preventive treatment. Diagnosing latent tuberculosis and preventing it makes more sense in high income and high middle income countries in an effort to eliminate tuberculosis. In low income developing countries its role has not been well defined.

Tuberculin skin test or Interferon gamma release assay can be used to screen these patients. Individuals should be asked about symptoms of TB before being tested for LTBI. Chest radiography can be done if efforts are intended also for active TB case finding. Individuals with TB symptoms or any radiological abnormality should be investigated further for active TB and Other conditions.[19]

New modalities in the molecular detection of TB, including nucleic acid amplification test (NAAT) and whole-genome sequencing (WGS), have led to a shorter time for diagnosis and faster TB treatments. The WGS can detect various types of mutations better than the Xpert MTB assay.[23]

4. Conclusion

JAK 2 inhibitor is widely used as main treatment modality of primary myelofibrosis, post polycythemia and post ET myelofibrosis, and also recommended after hydroxyurea resistance/intolerance in polycythemia vera. This case report indicates towards a possible life threatening complication of this treatment as reactivation of underling tubercular infection. The prevalence of tuberculosis is much higher in India compared to the west. A thorough pretreatment evaluation should ideally be done to rule out latent tuberculosis using Mantoux test or interferon gamma release assay (IGRA). Furthermore, the patients should be warned about the uncommon but real risk of reactivation of infections. Proper follow up is the needed in these patients with hematological malignancies receiving any kind of immunomodulatory treatment.

Conflicts of interest

The authors have none to declare.

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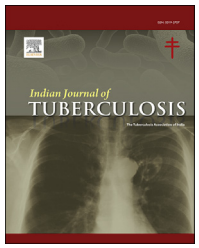

Case report

An unusual site of articular tuberculosis—A series of three conservatively managed cases

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ABSTRACT

Tuberculosis (TB) infection of the Sternoclavicular joint (SCJ) is a rare entity, with 1–2% of all osteo-articular cases reported. We report a series of three cases of TB of the SCJ, in the patients presented with swelling of SCJ. Cytology showed chronic granulomatous pathology in all three cases, with one patient having Cartridge base nucleic acid amplification test positive for TB and another one having acid fast bacilli positive on Ziehl Neelsen staining. All three were put on antitubercular treatment (ATT) that resulted in significant improvement. A high index of suspicion of TB to be maintained in cases with swellings at unusual sites especially in high burden countries like India. Similarly, gradually progressive osteoarticular swellings without systemic features should also raise suspicion of tubercular etiology, as diagnosis was delayed for about 4 months in two of our cases and about 1 year in the third case. The application of newer technologies such as CBNAAT can help in early microbiological confirmation of paucibacillary disease leading to early diagnosis and prevention of possible complications.

1. Introduction

Tuberculosis (TB) can affect any organ system of the body, most commonly affecting the pulmonary system followed by lymph node, pleural, ear nose throat, bone and joint, ocular, abdominal, cutaneous and CNS.[1] TB of Sternoclavicular joint (SCJ) is reported in 1–2% of all osteo-articular cases.[2] The pathogenesis of SCJ infection is debatable. Articular infection may remain latent for many years before any clinical presentation. Delay in diagnosis usually occurs due to lack of awareness and unusual site of presentation as happened in our cases. The increasing prevalence of tuberculosis in both immunocompetent and immunocompromised individuals makes tuberculosis a topic of universal concern especially in high burden countries like India. We report a series of three cases of SCJ TB without any evidence of active pulmonary TB.

2. Case series

Summary of three patients with sternoclavicular joint tuberculosis (Table 1).

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Table 1 – Compilation of three cases of sternoclavicular joint tuberculosis.

Case number	Age/ Gender	Chief complaints	Duration of symptoms	Side/Size of swelling/ Local examination/ any peripheral lymphadenopathy	Associated disease	Chest radiography	Contrast enhanced computed tomography (CECT)/ Magnetic resonance Imaging (MRI)	Diagnosis	Treatment
1	81/female	Dry cough, and swelling at right parasternal area	1 year	Right parasternal/ 5 × 3 cm/tender, soft, fluctuant,/none	k/c/o HTN	Grossly normal	CECT thorax showed osteomyelitis involving right sternoclavicular joint and first costo-sternal joint with mildly enhancing periartcular soft tissue, Lung parenchyma- normal, no mediastinal lymhadhenopathy (Fig. 1)	Fine needle aspiration cytology (FNAC) - Caseating granulomatous pathology and Catridge based nucleic acid amplification test (CBNAAT) for TB positive	Directly observed treatment, short course (DOTS) for 9 months. Patient stopped antitubercular drug therapy (ATT) after 9 months due to side effects. She is stable at 6 months follow up.
2	43/Female	Low grade fever and swelling at left parasternal area	4 months	Left sternoclavicular joint/7 × 4 cm/immobile, nontender and fixed to underlying tissue/none	History of oral and local steroid use on and off 6 years ago for uveitis	Grossly normal	CECT thorax revealed cortical erosion at medial end of left clavicle and along left lateral margin of sternum with associated soft tissue component, Lung parenchyma normal, no evidence of mediastinal lymohadenopathy (Fig. 2)	FNAC - AFB in ZN staining and Caseating granulomatous pathology on cytology	DOTS for 12 months
3	52/Male	Swelling in the left parasternal area for	4 months	Left/3 × 1 cm/non tender and soft in consistency/ none	None	Grossly normal	MRI thorax showed inflamed left sternoclavicular joint (Fig. 3)	FNAC-Caseating granulomatous pathology on cytology	DOTS for 12 months

