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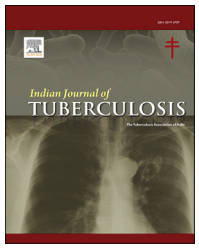

Editorial

Can Pan-TB shorter regimens be a promising hope for ending TB in India by 2025 in ongoing COVID-19 era?

Keywords:
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Shorter
Universal regimen

Tuberculosis (TB) remains a considerable public health burden with substantial morbidity and mortality worldwide. 10 million incident TB cases were reported globally with 2.9 million remain undiagnosed in 2019.[1] 1.2 million TB deaths occurred among HIV-negative people with additional 0.21 million among HIV positive ones. TB affects around 30,000 people every day with daily mortality of 4000 worldwide despite this disease is preventable and curable. Standardized 6 months regimen containing four anti-tubercular drugs- Rifampin (R), Isoniazid (H), Ethambutol (E) and Pyrazinamide (Z) in combination, remains the cornerstone of treatment for drug sensitive tuberculosis (DS-TB) with favourable treatment outcome of 85%.[1] However, drug-resistant TB (DR-TB) has evolved by development of acquired as well as transmitted resistance among strains of TB bacilli, creating important forms like rifampicin resistant-TB (RR-TB), multi-drug resistant tuberculosis (MDR-TB), and extensively drug resistant TB (XDR-TB). Multi-drug and Rifampicin-resistant tuberculosis (MDR/RR-TB) is now creating a potential hazard to control of TB. 3–4% of new and 18–21% of re-treatment TB cases worldwide had MDR/RR-TB since last one decade.[1] The World Health Organization (WHO) End TB Strategy has placed targets for eliminating TB with 80% and 90% reduction in incident rate as well as 90% and 95% reduction in mortality rate by 2030 and 2035 respectively.[1,2] The government of India aims to end TB by 2025 which is an admirable initiative.[3] The first step is to prevent emergence of new drug resistant cases. WHO has recommended universal drug susceptibility testing (DST) for rapid reduction of drug resistance by genotypic tests such as cartridge based nucleic acid amplification test (CBNAAT) and line probe assays (LiPA).[4,5] Another issue is that global treatment outcome of MDR/RR-TB cases remains sub-optimal. 186,772 MDR-TB cases were diagnosed among 500,000 notified cases of MDR-TB in 2019 with treatment success rate of 57%.[1] The favourable treatment outcome was achieved only in 48% even in India. Drug resistant cases are usually treated with conventional regimens containing a combination of second line drugs (SLDs') including injectables for duration of atleast 18–24 months.[4] The reasons for sub-optimal outcome are possibly due to prolonged treatment duration, expensive and toxic SLDs' particularly injectables leading to poor compliance. Fortification of regimens with newer drugs like Bedaquiline (Bdq) and Delamanid (Dlm) as well as repurposed drugs like Linezolid (Lzd) and Clofazimine (Cfz) in combination, has revolutionized management of DR-TB over last decade. WHO is working aggressively on enhancement of treatment success rate. All oral longer regimens containing newer drugs have been introduced for DR-TB patients with treatment duration of 18–20 months.[5] WHO has also introduced a shorter treatment regimen of 9–12 months duration with potential ability to curtail various aspects such as drug burden, culture conversion time, risk of infection transmission, incidence of adverse drug events, cost and treatment duration leading to improvement of adherence.[4,5] The regimen was introduced on the basis of STREAM (Standard Treatment Regimen of Anti-TB Drugs for Patients with MDR TB) Stage 1, a phase 3 randomized control trial (RCT) that reported non-inferiority of shorter regimen compared to longer regimen regarding primary efficacy outcome (78.8% versus 79.8%) and safety in patients with MDR/RR-TB having susceptibility to both FQs' and second line injectable drugs (SLID).[6,7] Shorter regimens

reported to have statistically-significant higher likelihood of treatment success than those received longer conventional regimens. (80%–83% versus 56%–75.3%)[4–9] However, there are various shortcomings associated with these regimens. The shorter regimen still requires a minimum 4 months of treatment in an intensive phase using drugs such as an SLID, Lzd and Cfz that have poor toxicity profile and also logistical challenges of multiple intramuscular drug administration leading to poor adherence. Another concern is that evidence remains sparse regarding potency of shorter MDR/RR-TB regimens in all settings with respect to DST pattern, HIV status, extrapulmonary involvement (except lymph node and pleura), disseminated or central nervous system involvement and pregnancy. Although higher success rate with shorter regimen was due to less default rate but was also associated with unfavourable outcome (failure or relapse) in the presence of documented resistance to medications included in the regimen especially FQs' and Z either at baseline or subsequently during ongoing treatment. Shorter regimens can be applied to 1/3 to 1/4 (5–25%) of MDR/RR-TB based on clinical criteria and prevalent drug resistance pattern in most countries.[10,11] All these inferences demand for enhanced approach to reliable DST. A search for innovative shorter regimens is essential that can overcome these limitations. A second stage of STREAM 2 trial is already evaluating two additional shorter regimens containing Bdq.[6] Many trials are ongoing and hunting for innovative shorter regimens for DS-TB (APT, CLO-FAST, PredictTB, TBHDT, TRUNCATE-TB) and DR-TB (BEAT TB, DELIBERATE, endTB, endTB-Q, MDR-END, NeXT, TB-PRACTICAL). Research Excellence to Stop Tuberculosis resistance (RESIST-TB), an initiative adopted by WHO is conducting all these trials for rapid control of DR-TB.[12] Pretomanid (Pa) is one of the promising newer drug that has shown to increase treatment success in M/XDR-TB.[13] The Nix-TB trial is evaluating Bdq-Pa-Lzd regimen of 6–7 months duration with minimum potential resistance in treatment of XDR-TB and also MDR-TB patients either non-responsive to treatment or not tolerating SLDs' requiring treatment discontinuation.[14] A cure rate of 90% was achieved after a 6 month course of treatment (MDR-TB- 92%; XDR-TB-89%). WHO has recommended that eligible XDR-TB patients can be treated with Bdq-Pa-Lzd regimen under programmatic research settings in whom design of effective regimen not possible and also no prior exposure to Bdq and Lzd over two weeks.[5] Acceptance and practical viability of this regimen is impressively high among TB stakeholders in Indonesia, Kyrgyzstan, and Nigeria as compared to that of individualized treatment regimen of 18–20 months duration (93% Vs 45%).[15] 88% of stakeholders are willing to implement Bdq-Pa-Lzd upfront among eligible patients despite various barriers related to long term efficacy, monitoring of adverse events and implementation at programmatic level.

Further, few trials are ongoing to design elusive shorter regimens that can serve the purpose to treat DS-TB in addition to DR-TB cases simultaneously favouring universal treatment approach as shown in Table 1.[12,16–19] These universal shorter regimens are also termed as Pan-TB regimens. Pa can be considered as backbone of pan-TB regimens. It has a distinct mechanism of action and is unaffected by bacterial mutations that confer resistance to other TB drugs, so it is equally effective against DR-TB as it is against fully DS-TB.[13] A multi-centric phase 2b trial included a non-randomized group for RR-TB patients treated with regimen containing Bdq-Pa-Mfx-Z.[19] The Pa containing regimen showed significantly higher bactericidal activity against DS-TB for the groups with the daily dose or loading dose of Bdq as compared to HRZE group. However, limitations exist with this trial such as shorter duration for assessment of bactericidal activity, non-placebo-controlled or blinded aspect and possibility of bias created by sponsor in methodology and data compilation. A modelling analysis from South Africa predicted that implementing the Bdq-Pa-Mfx-Z regimen universally could simultaneously improve cure rate for DR-TB patients from 60% to 90%, nearly 90% cure rate for DS-TB patients, curb treatment duration by atleast 2 months, and curtail transmission rate of infection by 3% for DS-TB to 50% for DR-TB.[20] The cure rate may remain exceptionally high after adopting this regimen even in settings having high prevalence of drug resistance or sparse DST coverage. Other advantages would include shorter treatment duration as well as culture conversion time, adequate infection control among all forms of TB patients including HIV co-infection and establishment of well-organized or co-ordinated health care delivery system between providers and patients.[21,22] Another mathematical modelling study has projected that if high burden countries like India implements pan-TB regimen by 2022, the annual incidence of TB will decline by 23.9% while treating all TB cases, and by 2.30% while treating only RR-TB cases in 2030.[23] However, economic feasibility must be kept in mind while implementing these regimens under programmatic conditions. It will be economically more productive if all forms of diagnosed TB cases should be treated with pan-TB regimen rather than treating only drug resistant ones considering cost around US dollar 360 on an average. Implementation of these regimens at national level could be epidemiologically purposeful and also cost-effective to TB control programmes on long run despite being more expensive than existing TB treatment.[23,24] Various theoretical advantages have been proposed like increased treatment initiation rates in public sector to 95%, treatment completion rate of DS-TB to 95%, improved adherence with less probability of missed dosing leading to 50% reduction in recurrence rates and also equal efficacy for both DS as well as DR-TB patients. Private sector should also be engaged in addition to public sector while implementing these regimens. Many disadvantages while using universal drug regimens have also been postulated such as rapid resistance amplification with loss of effective newer drugs due to strain variation, selection of drug resistant strains and pharmacokinetic variability, impairment of precise diagnostic tests and newer drug development due to lesser requirement for DST, encounter of more challenging management of drug resistance and toxicity with anti-TB drugs, lack of alternative regimens or rescue drugs, vigorous effort to maintain drug stocks by ensuring adequate supplies and scaling up productivity considering expenses, deviation from the patient centric or individualized approach recommended by WHO and probability of sub-optimal dosing in pediatric cases.[25] However, use of novel universal drug regimens should not be deferred in view of these uncertainties. The advantage of reduction of DR-TB transmission will be counterbalanced by development of resistance in DS-TB cases. It has been projected that the universal approach will remain only for limited duration due to

Table 1 – Ongoing trials working on PAN TB shorter regimens to treat DS-TB and DR-TB cases simultaneously favouring universal treatment approach.

Trial	Phase	Regimens compared	Study population	Primary objectives	Result	Outcome
NCT01215851[16]	Phase 2A, partially double-blinded, randomized trial	-Bdq (n = 15) -Bdq-Z (n = 15) -Bdq-Pa (n = 15) -Pa-Z (n = 15) -Pa-Mfx-Z (n = 15) -RHEZ (n = 10)	Treatment naïve uncomplicated DS-TB (n = 85)	Assessment of 14 day EBA as estimated from the daily fall in CFU of M. tb/ml of daily collected sputum	Mean 14 day EBA of Pa-Mfx-Z (0.23) significantly higher than Bdq (0.061), Bdq-Z (0.131), Bdq-Pa (0.114) but not Pa-Z (0.15) and comparable with RHEZ (0.14)	Pa-Mfx-Z is potentially suitable for treating both DS-TB and MDR-TB
NCT 01691534[17]	Phase 2A, two-center, open-label, randomized clinical trial	-Bdq-Pa-Z-Cfz (n = 15) -Bdq-Pa-Z (n = 15) -Bdq-Pa-Cfz (n = 15) -Bdq-Z-Cfz (n = 15) -Z (n = 15) -Cfz (n = 15) -RHEZ (n = 15)	Treatment naïve uncomplicated DS-TB (n = 105)	Assessment of EBA expressed as the rate of change in CFU counts over the 14 days of treatment	Mean 14 day EBA: - Bdq-Pa-Z (0.167), standard treatment (0.151), Bdq-Z-Cfz (0.124), Bdq-Pa-Z-Cfz (0.115), Bdq-Pa-Cfz (0.076) Z alone had modest activity Cfz had no activity alone (20.017) or in combinations	Bdq-Pa-Z is a potential new TB treatment regimen Regimen suitable for patients with MDR-TB with relatively high reported rates of phenotypical Z resistance in many areas
NCT01498419[18]	Phase 2b, multicentre, open-label, partly randomised clinical trial	-Mfx-Pa100-Z (n = 60), -Mfx-Pa200-Z (n = 62) -HRZE (n = 59) -DRMfx-Pa200-Z (n = 26)	Treatment naïve DS-TB (n = 181) MDR-TB patients (n = 26)	Assessment of 8 weeks EBA measured by mean daily rate of reduction in CFUs of M. tb/mL overnight sputum collected once a week	DS-TB:- mean BA of MPa200Z (0.16) and MPa100Z (0.13) were significantly greater than for HRZE (0.11) DRMPa200Z:- mean BA of 0·12	Mfx-Pa-Z showed superior bactericidal activity in DS-TB during 8 weeks of treatment Results consistent between DS-TB and MDR-TB Ready to enter Phase 3 trials
NCT02193776[19] NC-005	Multi-centre, open-label, partially randomized, phase 2b trial	Bdq_{load}-Pa-Z (59), Bdq200PaZ (60), HRZE (61) DRBdq-Pa-Mfx-Z (60)	DS-TB (n = 180) MDR/RR-TB (n = 60)	Daily percentage change in time to sputum culture positivity in liquid medium over 0–56 days in DS-TB population	Bdq200-Pa-Z highest daily percentage change in TTP (5·17%) followed by Bdq_{load}-Pa-Z (4·87%) and HRZE group (4·04%) In DRBdq-Pa-Mfx-Z group, the Z-susceptible RR-TB group showed the highest cumulative percentage of culture negativity in liquid culture medium compared to Z-resistant RR-TB group	Bdq200PaZ is a promising regimen to treat patients with DS-TB Bactericidal activity of these regimens have the potential to shorten treatment Simplified dosing schedule of Bdq200PaZ could improve treatment adherence in the field

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Table 1 – (*continued*)

Trial	Phase	Regimens compared	Study population	Primary objectives	Result	Outcome
SimpliciTB[12] NCT03338621 NC008	Phase 2c/3, multi-center, open-label partially randomized clinical trial	DS-TB (n = 150): Bdq-Pa200-Mfx-Z (4 months) DS-TB (n = 150) RHEZ (2 months)/RHE (4 months) DR-TB (n = 150)- DR Bdq-Pa200-Mfx-Z (6 months)	DS-TB patients (n = 300) DR-TB patients (n = 150)	Time to culture conversion to negative status over 8 weeks Proportion of participants experiencing bacteriologic failure or relapse or clinical failure (unfavourable outcome) at 52 weeks Incidence of bacteriologic failure or relapse or clinical failure at 104 weeks from the start of therapy Proportion of participants with sputum culture conversion to negative status in liquid culture (MGIT) at 4, 6, 12 and 17 weeks to be explored as a potential biomarker of outcome at 52 weeks from start of therapy	To be published	To be published
STAND[12] NCT02342886 NC-006	Phase 3 Open-Label parallel assignment Partially Randomized Trial	Mfx-Pa200-Z (6 months) (n = 67) Mfx-Pa200-Z (4 months) (n = 71) Mfx-Pa100-Z (4 months) (n = 65) RHEZ (6 months) (n = 68) DR-TB DRMfx-Pa200-Z (6 months) (n = 13)	DS-TB patients (n = 271) DR-TB patients (n = 13)	Incidence of combined bacteriologic failure or relapse or clinical failure at 12 months from start of therapy (modified ITT) Incidence of combined bacteriologic failure or relapse or clinical failure at 12 months from start of therapy (PPP) Incidence of bacteriologic failure or relapse or clinical failure at 24 months from the start of therapy Rate of change in TTP over time in liquid culture (MGIT) in sputum (at Screening, Day 1, 7; Week 2–7; Month 2–6, 9, 12, 15, 18, 24) Proportion of subjects with sputum culture conversion to negative status in liquid culture (MGIT) at 4, 8, 12 and 17 weeks	Favourable outcome (ITT/PPP) Mfx-Pa200-Z (6 months):- 43/56 (76.8%)/43/47 (91.5%) Mfx-Pa200-Z (4 months):- 46/61 (75.4%)/46/57 (80.7%) Mfx-Pa100-Z (4 months):- 38/57 (66.7%)/38/52 (73.1%) RHEZ (6 months):- 52/60 (86.7%)/52/53 (98.1%) DRMfx-Pa200-Z (6 months):- 10/11 (90.9%)/10/10 (100%)	Mfx-Pa200-Z is a promising regimen to treat patients with DS-TB Bactericidal activity of these regimens have the potential to shorten treatment

Abbreviations used:-Bdq- Bedaquiline; Cfz-Clofazimine; CFU-Colony forming unit; DS-TB-Drug sensitive tuberculosis; DR-Drug resistant; E-Ethambutol; EBA-Early bactericidal activity; H-Isoniazid; ITT-Intention to treat; Mfx-moxifloxacin; MDR-TB- Multi-drug resistant tuberculosis; MGIT-Mycobacterium growth indicator tube; M. tb-*Mycobacterium tuberculosis*; Pa-Pretomanid; PPP- Per protocol population; R-Rifampin; RR-TB-Rifampin resistant tuberculosis; STAND-Shortening treatment by advancing novel drugs; TTP-Time to culture positivity; Z-Pyrazinamide.

probability of gradual development (5–10 years) of acquired resistance to newer drugs like Bdq, Dlm or Pa by 5–10%.[26,27] These drugs can still be continued even if there is documented resistance. The regimens can be implemented but need to have backup with strong drug resistance surveillance and rapid DST for newer drugs as well. An important concern remains whether TB patients can be treated effectively with these upcoming shorter regimens in all settings particularly outside trial conditions or not requires robust evidence. Most of these investigational novel universal drug regimens in pipeline are currently undergoing through phase 2 trials and have to clear phase 3 trials for further approval. Phase 3 trials require larger sample size with thousands of patients and take atleast three to five years to commence. The Project to Accelerate New Treatments for TB (PAN-TB) collaboration among philanthropic, non-profit and private sectors has been launched with aim to fast-track development of these regimens through phase 2 clinical trials with further preparation for phase 3 trials.[28] This project is working on regimens that can be prescribed upfront with reduced requirement for drug resistance testing and also for baseline resistance to any component drug.

The National Tuberculosis Elimination Programme (NTEP) has introduced fixed dose combination (FDC) for treatment of DS-TB patients with potential advantages such as prevention of emergence of drug resistance, less probability of medication errors, better compliance, less adverse events, reduction of cost and proper maintenance of supply chain. The impact of FDC on treatment outcome still need to be defined. NTEP is also customizing treatment of DR-TB patients especially XDR and pre-XDR-TB ones with individualized regimens although it remains quite challenging to implement in settings with limited resources. These regimens require strong support of highly standardized laboratory facilities and expertise in analysis of DST results. Recently, the unprecedented COVID-19 pandemic has created a potential threat to healthcare system in managing patients with TB leading to undermining of global target of elimination of TB. The 2021 WHO global TB Report states that there is significant drop of 18% in notification of newly diagnosed TB cases worldwide as it remains only 5.8 million in 2020 as compared to 7.1 million in 2019.[29] India (41%), Indonesia (14%), the Philippines (12%) and China (8%) are the countries mainly responsible for this global fall in notification. A total of 157,903 DR-TB cases were notified with a drop of 22% in 2020 as compared to 201,997 in 2019. Global mortality due to TB among HIV-negative people is 1.3 million in 2020, up from 1.2 million in 2019 with an additional mortality of 0.22 million among HIV-positive ones as compared to 0.21 million in 2019. The global TB related mortality was double as caused by HIV. Mortality due to HIV continued to decline in comparison to that of TB from 2019 to 2020. TB was responsible for highest mortality among infectious diseases in 2019 but it was superseded by COVID-19 in 2020. The pandemic has derailed the momentum of global progress achieved by TB control programme from 2000 to 2019 and has created a setback as existing parameters in 2020 reversing to the level of 2012–2017.[29] The milestones of End TB Strategy for reduction in burden of TB by 2020 has been off tracked as these have not been achieved globally in most countries. A modelling analysis by STOP TB partnership predicted that numbers of TB including DR-TB cases will upsurge due to interruption of TB healthcare delivery services by COVID-19 between 2021 and 2025 resulting in unfavourable outcome. India has witnessed 25% drop in notification for both DS-TB and DR-TB cases within a span of one year (2019–2020).[30] The substantial reduction in TB case notification between 2019 and 2020 probably confined to imbalance between demand and supply for health services. More than 200 countries especially high TB-burden countries had to reallocate manpower, budget and other resources from TB control programmes creating acute shortage to combat COVID-19 pandemic. Factors responsible for such interruptions include reduced access to routine health care services due to imposed restrictions on movement during lockdowns, inability to provide direct services including medications to both DS as well as DR-TB patients, reluctance to avail health care facilities in view of fear of getting infected during a pandemic, under-reporting of data, re-prioritization of TB laboratories to enhance COVID-19 testing, lack of streamline infection control policies to protect vulnerable TB patients from COVID-19, shortage of ventilator beds for critically ill TB patients, lack of community participation due to social stigma associated with similarities in symptoms as well as myths created by media hype and apprehension due to COVID-19 infection even among frontline TB health care providers. In the current scenario, NTEP has to work on multi-dimensional domains in order to achieve the desired goal that still seems to be a herculean task. Therefore, it has become an utmost priority for proper allocation of budgets, attainment of target for TB control at double pace, hastening of newer TB diagnostics aiming for rapid detection and funding more on trials working on novel shorter regimens particularly pan-TB ones containing newer drugs in pipeline. Given the considerable global burden of TB accompanied with unfavourable outcome created by COVID pandemic, it is vital to evaluate these novel pan TB shorter regimens in various settings and implement under programmatic conditions as early as possible with aim to fulfil the goal of end TB strategy by 2025 in India.

Conflict of interest

The authors have none to declare.

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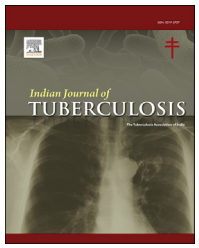

Viewpoint

Tuberculosis (TB) care challenges in post-conflict settings: The case of Afghanistan

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ABSTRACT

Tuberculosis (TB) is a huge global health concern, especially for low and middle-income countries. In Afghanistan, TB is highly prevalent that is attributed in part to, notable poverty, resource constraints, and a mismanaged health care system that engulf the country. This article describes unique challenges for TB care in Afghanistan. It concludes this endemic problem may now multiply due to COVID-19 and political challenges and transform into a disaster that may result in higher morbidity and mortality among TB patients. We recommend addressing the need for appropriate and timely TB-care amid the post-conflict setting. Additionally, the health workforce needs to play a vital role in policy advocacy and health service delivery that promotes TB care in this post-conflict and resource-limited setting.

Tuberculosis (TB) is a huge global health concern, especially for low and middle-income countries. Even though Afghanistan epitomizes a low-income country to the international society, the nearly five decades-long war and enduring political turmoil made it an exceptionally resource-limited country, particularly in the health domain.[1–3] Despite significant achievements in TB prevention and treatment in the last two decades due to international aid, Afghanistan is still home to a staggering number of TB patients on the global scale.[1,4] With 73,000 new cases in 2020, it ranked 22nd among the top 30 high TB burden countries.[1] This low ranking is attributed, in part to the enduring and immersing poverty, the overwhelming constraints of health care resources, and a mismanaged health care system that engulf the country.[1,4] In this article, we describe unique challenges for TB care in Afghanistan.

The COVID-19 pandemic has had profound health and economic implications for Afghanistan. During the pandemic, most health resources and workforce have been redirected to the COVID-19 response, leaving limited resources for other health programs such as TB care. Moreover, quarantine and limited transportation made it difficult for TB patients who reside in rural areas to access urban health facilities. Additionally, the authorities transformed some tertiary care centers into COVID-19 hospitals and allocated the laboratory equipment for COVID-19 screening.[2] Consequently, TB patients in Afghanistan are less likely to receive appropriate and timely TB care.

In August 2021, dethroning of the formally recognized and internationally assisted Afghan government led to deterring a large part of the international healthcare-related funds.[3] Hence, all healthcare services dependent on humanitarian non-

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governmental organizations (NGOs) are at their minimal functioning level. This unwarned resource-reduction critically affected TB prevention and treatment services and led to inadequate drug supply, non-functioning equipment, and fragile infrastructure.[3] Furthermore, during the last months, unprecedented challenges in the health workforce due to the fleeing of well-trained health professionals and unpaid salaries of the working staff are increasing in Afghanistan.[3,5] The multiple impacts of these challenges on the quality of healthcare services and the quality of TB care are beyond comprehension. Moreover, as the country has experienced decades of conflict, numerous Afghans are internally displaced due to socio-economic and political challenges. Such a displacement is bound to bring about the limited access to overall health care services.[3]

While TB is highly prevalent in Afghanistan, health resources and system capacities to address this endemic public health problem are scarce. Besides, this unaddressed endemic problem may now multiply due to the socio-economic and political challenges and transform into a disaster that may result in higher morbidity and mortality among TB patients. These challenges imply that Afghanistan is unlikely to meet Sustainable Development Goals (SDGs) 3.3 target as "end the global tuberculosis epidemic" by 2030.[1]

To, at least, preserve the TB-care gains that the Afghan health system obtained during the last two decades through the generous aid of the international society, national health policymakers and international donors have to address the need for appropriate and timely TB-care amid the post-conflict setting. Additionally, the health workforce needs to play a vital role in policy advocacy and health service delivery that promotes TB care in this post-conflict and resource-limited setting. However, the enduring reduction of financial resources in Afghanistan makes even the best policies and plans challenging to implement.