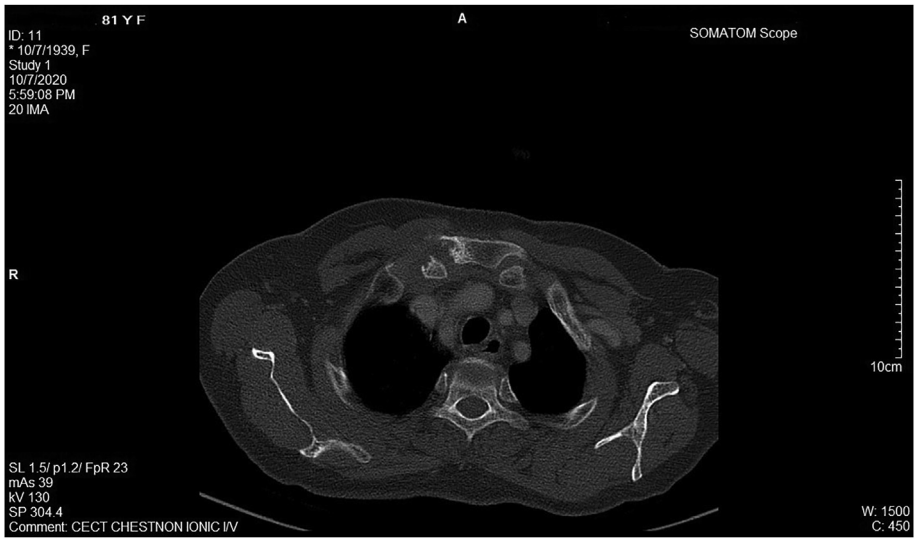

Fig. 1 – Case 1. CECT Thorax showing osteomyelitis involving right sternoclavicular joint and first costo-sternal joint.

3. Discussion

The SCJ is a saddle type joint, covered by fibrocartilages that functions as the only articulation between upper extremity and axial skeleton. SCJ is supplied by internal thoracic artery and suprascapular artery, innervated by medial supra-clavicular nerve and nerve to subclavius.[3] The proximity to important vasculature, nerves and organs in thoracic cavity such as the great vessels of mediastinum, trachea, esophagus, vagus nerve and phrenic nerve, makes SCJ both anatomically and clinically significant.[3] The pathogenesis of SCJ infection is debatable. The prevailing view is that the infection could originate from (1) a fresh or reactivated pulmonary focus which has spread hematologically,[4] (2) contagious spread from an apical pulmonary tuberculous focus of the sterno-clavicular joint[5] or (3) primary focus could be at the medial end of clavicle.[4] In our case series, the patients had primary infection of sternoclavicular joint with no evident foci of pulmonary or other extra pulmonary TB.

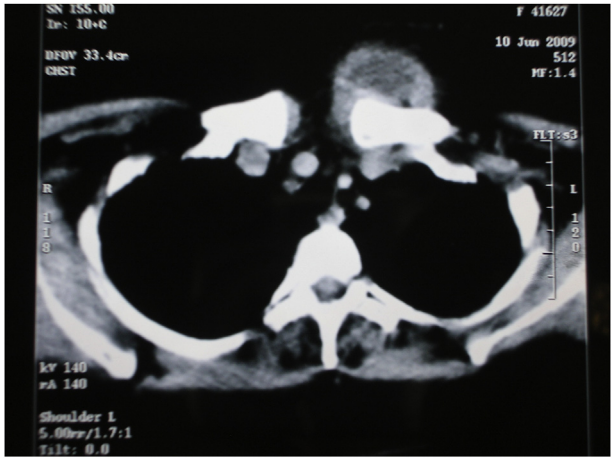

Fig. 2 – Case 2. CECT Thorax showing cortical erosion at medial end of left clavicle with associated soft tissue component.

Most common infectious organism causing SCJI in general population is *Staphylococcus aureus* whereas *Mycobacterium tuberculosis* is very rare (<5%).[3] Tuli et al reported only four cases of clavicle and sternoclavicular joint tuberculosis out of 1074 cases of osteoarticular tuberculosis.[4] Similarly, Martini et al reported only one case of sternoclavicular joint TB in the series of 642 cases of osteoarticular tuberculosis.[4] There are many risk factors for SCJI, such as immunocompromised status, diabetes mellitus, trauma, central venous catheter placement, intra-articular injections, arthropathies and rheumatoid arthritis. About 23% of cases have none of the risk factors.[3]

Most common symptoms in SCJ TB reported are pain and swelling of joint,[4] we have observed swelling of SCJ as most common finding followed by pain and fever. Unilateral SCJ infection can often present with fever, joint swelling, warmth and immobility. Bilateral infections can present as a butterfly rash on the chest. Some unusual presentations have also been reported in literature, such as a pressure like chest pain radiating to neck or shoulder.[3] High index of suspicion for TB infection should be there in cases with poor response to antibiotic therapy.

Diagnosis is often delayed by several weeks or months due to absence of constitutional symptoms, unusual site, and indolent nature of the disease. Final confirmation of SCJ TB is done on open biopsy or fine needle aspiration. Biological specimen from biopsy/FNAC should be subjected for ZN staining, Polymerase chain reaction (PCR) for TB, Liquid/solid culture for TB, cytology and histopathology for confirmation.

As osteoarticular tuberculosis is a paucibacillary disease, microbiological and histopathological tests may be negative.[2] In such cases where diagnosis is difficult, bacterial DNA detection from the specimen can be done by PCR such as CBNAAT.[6] We confirmed the diagnosis in all three cases by

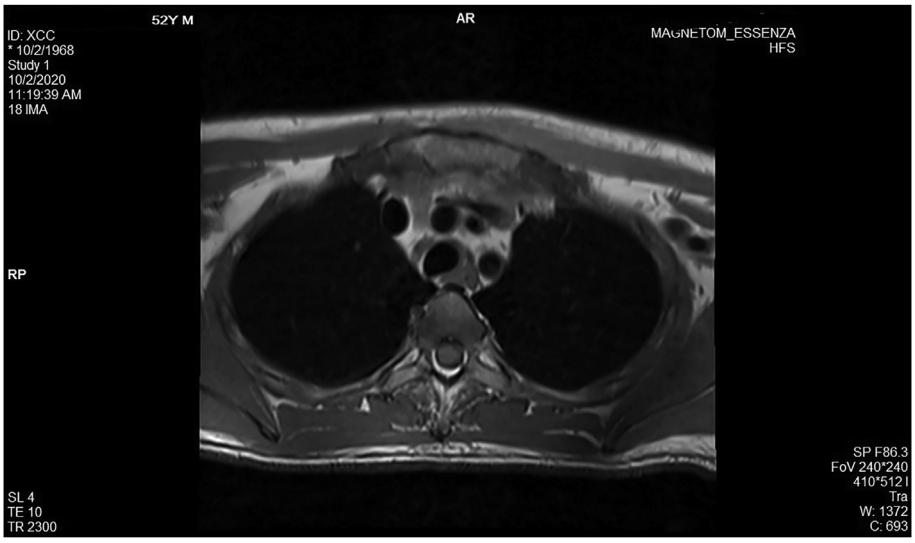

Fig. 3 – Case 3. MRI Thorax showing left sternoclavicular joint infection with surrounding inflammatory changes.

fine needle aspiration of material through swelling which revealed chronic granulomatous inflammation on cytology in all three cases, the detection of AFB by ZN stain in one case and by detection of bacterial DNA by CBNAAT in one case.

Conventional radiograph are not of much help in diagnosing this pathology.[5] Usually after 10–12 days, soft tissue swelling becomes evident on a typical X-ray. Demineralization and bony destruction appears much later.[3] Computed tomography (CT) scan is better modality having 83% sensitivity in diagnosing SCJ infection. CT can further elucidate the extent of anatomical involvement.[3] CT may show osseous destruction and sclerosis.[7] CT-guided aspirations are safe and have yielded positive cultures in more than 50% of cases. Magnetic resonance imaging (MRI) can detect osteo-articular changes as early as within 1–2 days of SCJ infection. Sensitivity and specificity of MRI is 88% and 93% respectively with focal enhancement on T1W fat suppressed enhanced images suggestive of osteomyelitis. MRI helps in determining extent of lesion, marrow involvement, soft tissue spread, abscess formation, destruction of articular cartilage and sinus tract formation. Shah et al, suggested that all the modalities complement each other though MRI is better in detecting marrow involvement and delineating soft tissue involvement.[8] Ultrasound (USG) is another safe diagnostic modality, can be used during pregnancy, but has low sensitivity. In cases with obvious fluid collection on USG guided aspiration can be performed.[3]

Differential diagnosis of swelling near the sternoclavicular joint are sternoclavicular hyperostosis, condensing osteitis, necrosis with infection, rheumatoid arthritis, myeloma and secondary deposits.

After clinical, microbiological and radiological confirmation of diagnosis, the patients were treated conservatively with antitubercular drug therapy consisting of Isoniazid(H), Rifampicin(R), Pyrazinamide(Z), Ethambutol (E) for 2 months (Intensive phase) followed by HRE for 10 months (Continuation phase) as per index TB guidelines recommendation.[1]

For extensive SCJI with bony involvement, abscesses, and periarticular fluid collection, surgical management is preferred along with medical management. The preferred surgical procedure is the *En bloc* resection, having a much better chance of resolving the infection compared to simple debridement or piecemeal resection.[3] Delayed/no treatment may lead to complications as compression or erosion of the large blood vessels at base of neck and migration of cold abscess in the mediastinum.[7]

TB of SCJ may follow slowly progressive, relatively painless disease without constitutional symptoms and minimal joint destruction due to differences in the virulence of the organisms and host resistance or more aggressive course leading to a painful and destroyed joint. To prevent such complications early diagnosis and management with appropriate therapy is important.

Author contributions

Dr. Aditi Gupta – Conception and design of the case series, drafting the work, analysis of cases, revising work critically for important intellectual content.

Dr. Gagandeep Kaur - Case diagnosis, data collection, literature search for publication, revising work critically for important intellectual content

Dr. Deepak Goyal – Case diagnosis, analysis of cases, literature search for publication, revising work critically for important intellectual content.

Dr. Vishal Chopra – Case diagnosis, the conception and design of the case series, analysis, and interpretation of data for the work, revising work critically for important intellectual content.