Authors' contribution

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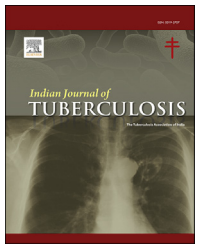

Viewpoint

Role of chest radiography in the diagnosis of pulmonary tuberculosis during nCovid19 pandemic

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ABSTRACT

Pulmonary tuberculosis and nCovid 19 share many common risk factors. nCovid19 may increase the risk to develop pulmonary tuberculosis. Pulmonary tuberculosis may precede, co-exist or follow nCovid19. Careful evaluation of chest radiography is useful to differentiate tuberculosis from nCovid19 bronchopneumonia. Symptoms of tuberculosis may be mistaken for long covid. A normal chest x ray in the absence of sputum production may help to rule out tuberculosis in such cases. All patients with nCovid19 bronchopneumonia should undergo a careful chest x ray evaluation for any lesions suggestive of tuberculosis. All patients with chest radiological abnormality should undergo sputum examination to rule tuberculosis as atypical radiological manifestations may be more common in patients with nCovid19. Symptoms, signs, clinical features and chest radiographic features of Pulmonary tuberculosis and nCovid19 bronchopneumonia may overlap in some cases. Correlation of chest radiographic findings with epidemiologic history, clinical presentation, and RT-PCR test results or in later stages antibody titres will help in confirming or excluding the diagnosis in suspected cases of nCovid19. In pulmonary tuberculosis definitive diagnosis should be established by bacteriological confirmation. Molecular diagnostic tools should be used to confirm or exclude tuberculosis in suspect cases as the results are rapid, accurate and reliable.

Healthcare resources and personnel throughout the world are busy in dealing with nCovid19 pandemic since last more than 20 months. Management of other chronic diseases has taken a backseat. This may lead to resurgence of these diseases. In countries with high prevalence of tuberculosis, this can lead to increase in the burden of tuberculosis. One of the key aspects in elimination of tuberculosis is early diagnosis and treatment of active pulmonary tuberculosis.[1] (see Table 1)

nCovid19 bronchopneumonia and pulmonary tuberculosis can coexist. Patients with pulmonary tuberculosis may be at

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Table 1 – Chest radiography in nCovid19 bronchopneumonia and Pulmonary TB.

	nCovid19 bronchopneumonia	Pulmonary TB
Typical	**Chest x ray** – Bilateral, peripheral, sub pleural, lower lobe predominant patchy opacities. **HRCT** – Ground glass opacities, crazy paving pattern and consolidation in early stages usually up to day 14. Band like opacities, fibrosis, traction bronchiectasis, organising pneumonia pattern in later stages usually after day 14. Lesions are bilateral, peripheral, sub pleural, lower lobe predominant.	Upper lobe predominant, unilateral or bilateral, cavity with surrounding infiltrates, fibrosis, no air-fluid level in cavity, patchy consolidation, fibonodular opacities, bronchiectasis. Pleural effusion, hydro pneumothorax with pulmonary infiltrates, mediastinal adenopathy
Atypical	Central lesions Upper lobe lesions Sub pleural sparing Lobar consolidation Unilateral lung involvement Solitary lesion	Lower lobe lesions Dense consolidation Collapse consolidation Cavity with air fluid level

higher risk to develop nCovid19 bronchopneumonia as both share the same risk factors.[2] Following nCovid19, susceptibility to develop pulmonary tuberculosis may increase. Cell mediated immunity plays an important role in the prevention of viral diseases and tuberculosis. Patients with reduced cell mediated immunity are more at risk to develop pulmonary tuberculosis and nCovid19 bronchopneumonia. Diabetes, advancing age, chronic renal disease, chronic liver disease, immunosuppression, underlying malignancy, malnutrition and immunosuppressant medications are the common risk factors for developing tuberculosis and nCovid19.[3] Medications like systemic steroid, immunomodulatory drugs used for treating nCovid19 bronchopneumonia can increase the risk for developing tuberculosis. Anosmia, anorexia due to nCovid19 can lead to weight loss and malnutrition which in turn can increase the risk for developing tuberculosis.

Economic constraints due to the pandemic can lead to malnutrition. Psychological stress, lack of work, idle time during the pandemic can increase smoking, alcoholism and drug abuse. All these in turn can increase the risk for developing pulmonary tuberculosis and nCovid19 bronchopneumonia.[3] Many patients on treatment for tuberculosis had defaulted during the covid pandemic.[4] Covid 19 pandemic has led to disruption of TB services in India leading to delay in diagnosis, surveillance and management of tuberculosis.[5] People suffering from both TB and COVID-19 may have poorer treatment outcomes if TB treatment is interrupted.[6]

Diagnostic challenges may arise in patients with active pulmonary tuberculosis with nCovid19 bronchopneumonia and in patients who develop pulmonary tuberculosis following nCovid19 disease.[3] Pleural complication like pneumothorax and pleural effusion due to tuberculosis can present with acute onset of dyspnea which can be mistaken for nCovid19 symptoms. Symptoms of pulmonary tuberculosis like fever, cough, nonspecific chest pain, breathlessness, anorexia, fatigue and weight loss may be mistaken for symptoms of long covid and may be ignored. This may lead to delayed diagnosis of tuberculosis.

Gold standard for diagnosis of pulmonary tuberculosis is sputum examination and bacteriological confirmation.[1] Chest radiography is useful to detect early lesions in pulmonary tuberculosis and to diagnose smar negative cases.[1] Advantages of chest x ray include widespread availability, low cost, early identification of lesions and high sensitivity.[7] Hence chest x ray is a useful screening tool for pulmonary tuberculosis. Main disadvantage of chest x ray is low specificity. Many other diseases can mimic tuberculosis in chest x ray and vice versa. Active tuberculosis and post tubercular sequelae can't be differentiated with chest x ray.[8] Hence bacteriological confirmation is always preferred for definitive diagnosis of pulmonary tuberculosis. The outcome of chest x ray as a screening tool for pulmonary tuberculosis can be improved by training the healthcare providers in identifying the radiological abnormalities and triaging the patients who require further evaluation.

Some patients with pulmonary tuberculosis may not have significant respiratory symptoms suggestive of tuberculosis. Patients with advanced age, multiple comorbidities, poor general condition, neurological illness, psychiatric illness, other severe diseases, deaf mute and dementia may not report classical respiratory symptoms suggestive of pulmonary tuberculosis.[9] Pulmonary tuberculosis may be overlooked in presence of other respiratory diseases like nCovid19 bronchopneumonia. Careful evaluation of chest radiology will be useful in such patients. Normal chest x ray in the absence of any productive cough may rule out pulmonary tuberculosis in such patients.

All patients with nCovid19 bronchopneumonia usually undergo chest x ray. A careful evaluation of chest x ray should be done in all these patients for any lesions suggestive of pulmonary tuberculosis. Miliary tuberculosis can mimic nCovid19 bronchopneumonia in chest x ray. When diagnosis is uncertain high resolution chest CT scan may be useful in such cases.

In patients who undergo CT scan of the thorax during nCovid19 bronchopneumonia, a careful evaluation of chest

radiology should be done for possible coexistent pulmonary tuberculosis.

Susceptibility to develop pulmonary tuberculosis may increase following nCovid19.[2] Hence patients who had nCovid19 should be kept under observation for at least 6 months for any recurrence of respiratory symptoms. Further evaluation for pulmonary tuberculosis should be done in such patients in case of any symptoms. Chest x ray in such patients may show lesions suggestive of pulmonary tuberculosis (Fig. 1). A normal chest x ray in the absence of any sputum production in such patients may exclude active pulmonary tuberculosis.

Some patients may continue to have respiratory symptoms following nCovid19. They should be screened for pulmonary tuberculosis. A negative sputum examination and normal chest radiology will rule out pulmonary tuberculosis in such patients.

It should be remembered that atypical radiological manifestations of tuberculosis are more common in patients with diabetes, advancing age, chronic renal disease, chronic liver disease, immunosuppression, underlying malignancy, malnutrition and immunosuppressant medications.[9] Above are the risk factors for nCovid19 bronchopneumonia as well. Hence we suggest patients with any form of chest radiological abnormality should undergo sputum testing to exclude pulmonary tuberculosis.

Typical chest radiographic findings in pulmonary tuberculosis include unilateral or bilateral upper lobe lesions, cavity with surrounding infiltrates where cavity usually devoid of any air fluid level, patchy consolidation, fibro nodular lesions.[7] Typical chest radiographic findings in nCovid19 bronchopneumonia include bilateral, peripheral, lower lobe predominant patchy opacities. Lung parenchymal lesions in nCovid19 bronchopneumonia usually progress from ground glass opacities to crazy paving pattern and consolidation.[10] These

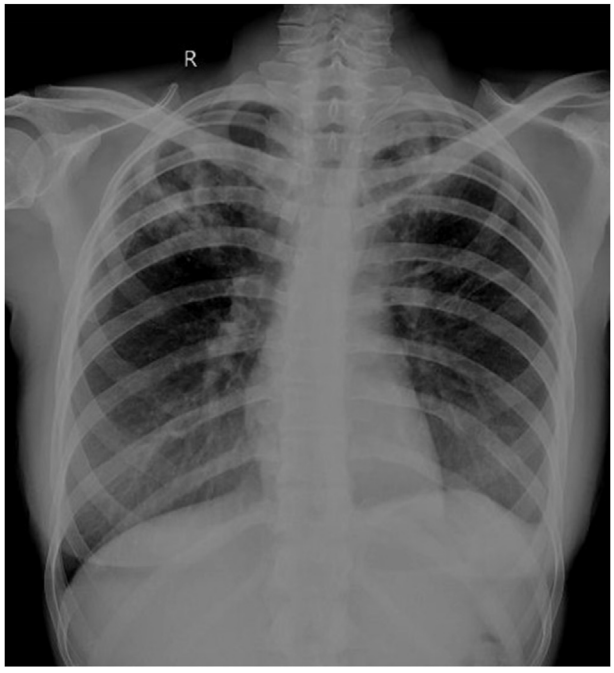

Fig. 1 – showing features suggestive of pulmonary TB in a patient who came with respiratory symptoms 4 weeks after.

lesions usually progress up to 10 to 14 days after which radiological resolution starts in patients who respond to medications. In the stage of resolution band like opacities, fibrosis, traction bronchiectasis and organising pneumonia occur in various combinations.[10] Hence pulmonary tuberculosis and nCovid19 bronchopneumonia can be differentiated when symptoms, clinical features and chest radiographic findings are typical.

But atypical chest radiographic findings are not uncommon in pulmonary tuberculosis. Atypical chest radiographic findings are more common in patients with diabetes mellitus and other immunosuppressive conditions. Atypical radiographic findings in pulmonary tuberculosis include predominant lower lobe lesions, consolidation, collapse (in endobronchial TB, Broncho stenosis), cavity with air-fluid level and cavity in lower lobes.[11] Lower lobe lesions in TB may mimic nCovid19 bronchopneumonia. Chest x ray may appear normal in endobronchial TB and early cases of parenchymal TB and early cases of nCovid19 bronchopneumonia. High resolution computerised tomography scan (HRCT) may be helpful in such cases. HRCT in early nCovid19 bronchopneumonia may show typical ground glass opacities which are usually bilateral, lower lobe predominant and sub pleural.[12] Tree in bud appearance may be seen in endobronchial TB. It should be remembered that patients with diabetes mellitus and other immunosuppressive conditions are more at risk to develop nCovid19 bronchopneumonia. Hence in patients with diabetes mellitus and other immunosuppressive conditions chest radiography may not be ideal to differentiate nCovid19 from pulmonary TB. Moreover, nCovid19 and pulmonary tuberculosis can coexist.

Cavity, intrathoracic lymphadenopathy and pleural effusion can occur in tuberculosis but are uncommon in uncomplicated nCovid19 bronchopneumonia. In some patients, atypical radiological features can occur in nCovid19. These include central involvement, sub pleural sparing, peribronchovascular involvement, isolated upper lobe involvement, nodular involvement, lobar consolidation, solitary involvement and unilateral lung involvement.[13] In such cases chest radiography may mimic pulmonary tuberculosis.

Interpretation of chest radiography should be always done with background history and clinical findings. Patients with nCovid19 bronchopneumonia usually give history of fever, upper respiratory symptoms which is followed by cough and breathlessness within 4–7 days.[14] Majority will show hypoxia. There may be history of similar symptoms in family members or contacts. Acute onset of breathlessness and hypoxia is uncommon in pulmonary tuberculosis. Pleural effusion, pneumothorax, miliary TB, extensive pulmonary TB, ARDS, pre-existing parenchymal lung disease and associated obstructive airway disease can lead to breathlessness in patients with pulmonary tuberculosis.[15]

During the stage of resolution in nCovid19 bronchopneumonia, which usually occurs after 14 days, band like opacities, fibrosis, traction bronchiectasis and organising pneumonia occur in various combinations.[10] These lesions may mimic tuberculosis radiologically (Fig. 2).

It should be remembered that chest radiography is not diagnostic in pulmonary TB as well as nCovid19 bronchopneumonia. Correlation of chest radiographic findings with

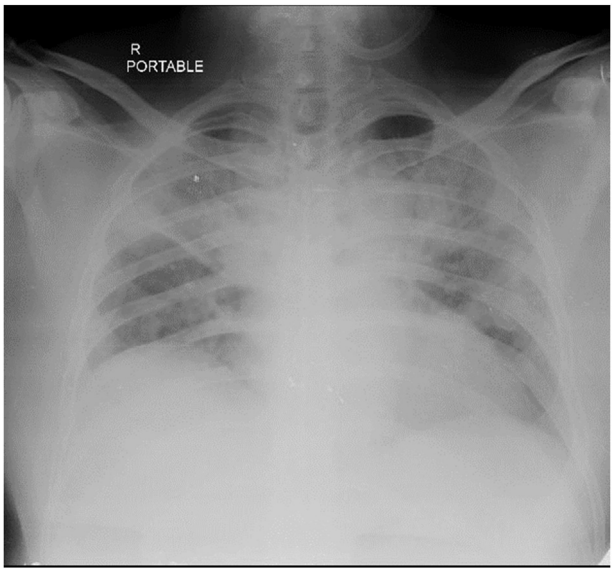

Fig. 2 – Showing upper lobe lesions in a patient with nCovid19 bronchopneumonia. Further evaluation proved the lesion was due to nCovid19 bronchopneumonia.

epidemiologic history, clinical presentation, and RT-PCR test results or in later stages antibody titres will help in confirming or excluding the diagnosis in suspected cases of nCovid19 bronchopneumonia.[10] In pulmonary tuberculosis definitive diagnosis should be established by bacteriological confirmation. Molecular diagnostic tools should be used to confirm or exclude tuberculosis in suspect cases as the results are rapid, accurate and reliable.[1]

Authors contributions

Dr. Vishnu sharma. M. - Conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. Dr. Vijay Kumar Arora. - Conception and design of the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. Dr. Anupama. N - Analysis and interpretation of data, drafting the article and revising it critically for important intellectual content.

Conflicts of interest

The authors have none to declare.

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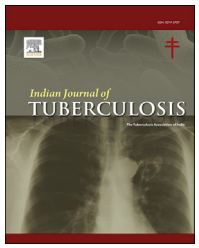

Review article

Changing paradigms in the treatment of tuberculosis

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ABSTRACT

Tuberculosis, caused by *Mycobacterium tuberculosis*, is a disease long dealt with, but still remains the second leading cause of death world-wide. The current anti-tubercular chemotherapy primarily targets the microbial pathogenesis, which however, is failing due to the development of drug resistance. Moreover, with fewer new drugs reaching the market, there is a need to focus on alternate treatment approaches that could be used as stand-alone or adjunct therapy and the existing drugs, referred to as Track II chemotherapy. This article is an attempt to review the changing global patterns of tuberculosis and its treatment. Further, newer drug delivery approaches like multi-particulate drug carriers which increase the therapeutic efficacy and bring down the systemic toxicity associated with drugs have also been discussed. There is also a need to use interventions which can be used as Track II therapy. Host-directed therapeutics (HDT) is an emerging area concept in which host cell functions and hence the response to pathogens can be modulated, which can help manage TB. HDT decreases damage induced due to inflammation and necrosis in the lungs and other parts of the body due to the disease. Various immuno-modulatory pathways have been discussed in this review which could be explored further to treat TB. An in-depth understanding of multi-particulate drug carriers and HDT could help in dealing with tuberculosis; however, there is still a long way to go.

1. Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*M. tb*) which was isolated in 1882 by Robert Koch, a German physician, who was also awarded Nobel Prize for this discovery. *M. tb* is one of the most successful bacterial human parasites in causing latent infection.[1] The bacteria can live on up to decades in a dormant state in hypoxic tubercles in the lungs, and drive recurrent and resistant forms of infection, thus posing an obstacle to full eradication of TB.[2] As recommended by World Health Organization (WHO), an initial intensive phase of four months involving administration of first-line drugs: rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ), and ethambutol (ETH) daily for two months is followed, which is further extended by a continuation phase of four months, in which only rifampicin and isoniazid are administered either daily or thrice weekly. For the treatment of multi-drug resistant (MDR) TB, WHO recommends combination therapy using second-line drugs combined with pyrazinamide, and new generation drugs like parenteral fluoroquinolone, ethionamide, prothionamide, cycloserine or p-

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aminosalicylic acid (PAS). However, these treatment regimens show a narrower therapeutic effect/toxic effect ratio compared to first-line anti-tubercular drugs. Therapeutic drug monitoring can visualize the fluctuations in patient responses and ensure the success of second-line therapeutics. The third-line treatment regimen has also been proposed to administer a combination of ethionamide, pyrazinamide, ofloxacin, and aminoglycosides. These third-line drugs are administered during the initial phase, followed by administration of ofloxacin and ethionamide.[3]

1.1. *Role of the World Health Organization*

TB, a major global health problem, causes ill-health among millions of people each year and is the second leading cause of death after human immunodeficiency virus (HIV). Globally, 10.0 million people (5.8 million men, 3.2 million women and 1.0 million children) were infected by TB in 2017 (Fig. 1). In 1993, despite the availability of short-course regimens of first-line drugs, WHO declared TB as a global health emergency.[4] South Africa, Swaziland, Lesotho, Namibia, Botswana, Mozambique, Zambia, Zimbabwe and Malawi are the nine countries which account for nearly 50% of the global burden of TB/HIV, with southern Africa being the epicentre of the dual epidemic.[5] TB remains one of the most typical causes of morbidity and the leading cause of death in HIV-infected adults in sub-Saharan Africa.[6] End TB program supports different countries across the globe to fill gaps in detection and treatment of TB, alongside, collaborating with 'Stop TB Partnership' and 'Global Fund to Fight AIDS, Tuberculosis and Malaria' programs for the initiative, 'Find. Treat. All'. The joint initiative has set a target for detecting and treating 40 million people with TB during the period 2018–2022.[7]

WHO developed the Directly Observed Therapy–Short Course (DOTS) strategy way back in the 1970's to control TB. DOTS is a five-component package comprising political commitment, diagnosis using sputum smear microscopy, a regular supply of first-line anti-TB drugs, short-course chemotherapy and a standard system for recording and reporting the number of cases detected by national TB control programs (NTPs) and the outcomes of treatment.[7]

In 2018, a high-level UN meeting attended by the heads of various governments and non-government agencies worldwide pledged their commitment to end tuberculosis globally by 2030 according to Sustainable Development Goal. The Sustainable Development Goal 3, Target 3.8 aims to achieve Universal Health Coverage (UHC) including financial risk protection, access to quality essential healthcare services and access to safe, effective, quality and affordable essential medicines and vaccines for all.

The End TB Strategy milestones for 2020 and 2025 can be achieved only if TB diagnosis, treatment and prevention services are provided within the context of progress towards universal health coverage (UHC). To achieve this goal, TB incidence rate should fall at a rate of 10% per year by 2025; along with a fall at the rate of 6.5% per year in the TB induced death rates during the same period. So, fundamentally UHC must be received by everyone, irrespective of their living standards, and that these health services do not cause financial hardship on the receiver.[7]

The development of new strategies for diagnosing and treating TB is underway but progressing at a languid pace. Currently, there are several new drugs, vaccine and treatment regimens in various phases of clinical trials. As per the annual reports published by Treatment Action Group, funding for TB research and development has been drastically increased, peaking at US$ 724 million in 2016.[8]

1.2. *Drug resistance*

Control of TB has been slowed down further by detecting drug-resistance (DR) for Anti-tubercular drugs (ATDs). Multi-DR (MDR) TB occurs when resistance to at least two main first-line ATDs (isoniazid and rifampicin) develops. Extensive-DR, also referred to as extreme drug resistance, is a case of MDR TB resistant to three or more of six classes of second-line drugs. The first of the totally drug-resistant tuberculosis patients (TDR-TB) was diagnosed in India. WHO defines TDR–TB as a virulent strain of tuberculosis that is resistant to all known treatments.[6]

Development of resistance to one or more first-line drugs is also a significant issue associated with TB. According to WHO report of 2017, approximately 5,58,000 people were resistant to rifampicin, the most effective first-line drug, and of these, 82% of the patients also had multi-drug resistance. TB has also emerged as a leading killer among HIV-infected people with compromised immune function. The majority of TB deaths occur in the developing world (countries of Latin America, Asia, and Africa), affecting mostly young adults in their most productive years, creating an adverse impact on the global

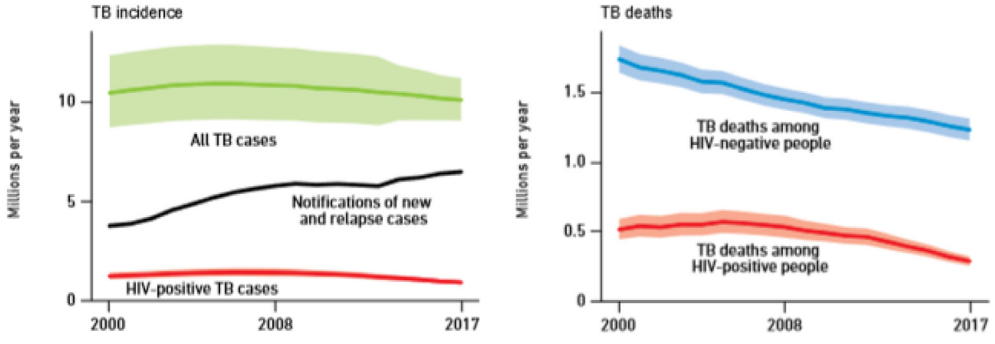

Fig. 1 – Global trends representing estimated number of incident cases and deaths due to TB (in millions) for the period 2000–2017. Shaded areas represent uncertainty intervals [Global tuberculosis report 2017 by W.H.O].[1]

economy.[3] Without treatment, the mortality rates are high, around 70% of pulmonary TB patients with sputum smear-positive and HIV-negative symptoms died within ten years. During the same period, the mortality rate amongst culture-positive (but smear-negative) patients was 20%.[5]

2. Pathogenesis of tuberculosis

M. tb is a gram-positive aerobic, rod-shaped acid-fast bacillus, with a lipid cell wall that allows it to survive in hostile environments and provides the organism with a resistant barrier against many conventional drugs. Infection from *M. tb* occurs through inhalation of droplet nuclei containing *M. tb*, generated when patients having pulmonary or laryngeal TB cough, sneeze, shout or sing. These particles remain airborne in the form of a mist which upon inhalation traverse mouth or nasal passages, and through the upper respiratory tract and bronchi reach lung alveoli. However, larger particles containing the bacilli settle faster and do not serve as useful vehicles for transmitting infection. Somehow, if the particles reach the respiratory tract, they are expelled via mucociliary blanket.

The bacteria infecting the lungs cause pulmonary tuberculosis. Those moving to other body parts cause extrapulmonary tuberculosis such as cerebral, abdominal, lymphatic, genitourinary, bone and joint, miliary and meningeal tuberculosis (TBM). TBM generally affects children less than five years of age.[9] Besides children, patients co-infected with HIV, are at high risk of TBM, and as the studies indicate, TBM is also proposed as a precursor for initial presentations of AIDS.[10,11] According to a survey, 13% to 23% mortality rate was observed in children with TBM and in HIV co-infected group, it was around 79%.[12] The mycobacterium can remain in "dormant state" inside in granulomas for years, varying the risk of developing active disease with the time of infection, age, and immunity of the host organism.[13] However, the lifetime risk of illness for a newly infected young child has been estimated at 10%.[14]

2.1. Role of endogenous phagocytes

After inhalation, *M. tb* resides in deep lung alveoli space primarily within the alveolar macrophages, alveolar pneumocytes and dendritic cells. These cells serve as bio-reservoirs for the bacteria and; from here trafficking of mycobacteria to lymph nodes and bloodstream occurs, resulting in extra-pulmonary dissemination of infection.[15] Macrophages also play a significant role in initiating and maintaining sustained immune response that suppresses, but fails to eradicate *M. tb*. The host response involves many cellular immune system limbs but predominantly consists of *M. tb* specific Th1-type IFN-γ–secreting CD_4 and CD_8 effector T-cells. The clinical manifestations of the activated immune response are suppressing effecter response to *M. tb* antigen-specific T-cells by the subsets of cells infected with *M. tb*. So, patients with active TB have reduced *M. tb* antigen-specific IFN-γ production compared to healthy subjects.[16] Also, *M. tb* secretes molecules that prevent phagosome (containing the bacteria)-lysosome (granules present in the cell cytoplasm) fusion, which occurs following phagocytosis of microorganisms within the macrophages. The fusion of phagosome-lysosome is also responsible for oxygen-dependent and the oxygen-independent killing of bacteria, wherein the enzymes and cationic peptides present in the granules play a significant role.[17]

2.2. Mechanism of extrapulmonary dissemination

The deep-seated alveolar tissues provide bacteria with a less hostile niche than macrophages for bacterial replication and allow them to undergo cell–cell spreading through the epithelial monolayer.[18] The bacteria interact with the alveolar epithelium and generate local inflammatory actions by accumulating various immune cells. *M. tb* can invade both alveolar epithelial cells and polarized alveolar epithelial cells. The passage through the epithelial cells is a normal phenomenon, but, the movement through polarized alveolar epithelial cells occurs when the bacteria develop an invasive property after a span of intracellular stay either in macrophages or alveolar epithelial cells. The enhanced efficiency is the result of a change in phenotype within the bacterial cells. These findings have been supported by similar studies suggesting a five-to nine-fold increase in invasive efficiency of *Mycobacterium avium* present in the monolayers of epithelial cells or macrophages. The mechanism of movement of *M. tb* in the body has been shown in Fig. 2.

Alveolar epithelial cell translocation is facilitated by Heparin-binding haemagglutinin adhesin (HBHA), which helps mycobacterium bind to sulfated glycoconjugates on epithelial cells, for endocytosis and transcytosis across the epithelial cell layer.[19] HBHA is an essential bacterial factor involved in the extra-pulmonary dissemination of *M. tb* through interaction, primarily, with alveolar epithelial cells. The mycobacteria can also invade and traverse the monolayers by an alternative process involving actin cytoskeletal rearrangements.[20]

Granulomas are formed within the lungs by the macrophages, in which the pathogens remain in "dormant state" for years.[21] In human beings, the granuloma consists of a central necrotic core surrounded by concentric layers of macrophages, T- and B- lymphocytes, neutrophils, epithelioid cells, foamy macrophages, multinucleated Langerhans giant cells and extracellular matrix components. The bacteria utilize the granuloma as foci to recruit uninfected macrophages and disseminate infected cells to distant sites.[22]

Initially, the granuloma is a rigid mass. Still, after 10–12 days of infection, bacilli inside the granuloma are attacked by activated T-cells (CD_4^+ helper T-cells) interferon IFN-γ and CD_8^+ suppressor T-cells directly), which degrade to form a cheese-like liquefied material called caseum. Caseum is a soft mass characterized by the presence of lipids, a large concentration of nuclear debris, and dead macrophages and eosinophils. *M. tb* remains dormant within granuloma but may replicate exponentially under favourable conditions.[23]

Dendritic cells are also involved in spreading *M. tb* and actively transport antigens from the periphery to secondary lymphoid organs to primate the adaptive immune system. As the dendritic cells are less efficient in killing mycobacteria, they provide an environment better than that of macrophages for intracellular survival and growth.[24] Also, dendritic cells containing *M. tb* play a vital role in priming IFN-γ to produce T-

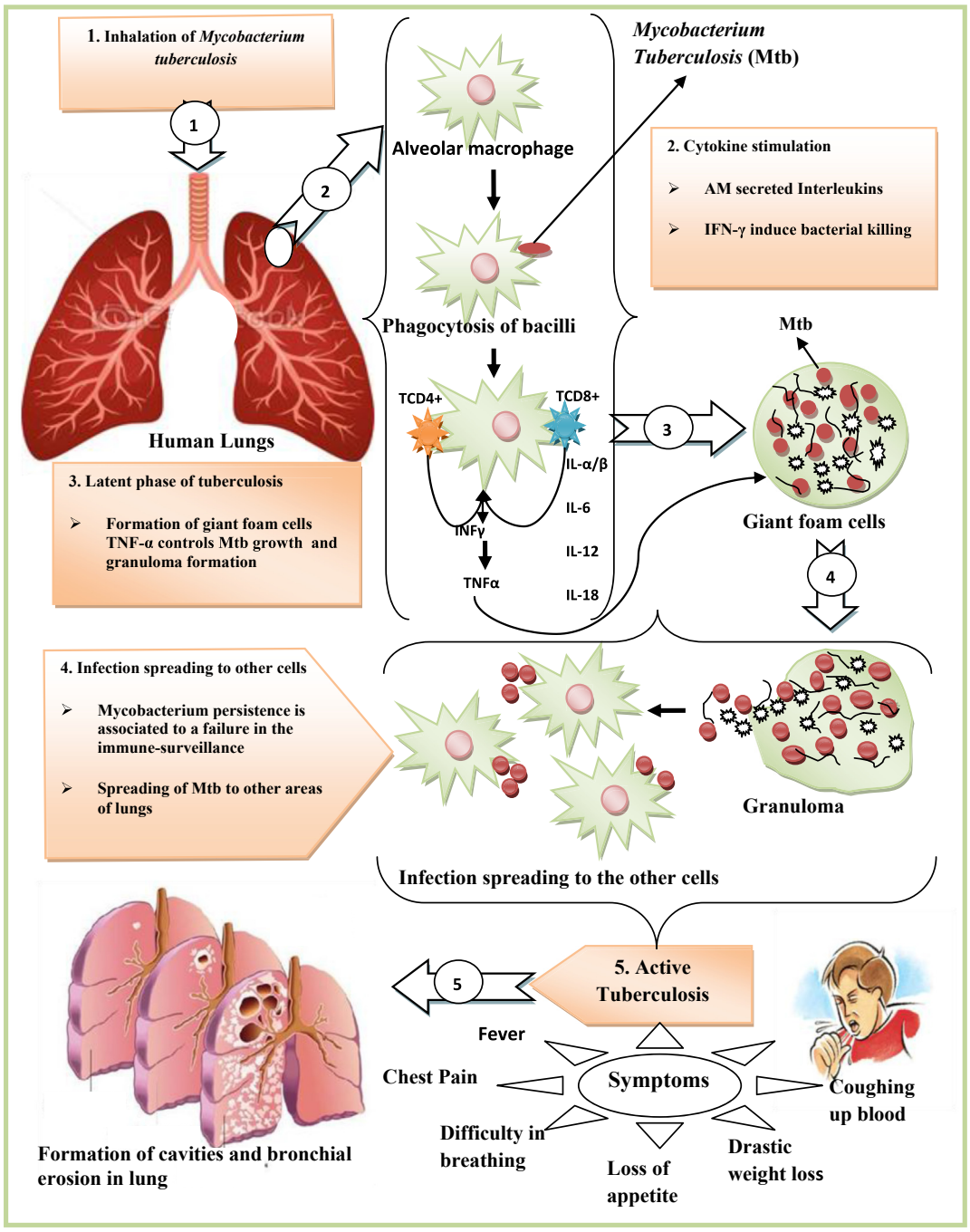

Fig. 2 – Schematic representation of series of events happening in human body due to infection by *Mycobacterium tuberculosis*.

cells and initiate the cell-mediated response to infection.[25] The dendritic cells also, facilitate circulation of bacteria via lymph nodes and blood.[3] Dissemination triggers T-cell-mediated immune response much before it has been controlled by the adaptive immune response.[26] Before dissemination to cervical lymph nodes, hilar and mediastinal lymph nodes are infected, subsequently forming caseous pus, abscess and finally sinus. Rupture of the caseous focus results in pus and mycobacterium's effusion into the pleural space causing empyema thoracic.[13]

In HIV-TB co-infection, the central nervous system's involvement is five times higher in seropositive than in sero-negative patients.[27] HIV targets and depletes CD_4^+ T-cells and humans with advanced HIV infection and low peripheral blood CD_4 counts (<50 cells/mm^3) are potentially at higher risk of persistent bacteremia with *M. tb* and other environmental mycobacteria.[28] The release of tumour necrosis factor (TNF-α), though at elevated levels in TB, is suppressed in HIV–TB co-infection, leading to the impairment of TNF-α mediated macrophage apoptosis. Apoptosis is a host defence response

to *M. tb* infection, impairment of which leads to higher susceptibility of *M. tb* in TB- HIV co-infection.[29] Moreover, the risk of dying from other opportunistic infections is drastically increased in HIV-TB co-infection.

2.3. *Conventional treatment methods*

Treatment regimen as recommended by WHO consists of first-line chemotherapeutic agents, isoniazid, rifampicin, and pyrazinamide (Fig. 3). Alongside a fourth drug, usually ethambutol (ETH) is administered. Ethambutol can be substituted with streptomycin during the initial phase of treatment if the organism is susceptible to the former.

Resistance to one or more first-line drugs is a significant obstacle in the treatment of TB. MDR-TB (resistance to isoniazid and rifampicin) cases are treated with more toxic, more costly, and less effective agents than first-line drugs. Pyrazinamide is generally used in drug-resistant cases to increase the therapeutic effectiveness of the regimen. In the case of non-responsiveness to first line treatment, second-line drugs, aminoglycoside antibiotics (amikacin, kanamycin, and streptomycin), cycloserine, ethionamide and fluoroquinolones (levofloxacin and moxifloxacin) are administered to patients.[30] As a preventive therapy or measure against tuberculosis, bacille Calmette -Guérin (BCG) vaccine is administered to children. Though a century old, the vaccine is still widely used and boosts the immune system to protect children from tuberculosis meningitis during the first five years. Further, it has been observed that in children affected with tubercular meningitis, the vaccinated children respond better to anti-tubercular drugs in comparison to the unvaccinated group.[27]

The current treatment regimen of Mycobacterium infections is complicated, requiring long and strict schedules of multi-drug regimen, which results in lower compliance, therapeutic failure and development of multi-drug resistance.[31,32] Moreover, other factors such as socio-economic conditions (poverty, crowding, malnutrition and poor hygiene conditions) also contribute to poor treatment outcomes.

Also, the current therapy cannot deliver sufficient concentrations of antimicrobials within the intracellular compartment where the mycobacteria reside. The course of tuberculosis therapy should necessarily take care of some facts such as (a) regimens should ideally have a low level of toxicity for long-term systemic administration, and (b) drugs should also be able to penetrate and target the intracellular environment in which the tubercle bacilli are found.[33]

3. Therapeutic potential of nanocarriers in the treatment of TB

In view of the challenges being faced by the current treatment methodology used for TB, alternative methods, as mentioned

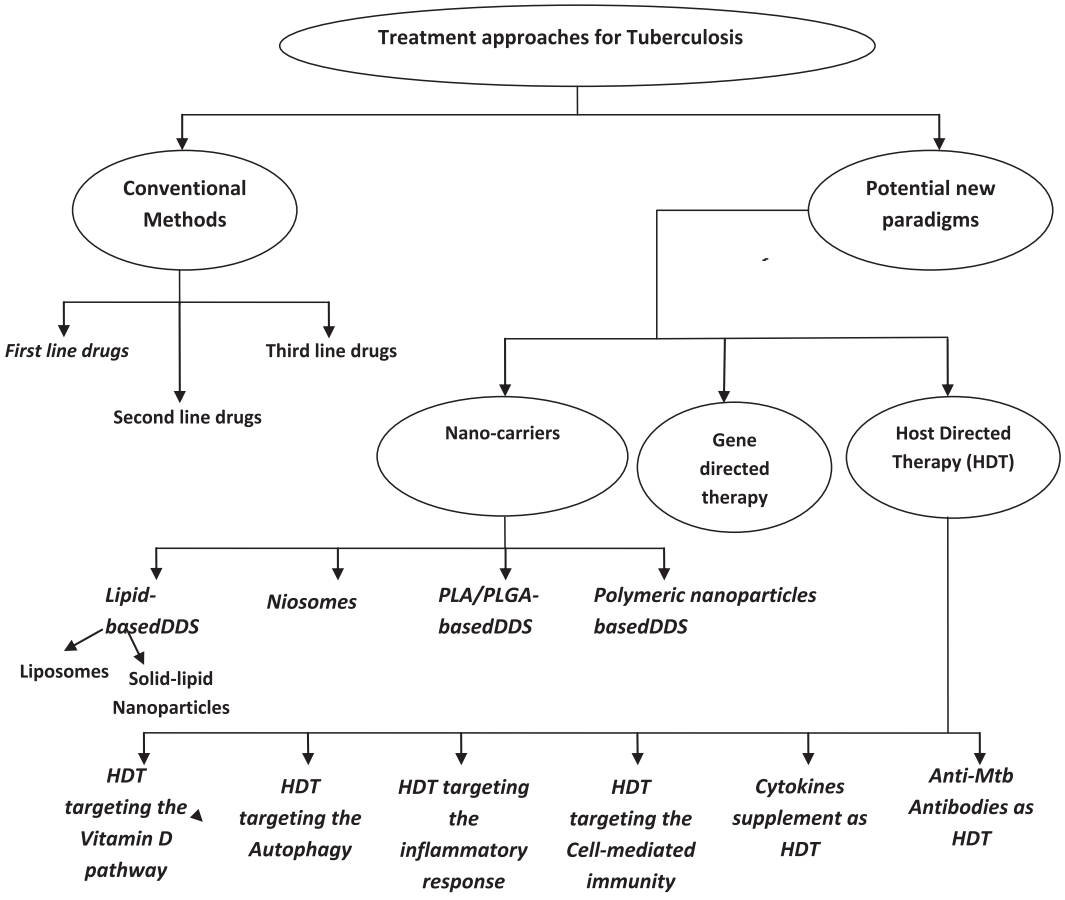

Fig. 3 – Conventional and emerging drug delivery approaches for the treatment of Tuberculosis.

in Figs. 3 and 4 can be potentially used to treat TB. These drug delivery approaches offer targeted treatment benefits, improved bioavailability, reduced systemic toxicity, reduced local irritation, predictable gastric emptying, and enhanced pharmacokinetic behaviour compared to conventional (monolithic) formulations.[34] In specific, nanocarriers' sub-cellular and sub-micron size facilitates uptake and sorting into different intracellular compartments or cell organelles such as macrophages, dendritic cells, etc.[35] Phagocytes take up these drug carriers by endocytosis and upon intra-lysosomal degradation; drugs are released from these carriers in the cytosol. For the treatment of tuberculosis, sequestration of carriers containing ATDs into phagocytic cells containing the bacteria co-localizes the drugs with bacteria, thereby increasing treatment efficacy (Fig. 5). The first report on evaluating nanoparticulate-based delivery systems loaded with anti-tubercular drugs isoniazid, rifampin, and streptomycin was published by Anisimova et al in 2000.[36]

3.1. *Polymeric nanocarriers*

Dry powder inhalation of spray-dried poly-lactic acid (PLA) microparticles with high payloads of isoniazid and rifabutin demonstrated micro particles' delivery to macrophages and not epithelial cells, upon inhalation by mice. The drug concentrations in macrophages were approximately 20 times higher as compared to when the drug solution was administered.[37] This research group also studied the defence strategies initiated in macrophages when microparticles were administered. The microparticles were able to target lung macrophages *in vivo*, induce intense Golgi activity in the vicinity of micro particle-containing phagosomes, activate

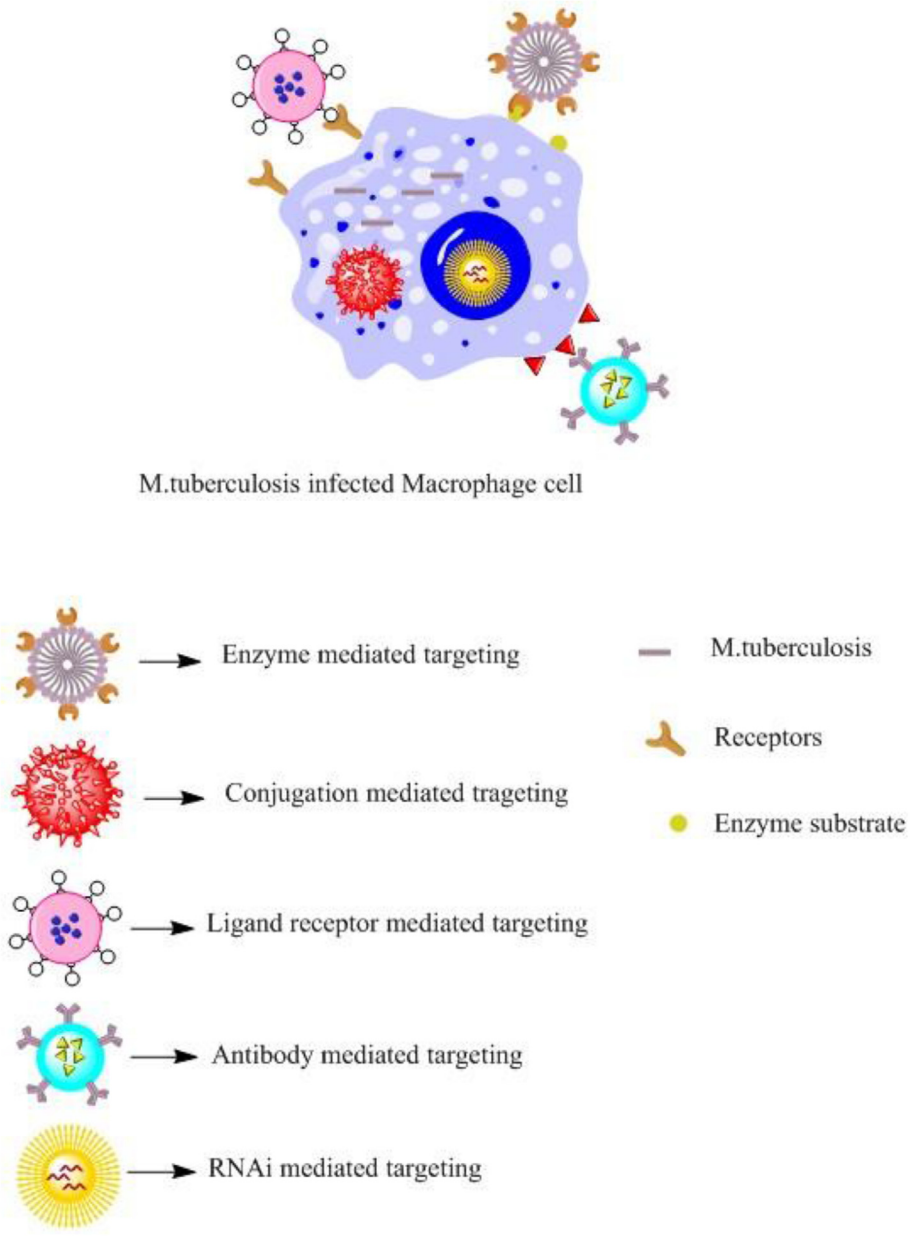

Fig. 4 – Alternative nanocarrier mediated treatment of Tuberculosis.

NADPH oxidase and enhance nitric oxide production by infected macrophages, and also, induce secretion of tumour necrosis factor-α by macrophages during infection.[38] Further, it was found that alveolar macrophages migrate to secondary lymphoid organs after taking up particulate material in the lungs indicating that mycobacteria disseminate to the bloodstream (hematogenous dissemination) but also to other sites in the body.[39] Similar results were observed by another research group when PLA microparticles of an ionizable prodrug of INH, isoniazid methanesulfonate (INHMS), were administered intra-tracheal installation to rats. A substantial reduction in acetyl isoniazid (AcINH) blood levels, a prominent and potential toxic metabolite of INH was observed during the study.[40] Tween-based microemulsion systems of rifampicin prepared from oleic acid, phosphate buffer, Tween 80 and ethanol have also been studied as a potential drug carrier of anti-TB drugs.[41]

Pandey and co-workers have extensively studied single or multiple encapsulated anti-tubercular drugs for their therapeutic efficacy compared to a free drug suspension. PLGA NPs of RIF, INH, and PYZ administered orally to guinea pigs showed considerably fewer lung lesions characterized by discrete foci of fibrosis with minimal lung parenchymal involvement. Especially, in mediastinal lymph nodes (MLN) extensive bacterial clearance was observed, showing the efficiency of PLGA NPs to sterilize MLN, which can otherwise act as reservoirs for bacterial distribution.[42] PLGA NPs containing rifampicin, isoniazid, pyrazinamide and ethambutol studied in rat tuberculosis model, showed sustained drug levels in plasma for five days and in organs (lungs, liver, and spleen) for 7–9 days, resulting in higher half-life and mean residence time of NPs; and increase in relative bioavailability of ATDs. Further, no hepatotoxicity was observed on repeated administration of the formulation.[43] An overall increase in rifampicin and isoniazid bioavailability was observed when PLGA nanoparticles of these drugs were administered via intraperitoneal route. Besides exhibiting sustained drug release pattern, enhanced intracellular drug concentrations were observed, due to uptake by peritoneal macrophages.[44] Biodistribution studies of ATDs delivered through oral route showed intense intracellular concentrations' of ATDs within alveolar macrophages.[45]

Nanoparticles (NPs) prepared using poly-n-butyl cyanoacrylate, and poly-isobutyl cyanoacrylate exhibited an average particle size of 250 nm and showed accumulation in human monocytes with significant antimicrobial activity. Even the second-line ATDs have shown enhanced therapeutic potential when delivered as nanoparticles. Pharmacokinetic evaluation of ciprofloxacin-loaded poly-isobutyl cyanoacrylate nanoparticles administered as intravenous infusion to rabbits, showed increase in AUC, half-life, and V_d; and a decrease in clearance of drugs in comparison to drug solution. Also, NPs showed better antimicrobial activity against *Mycobacterium avium complex* (MAC) in human macrophages compared to drug solution.[46] Dry powder aerosol of nano-ciprofloxacin administered as monodisperse porous PLGA particles (~10–15 μm) exhibited smaller particles' aerodynamics and showed significant deposition in infected lung compartments along with the sustained release of drug up to 4 weeks. The larger particles, however, resisted uptake by alveolar

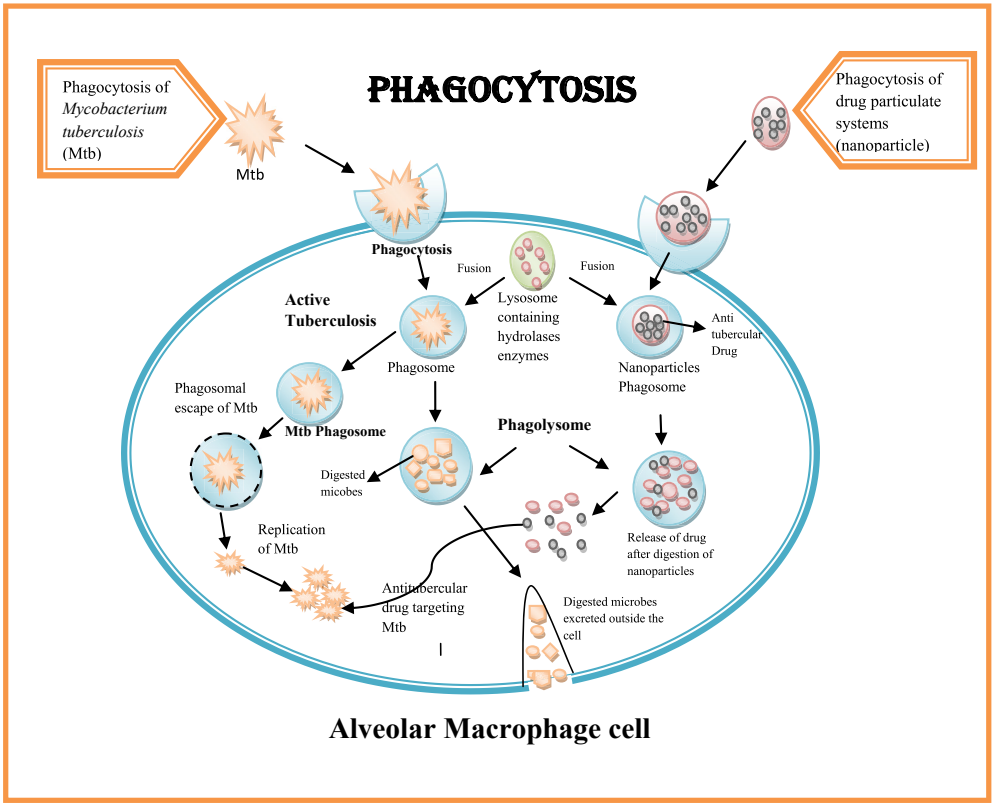

Fig. 5 – Schematic representation of phagocytosis of mycobacterium and nanoparticles by the alveolar macrophages.

macrophages.[47] Reduction in the minimum inhibitory concentration of drugs has also been reported by the use of NPs. Cyanoacrylate nanoparticles of moxifloxacin inhibited *M. tb* at one-tenth of the concentration (1 μg/ml) required for its inhibition by free moxifloxacin. The results of the study also indicated three-time higher accumulation, and six-time more extended residence of NPs in the cells in comparison to free moxifloxacin at the same extracellular concentration.[48]

Nanoparticles prepared from natural polymers have also shown promising results. Alginate nanoparticles of first-line ATDs prepared by cation-induced gelification process demonstrated high drug encapsulation efficiency (70%–90%). A single oral dose of alginate nanoparticles maintained therapeutic drug concentrations in plasma up to 11 days and in organs (lungs, liver, and spleen) for nearly 15 days. In contrast, free drugs were cleared from plasma/organs within 12–24 h of administration. Three oral doses of formulation given fortnightly, resulted in complete bacterial clearance from the organs, compared to 45 regular doses of orally administered free drugs.[49] Gelatin nanoparticles of RIF demonstrated 6-, 2.5- and 3- fold accumulation in lungs, liver, and spleen, respectively. Also, approximately 2- fold reduction in colony-forming units (CFU) of mycobacteria in lungs and spleen was observed for the nanoparticulate drug.[50]

Negligible cytotoxicity of PLGA microparticles of RIF towards alveolar epithelial cells and higher intracellular concentrations compared to free RIF has also been reported by another research group.[51] Besides, multi-particulate carriers can initiate various innate bactericidal responses, including induction of free radicals, alteration of mitochondrial membrane potential and apoptosis. Koch's dictum for curing tuberculosis: "*stimulate the phagocyte*" is very well justified by the use of multi-particulate carriers.[52]

Poly (lactic-co-glycolic acid), which is a biodegradable polymer, gets hydrolyzed in the body to form lactic acid and glycolic acid (endogenous molecules), which are readily metabolized via Krebs cycle, thereby causing minimal systemic toxicity.[53] PLGA can be taken up by the mononuclear phagocyte system from the bloodstream and transported to the liver, bone marrow, lymph nodes, spleen, and peritoneal macrophages. Moreover, opsonin proteins present in the body bind to these nanoparticles and facilitate phagocytosis.[54] As a result, PLGA is widely being used as a carrier material (drug carrier) to administer drugs via different routes. In contrast to the free drug, encapsulated drugs are safer and non-toxic; and show higher accumulation in macrophage rich organs like liver, lungs, and spleen.