Final version of the paper was approved by all the authors All authors agreed for the accountability for all aspects of the work regarding accuracy and integrity of study work.

Conflicts of interest

The authors have none to declare.

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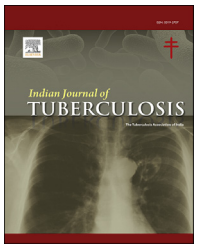

Correspondence

Effect of COVID-19 pandemic on tuberculosis notification

Keywords:
COVID-19
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Notification

To The Editor

With the emergence of COVID-19 as a pandemic in January 2020, India has witnessed two waves of the pandemic. The second wave was worse in terms of magnitude and severity of disease, bearing severe constraints to the overburdened healthcare system. COVID Pandemic has affected all walks of life but its impact has been profound on the vulnerable groups such as Tuberculosis (TB) Patients. The TB Programme in India was renamed in 2019 as National TB Elimination Programme (NTEP) with a mission to end TB by 2025 i.e, five years ahead of Sustainable Development Goals. Intensive measures were taken in 2018, 2019 and early parts of 2020 to improve TB case finding in public as well as private sector due to which India had made significant progress towards the goal of Ending TB till 24th March, 2020 when the nation-wide lockdown affected all the key strategic interventions resulting in decline in TB case notification.[1]

India witnessed the first decline in TB notification in 2020 which was closely associated with the rise in COVID cases and subsequent lockdown across the country (Fig. 1). Modelling studies to understand the potential effect of the COVID-19 response on TB epidemiology has been published by Stop TB Partnership indicates that for every month of Lockdown, 2,32,665 excess Cases and 71,290 Deaths will be added in India.[2]

A similar pattern is evident again this year in 2021 during the second wave where we see a sharp decline in the notification of TB cases. May 2021 has witnessed the lowest number of TB cases reported cumulatively across India in the past 3 years (Fig. 1).

There are always two sides of the coin. A positive thought could be that there is an actual decline in the number of TB Patients. COVID appropriate behaviour is not only helpful in tackling the Pandemic but mask, hand hygiene, social distancing has led to a major behavioural shift in Indian population which could be a potential factor in limiting the spread of Tuberculosis during the Pandemic leading to an actual decline in number of TB Patients.[3]

However, the other side of the coin is there is only a decline in the notification of TB cases. There are several potential factors (like closure of health facilities, fear of contracting COVID in Healthcare Centres, TB Care Providers involved in COVID activities, Patients stranded in different locations, non-availability of transport services and restricted movement, COVID-19 and TB coinfection,[4] use of NTEP labs for COVID-19 testing) which could have led to the decline in the notification of TB cases.

It is extremely important to tackle this unfavourable trend. Suggested solutions include TB-COVID Bidirectional screening and research,[5] contact tracing of all notified TB cases, door-step collection of samples wherever required, continued services at laboratories for providing both TB & COVID services along with continuous drug supply of anti-tubercular therapy. The social norms of following COVID appropriate behaviour while continuing TB services is the need of the hour.

A learning lesson which can also be identified from Fig. 1 is that due to intensified efforts of TB Care workers & strong commitment towards TB Elimination, India was successfully able to reach the pre-COVID level of notified cases by the end of 2020. The second wave in 2021 has created a similar challenge, but with the added advantage of lessons learnt from managing TB Cases during a similar situation last year. It is not hard to imagine that with all the steps taken to mitigate the impact of COVID-19 on Tuberculosis, India can not only achieve the pre-COVID level of notified cases but even beyond that!

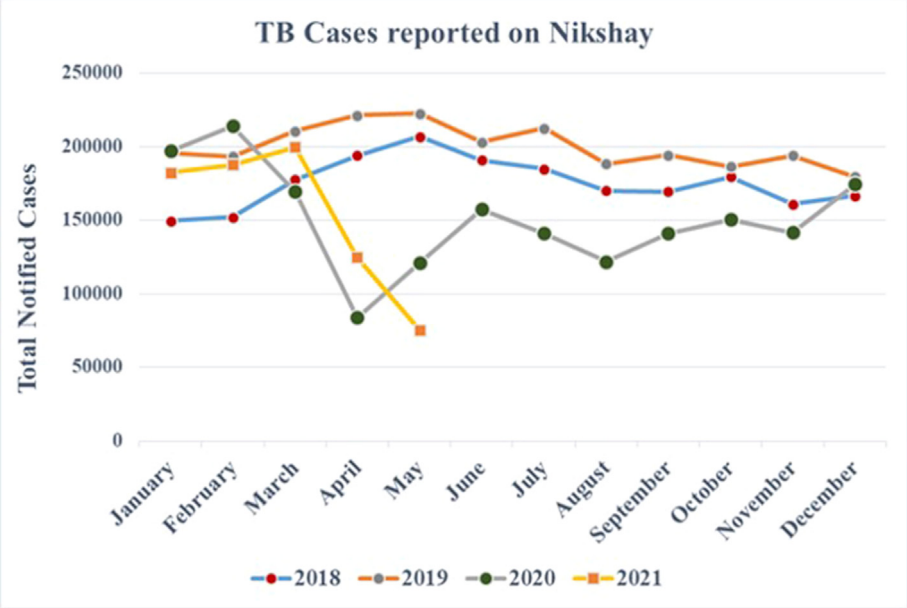

Fig. 1 – Total notified TB cases from India over 2018–2021 [Source: Nikshay Dashboard].