3.2. *Lipid-based nanocarriers*

Lipid-based carriers can also be taken up readily by phagocytes, resulting in higher intracellular drug concentrations.[55] Liposomes of usnic acid, a secondary lichen metabolite, prepared using soya phosphatidylcholine, cholesterol, and stearyl amine/phosphatidic acid (7:2:1 molar ratio), showed improved intracellular uptake and duration of stay (up to 30 hours) inside the macrophage. Also, up to two-times reduction in minimal bactericidal concentration (MIC) of usnic acid when encapsulated in liposomes (MIC:16 μg/ml) was observed in comparison to the unencapsulated drug (MIC: 32 μg/ml). An overall increase in the anti-mycobacterial activity of usnic acid was observed on liposomes' encapsulation.[56]

Egg phosphatidylcholine liposomes of RIF, surface grafted with tetrapeptide tuftsin (a macrophage activator) were found to be 2000 times more effective than the free drug in lowering the load of lung bacilli in infected animals when administered twice weekly for two weeks.[57] Liposomes of RIF-containing maleylated bovine serum albumin, and alveolar macrophage-specific ligand delivered as pressurized aerosols showed 1.5–1.8 times higher airway penetration efficiency and also, greater drug accumulation in lung macrophages as compared to plain drug and placebo liposome-based aerosols.[58] Site-specific delivery of liposomal pyrazinamide delivered parenterally has also been demonstrated, resulting in reduced intracellular bacteria.[59]

Liposomes containing a wide variety of second-line ATDs drugs such as streptomycin[60] rifabutin,[61] ofloxacin/clarithromycin,[62] resorcinomycin A,[63] sparfloxacin,[64] capreomycin[65] and azithromycin[66] have also exhibited better efficacy in terms of bacilli clearance as compared to free drugs for the treatment of *M. tb.* Infections. Research has suggested that surface modification of aerosolized liposomes with 4-aminophenyl-α-D-mannopyranoside (mannose) increases uptake of liposomes in alveolar macrophages and various formulations for mannose specific receptors have been designed.[67]

Solid lipid nanoparticles of rifampin for oral administration prepared using cetyl palmitate as lipid concentrated at the centre and; tween 80 and poloxamer 188 as surfactants were found to be eightfold more efficacious than RIF solution against *Mycobacterium fortuitum*.[68] Rani et al prepared niosomes of rifampicin and gatifloxacin by lipid hydration technique. Studies have shown that the bactericidal effect is enhanced by the BACTEC radiometric method for resistant strains of *mycobacterium tuberculosis* (RF 8554) and susceptible strains (H37Rv).[69]

4. Potentials of gene-directed targeted therapeutics in the treatment of TB

Many research groups explored gene-directed therapy for detection and treatment of mycobacterial infections (Fig. 3). Different genes have been reported to play a significant role in the pathogenesis of tuberculosis, and scientists have used them as potential targets for TB treatment.[70–73] Kumar et al, performed enzyme-linked immunosorbent assay (ELISA) to evaluate the serodiagnostic efficacy of 30/32-kDa mycolyl transferase protein complex (Ag85 complex) and proteins ESAT-6 and CFP-10 (which are specific to mycobacterium tuberculosis).[74] *Mycobacteria* secretes 30/32-kDa mycolyl transferase protein complex also known as antigen 85 complex (antigen 85A (32A), antigen 85B,[29] and antigen 85C (32B)) which is involved in the catalytic transfer of mycolic acid from

one trehalose-6-monomycolate to another leading to the formation of trehalose-6,6′-dimycolate and one free trehalose.[70] Early secreted antigenic target 6-kDa, i.e. ESAT-6 plays a vital role in the virulence of *M. tb*. ESAT-6 and its complex with the chaperone culture filtrate protein (CFP-10) modulate the immune responses (both innate and adaptive) of the host.[71] Kumar et al reported that 30/32-kDa mycolyl transferase protein complex showed higher sensitivity (84.1%) as compared to ESAT-6 (64.9%) and CFP-10 (66%). It was observed that the specificity was almost similar for all the three (330/32-kDa mycolyl transferase- 85.2%; ESAT-6- 88.9%; and CFP-10-85.2%) tests. Diagnostic potential of Ag85complex was further evaluated through immunoblot analysis by the same research group, and reported that clinical isolates and H37Rv strain of *mycobacterium tuberculosis* exhibited strong reactivity to sera collected from individuals infected with *mycobacterium tuberculosis* to Ag85 complex.[74] Another research group explored the therapeutic potential of 30/32-kDa mycolyl transferase protein complex in the treatment of TB. Harth et al, silenced the genes encoding 30/32-kDa mycolyl transferase protein complex by sequence-specific antisense oligonucleotides technology. They targeted different sites of the protein complex by phosphorothioate-modified oligodeoxyribonucleotides (PS-ODNs). They suggested that once or weekly administration over the 6-wk observation period of single PS-ODNs (targeting one of the three transcripts of 30/32-kDa mycolyl transferase protein complex) inhibited the growth of bacteria by 1 log unit and a combinatorial approach consisting three PS-ODNs targeting all the three transcripts of 30/32-kDa mycolyl transferase protein complex inhibited the growth of bacteria by 2 log units. The research group also concluded that the weekly administration of PS-ODNs for a 6-wk period was more effective in silencing the genes than a single time administration.[70]

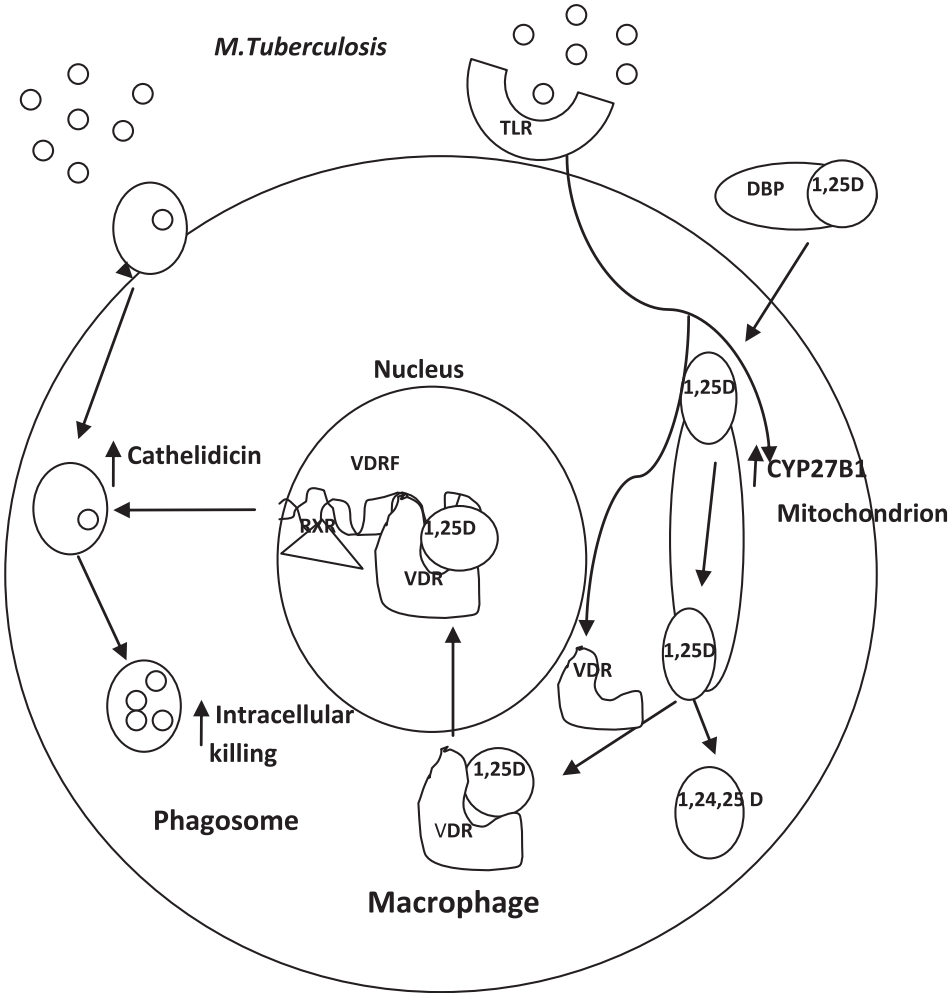

Fig. 6 – Vitamin D-triggered pathway for stimulation of the innate immune system in macrophages; activation of toll-like receptors (TLR) on the macrophage membrane due to the attack of *M. tb* is required to enhance the expression of CYP27B1 and vitamin D receptor (VDR), which gradually synthesizes 1,25D and VDR. The VDR-dependent pathway helps to up-regulate the cathelidicin expression, which has an antimicrobial property to destroy the invading pathogen inside the phagosomes.[2]

Table 1 – Host directed therapeutics for the treatment of tuberculosis.

HDTs compound	Host target	Mechanism of action	Effect	References
Autophagy Lithium, Valproic acid, Prochlorperazine	Autophagy	Reduction phosphorylation of mTOR and PI3-Kinase	Inhibition TBK-1, reduction TNF-mediated tissue damage	71
Rapamicin	Autophagy	Inhibition of mTOR	Promotion of Autophagy	72
Vitamin D3	Autophagy via cathelicidin	Stimulation of VDR (Vitamin D receptor) to induce cathelicidin expression; upregulation the expression of Atgs and Beclin-1	Immunomodulation and direct antimicrobial activity	73
Metformin	Autophagy	Reduction phosphorylation of mTOR and p70s6k	Induction of mitochondrial ROS, phagolysosome fusion and increase MTB-infected cell apoptosis	74
Immunometabolism statin	Cholesterol	Inhibition of	Promotion phagosomal maturation, phagolysosome fusion and increase MTB-infected cell apoptosis	75
Aspirin	Eicosanoids	Unselective COX-inhibitor	Inhibition of bacterial DNA synthesis	76
Diclofenac	Eicosanoids	Unselective COX-inhibitor	Inhibition of bacterial DNA synthesis	77

5. Targeting secondary pathways involved in the pathogenesis of TB- new insights in the treatment

5.1. *Host-directed therapeutics (HDTs)*

Besides working on developing novel drug carriers, the focus of various research groups is also shifting towards novel treatment strategies in anticipation of overcoming obstacles faced by antibiotic therapies for TB. The potential host-directed therapeutics can be foreseen as Track II strategy to address the issues related with the current therapeutics of TB (Fig. 3). The aim of HDTs is to curtail the course of treatment, diminish several agents required in combination drug therapy, simplify the treatment of drug-resistant TB by improving the efficacy of second-line treatment, and/or preserve lung function of TB patients.[75]

HDT is a promising treatment strategy for managing MDR- and XDR-TB which operates by directly modulating host cell functions; thereby preventing the development of drug resistance by *M. tb*. In cases of co-infection of MDR- and XDR-TB and HIV, host-directed therapy can be of immense potential. HDT drugs modify the antimicrobial activities of host immune cells and reduce the inflammation and tissue damage associated with TB.[76] Various host-directed therapeutic approaches that have gained considerable research interest as an adjunct to antibiotic-based anti-TB treatments have been shown in Table 1.

5.1.1. *Vitamin D receptor signalling as HDT*

The vitamin D receptor (VDR) signalling has been most widely studied in the context of autophagy and host defence (Fig. 6). VDR signalling facilitates antimicrobial host defences against *M. tb* infection through innate immune activation. Research shows that activation of VDR signalling leads to the induction of cathelicidin, a cationic antimicrobial protein that helps kill *M. tb*.[77] Further studies have shown that 1, 25-D3 enhances antibacterial autophagy activation and the elimination of intracellular *M. tb* in human monocytes/macrophages via cathelicidin induction and autophagy gene activation. The TLR signalling activation during *M. tb* infection triggers a complicated intracellular signalling pathway that induces the expression of the Cyp27b1 gene (1α-hydroxylase) in human monocytes/macrophages, thereby activating functional VDR signalling to amplify cathelicidin induction and antimicrobial responses.[78] 4-phenylbutyrate (PBA), or in combination with vitamin D3, helps to overcome the *M. tb*-induced inhibition of cathelicidin LL-37 expression and facilitates anti-mycobacterial effects.[79]

5.1.2. *Effect of iron in the inhibition of lysosome formation*

Iron is an auxiliary factor for the action of *M. tb*-encoding enzyme; it is involved in electron transport and oxidative metabolism; and it is also required for the synthesis of amino-acids, pyrimidine nucleotides and a series of nutritional and genetic substances. A natural resistance-related membrane protein 1(Nramp1) is primarily expressed in the membranes of late endosomes, phagosomes, and lysosomes of macrophages. Nramp1 is responsible for an early microbicidal

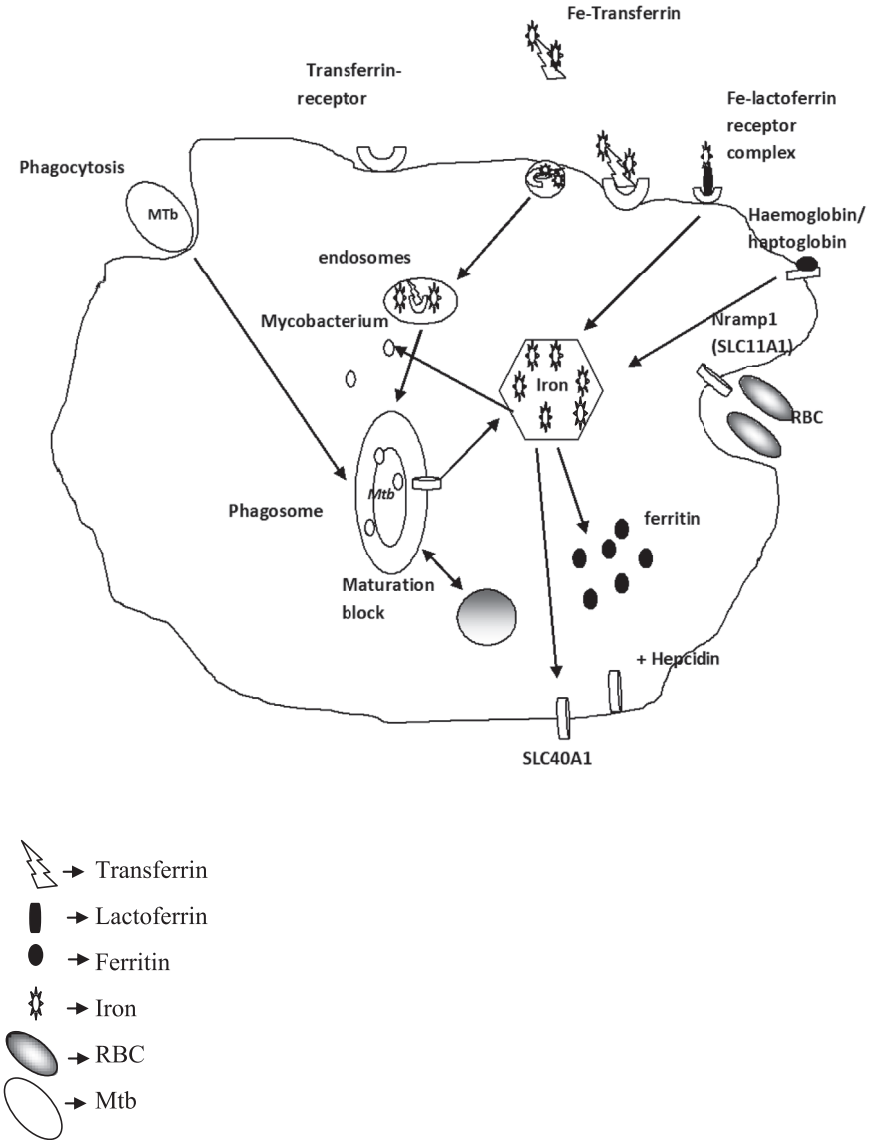

Fig. 7 – Schematic representation of iron metabolism in *Mtb* infected macrophage. Presence of *Mtb* limits the maturation of early phagosome to late phagosome and phagolysosome. Red blood cells containing Heme-bound iron are the primary source of iron for macrophages. Iron is also taken up via the receptor CD163 (hemoglobin scavenger) which binds with hemoglobin-haptoglobin complex. Further, transferrin and lactoferrin get bound to iron present in the cytoplasm and integrate to form proteins like ferritin (iron storage protein). SLC40A1 (ferroportin 1) is a transporter protein which expels the iron from the cell at low serum concentration of hepcidin; hepcidin binds to ferroportin 1 when serum hepcidin is high in presence of surplus iron or during inflammatory condition, then the iron export is barred due internalization of the ferroportin-hepcidin complex, which leads to the iron-retention inflammation. Iron-bound transferrin binds to the transferrin receptor present on the cell surface and during early endosome this complex gets internalized releasing iron from the complex at low pH, allowing the recycling of iron-free complex to the cell surface and separated iron get delivered into the cytoplasm via transporter-like SLC11A2 (DMT1). Inside the phagosomes, *Mtb* can get iron either from the cytoplasm or from the iron-transferrin/transferrin receptor complex through interaction with the early endosome. The entrapment of iron by *Mtb* is due to mycobacterial siderophore system made of mycobactins ([3]with modification).

mechanism which provides resistance to several intracellular microorganisms, including *M. avium* and *M. bovis* (bacille Calmette-Guérin [BCG]) but not *M. tuberculosis*. The substitution of G169D renders NRAMP1 nonfunctional, increases the susceptibility to these infections. The expression of Nramp1 increases with the stimulation of IFN-γ, which facilitates the transport of ferrous iron across the cell membrane and limiting the access of *M. tb* to iron and resisting the invasion of extracellular pathogenic bacteria from pumping iron out of the phagosome into the cytosol. Accordingly, host cells can

prevent the growth of *M. tb* by limiting their iron intake and exogenous iron required for developing *M. tb*; this is accomplished by the activation of Rab5 and via the mediation of the transferrin receptor. Nramp1 of *Leishmania*, *M. tb* and other pathogens which resist their invasion by inactivated macrophages in alveoli lead to weak antibacterial activity, but it cannot inhibit the growth of *M. tb*. *M. tb* can be transported to other places and present antigens, sensitizing the surrounding T lymphocytes. The Sensitized lymphocytes produce multiple lymphokines, such as IL-2, IL-6 and INF-γ, whose interaction with TNF-α can kill *M. tb* in the lesion.[80] Besides, the growth of *M. tuberculosis* is reduced by interrupting iron availability either by treatment with an exogenous iron chelator, such as deferoxamine, silybin and a phytosiderophore or exposure to the endogenous iron chelators apotransferrin and apo-lactoferrin. Furthermore, an anti-TfR antibody of *M. tuberculosis*; or gallium salts or gallium transferrin, occupies iron pathways with a metal that cannot be utilized by *M. tuberculosis*. A schematic representation of the mechanism has been shown in Fig. 7.

5.1.3. *Use of cytokines as HDT*

Interferon-γ, a cytokine, activates innate immune functions and mediates antigen-specific T cell immunity in response to *M. tb* infection. A randomized controlled clinical study on aerosolized recombinant IFN-γ as an adjunct to standard anti-TB therapy showed suppression in the production of pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-8.[81] Administration of200 μg recombinant IFN-γ either by nebulization or by subcutaneous injection three times per week over 4 months helps to elevate the CD4+ lymphocytes' and improve the response to treatment in the cavitary-TB patient through enhanced *M. tb* clearance in sputum. A clinical study conducted on 50 MDR-TB patients showed improved immunity status and sputum smear conversion through adjunct supplementation of recombinant human IL-2 (500,000 IU in alternative days and up to 7 months).[82] These researches highlight the progressive role of cytokines in regulating immune cell functions and modulating cytokine-stimulated activities as HDT for TB treatment.[83]

5.1.4. *Use of antibodies as HDT*

Studies have shown that serum rich in anti-lipoarabinomannan (LAM) antibodies enhances the expression of CD4+ and CD8+ T cells by modulating IFN-γ, thereby improving phagolysosomal fusion. Further, a significant reduction in bacterial burden in the lungs and spleen of mice infected with *M. tb* was observed on administering 9d8 antibodies to antigen arabinomannan (AM), the LAM-specific SMITB14 antibody.[84] It has also been reported that elevated levels of anti-M. tb IgG3 antibodies prevent reactivation of TB in high-risk individuals.[85] Significant decrease in bacterial load in the lungs of *M. tb*-infected mice following intranasal administration of human gamma globulin has also been reported.[86] A novel human monoclonal IgA1 (constructed using a single-chain variable fragment clone 2E9) showed a high binding affinity for the mycobacterial α-crystallin Ag the human FcαRI (CD89) IgA receptor. Intranasal administration of 2E9IgA1 and recombinant mouse IFN-γ significantly inhibited pulmonary H37Rv infection in mice transgenic for human CD89.[87] However, further detailed studies are required to validate these approaches.

5.1.5. *HDT targeting cell-mediated immunity*

Statins, HMG-CoA reductase inhibitors, have been known as lipid-lowering drugs. Still, recent studies have shown their protective and immune-modulatory mechanisms by inhibiting the host mevalonate pathway's intermediates, thereby compromising the immune evasion strategies of pathogens and their survival.[88] In a similar study, simvastatin, a statin showed a significant increase in tuberculocidal activity of first-line anti-tubercular drugs isoniazid, rifampicin and pyrazinamide in chronic TB infection in BALB/c mice.[89]

5.1.6. *Modulation of inflammatory response through HDT*

Maintaining a sustained immune response is essential to maintain a balance between the pro-and anti-inflammatory reactions. Lipoxin A4 (LPX4) and leukotriene B4 (LTB4) play opposite roles in maintaining the balance. LPX4 controls the progression of TB by maintaining inflammatory balance, whereas, LTB4 causes hyper-inflammation and increases disease severity.[86] Aspirin (acetylsalicylic acid) stimulates the production of LXA4, which in turn regulates inflammatory pathology for mycobacterial infection, especially tuberculous meningitis. It also works by down-regulating the machinery required for transcription and translation in *M. tb*.[90] Vasoconstrictors like prostaglandin and thromboxane enable accumulation of platelet and control the inflammation. Ibuprofen suppresses cyclooxygenase-1 (COX1), COX2, prostaglandin H2, and thromboxane production resulting in immunomodulatory activity during TB.[91,92] Indomethacin could also play a potential role in tubercular pathogenesis as it inhibits COX1/2, and regulates CD4+, CD8+ and regulatory T cells.[93] Celecoxib, a selective COX-2 inhibitor has been reported to regulate the homologous MDR-1 pumps in humans. It thus can be used in combination with the anti-tubercular drugs to reduce the dose or shorten treatment duration.[94]

6. Recent advances in the treatment of TB

Recently, on August 14, 2019, U.S. FDA approved Pretomanid tablets to be administered in combination with Bedaquiline and Linezolid for the treatment of highly -resistant cases of pulmonary tuberculosis.[95] Pretomanid, a nitroimidazooxazine based prodrug, needs to be activated by the enzyme nitroreductase to produce its active metabolites responsible for the therapeutic action.[96]

7. Conclusion

Even though tuberculosis has been a disease long dealt with, the treatment outcomes have not been very encouraging. Prolonged treatment duration involving the administration of high doses of multiple drugs leads to patient non-compliance. Therapeutic non-compliance in infectious diseases not only causes treatment failure but, somehow, also creates a favourable ground for

the evolution of drug-resistant microorganisms. Besides being a healthcare issue, non-compliance also levies an enormous financial burden on society because of the costly and complicated treatment of relapse cases. Moreover, the pace at which new drug molecules is reaching the clinical trials shows a little reason to expect a miraculous solution to improvement in the present situation. Development of newer anti-TB drugs though seems to be the only possible solution to deal with the case, but, till the time newer drugs reach the market, a plausible alternative could be in the line of modifying the drug delivery carriers or therapeutic targets. The HDT drugs can plausibly regulate the antimicrobial activities of host immune cells and bring down the inflammation and tissue damage associated with TB.

It envisaged that these tailor-made systems might help in dose minimization due to slow and controlled/sustained release of the drugs over a prolonged period. Also, improved half-life and targeted delivery of therapeutic compounds from these carriers might replace large daily doses of medications with smaller ones. These benefits are likely to relieve both the patient and health professionals from complicated prescription patterns. It might also supplement the DOTS program, making it more manageable and probably affordable.

So far, the outcome of studies on these lines on small animals has been quite promising. But there is a dire need to work towards clinical translational models, so that in-depth understanding of pharmacokinetics and biodistribution patterns. Also, detailed information on toxicity, accumulation, immuno-modulatory, and inflammatory response on the use of these strategies is still lacking. The future of these alternative Track II therapeutic approaches is promising, especially for tuberculosis, wherein there is a dearth of innovations and successful interventions.

Author contributions

Dr. Ruchi Chawla - Review planning, administration, Manuscript revision and supervision.

Varsha Rani – Methodology, Manuscript writing, Manuscript revision.

Mohini Mishra- Review designing, Manuscript writing and detailing.

Conflicts of interest

The authors have none to declare.

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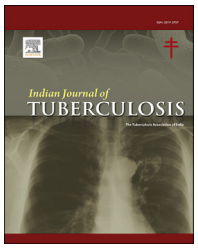

Review article

Insights into development of Decaprenyl-phosphoryl-β-D-ribose 2′-epimerase (DprE1) inhibitors as antitubercular agents: A state of the art review

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ABSTRACT

Mycobacterium tuberculosis is a causative agent for the world threatening infectious disease known as tuberculosis. M. tuberculosis is also referred as Koch's bacillus as it was first defined by Robert Koch in 1821. In the entire history of M. tuberculosis infection, several different targets were identified and explored with a hope of effective therapeutic treatment against tuberculosis. Drug-resistant tuberculosis is the major obstacle for researchers and letting them fail continuously to discover new drug candidates. Among the numerous antitubercular targets, Decaprenyl-phosphoryl-β-D-ribose-2′-epimerase (DprE1) is novel target identified in the year 2009. The present article portrays insights of DprE1 enzyme in all the aspects i.e., identification, structural elucidation to designing strategies and synthesis of potential drug candidates to combat resistant strains. Along with the synthesis and biological activity of novel compounds, structure–activity relationship (SAR) data is given to help medicinal chemists and researchers working in this area for the development of new inhibitors to fight against M. tuberculosis. DprE1 is new ray of hope for antitubercular treatment. No single drug candidate (DprE1 inhibitor) has passed clinical trial yet and hence it nullifies the risk of development of resistance or mutations at specific residues. Researchers working in this area have to design and come up with new potent candidates with less dose, no toxicity to combat this deadly infection. This review emphasized on year wise systematic development and progress of DprE1 inhibitors.

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1. Introduction

Tuberculosis (TB) is a life-threatening infection and has spread among millions worldwide.[1] Including the World Health Organisation (WHO), many leading organizations are putting their hard efforts to reduce global burden of the infection.[2] WHO's approach is based on the needs and potential to contribute knowledge in the low and middle-income nations, which bears an enormous burden of human grief due to TB. The researchers and all the people who are working in this area must come out with the appropriate solutions and remedies to combat TB.[3] There are significant and crucial steps are required to achieve this goal, and hopefully, this will help to eliminate TB as a public health problem in the coming years. The research strategies have been built on a global TB research plan, developed through consensus and consultation of researchers and stakeholders from around the world.[4]

In past several decades, there were several reviews and surveys were carried out based on scaffolds used in antitubercular drug discovery, antitubercular agents, different targets used in antitubercular drug discovery, etc. Among the various explored and unexplored targets in TB, DprE1 (Decaprenyl-phosphoryl-β-D-ribose-2′-epimerase) is a flavoenzyme enzyme which plays an essential role in the biosynthesis of arabinan (one of the constituents of mycobacterial cell wall). However, till date no detailed review was published on specific DprE1 enzyme as target and no collective information is available for researchers working on tuberculosis research targeting proposed enzyme. This review has focused specifically on the DprE1 enzyme as a target. The specific year-wise literature survey is presented here (Fig. 1), which will help the researchers targeting DprE1 as antitubercular agents. While going through the literature, there were few parameters considered such as antitubercular activity, DprE1 enzyme inhibition, various chemical scaffolds used in the synthesis of antitubercular compounds, etc. Structure–Activity Relationship (SAR) was described to make readers understand the advantages and disadvantages of chemical structure modifications. Substantial literature was found, but the information related to the topic such as introduction to the TB, chemical synthesis, SAR, DprE1 enzyme, medicinal and synthetic chemistry, chemical biology, and some papers having biological relevance related to DprE1 were taken into consideration.

2. Decaprenyl-phosphoryl-ribose 2′-epimerase (DprE1)

The protein decaprenyl-phospho-ribose 2′-epimerase catalyzes the epimerization reaction of decaprenylphosphoryl-D-ribose (DPR) into decaprenylphosphoryl-D-arabinose (DPA). The reaction takes place through a continuous oxidation-reduction with the involvement of intermediate, decaprenylphosphoryl-2-Keto-ribose (DPX), which is a result of DPR oxidation and a precursor of DPA (Fig. 2).[5,6]

3. Development of DprE1 from 2009 – 2020

In 2009, Makarov et al investigated and synthesized 1,3-benzothiazin-4-ones (BTZs), as a novel class of new chemical entities (Fig. 3), which kills the *Mycobacterium tuberculosis*. Through various studies such as *in vitro*, *ex vitro*, mouse models of TB, genetics; it has been found that these compounds were able to inhibit the synthesis of decaprenyl phosphoryl arabinose essential precursor is required for the synthesis of the cell-wall arabinans). The more potent compound was BTZ043, which is promising drug candidate for drug-resistant TB.[7]

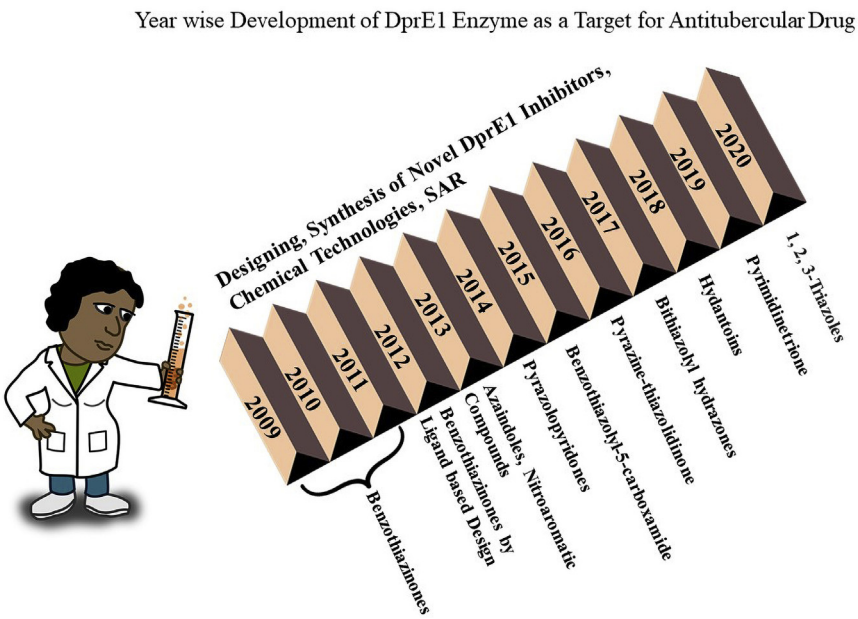

Fig. 1 – Development of DprE1 Inhibitors over the years.

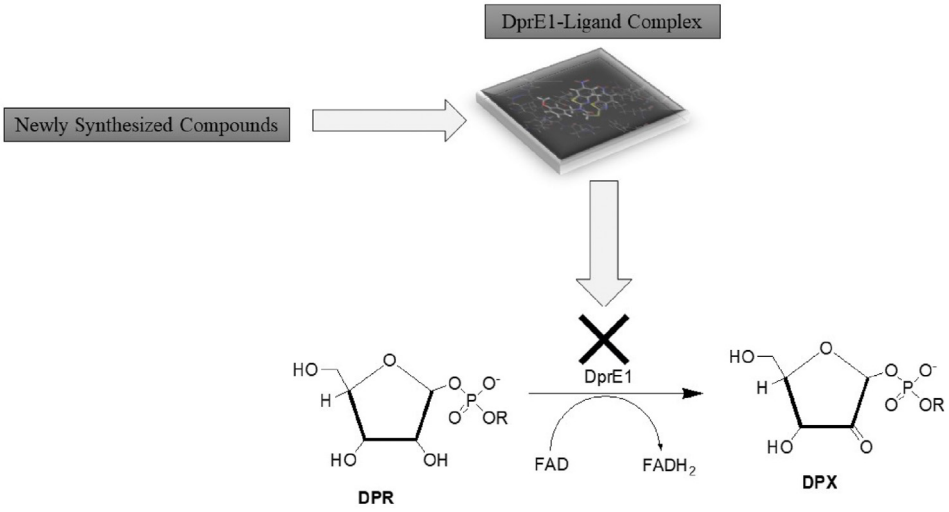

Fig. 2 – DPX is an intermediate in biosynthesis of Decaprenyl phosphoryl-β-D-arabinofuranose (DPA).

Later, Christophe et al presented the development of cell-based assay. The library of more than 57,000 small molecules was screened using cell-based assay. Finally, 135 potent compounds were found with antitubercular activity. Among these, DNB exhibited high activity against XDR strains of TB.[8]

In 2010, Maria Pasca et al reported novel derivatives of BTZ043, which target the DprE1 enzyme. To study the development of resistance for BTZ043, around 240 clinical samples from different hospitals in European continent were studied for mutations of DprE1 gene.[9]

In 2011, British microbiologists Stewart Cole et al described that the most promising antitubercular compounds in clinical trials and also introduced some aromatic compounds bearing nitro group that inhibit the DprE1 enzyme, which is crucially involved in mycobacterial cell wall synthesis.[10]

In the beginning of 2012, Trefzer et al demonstrated that compounds from BTZ class exhibited nmol bactericidal activity against tubercle bacilli, and BTZs are suicide substrates for the DprE1 enzyme. Role of DprE1 enzyme in epimerization was illustrated (Fig. 4).[11] In the same year, Lechartier et al with checkerboard method and cell viability assays, studied the different interaction profiles of BTZ043 with several antitubercular agents and antimicrobials. No antagonism was found between BTZ043 and other compounds. Interaction between TMC207 and BTZ043 (Fig. 5), was observed with further studies, and novel targets were introduced to tuberculosis research.[12]

Stanley et al presented their research by whole-cell screening of various molecules as a method for the identification of novel candidates that will target a new mechanism for mycobacterial drug discovery. The primary screening had limitations for the identification of new inhibitors against *M. tuberculosis*. Characterization of two novel compounds (Fig. 6), benzimidazole (MmpL3) and nitro-triazole (DprE1), was performed.[13]

After significant contributions, in 2013 a group of Italian researchers, Riccardi et al again focused their studies on the DprE1 enzyme. They also summarize structure, enzymatic activity, and inhibitors, this enzyme was termed as one of the most vulnerable targets for drug discovery.[14] Shirude et al wrote 1,4-azaindoles (Fig. 7) as a new series of antitubercular agents, which were effective in tuberculosis mouse models. This series was developed from the scaffold morphing method. Three points of modification were recognized for the 1,4-azaindoles, namely the amide side chain, a hydrophobic group, and core ring substitutions. A secondary amide is an essential group for maintaining potent MIC, and that could be involved in either intramolecular hydrogen bonding with nitrogen (N4) of the azaindole ring or hydrogen bonding with the target enzyme. Small hydrophobic or hydrophilic amides such as methyl cyclopropyl, fluoro-ethyl, and hydroxyethyl amides were preferred for cellular potency. The hydrophobic group tolerates various disubstituted benzyl and disubstituted heteroaryl-methyl groups; however, monosubstituted benzyl groups were less favoured. Addition of a methyl group at the C-6 position of the 1,4-azaindole improved cellular potency. This class can develop a therapy for drug-resistant tuberculosis.[15]

Tiwari et al synthesized newer antitubercular agents (Fig. 8) by considering structural features of BTZs and some other nitro-aromatic derivatives. Computational studies revealed that unsubstituted carbons of BTZs and other nitro-aromatic compounds are most electron deficient. Non-enzymatic reduction of the nitro group was reported, which further converted into nitroso in a similar manner that of von richter reaction and which can also interact with Cys387.[16]

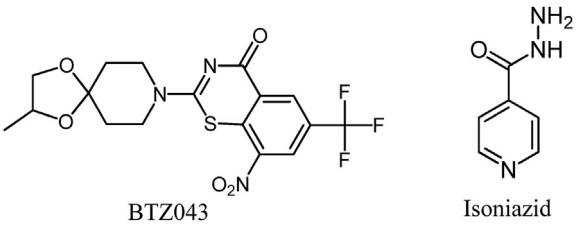

Fig. 3 – Chemical structure of BTZ043 and Isoniazid.

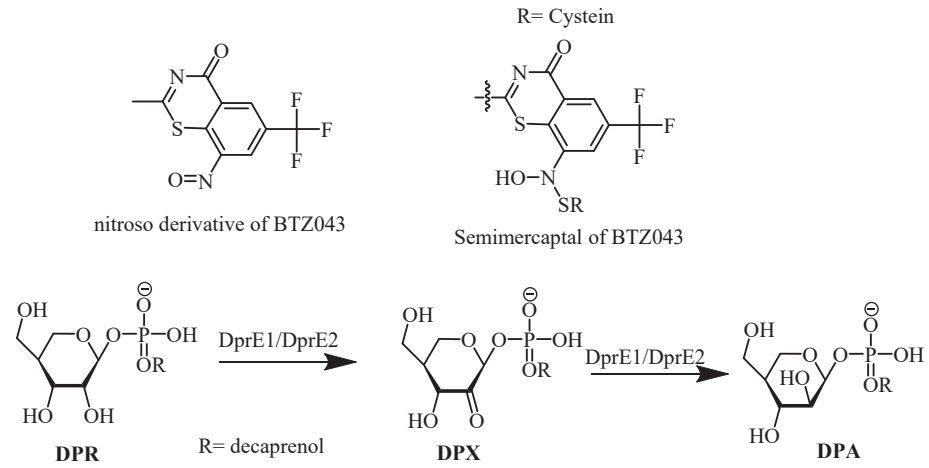

Fig. 4 – BTZ043, its nitroso and semimercaptal derivatives along role of DprE1 in epimerization reaction.

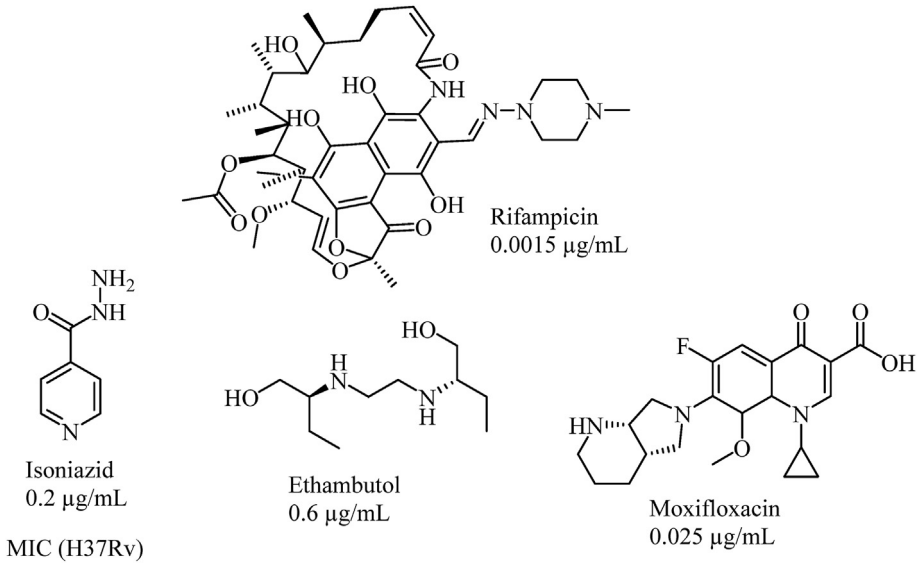

Fig. 5 – MICs of selected compounds for study by REMA method.

Friggeri et al synthesized a new series of R-4-amino-3-isoxazolidinone derivatives (Fig. 9) found to be active at a micromolar level against M. tuberculosis was used, and the predicted bioactivities were acceptable.[17]

In 2014, Chatterji et al reported that the 1,4-azaindoles (Fig. 10) as potential DprE1 inhibitors with improved physicochemical properties in mice. Compound (2) has better cellular activity, efficacy in animal models. This compound does not have an antagonistic effect with any other antitubercular agent/drug. Compound (2) has shown synergy with PA824 and TMC207 *in vitro*. 1,4-azaindoles are a promising candidate for developing new anti-TB drugs. The potency of these derivatives against *M. tuberculosis* was mostly improved through the MIC based SAR in following manner, amide side chain, a hydrophobic group, and center ring replacements. The secondary amide referred to as the amide side chain was essential for activity. It might be associated with the hydrogen-holding formation. Little hydrophobic or hydrophilic amides, similar to methyl cyclopropyl, fluoroethyl, or hydroxyethyl amides, were suggested for more potency. The replacement of a methyl or methoxy at the C-6 situation of the 1,4-azaindole improved the potency.[18]

Panda et al stated a novel pyrazolopyridone class (Fig. 11) from whole-cell screening. This series has come up better

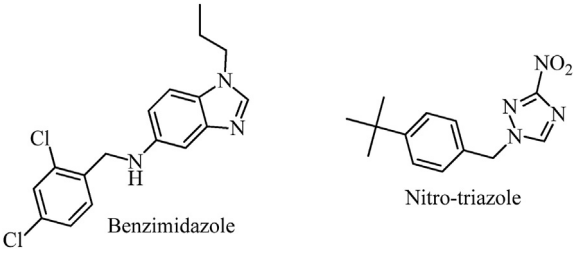

Fig. 6 – Chemical structures of benzimidazoles and nitrotriazole.

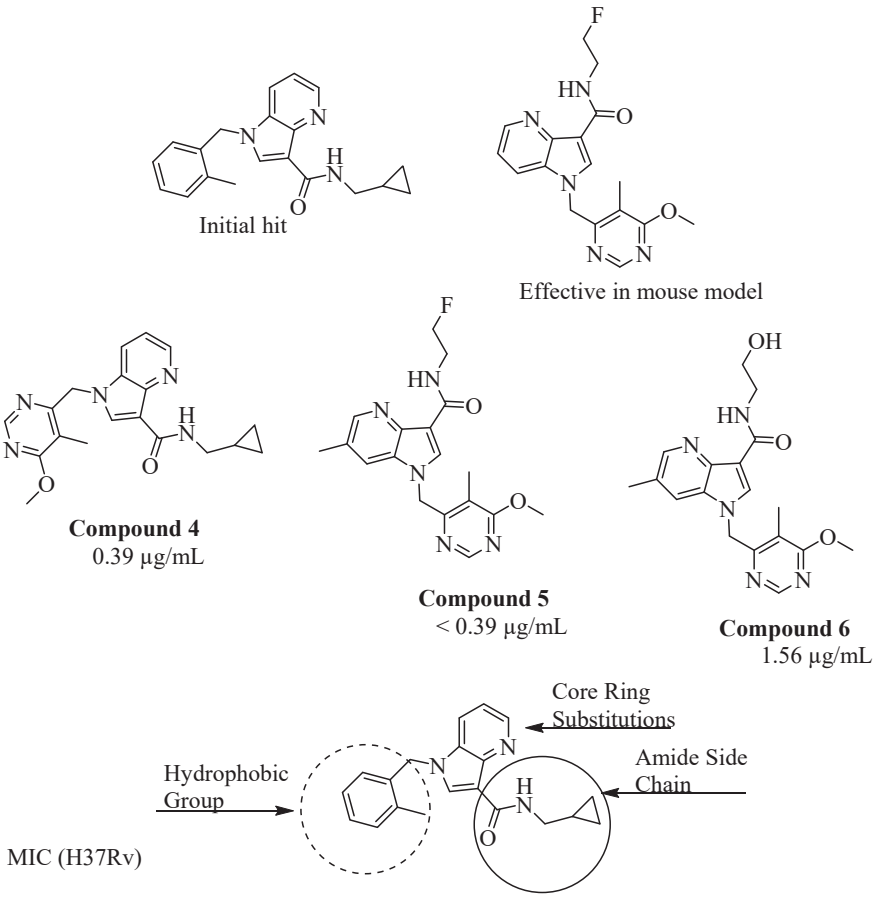

Fig. 7 – Representative compounds from the 1,4-azaindole class.

bactericidal activity. Detailed biochemical studies reveal that this is a noncovalent inhibitor of DprE1. Pyrazolopyridine class is a non-nitro containing series which targets the DprE1 enzyme. They have mentioned important points related to SAR for the replacement at the phenyl ring at R1; they began with unsubstituted phenyl ring, which diminishes the activity. Groups such as, methyl and electron-withdrawing nature, for example, nitrile at the meta position resulted into decrease in activity. Substituting pyridone with N-methylpyridone or its isomeric structure methoxypyridine likewise led to loss of activity hence it was concluded that the pyridone moiety is mandatory. Replacing the - NH linker with an amide group led to the loss of activity.[19]

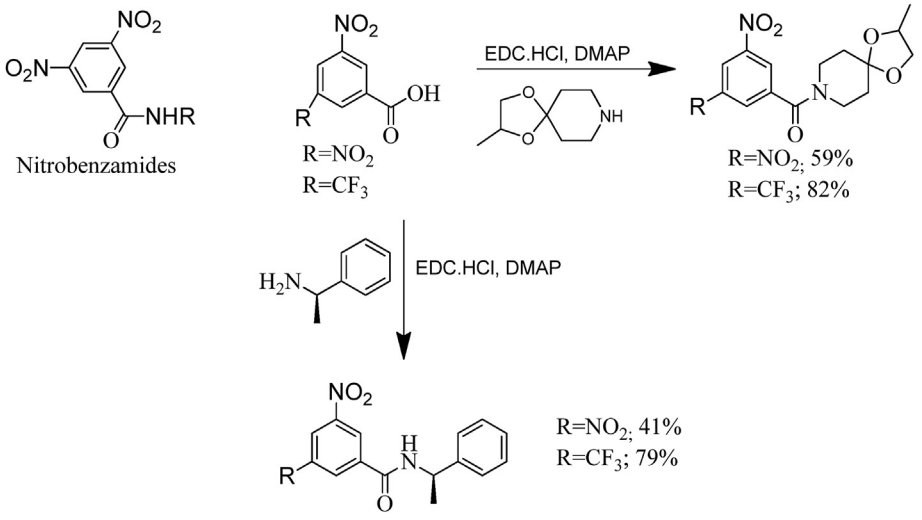

Fig. 8 – Synthesis of some nitro-aromatic compounds.