Conflicts of interest

The authors have none to declare.

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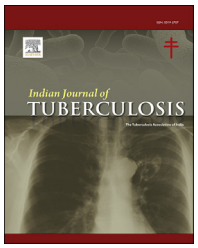

Correspondence

Casirivimab - Imdevimab in Covid 19 – Early Indian experience

Sir,

Corona viruses have traditionally been known to cause disease in humans and animals. By the fag end of the year 2019, medical attention was drawn to an outbreak of pneumonia cases in Wuhan, a city in the Hubei Province of China.[1] A novel corona virus was identified as the culprit which was subsequently designated severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). The disease rapidly spread to vast territories across the globe and has become the worst pandemic of the 21st century. With mounting evidence and clinical experience, if has become clear that most persons who get infected have few or no symptoms despite harboring high viral loads and their condition can be managed on an outpatient basis.[2] Disease progression with development of pneumonia and respiratory failure occurs in a smaller number of persons necessitating hospitalization and administration of supplemental oxygen. Remote (telehealth) management without the need for the patient directly coming into contact with healthcare personnel is an attractive option and is gaining popularity in mild cases.

Risk factors for disease progression and worse outcomes are increasingly being spelt out and subgroup of patients at added risk merit careful monitoring and therapy.[3] It has been postulated that complications and death from Covid-19 are a direct off-shoot of SARS-CoV-2 viral burden and reducing this burden early in the course of disease leads to clinical benefit. Monoclonal antibodies that target spike proteins of SARS-CoV-2 have been evaluated in outpatients with mild to moderate disease and risk factors for severe disease.[4,5] Trial results suggest a benefit from the use of these agents in the form of decreasing need for hospitalisation. In the United States, the monoclonal antibody therapies that have been authorised for emergency in select outpatients at risk for severe disease include Bamlanivimab-etesevimab, Casirivimab-imdevimab and Sotrovimab.[6,7]

Casirivimab – imdevimab is a cocktail made up of two noncompeting, neutralizing human IgG1 antibodies that target the receptor binding domain of the SARS-CoV-2 spike protein and block viral entry into human cells.[8] A "cocktail" approach may prevent the emergence of treatment-resistant mutant virus with usage of a single antibody as has occurred previously when suptavumab, was used to target respiratory syncytial virus. FDA issued an emergency use authorization for the investigational monoclonal antibodies casirivimab and imdevimab for the treatment of mild to moderate coronavirus disease 2019 (COVID-19) in adults and pediatric patients with positive results of direct SARS-CoV-2 viral testing who are ≥12 years of age weighing ≥40 kg, and who are at high risk for progressing to severe COVID-19 and/or hospitalization. Regeneron Pharmaceuticals introduced this antibody cocktailunder the name REGEN-COV (casirivimab with imdevimab) and is globally marketed by Roche limited and in India by Cipla pharma under the trade name Ronapreve. Central Drugs Standards Control Organisation (CDSCO) has provided an Emergency Use Authorisation (EUA) for Roche's antibody cocktail (Casirivimab and Imdevimab) in India since May 2021.

The specific challenges regarding the use of this cocktail foreseen in Indian circumstances include

a. The efficacy of the agent against variants of the SARS-CoV-2 that are prevalent in the country,
b. The economic burden of procuring the drug in our patients, considering the fact that most of our patients do not have third party assistance and pay out of pocket for health care related expenses
c. The potential difficulties of administering the agent in an outpatient setting observing full infection control precautions.[9]

The cocktail is available as a vial which has adequate dose for administration to two individuals. Once opened and reconstituted, it should be administered within 48 hours after storage at 4–8C. So, the clinician has the added task of identifying a second patient with indications who can share the vial.

We share the clinical data and results of our first 29 patients in whom the agent was administered. The drug was offered to 146 patients of whom 29 decided to go ahead with administration of the agent. Reasons for opting out included

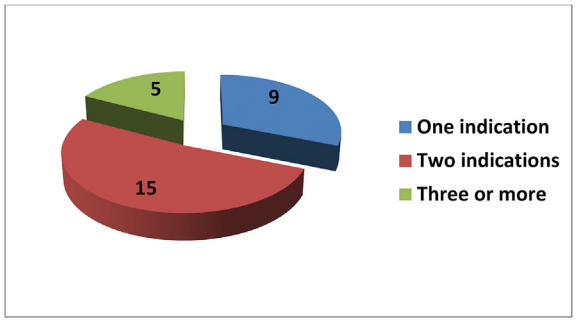

Fig. 1 – Number of high risk factors for administering casirivimab imdevimab in individual Covid 19 outpatients.

cost of therapy and lack of concern towards disease progression. The patients included out patients and covid patients admitted for reasons other than covid. The drug was administered in the Covid 19 in-patient ward (for out-patients also) and they were observed for a period of four hours for any adverse reactions. Of the patients in whom the drug was used, more than 50% had two indications. Fig. 1 depicts the number of indications that the patients had. Diabetes mellitus, chronic kidney disease, age >65 years, COPD etc were the common risk factors which necessitated antibody cocktail treatment. Table 1 summarises the indications in those patients who were given the antibody cocktail.

The agent was well tolerated in all patients in whom it was administered. No anaphylaxis was reported and no treatment related adverse event was noted. One patient with coronary artery disease, who was admitted with left ventricular failure turned out to be Covid 19 antigen positive at admission and received the drug. He expired after 48 hours with worsening pulmonary edema. Two patients had worsening of covid disease and hypoxia necessitating admission. The drug had good results in 26 patients who did not have worsening of covid disease, emergency visits or hospitalisation. 24 out of this 26 patients reported significant relief of their symptoms (fever, myalgia or fatigue) within 48 hours of drug administration. Fig. 2 summarises the results of therapy with casirivimab imdevimab in our patients, Overall, our preliminary experience suggests that casirivimab imdevimab monoclonal antibody cocktail for covid 19 is safe and efficacious in Indian patients. Larger experience in terms of patient numbers and participating centres is expected to throw more clarity in this subject.

Table 1 – Common indications for administering monoclonal antibody cocktail in covid 19 outpatients.

Indications	Number of patients	Percentage
Diabetes mellitus	14	48.27
Chronic kidney disease	17	58.62
Age >65 years	12	41.37
COPD	11	37.93
Immunosuppressive treatment	5	17.24
Obesity	4	13.79
Cardiovascular disease	5	17.24

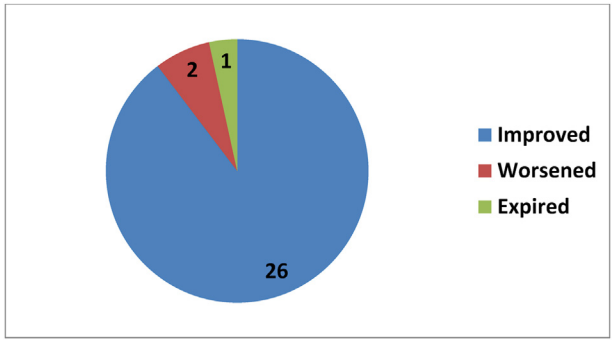

Fig. 2 – Results of therapy with casirivimab imdevimab in Covid 19 outpatients.

Conflicts of interest

The authors have none to declare.

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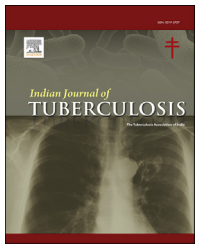

Letter to the Editor

Indeterminate mycobacterium tuberculosis QuantiFERON post Moderna mRNA Covid-19 vaccination

Keywords:
Mycobacterium tuberculosis
QuantiFERON
Moderna
mRNA Covid-19 vaccination

A B S T R A C T

We report an interesting case of an indeterminate MTB QuantiFERON for a 26-year-old healthy soldier planned for a routine field exercise to Brunei. Further medical history revealed that the patient had a Moderna mRNA Covid-19 vaccine the day before his MTB QuantiFERON test. The patient was subsequently asked to repeat a T-spot test which was non-reactive, there were no longer any issues with the positive control for the T-spot test.

Current Covid-19 research suggests that infection causes a dysregulation of the immune system, perhaps this might also be extrapolated where a Covid-19 vaccine might provoke an immune response which might interfere with some immunological assays. In summary there should be more research invested into the immunological interactions that the newly developed Covid-19 vaccinations have with our existing immunological tests such as QuantiFERON tests which forms a key cornerstone in our fight against tuberculosis.

Dear Editor

We read with interest the recently published article positive QuantiFERON test and the severity of COVID-19 disease: A prospective study. It was interesting to note that a negative mycobacterium tuberculosis (MTB) QuantiFERON test was a strong predictor of mortality in severe Covid-19 diseases.

In response, the authors would like to report an interesting case of an indeterminate MTB QuantiFERON for a 26-year-old healthy soldier planned for a routine field exercise to Brunei. As part of the pre-departure health screening, soldiers in Singapore are supposed to be screened for MTB, of which the preferred method is the MTB QuantiFERON assay. This patient was referred by the military medical officer due to the indeterminate (MTB) QuantiFERON result. The reason for the indeterminate result was due to the positive control being not interpretable as the mitogen minus tube did not have a sufficient amount of activation. The patient was asked multiple screening questions regarding recent infective symptoms, recent illness/Covid-19 infection, chronic medication or complementary medication usage. A thorough history of sexually transmitted infections, risky sexual behavior and risky sexual behavior was also performed. All of the above questions were negative. Further questioning about recent vaccinations did reveal that the patient had a Moderna mRNA Covid-19 vaccine the day before MTB QuantiFERON test. The patient was subsequently asked to repeat a T-spot test which was non-reactive, there were no longer any issues with the positive control for the T-spot test.

This is an interesting and likely first reported case of an indeterminate MTB QuantiFERON result post mRNA vaccination in an otherwise healthy patient. Currently the Centre for Disease Control in the United States of America stipulates that a QuantiFERON test be delayed 4–6 weeks if a patient has been vaccinated with a live attenuated virus.[1] As current evidence shows that vaccination with live viruses (such as the MMR vaccine) can cause mild immune system suppression. This may reduce the reactivity of the tuberculin skin test and possibly causing a false–negative reaction.[2] While inactive vaccines do not interfere with TB test results. Currently there is little data regarding mRNA vaccines in the setting of a QuantiFERON test. There have been studies

regarding QuantiFERON in the setting of severe Covid-19 infection. Current evidence suggests that there is an increased rate of indeterminate QuantiFERON results in critically ill COVID-19 patients with most showing huge reduction in mitogen stimulus thus suggesting gross general unresponsiveness of T cells.[3–5] It seems that Covid-19 infection causes a dysregulation of the immune system, perhaps this might also be extrapolated where a Covid-19 vaccine might provoke an immune response which might interfere with some immunological assays.