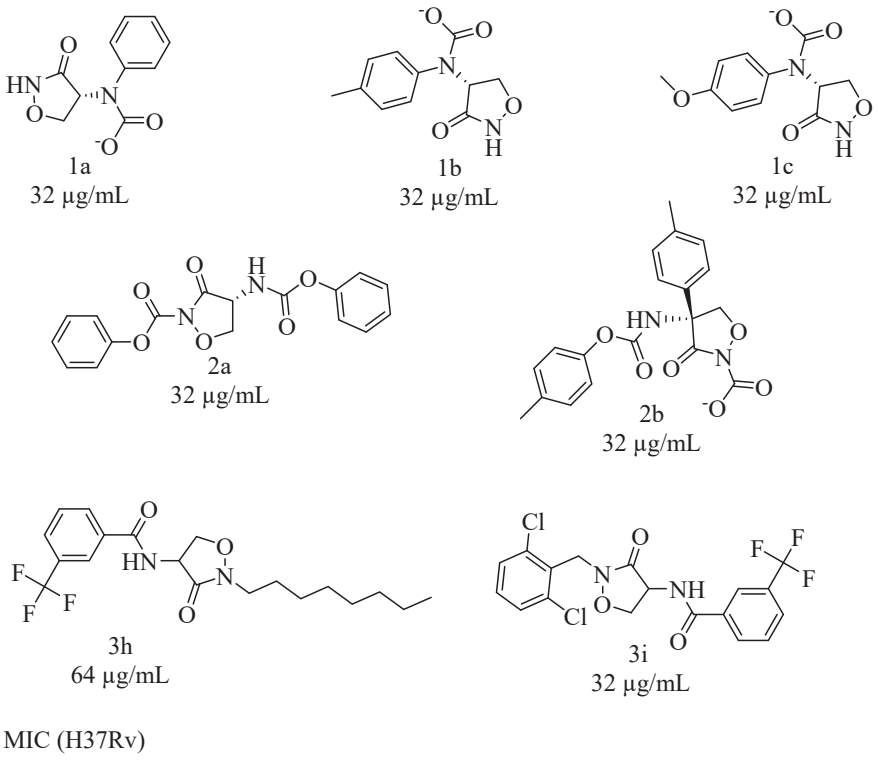

Fig. 9 – Representative 4-amino-3-isoxazolidinone derivatives.

Shirude et al reported lead optimization of previously reported 1,4-azaindoles (Fig. 12) resulted in more potent compounds with more robust physicochemical properties. 1,4-azaindole series would meet for further safety studies.[20]

In 2015, Peng et al synthesized 4-carbonyl piperazine substituted 1,3-benzothiazin-4-one (Fig. 13), studies for antitubercular activity against H37Rv. Derivatives with alkyl side chains had shown promising activity with no toxic effects with MIC 0.008 µg/mL, which is much more potent than BTZ043 and PBTZ169. They explored the effect of various substituted phenyl rings.[21]

Chikhale et al used SBDD approach to design and synthesize twenty new derivatives of benzothiazolylpyrimidine-5-carboxamides (Fig. 14). These derivatives were tested for antitubercular activity. Four compounds were found potentially active. With computational studies, interacting amino acid residues were studied.[22]

Makarov et al revealed that $-NO_2$ group at 8th position is required for DprE1 inhibition, which further interacts with Cys387 residue.[23] Landge et al reported a series of compounds using whole cell-based screening (Fig. 15). These studies provide sufficient information for the development of future potent antitubercular drug candidates. They had a series of amides. From the IC_{50} data, it proves that, BTO analogues are more potent than BT analogues which in turn are more potent than cBT analogues. The weaker IC_{50} of cBTs can also be attributed to the steric bulk around the NO_2 group that could affect its reduction to a nitroso group, required to form a covalent interaction with the active site cysteine of DprE1.[24]

Pore et al investigated a series of novel 11α-triazole bile acid compounds, also reported *N*-alkyl and *N*-acyl derivatives of C-11 amino bile acid esters. Synthesized compounds were studied for antitubercular potency against strain H37Rv. Four compounds were studied for the dose-dependent effect against tuberculosis. Molecular docking studies revealed that the compounds had shown better binding with residues of DprE1. ADME evaluation confirmed that the series has the potential to develop oral drug candidates.[25]

Neres et al further investigated phenotypic screening of the quinoxaline library lead to the identification of Ty38c with MIC 3.1 µg/mL, and Ty38c is bactericidal. Additionally, they investigated their mode of action. They isolated mutants to Ty38c and sequenced their genome. Genetic studies, biochemical validation, SAR studies concluded that Ty38c is a non-competitive DprE1 inhibitor.[26]

In 2016 Karabanovich et al reported 5-substituted 1,3,4-oxadiazoles as a new class of antitubercular agents. Few analogues have shown excellent MIC values as low as 0.03 µg/mL against resistant strains. Synthesized compounds did not

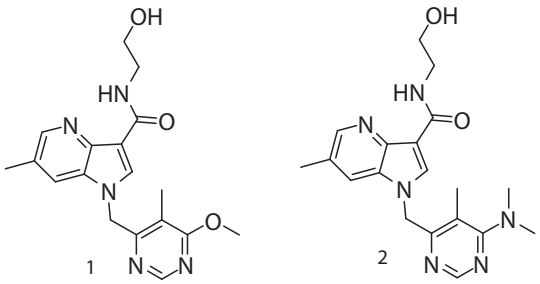

Fig. 10 – Selected compounds for synthesis from 1,4-azaindole Series.

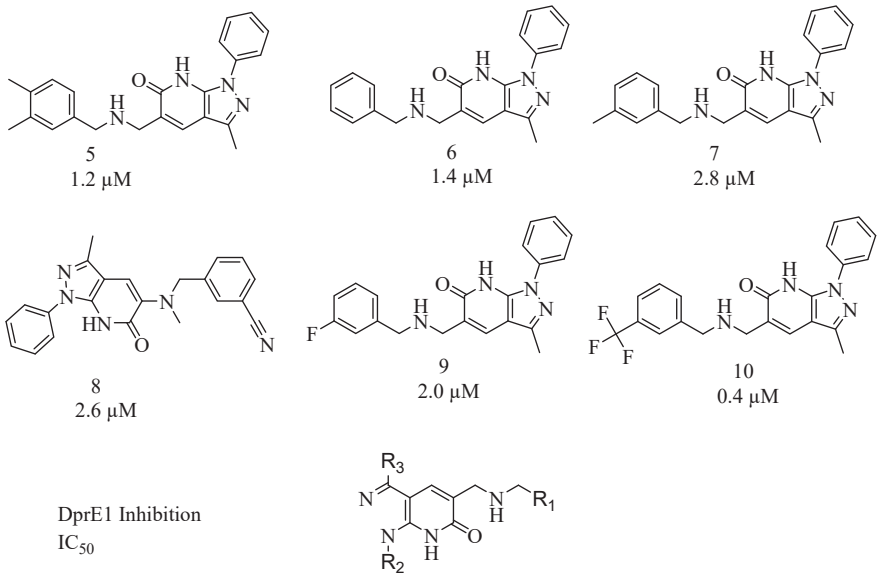

Fig. 11 – Examples of novel pyrazolopyridone class.

exhibit any toxic effects on mammalian cells and isolated hepatocytes. Genotoxicity assays confirmed that there was no mutagenic activity. In conclusion, oxadiazoles and thiadiazoles were proved better compared to rifampicin with excellent therapeutic and toxicity profiles. 3,5-dinitro substitution has an important role in the antimycobacterial activity. Changes in positions led to decrease in antitubercular activity. The 2,4-dinitrobenzylsulfanyl derivatives (Fig. 16) possessed significant low antimycobacterial effect, and the 4-nitrobenzylsulfanyl analogues practically loses activity. The main drawback reported was the compounds had low solubility in aqueous media. The replacement of one nitro group with a trifluoromethyl moiety resulted in compounds with diminished antimycobacterial activity.[27]

Gao et al identified new analogue SKLB-TB1001 has shown excellent antitubercular activity in the Microplate Alamar blue assay and intracellular model. No antagonism was reported when tested with rifampicin. SKLB-TB1001 has shown excellent efficacy than BTZ043. $-NO_2$ at 8th and sulphur at 1st positions were important for activity also trifluoromethyl at

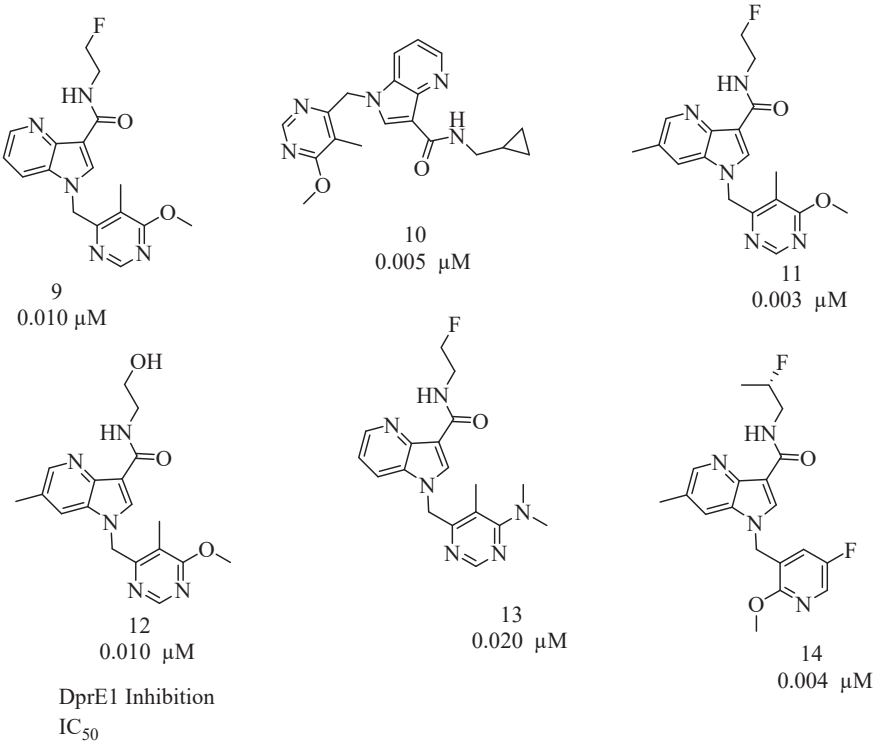

Fig. 12 – Optimized compounds from 1,4-azaindoles.

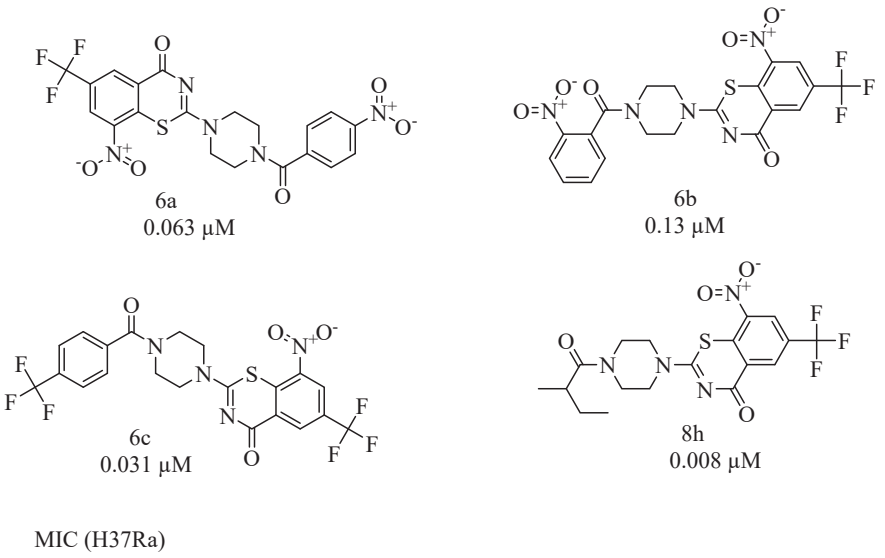

Fig. 13 – Representative 4-carbonyl piperazine substituted 1,3-benzothiazin-4-one compounds.

6th position is essential to retain the activity.[28] Yan Foo et al explained that benzothiazinones (BTZs) are more potent against resistant tuberculosis. BTZ mutants were characterized, and biochemically studied the effects of these mutations on DprE1 functions. By using various parameters, alterations in the DprE1 enzyme were studied. This study also focuses on the importance of binding with Cys387 residue of DprE1 and covalent inhibitors and other recently identified nitro-aromatic.[29] Mahajan et al synthesized derivatives of benzo[b] thiophene-2-carboxylic acid (Fig. 17) and tested against strain H37Rv and also against resistant strains. Compound 7c was found to be highly effective against drug-resistant tuberculosis. Compound 8c and 8g exhibited excellent activity against dormant strain with MIC 0.60 and 0.61 μg/mL. Synthesized compounds were also analyzed for toxicity along with molecular docking studies which proved that of benzo[*b*]thiophene-based 1,3-diketones and flavones as potential leads against DprE1 enzyme.[30]

Chitre et al designed and synthesized pyrazine-2-carbohydrazide derivatives (Fig. 18) using a molecular hybridization approach with pyrazine and thiazolidinone skeletons. Most of the synthesized compounds were lies in the range of MIC: 0.3–1 μg/mL. Molecular docking studies revealed that newly synthesized hybridized molecules can provide novel pharmacophore to develop novel compounds against dormant TB.[31]

Shaikh et al prepared benzothiazinone based 1, 2, 3-triazoles (Fig. 19) by click chemistry approach. Among these compounds 6c and 6e were found as most effective antitubercular agents. Their study revealed that activity depends on various substituents present on phenyl rings. –F at third position in compound 6c and –Cl in compound 6e at second position has shown very promising antitubercular activity. Molecular docking studies revealed that triazole containing benzothiazinone might have ideal structural requirements for the development of future therapeutic agents.[32]

In 2017, Kloss et al reported reduction processes of clinical drug molecules BTZ043 and PBTZ169. Nitrobenzothiazinones are excellent antitubercular agents. The reduction performed was reversible and took place in all mammalian cells. The reduction was confirmed by biochemical studies with a complete study of the Meisenheimer complex and its biochemical aspects as well. Through *in vivo* data, chemical studies, and LC-MS studies, it was concluded that this is a very stable class in the development of antitubercular agents.[33] Bhalerao et al pioneered the synthesized bithiazolyl hydrazones (Fig. 20) with one pot cyclocondensation process and diisopropyl ethyl ammonium acetate (DIPEAc) at room temperature. Most of the synthesized compounds have shown remarkable antitubercular activity compared to rifampicin with less toxicity. Molecular Docking studies have shown good binding of synthesized compounds with the receptor. Amongst the studied compounds, one compound has phenyl moiety in one of the thiazoles, and 4-methoxy phenyl in other thiazole ring

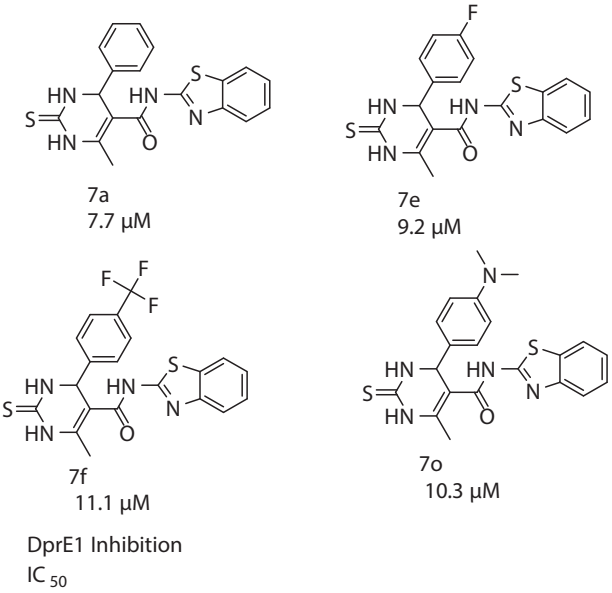

Fig. 14 – Representative derivatives of benzothiazolyl pyrimidine-5-carboxamides.

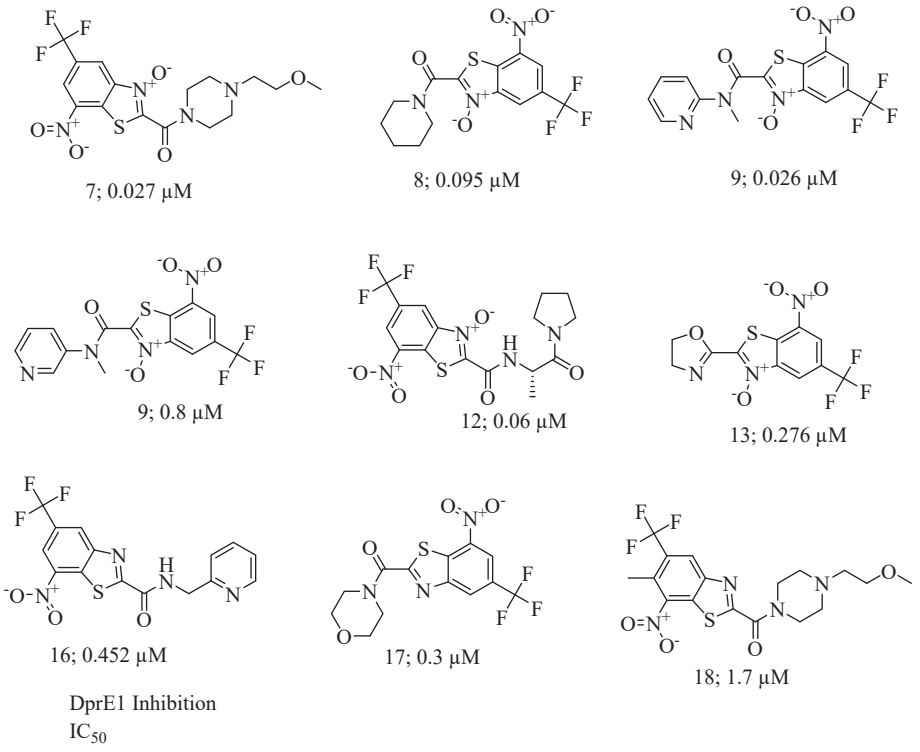

Fig. 15 – Representative compounds from benzothiazole Series.

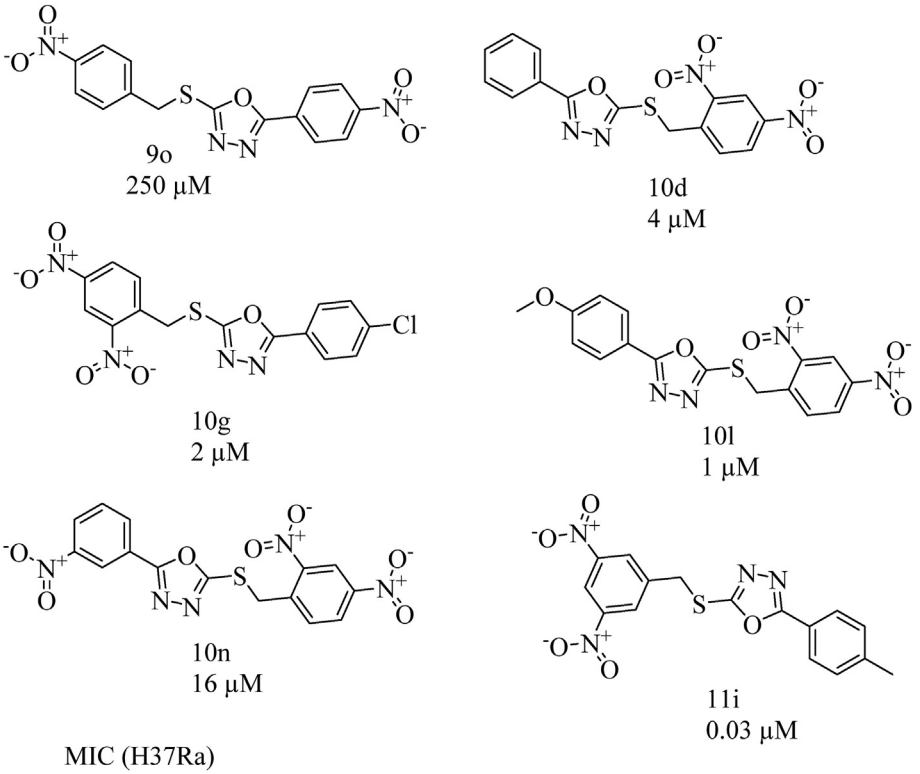

Fig. 16 – Representative 3,5-Dinitrobenzylsulfanyl-1,3,4-oxadiazoles and thiadiazoles.

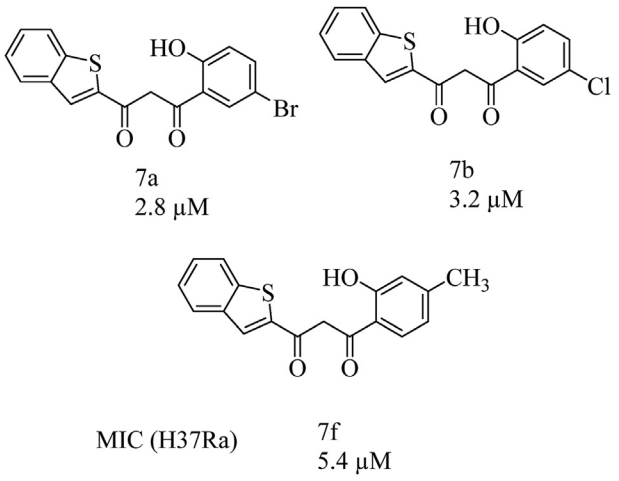

Fig. 17 – Some examples of new benzo[*b*]thiophenes.

system has displayed better inhibition against *M. tuberculosis* H37Rv.[34]

In 2018, Rogacki et al reported a novel hydantoin based (Fig. 21) family with antitubercular activity with inhibition of DprE1 enzyme. The library of more than 100 compounds was prepared and studied for biological and physicochemical properties. Synthesized compounds were tested for toxicological studies. Overall, the novel group of compounds could be used for the future development of effective drug candidates.[35]

Yalchin et al performed docking studies on fluoro substituted chalcones with DprE1 enzyme inhibition. It shows that chalcone derivatives exhibited better binding affinity. Few compounds have shown good inhibition, compounds explicitly with a double bond. Structure–activity relationships proved that these compounds might be an excellent platform for designing new DprE1 inhibitors. Docking study provides an understanding of the possible binding conformations, displaying the hydrogen bonds, pi interactions, and close relationships with critical residues such as Lys 418, Gln 334, Tyr 314, and Ser 228.[36] Gawad et al synthesized 23 new imidazo [4,5-b] pyridine derivatives from the computational chemistry approach. Hydroxyl, methoxy, nitro, and bromo substituted compounds exhibited better antitubercular activity and docking score (−8.825) with employed DprE1 protein.[37]

Recently in 2019, Shiva Raju et al synthesized substituted pyrimidine-1, 2, 3-triazole derivatives (Fig. 22) through copper-catalyzed azide–alkyne cycloaddition. Synthesized derivatives were evaluated for their antitubercular potential using H37Rv. Molecular docking studies have shown a good score and might be useful for the development of antitubercular drug development.[38]

Manjunatha et al reported novel substituted benzimidazole derivatives (Fig. 23) as DprE1 inhibitors from scaffold morphing of 1,4-azaindole series. Few compounds were studies and resulted in improved solubility with potent DprE1 inhibition. Molecular modeling studies explored the possible binding mode with a target to exhibit desired inhibitory activity, which can be considered for further exploration of compounds. They have also established MIC-based SAR against Mtb. Three points were taken into consideration to explore structure activity relationship, namely, the amide side chain, hydrophobic group, and core ring substitutions. The secondary amide of the amide side chain is critical for maintaining a potent MIC and is hypothesized to be involved in hydrogen bonding with the DprE1 enzyme. Small hydrophobic amides such as fluoro-ethyl and difluoro ethyl amides are preferred for antimycobacterial activity. Substitution at the C-6 position of the benzimidazole core with methyl or methoxy groups also enhanced antimycobacterial activity, as observed for azaindoles.[39]

Bodige et al synthesized novel compounds containing substituted pyridine-3-carboxamide derivatives (Fig. 24). All the compounds were characterized and screened for antitubercular activity using a REMA method. The halogen-substituted compounds have shown promising DprE1 inhibition. Molecular docking studies were also supported in activity

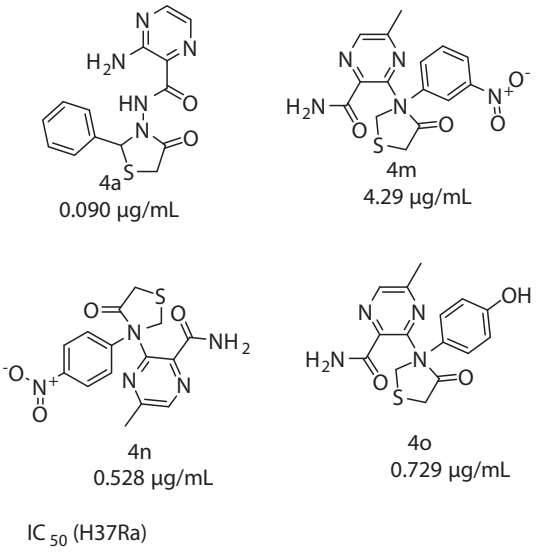

Fig. 18 – Pyrazine–thiazolidinone hybrid scaffold.

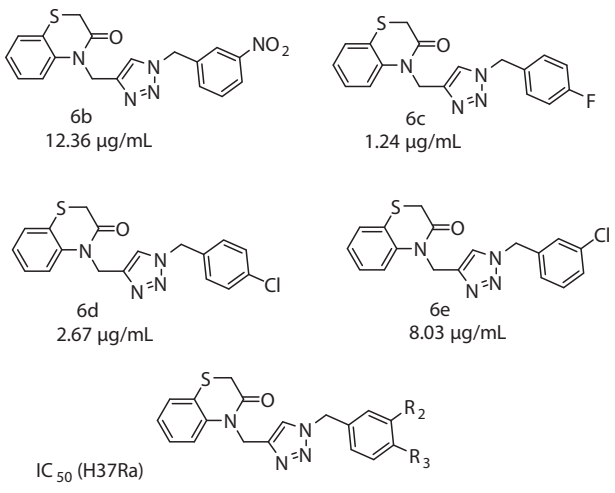

Fig. 19 – Triazole containing benzothiazinone derivatives.

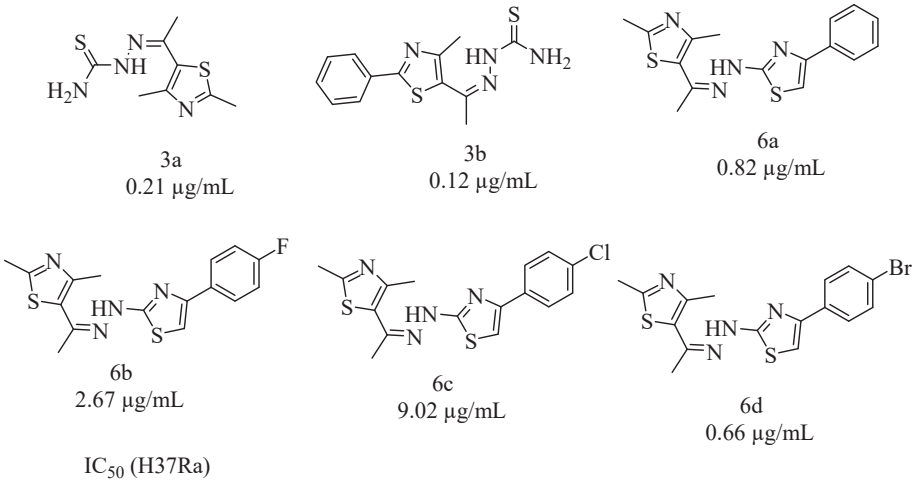

Fig. 20 – Synthesized bithiazolyl hydrazones with cycloaddition reaction.

data. Comparatively, it has shown similar kind of binding modes that of previously reported analogues.[40]

Gao et al applied the systematic strategy to identify a more potent drug candidate that can inhibit DprE1 more efficiently. Rational drug design and synthetic strategy were reported, and this compound has no toxicity. General structure of pyrimidinetrione is given below (Fig. 25).[41] Gawad et al reported newly synthesized substituted benzothiazole (BTZ) compounds which have exhibited good antitubercular activity and promising DprE1 inhibition.[42]

In 2020, Borthwick et al describe new compounds with morpholino-pyrimidine backbone (Fig. 26) as a DprE1 inhibitor. This series was derived from the HTS technique. The compounds have shown improved physicochemical properties and also shown efficacy in a murine infection model.[43]

Balabon et al reported hydantoin based compounds (Fig. 27) and which has shown good antimycobacterial activity. SAR of these compounds was explored using more than 80 compounds. Most active compounds were studied for physicochemical properties and human cytotoxicity. At last, the hydantoin series is a group of compounds with DprE1 inhibitory activity and possesses a noncovalent set for the process of drug discovery.[44]

Ma et al discovered new BTZs containing hexahydropyrrolo[3,4-c] pyrrole moiety (Fig. 28). Among these compounds, two compounds (compound 2 and 6) have shown excellent activity with good solubility and low cytotoxicity. This study suggested that these two compounds may serve as better candidates for drug discovery.[45]

Gawad et al successfully designed and synthesized quinazoline-2-carboxamide derivatives (Fig. 29) as DprE1 inhibitors. Hydroxy, Bromo, and nitro substituted compounds have shown promising antitubercular activity (1.12 μg/mL and 0.96 μg/mL) and DprE1 inhibitory activity.[46]

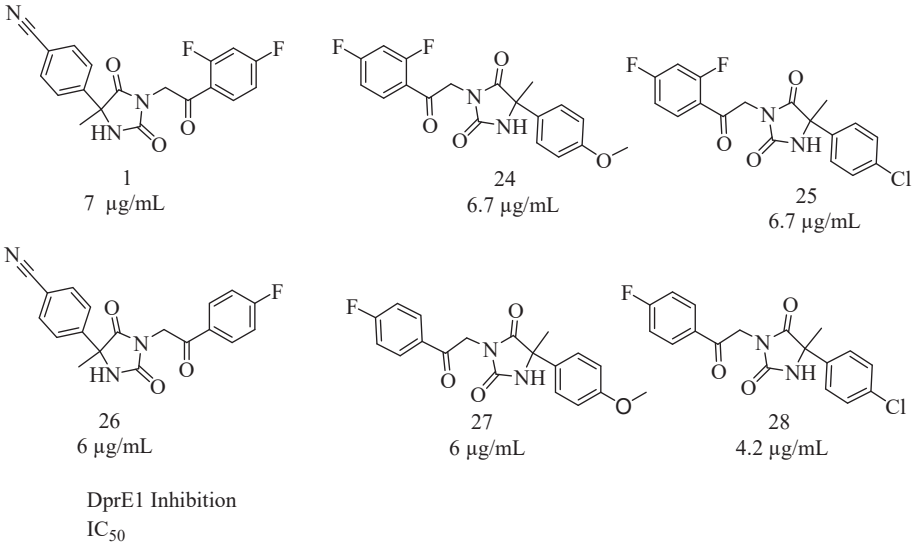

Fig. 21 – Novel hydantoin derivatives.

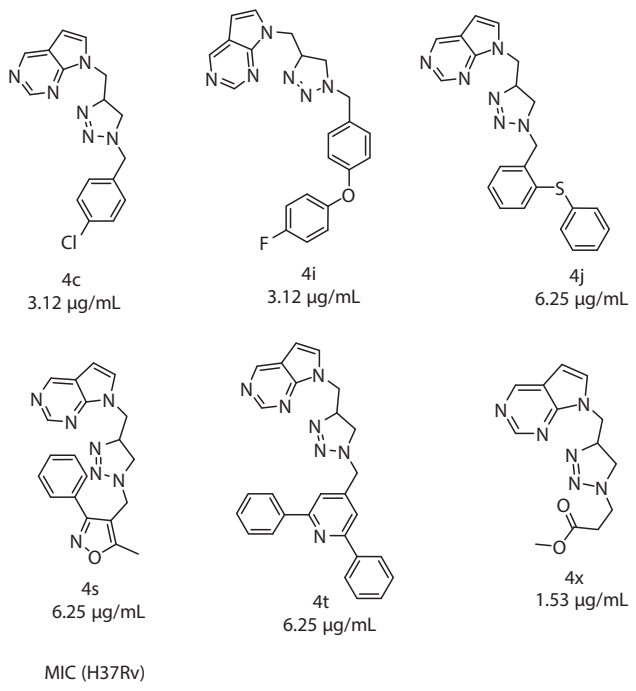

Fig. 22 – Substituted pyrimidine-1, 2, 3-triazole derivatives.

Suma et al, optimized azaindole class for DprE1 enzyme inhibition. Precise pharmacophore model was developed. On the basis of fitness score pharmacophore model was selected and screen and proceed for molecular docking studies. Molecular dynamics studies were preformed and confirmed binding affinity and stability.[47]

Niranjan Kumar et al, identified the inhibitors for DprE1 using virtual screening technique. ChEMBL database was used for screening. Molecular docking and dynamics were performed to confirm binding affinity.[48] Beteck et al, synthesized novel nitro quinolone-based compounds and tested them *in vitro* against Mtb and some other species for antibacterial activity.[49] Garg et al, reported an efficient, green synthesis of 1,4-disubstituted-1,2,3-triazole under solvent free conditions. By *in silico* studies, it was confirmed that some of the synthesized compounds were effective against DprE1 enzyme.[50]

4. Expert opinion

The name Tuberculosis threatens the society even in the 21st century. The advent of latest technology and advancements in science has made our lives easier and simpler than previous but humans are still struggling for remedy of several dreadful diseases like tuberculosis. First line drugs were effective for several decades but latter mutations and development of resistance has made this journey critical and even more tough. With the available treaties for tuberculosis, it can be treated temporarily but still high risk of reoccurrence of infections in due time. Development of resistance has also created major obstacle in the entire drug discovery process. The researchers working in this area should think of getting out of these hurdles like resistance. This will definitely lead to discovery of novel and safer lead molecules/drug candidates to treat TB. The use of computational tools must be increase to understand various aspects of protein structure, chemical

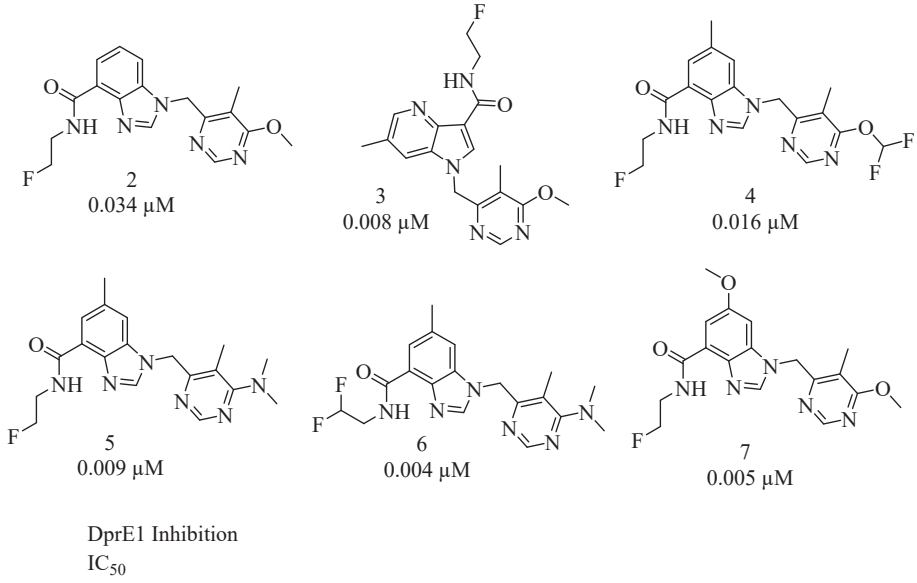

Fig. 23 – Novel substituted benzimidazole derivatives.

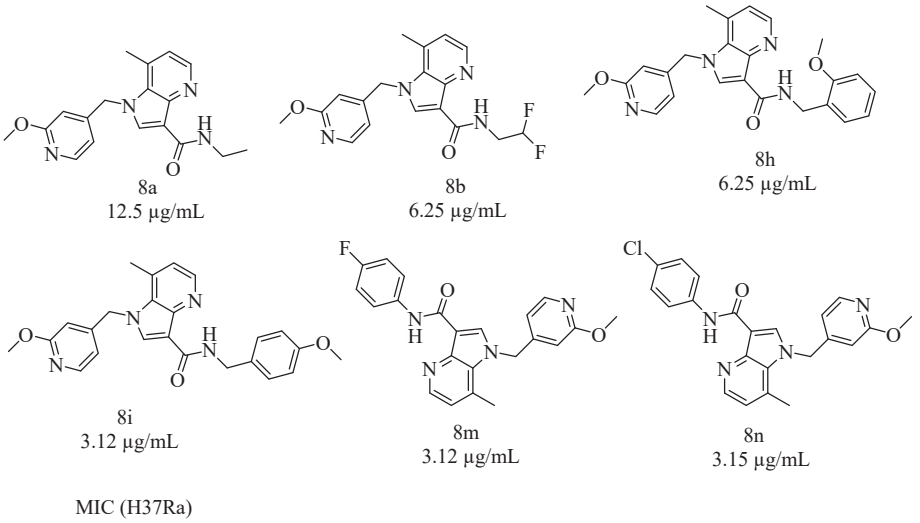

Fig. 24 – Novel series of substituted pyridine-3-carboxamide derivatives.

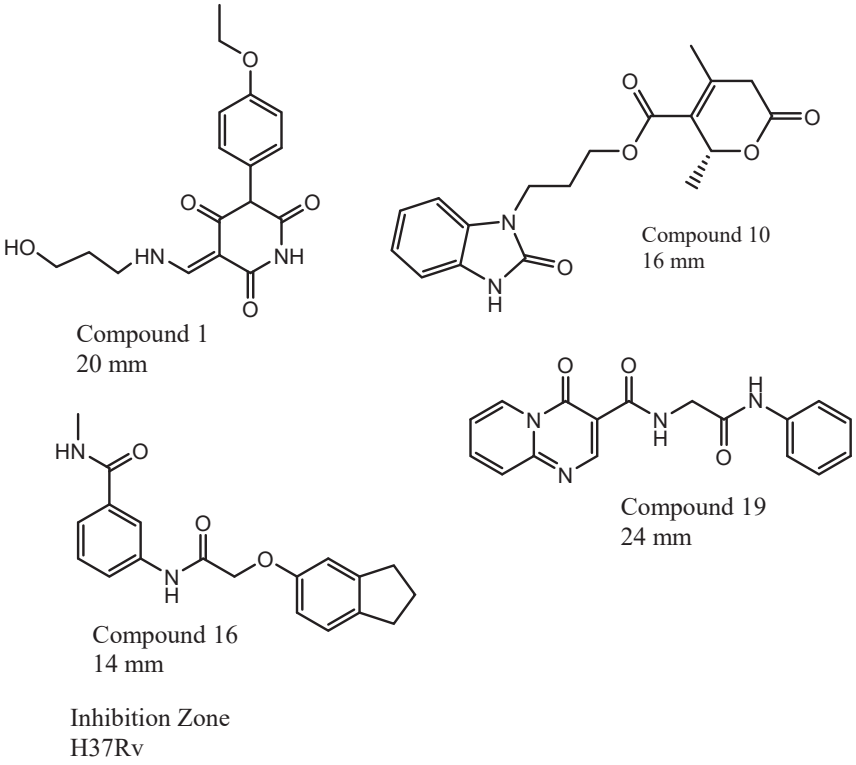

Fig. 25 – Chemical structures of the selected compounds.

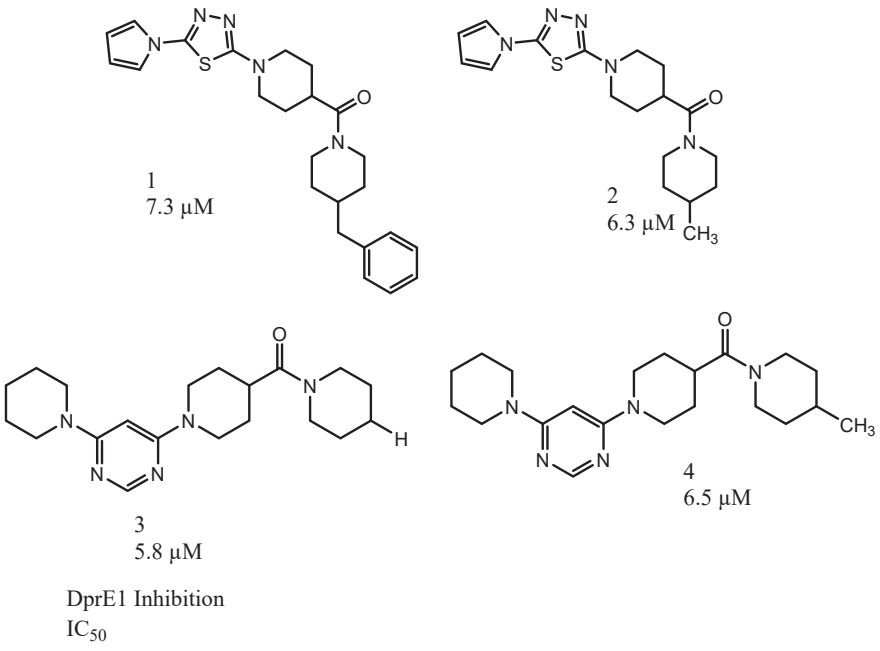

Fig. 26 – Representative compounds from morpholino-pyrimidine series.

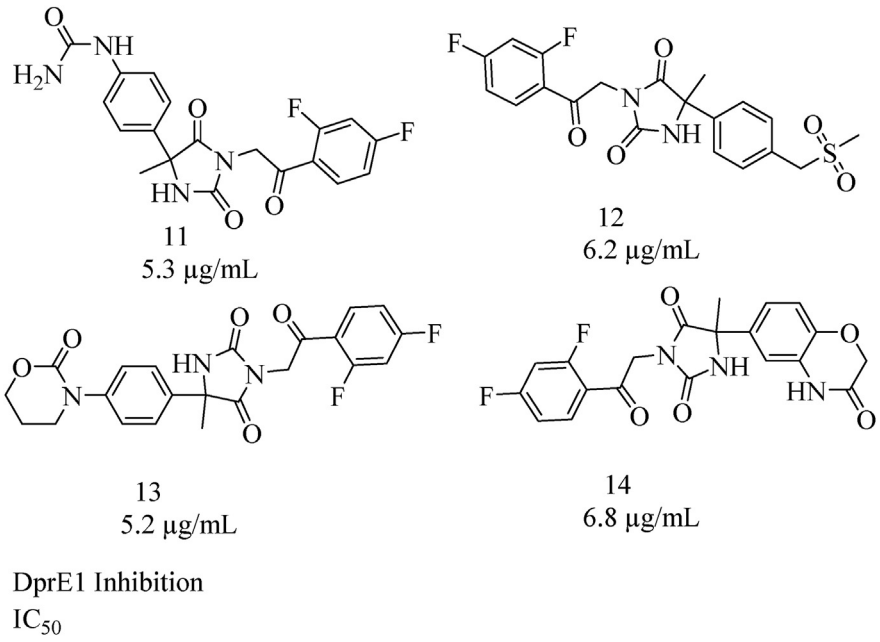

Fig. 27 – Some hydantoin based compounds.

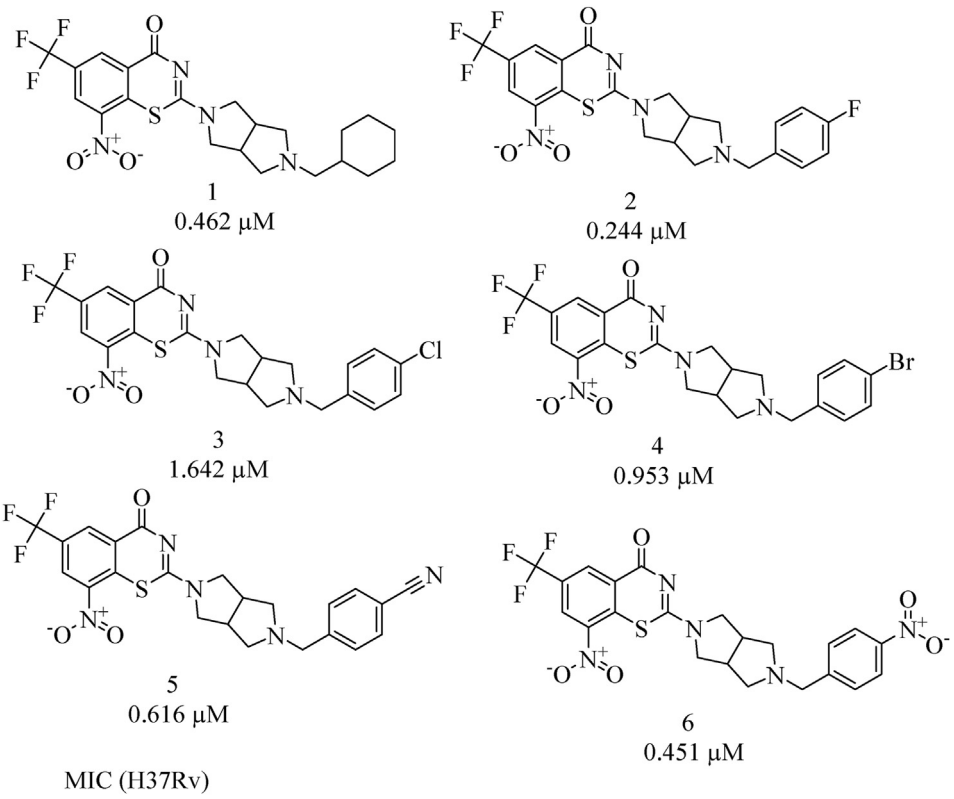

Fig. 28 – Some hexahydropyrrolo[3,4-c] pyrrole Derivatives.

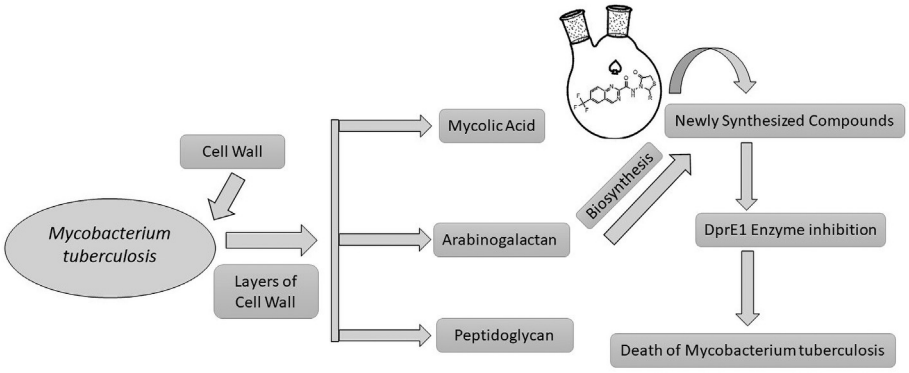

Fig. 29 – Mechanism of quinazoline-2-carboxamide derivatives as DprE1 inhibitors.

structure, to make structural modifications of the existing drugs to overcome drawbacks and side effects.

5. Conclusion

Conclusively, tuberculosis is an air borne respiratory disease. WHO has declared TB as a major severe epidemic of the past several decades. There is a significant gap in the development of novel antitubercular drugs. Recently, a drug bedaquiline has been approved for use against XDR-TB in 2012 (USA). The drug came after 40 years of research and development since the last drug was discovered in the field of tuberculosis. Detail literature survey has been carried out to understand the current research on TB, various targets for antitubercular drug discovery and specifically, development of DprE1 as an upcoming target for antitubercular drug discovery. Antitubercular drug discovery remains a major challenging area for researchers across the globe. After first and second-line antitubercular drugs, i.e., isoniazid, rifampicin, pyrazinamide, ethambutol, etc., no new agent has been reached successfully to market. Due to the advent of drug-resistant tuberculosis, from the past couple of decades, researchers were struggling with MDR and XDR tuberculosis. Several antitubercular

targets were discovered and explored to eradicate this infection. DprE1 is a new ray of hope for resistant tuberculosis. This review was totally emphasized on year wise developments in DprE1 enzyme. Role of DprE1 enzyme and mechanism, was reported for the first time in 2009. From the year 2009, DprE1 has dragged the attention of various researchers working in antitubercular drug discovery research. In past decade, every year researchers were able to find out new scaffolds and NCE which were effective against DprE1 inhibitors but not reached into the clinical trials. Hence young and experienced researchers both have more scope to identify the gaps in the research to design and develop more potent novel inhibitors. Number of chemical scaffolds were discovered and explored over the time i.e., benzothiazoles, azaindoles, thiazoles, quinoxalines, dinitrobenzamides, pyrazolopyridines, aminoquinolones to design and synthesized DprE1 inhibitors. With the progress of around more than a decade of DprE1 inhibitors, it can be concluded that in coming years further investigations on DprE1 inhibitors will happened and soon DprE1 will reach to the market to serve the mankind. Since the past decade, DprE1 exploring in various ways like protein structure and crystallography, several compounds with different scaffolds were constructed and tested for enzymatic studies. Till 2021, every year, significant discoveries and novel aspects of the DprE1 enzyme were reported by the researchers.