In summary there should be more research invested into the immunological interactions that the newly developed Covid-19 vaccinations have with our existing immunological tests such as the tuberculin skin tests, QuantiFERON tests and T-spot tests which forms a key cornerstone in our fight against tuberculosis.

Author contributions

All authors contributed to (1) concept or design, (2) acquisition of data, (3) analysis or interpretation of data, (4) drafting of the manuscript, and (5) critical revision for important intellectual content.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

The authors have none to declare.

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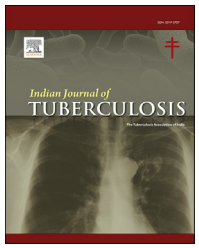

Correspondence

Comparison of performance indicators of MGIT primary culture to assess the impact of implementation of ISO 15189:2012 standards

Keywords:
MGIT 960
M. tuberculosis
NABL
Indicators
Performance

A B S T R A C T

Performance indicators are key component and plays a major role for monitoring and continuous quality improvement of the test results. The NABL certificate of accreditation is issued in accordance with the standard ISO 15189:2012 requirements. As part of the accreditation process, the laboratory has acquired knowledge and implemented the quality system procedures. Present study analyzed the impact of the accreditation process on the "performance indicators" of MGIT primary culture and found that performance indicators have been improved significantly after implementation of NABL for almost all indicators which clearly indicate the importance of accreditation and implementation of quality procedures for reliability of valid test results.

Dear Editor,

Performance indicators are key component and play a major role for monitoring and continuous quality improvement of the test results.[1,2] The Department of Bacteriology at ICMR-National Institute for Research in Tuberculosis, Chennai, India is accredited by National Accreditation Board for Testing and Calibrating laboratories (NABL), a constituent board of quality council of India, for quality and competence under medical laboratory testing. The certificate of accreditation is issued in accordance with the standard ISO 15189:2012 requirements. As part of the accreditation process, the laboratory has acquired knowledge and implemented the quality system procedures (QSP's), revised the standard operating procedures (SOP's) and the staff was trained according to the protocol for mycobacterial culture and sensitivity testing. To analyse the impact of the accreditation process the "performance indicators" of MGIT primary culture was compared before and after implementation of NABL and presented herewith (Table 1). The performance indicators have been improved significantly after implementation of NABL for almost all indicators except for very few. Z proportion test was used to assess the difference in the performance indicators. The difference has attained statistical significance ($p < 0.001$) which clearly indicates the importance of accreditation and implementation of quality procedures for reliability of valid test results.

The laboratory receive specimens (pulmonary and extra pulmonary) from the city of Chennai and sub centers of the institute which is located within 500 km radius. Once collected, the specimens were refrigerated and transported under cold chain to the laboratory for processing. Under this scenario, the serial number 1 in Table 1 was used to capture the data whether the specimens received to lab within 72 hours of sputum collection. A significant number of specimens were received within 72 hours both before and after NABL but the difference attained statistical significance (Table 1). The specimens received under 4–8 °C with proper packaging instructions (Sr. No. 2 of Table 1) were significantly increased (68.8% Vs 97.9%) which aided in reduction of specimen rejection rate (Sr. No: 3 of Table 1; 0.9% Vs 0.3%), to increase rate of recovery of mycobacteria (Sr. No: 4 of Table 1; 92.1% Vs 97.5%) and also to reduce the rate of contamination (Sr. No: 5 of Table 1; 11.3% Vs 9.6%). The difference for allthese indicators attained statistical significance. No significant difference was observed for specimens reported as *M. tuberculosis* complex (Sr. No: 6 of Table 1) whereas specimens with culture results reported as NTM had reduced significantly and the difference was found to be significant (Sr. No: 7 of Table 1; 7.1%

Table 1 – Performance indicators of MGIT primary culture before and after implementation of ISO 15189: 2012 Standards.

S. No	Indicators	Before NABL (August 2017 to July 2018)			After NABL (August 2018 to July 2019)			Difference	Significance
		Numerator	Denominator	Percent	Numerator	Denominator	Percent		
1	Specimens received within 72 hours of sputum collection	7312	7717	94.8	7987	8084	98.8	4	<0.001
2	Specimens received under 4 –8 °C	5312	7717	68.8	7922	8084	97.9	29.1	<0.001
3	Number of specimen rejected at the lab due to various reason (eg. Leakage, inadequate quantity, etc)	73	7717	0.9	22	8084	0.3	−0.6	<0.001
4	Smear-positive diagnostic specimens reported as culture-positive	785	852	92.1	921	945	97.5	5.4	<0.001
5	Specimens with culture-contaminated results	862	7644	11.3	776	8062	9.6	−1.7	<0.001
6	Specimens with cultures reported as *Mtb.* complex	3172	7644	41.5	3400	8062	42.2	0.7	0.404
7	Specimens with culture results reported as NTM	546	7644	7.1	405	8062	5.0	−2.1	<0.001
8	Specimens with culture results completed within the benchmark turn-around time	7538	7644	98.6	8023	8062	99.5	0.9	<0.001
9	Patients with final culture results reported to providers within 1 days of declaration of result	7591	7644	99.3	8034	8062	99.7	0.4	0.004

Vs 5%). Though not much difference for specimens with culture results completed within the benchmark turn-around time, it attained statistical significance (Sr. No: 8 of Table 1). Finally, no statistical difference was found for patients with final culture results reported to providers within 1 days of declaration of results (Sr. No: 9 of Table 1). The data will be helpful for the other laboratories to acquire accreditation and to improve the quality of work especially for medical testing.

Ethical statement

Ethical clearance is not required for the study as this is the retrospective analysis of the data.

Conflicts of interest

The authors have none to declare.

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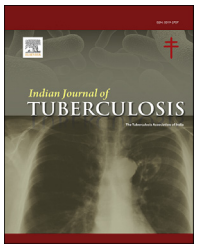

Correspondence

Are we ready with fluroquinolone based treatment regimen for drug resistance tuberculosis in a resource limited country?

Sir,

Tuberculosis (TB) is a significant contributor to global morbidity and mortality. The emergence of drug resistant TB has globally threatened the efforts towards the goal of TB elimination.

Treatment of Multi-drug resistance tuberculosis (MDR-TB) is immensely long and expensive, along with significantly lower success rates compared to drug-sensitive TB. According to World Health Organization (WHO) report (2017), the global treatment success rate for new TB cases was 85%; whereas it remains poor for MDR-TB (57%).[1]

The WHO upgraded classification of drugs used in Drug resistance TB represents Fluoroquinolones (FQs) as one of the most effective drugs and remains a core drug in regimen for MDR/XDR TB and H mono-resistance TB. But there are several questions need to be answered before including FQs in DRTB regimen.

- Do we have enough lab infrastructure to performed First line LPA for drug sensitive and Second line LPA for Rifampicin resistance TB to detect FQ, and second line injectable drug resistance?
- Are we really ruling out FQ and second line drug (SLD) resistance before putting on shorter course RR/MDR regimen?
- Is there a need to restrict use of FQ and availability as OTC drug to reduce increasing resistance?

There are several studies showed alarming figure of FQ resistance by mycobacterium tuberculosis in our country. One of the study showed, resistance to any fluoroquinolones reported in MDR TB cases in India was 21.82%.[2] Liquid culture drug sensitivity test (DST) using the BACTEC MGIT 960 of 100 Rifampicin Resistance tuberculosis (RR-TB) patients showed a 65% resistance to fluoroquinolones (Levofloxacin 56%; Moxifloxacin 44%) a study by Nishtha et al.[3] Various study done in different states of our country showed additional FQs resistance in almost 50% patients of RR TB[4] and showed an increase from 3% in 1996 to 35% in 2004 for FQs resistance among newly detected tuberculosis.[5]

There could be several reasons attributing for such a high prevalence of FQs resistance including widespread and injudicious use, easy availability as over the counter (OTC) drug, low cost of drug. Due to widespread prescription of FQs for trivial infections, resistance to these drugs has remarkably increased, and this poses a major challenge to the clinicians, as it takes away a very potent weapon from the armamentarium against M/XDR-TB treatment.[6] It is even prescribed for drug sensitive tuberculosis in case of drug induced hepatitis or as a modified Anti-tubercular regimen. Last but not the least, transmission of fluoroquinolone-resistant strains can also be one of the reason for increasing FQs resistance.

In 2016, the WHO recommended a shorter drug regimen (9–12 months) for MDR TB or RR-TB patients who had not received second-line drugs (SLDs) and in whom resistance to fluoroquinolones and injectable SLDs is considered highly unlikely and should be used after ruling out FQs, injectable anti TB drugs resistance through SL-LPA. Studies done on shorter regimen showed that FQ resistance is associated with worse treatment outcome.[7] According to latest TB INDIA 2021 report,[8] 72% of RR/MDR TB patients have been put on shorter regimen but if we follow strict clinical and DST exclusion criteria of shorter regimen, less than 40% patients are eligible for shorter course regimen which has been assessed in a study from Mumbai showing less than 5% patients are eligible for shorter regimen if we performed full phenotypic DST.[9] Another study showed that almost 50% patients were not eligible for shorter course due to FQ resistance.[iv] Almost half of patient from Pakistan, Brazil is not eligible for Short Course treatment.[10,11] In areas with higher disease burden like Eastern Europe, these figures are even lower and less than 5% are eligible for short course treatment.[12]

So, it is urgent need to find out and rectify the imminent causes leading to over treatment with shorter course regimen. These could be primarily attributed to poor adherence to exclusion criteria of shorter course regimen. Resource limited

country like ours lacks a good lab infrastructure, lack easy availability of LPA, poor availability of culture at periphery and high turnaround time to get results.

So, the need of the hour is to make certain changes related to FQs in our country. Owing to the high proportion of resistance to fluoroquinolones, the empirical use of FQs in various ailments should be discouraged, should not be available as OTC drug for easy use by physicians. It should be only considered in DRTB regimen when susceptibility is evidenced microbiologically and should not be used as empirical or 1st line drug for non-tubercular indication. The cost of the drug can be increased in order to reduce its use in non-classical indication. There is a die-hard need to increase the facility of second line DST specially LPA to reduce turnaround time. Shorter MDR regimen should not be used as an empiric treatment of MDR TB or rifampicin-resistant TB patients in our country.

Conflicts of interest

The authors have none to declare.

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