6. Future perspective

In upcoming years, researchers should focus on synthesizing more potent analogues with minimum dose (from μg-ng), less toxicity, and side effects. Organic and medicinal chemists, structural biologists should develop more and more inhibitors to combat drug-resistant tuberculosis to eradicate this deadly respiratory airborne infection.

Conflicts of interest

The authors have none to declare.

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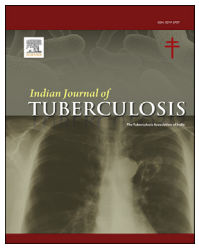

Review article

Gall bladder tuberculosis: Review of literature

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ABSTRACT

Gall bladder tuberculosis (GB TB) is a very rare disease and scarce data is available on exact incidence and clinicopathogenesis even in endemic areas. The aim is to provide an insight into epidemiology, pathophysiology and management for better understanding of gall bladder tuberculosis. We collected data available from the literature on all histologically proven gall bladder tuberculosis. Case reports with either no article or only abstracts were available excluded from the study. Fifty two case reports and series with total 73 patients were included in this study. Mean age of patients was 48 years (Range 8–86 years) with male: female ratio of 1:1.7. 53 (73%) patient had isolated disease and 18 (24%) had associated abdominal tuberculosis. 3 (4%) of patients had concomitant and 7 (9%) had past history of pulmonary tuberculosis. 39 patients presented as cholecystitis and 25 as gall bladder mass. 44 (60%) patients had gall stones and majority of them (56%) are multiple. Granuloma and caseous necrosis was found in 80% & 60% of patients respectively. In conclusion, Gall bladder tuberculosis is a very uncommon presentation of abdominal tuberculosis. Pre-operative diagnosis is not possible due to lack of specific diagnostic test so increase in awareness and a high index of suspicious is required.

1. Introduction

Hepatobiliary tuberculosis (HB TB) is a rare extrapulmonary manifestation of pulmonary TB (PTB).[1–3] Gall bladder tuberculosis (GB TB) is still rarer and very uncommon even in areas where tuberculosis is endemic and continues to represent a diagnostic challenge to clinicians.[4] The clinical presentation of GB TB is vague and diagnosis is often delayed due to lack of specific symptoms and diagnostic test. Histopathological examination is necessary for the definite diagnosis. Increase in awareness and proper investigation might help in adequate diagnosis and management of gallbladder tuberculosis.

Only case reports/series has been published in the literature and exact incidence of GB TB is still unknown. We have tried to collect the data of HPE confirmed cases of GB TB. The aim of this manuscript is to provide an insight into epidemiology, pathophysiology and management of this rare pathology for better understanding, from the available literature. Till now, around 180 cases of GB TB have been reported in literature although few of them are not histologically proven.

2. Methods

We have searched the literature for confirmed cases GB TB either by fine needle aspiration cytology (FNAC) or

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histopathological examination (HPE) of gall bladder. We have excluded the case reports on hepatobiliary and lymphnodal (LN) TB which had no comment on histological GB involvement. We have also excluded the case reports on which full article was not available. We have collected information on demographic profile, clinical presentation, investigation, management and histology of all cases of confirmed GB TB.

3. Results

We found 113 case reports/case series on GB TB reported in the literature till now involving about 180 patients. Unfortunately detailed information were not available in several reports. Fifty four case reports excluded due to either no article or only abstracts were available. Another 7 case reports also excluded because GB TB was not confirmed on HPE. These includes cases of LN TB, Biliary TB and Hepatic TB. So finally, 52 case reports with total 73 patients of HPE confirmed cases of GB TB were included in this study. About half of these patients were reported from the India where tuberculosis is endemic.

On seeing demographic profile we found that mean age of the patient was 48 years (Range 8–86 years) with about two third of patients were having age more than 40 years. Patient presented with pre op diagnosis of suspected carcinoma GB are older with half of patient having age more than 50. Women are more commonly involved with Male:Female (M:F) ratio of 1:1.7 but man are twice more likely affected when pre op diagnosis is suspected carcinoma GB with M:F ratio 2.1:1.

Only 20% of the patients had reported comorbidity with most common being the diabetes mellitus (DM) in 10% of patients and human immunodeficiency virus (HIV) is associated with 3 patients. Approx. half of these cases have documentation on past history of PTB and only 8 patient (9%) have reported past history of PTB. Majority of patient (73%) presented as isolated GB TB and only 20 (27%) patients found to have associated site TB at the time of presentation. Most common site associated with GB TB was abdominal TB in 18 patients (24%). Only 3 (4%) patients found to have active PTB at the time of presentation.

Abdominal pain (90%), fever (30%) and abdominal lump (25%) were the most common symptoms. Anorexia and weight loss present in about 20% of the patients. Only 15% of patients had jaundice at the time of presentation. Patient with diagnosis of carcinoma GB (CA GB) had more likely to present with jaundice and lump abdomen. 4 (5%) patient presents as acute abdomen with peritonitis, 2 were diagnosed as perforated GB intraoperatively, and they were preoperatively diagnosed as peptic and duodenal perforation. One patient presented with persistent post-cholecystectomy fistula.

70% had ultrasonography (USG) abdomen done and 46% underwent cross sectional imaging as diagnostic workup. The commonest USG findings includes features of chronic cholecystitis with thick wall and stones, or GB mass suspected of malignancy. 45 (60%) patients found to have gall stone on USG and majority of them (56%) are multiple. 18% patients are without gall stones and another 15 case report did not comment on gall stones. Most common preoperative diagnosis was either acute or chronic cholecystitis 53% (39) followed by carcinoma GB in 34% (25) patients.

Open cholecystectomy with or without biopsy was the most common surgical procedure done in 56% (41) patients. 16% (12) of patients underwent lap cholecystectomy. Out of 25 patient with suspected CA GB, 8 patient underwent radical cholecystectomy and 12 patient underwent cholecystectomy with intra operative biopsy. 5 patients did not underwent surgery and out of which one found GB TB at autopsy and rest 4 (5%) had FNAC from GB mass as suspected CA GB detected as GB TB.

Almost in all patients GB TB was diagnosed after the histology and most common histology findings were based on presence of epithelioid cells, Langhans' giant cells, peripheral lymphocytes, and caseous necrosis. Granulomas with epithelioid and Langhans' giant cells was found in 80% of the patient. 60% of patient had caseous necrosis on histology. Only 22% (16) of patient was found to have acid fast bacilli (AFB) on ziehl–neelsen (ZN) staining. 80% patients had comment on post-operative ATT for about 6–9 months. Majority of patients had good post-operative recovery and were asymptomatic on follow up.

4. Discussion

Gall bladder TB is a rare infectious disease traditionally included with HB TB but now with increasing number of cases now it has been identified as an independent entity. Simmonds in 1908 first differentiated 2 types of cholecystitis, acute and chronic based on whether cholecystitis is caused by an infection through the biliary passage or due to secondary infection of a gall-bladder that has already been damaged by non-specific inflammation.[5] Bergdahl (1970) first reported the review of 41 cases GB TB.[6] Majorities of cases till now are reported as isolated case reports but few case serious are also published.[7–10] This is the first article witch collected the all the article published on GB TB and describe its demography, clinical presentation and its management.

4.1. *Epidemiological features*

GB TB is a rare presentation of extrapulmonary tuberculosis (EPTB). The most common form of EPTB is the involvement of lymph nodes (30%–40% of cases).[11–13] Abdominal TB is uncommon and reported prevalence is comprising upto 12% of EPTB.[14] Abdominal lymphadenopathy is the most common manifestation of the abdominal TB and isolated involvement of abdominal solid organs is present in 15%–20% of all patients with abdominal TB.[15] The genitourinary system is the most commonly involved followed by liver, spleen and pancreas.[16] Reported prevalence of primary HB TB is only 1% of the abdominal tuberculosis and tuberculosis of gall bladder is extremely rare.[1–3]

Various studies suggest wide range of concomitant pulmonary and abdominal TB however, active pulmonary TB has been reported in only a minority (6–38%) of these cases of abdominal TB.[17] Horvath KD et al and Akhan O et al showed approximately 15%–25% of cases with abdominal TB have concomitant pulmonary TB.[18,19] Although no clear data available on prevalence of concomitant gall bladder TB with abdominal or other site TB. Our study showed majority of

patient (73%) presented as case of isolated case and 27% patients found to have TB at other site along with gall bladder tuberculosis at the time of presentation. Most common site associated with GB TB was abdominal TB (24%) with mostly as peritoneal nodules. Only 4% patients found to have active pulmonary TB at the time of presentation with 9% of patient had past history of PTB. In contrast, abnormal chest x-rays was found in approximately 75% of patients with HBTB demonstrating pulmonary TB.[20] In a 6-year study by Essop et al, 10 patients out of a total of 96 patients had localized HB TB.[3] In a study by Amarapurkar et al, concomitant pulmonary TB was seen in 28.9% cases of HB TB, whereas in the world literature it varies from 10% to 65%.[4,20–22] In the last few decades the incidence of gallbladder tuberculosis has increased and majority of the case reports are of isolated gallbladder tuberculosis. The incidence of tuberculosis is also on the rise due to increase in incidence of HIV, intravenous drug abuse and increased immunocompromised states.[23]

4.2. *Classification*

GB TB has been classified on the basis of clinical presentation and radiological findings. Based on 40 cases published in world literature up to 1955, Weitz classified four types of gall bladder TB: I. Miliary tuberculosis in children with ulcerating tubercles in the gall-bladder. 2. Gall-bladder tuberculosis in association with severe general tuberculosis. 3. Tuberculosis limited to the gall-bladder. 4. Gall-bladder involvement in association with tuberculosis in other peritoneal organs. According to this classification, group 1 accounts for 14%, group 2 30%, group 3 45% and group 4 11% of the cases.[24] In our study type III (isolated TB) was the most common type of presentation with 73% of patients which was much higher than as described by weitz (45%). Type IV being the second common presentation in 24% of patients. This can be explained by to two factors first, the definitive management of Pulmonary TB along with BCG vaccination decreased the overall prevalence and severity of TB all over the world and second, more cases now operated for symptomatic GSD in last few decades are being diagnosed as GB TB on histology. However, in contrast Xiu-Fang Xu et al reported that among the seven cases of gallbladder TB, six cases were accompanied by abdominal extra-gallbladder TB.[9]

Although radiological findings are not very characteristic of GB TB, radiological classification was described by Xiu-Fang Xu et al into three different computed tomography (CT) findings of gallbladder tuberculosis and correlated them with pathologic findings. Three different CT findings were: 1) micronodular lesion of the gallbladder wall which may mimic gallbladder polyp or early carcinoma, 2) an irregularly thickened wall and 3) a gallbladder mass with multiple-focus necrosis or calcifications accompanied by the typical CT findings of abdominal tuberculosis.[9]

4.3. *Pathophysiology*

Mycobacterium tuberculosis is an aerobic intracellular pathogen, which grows well in oxygen abundant environment. Tubercular bacilli may reach hepatobiliary system through several possible routes. Vojtisek and Zrustova described the haematogenic or lymphogenic dissemination from neighbouring or distant organs. This may reach GB through Hepatic artery causing miliary tuberculosis or through portal venous system from other intra-abdominal organs.[25] Simmonds and Ajello suggest an ascending canalicular infection via the biliary passages.[5,26] Although direct involvement from a nearby viscera is also postulated by Pohl and Weitz.[24,27] Differentiation of gallbladder tuberculosis into two types is possible according to routes of spread into canalicular or hematogenous and lymphatic spread and its correlation with involvement of mucosa. If the dissemination is through canalicular route gallbladder mucosa usually shows tubercles, but tubercle will be present on peritoneal surface if the dissemination was through transcoelomic spread.[28]

Normal gallbladder is highly resistant to the tubercular infection due to relative hypovascularity of gallbladder sac and high alkalinity of concentrated bile.[8,29,30] Prior gallstone disease, diffuse papillomatosis or cystic duct obstruction makes gallbladder susceptible to tubercular infection.[31] Gall stones cause damage to the gall bladder wall which seems to be the risk factor for development of cholecystitis. Cystic duct obstruction causes decreased bile acids in gallbladder bile and decreased alkalinity which predisposes gallbladder for infection.[29,30,32] Literature showed cholelithiasis is associated with more than 70% of cases of tubercular cholecystitis.[8,29,33] Bergdahl reported that out of the 44 cases of tuberculosis of the gall-bladder, only 2 are acalculous.[6] Our study showed presence of gall stones in 45 (60%) patients which is less than the reported literature. This study also showed that majority of gall stones (56%) were multiple with 18% of patients were without gall stones.

4.4. *Patients characteristics*

GB TB is distinct from abdominal TB in that it affects more often elder population with mean age 48 years whereas abdominal TB is more common in young patients. Few studies reported gall bladder tuberculosis occurs most commonly in women over 30 years of age but in our study half of patients were $\geq$50 year of age.[30,31] In contrast to HB TB which is more common in man with a male to female ratio of 2:1 but our study showed that in GB TB women are more commonly affected with M:F ratio of 1:1.7.[34,36] Interestingly our study showed that patients with diagnosis of CA GB had reverse male to female ratio of 1.7:1, so man with GB TB were more likely diagnosed as CA GB.

4.5. *Clinical presentation*

The clinical presentation of GB TB is slow and insidious, usually with non-specific symptoms and signs. It may present with features of cholecystitis (53%), a gallbladder mass (34%) or obstructive jaundice (15%). Acute presentation of GB TB also has been reported in few cases caused mainly by GB perforation.[35–38] Kumar P et al reported a case of GB TB presented as localised GB TB perforation with persistent fistula.[41] Abdominal pain (90%), fever (30%) and abdominal lump (25%) were the most common presenting symptoms. In comparison, the clinical manifestations of hepatobiliary TB are mainly those of the extrahepatic disease, as hepatic involvement is usually asymptomatic. A study showed that in HB TB,

right upper quadrant pain appeared to be the most common symptom present in 65–87% and 20–35% of patients presented with jaundice.[34] High index of suspicion has to be there especially in patients of high risk group such as from high endemic area, active or prior pulmonary tuberculosis, immunosuppression etc. HIV and DM are the potent risk factor and TB is the most common opportunistic infection in patients with HIV in developing countries. The relation between DM and TB is more prominent in younger people and patients with type 1 DM.[39] In our study only 10% of patient was having DM and HIV was associated in only 3 cases.

4.6. *Investigations*

Due to the lack of specific symptoms and other pathognomonic findings GB TB poses a considerable diagnostic challenge. Routine blood investigation may show features of chronic inflammation like low hemoglobin level, raised ESR etc and deranged liver function tests in case of jaundice. Tubercular skin test may be positive. Radiological investigations also shows nonspecific findings and contributes little to the diagnosis of gallbladder tuberculosis. USG shows features of either acute or chronic cholecystitis with thick wall and stones, or GB mass with lymph nodes.[7,32] 46% underwent cross sectional imaging including those who are suspected of GB malignancy or if there is diagnostic dilemma.

The largest case series by Xiu-Fang Xu et al in 2011 suggested that a large mass with multicentric necrosis may be the typical CT features of gallbladder TB. The most common form of GB TB is the thickened-wall type, which can be frequently misdiagnosed as CA GB or cholecystitis. The wall is mostly thickened, uniform, and diffuse. The edema "halo" in GB TB patients can be observed on CT scan unlike that in CA GB patients.[9]

Both tuberculosis and carcinoma can give rise to lymphadenopathy and ascites. Few features which can favour the malignancy were contiguous infiltration of the liver by the gallbladder lesion, and hepatic metastases whereas associated mesenteric thickening and adenopathy, and pulmonary infiltrative lesions would favour tuberculosis. Mahendran et al in 2011 showed in contrast to those with malignancy, patients with biliary tuberculosis had a longer history (122 vs 44 days), an abdominal mass was present less frequently (28% vs 57%), the serum bilirubin was lower (1.6 vs 6 mg/dl), and they also had evidence of tuberculosis elsewhere in the body (28.5%).[40] Gulati et al reported the atypical presentation of GB TB on CT with appearance of multiloculated, thick walled GB.[41] Positron emission tomography – computed tomography scan can be falsely positive in gallbladder tuberculosis and is not of much benefit.[42]

Preoperative FNAC of the gallbladder mass is usually not performed routinely. In our study 4 patient with GB mass and suspicious of malignancy had preoperative FNAC which was s/o GB TB.[32,41,43,44] FNAC is also not conclusive in all cases and two cases of gallbladder tuberculosis were found where preoperative FNAC was inconclusive.[7,45] Endoscopic ultrasound (EUS) guided FNAC which is of great help in many cases of HB TB especially in lymph nodal/pancreatic TB but has a little role for the diagnosis its role in GB TB. Few reports are available on use of EUS FNAC for evaluation GB mass. In a recent largest study by singla et al EUS FNA done in 101 cases of GB mass and found confirm diagnosis done in 99 patient with a sensitivity 90% and specialty of 100%. So they concluded EUS-FNA appeared safe, without any serious complications.[46] So EUS FNAC can be a tool for diagnosing GB TB in patients with clinical or radiological suspicious of TB and surgery can be avoided in such patients. Other modalities like USG or CT-guided FNAC of the enlarged lymph nodes can be useful in diagnosis but since majority of patient present as isolated GB TB there role is also limited. With the advent of image-guided fine needle aspiration cytology, endoscopic brush cytology and PCR testing of bile diagnosis of hepatobiliary tuberculosis can be made without the need of laparotomy.[47] But role of these investigations is still unclear in diagnosis of GB TB. Rarely GB TB was diagnosed in a chronic cholecystitis specimen by TB-PCR.[48]

4.7. *Differential diagnosis*

Most common differential diagnosis includes chronic cholecystitis and GB carcinoma. Chronic cholecystitis can be differentiated with tuberculous cholecystitis only on histology after cholecystectomy. Although GB carcinoma cannot be completely ruled out during the preoperative evaluation but young age, diffuse GB wall thickening without liver infiltration, associated mesenteric thickening with adenopathy, and pulmonary infiltrative lesions with would favour tuberculosis. Preoperative FNAC and intra operative frozen is really helpful to establish the diagnosis if there is suspicious of TB.

4.8. *Histology*

Almost in all patients GB TB was diagnosed as histological surprise. On gross examination majority of patient had thick wall GB. However, Tanwani reported TB in a normal-looking gall bladder wall and mucosa.[49] Most common histology findings were based on presence of Granulomas with epithelioid and Langhans' giant cells was found in 80% of the patient. 60% of patient had caseous necrosis on histology. Presence of acid fast bacilli on ZN staining will further confirm the diagnosis but not all GB TB patient will demonstrate that. In our study only 22% of patient found to have AFB bacilli in the Gall bladder. Kapoor et al, revealed acid fast bacilli in only one case out of five.[8] In the world literature, 9–60% cases of HB TB show the presence of AFB in biopsy smear/culture, more so with the presence of caseation.[3,4] Compare to HB TB, 33–100% of liver biopsy specimens showed Caseation, a hallmark finding of TB granulomas, and AFB stains was positive in about 60%.[34]

4.9. *Management*

Since preparative diagnosis was not possible, majority of patient treated as acute or chronic cholecystitis and open cholecystectomy with or without biopsy was the most common surgical procedure done in 56% of patients. Laparoscopic cholecystectomy was done in 16% of patients. Out of 25 patient diagnosed as suspected CA GB, 8 patient underwent radical cholecystectomy and 12 patient underwent cholecystectomy with intra operative frozen/biopsy. Intraoperatively most common finding include presence of adhesion and thick wall GB with exception for few case reports with a normal GB and no evidence of peri-cholecystic adhesions.[49] Majority

(80%) of patients had comment on post-operative antitubercular treatment for about 6–9 months and most of them had good post-operative recovery and were asymptomatic on follow up. Conventional antitubercular therapy remains the cornerstone of post-surgery treatment. Treatment consists of a combination of isoniazid, rifampicin, pyrazinamide and ethambutol generally for 6–9 months. GB TB is now more likely diagnosed and managed as isolated tuberculosis with very low morbidity and mortality compared to 15%–42% of cumulative mortality for hepatic TB.[34]

5. Conclusion

Gall bladder tuberculosis is a very uncommon presentation of abdominal tuberculosis. Elderly women with multiple gall stones are more commonly affected. Abdominal pain, fever and abdominal lump were the most common symptoms. Preoperative diagnosis is not possible due to lack of specific diagnostic test and a high index of suspicious is required.

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Conflicts of interest

The authors have none to declare.

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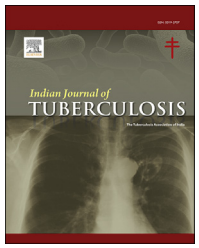

Review article

Health system resilience: Ensuring TB services during COVID-19 pandemic in Kerala, India

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ABSTRACT

COVID-19 pandemic has affected TB case detection and continuity of care globally. Kerala, the southern Indian state has experienced a reduction in TB notification during second and third quarter of 2020. Through (1) causal analysis (2) meticulous planning and establishment of systems (3) locally customised guidelines (4) better management of resources (5) integration with other programs and (6) good partnership with private sector, Kerala was able to catch up the TB notification and ensure that TB services remain intact even during the COVID-19 pandemic. Approach to catch up TB diagnosis included (1) Field based active case finding among the vulnerable individuals, (2) bilateral screening for TB and COVID-19, (3) enhancement of biosafety in laboratories, (4) strengthening of specimen collection and transportation systems, (5) targeted advocacy and communication to find out missed cases and (6) effective partnership with the private sector.

Current experiences also show that TB case finding could be improved and delay in diagnosis could be averted by integrating TB case finding into the screening and testing systems established for COVID-19. The experiences of ensuring TB services during pandemic in Kerala also affirms the importance of maintaining an integrated and strong TB control component in the public health sector and vesting ownership of the TB control programme with the primary health care team. Community-based and community-led responses that take diagnosis, care, and support to the doors of those affected have much potential in delivering TB services in the subsequent years of pandemic.

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1. Introduction

COVID-19 has challenged health systems and restricted essential health service delivery. Health system infrastructure, from diagnostic tools to the workforce, has pivoted towards COVID-19 and away from competing priorities, including tuberculosis. Health-care access has been constrained due to many reasons including lockdowns, redeployment of health staff, polyvalent molecular diagnostic platforms, health program management processes and fear to access health. Nine of the countries with the most tuberculosis (TB) cases—representing 60% of the global TB burden—-saw a drastic decline in diagnosis and treatment of TB infections in 2020, ranging from 16% to 41%.[1]

2. Local setting

Kerala, the southern Indian state has made remarkable progress in improving the people's health as evidenced by a higher life expectancy and a lower infant mortality rate.[2] Primary health care delivery system is well established with a primary health centre manned by a modern medicine practitioner for every 25,000 population. For every 5000 population, there are two multi-purpose health workers and for every 1000 population there is a community health volunteer (ASHA). TB Program is well integrated with the primary health care delivery. Experiencing a 7.5% annual decline in the incident TB, state has notified 72 TB cases per 100,000 population in 2019.[3]

To flatten the Covid-19 incidence curve, India has locked down the country from 25th of March 2020 to 31st of May 2020. Kerala state which reported the first COVID-19 case in India, had 22,314 cumulative confirmed cases per million population by Dec 31, 2020. Peak of COVID-19 pandemic in the state was in October 2020 (260 cases per million per day).[4]

Government of Kerala has issued advisory, which was a locally customised version of advisory by NTEP (National TB Elimination Program), as early as on April 2020 and established system to ensure that all services to the TB patients remains uninterrupted during and after the lockdown.[5] All TB laboratory staff were provided with adequate additional personal protective equipment. Extra efforts were taken by the health system to deliver the services for TB patients at their door steps including medicines for TB and co-morbidity management, providing proactive fortnightly clinical reviews through tele consultations and ensuring public health actions including offering Rifampicin sensitive testing at baseline, TB Preventive Therapy to the eligible household, contact investigations, Direct Benefit Transfer of INR 500 (7USD). All TB related activities were monitored daily through NIKSHAY- a case-based patient management system through the e-platform.[6]

3. Problem

While all services to the diagnosed TB patients were ensured, Kerala experienced a reduction in TB notification during lock down period (82% of estimated cases notified in second quarter Vs 99% of estimated cases notified in the first quarter). A more severe reduction in TB notification was observed during the period when the COVID-19 cases started increasing rapidly (76% of estimated cases notified in third quarter). An unusual delay in diagnosis of TB was reported by clinicians as some of the cases presented to the health facilities with haemoptysis and chest x-rays revealing cavity.

4. Approach

Causal analysis was done for non-improvement in TB notifications through 10 key informant interviews with primary health care providers and middle level program managers. Identified problems and suggested solutions were listed in Table 1. Based on the potential solutions, an action plan was prepared to catch up the TB notification in Kerala. The approach included.

4.1. *A field-based active/intensified TB case finding*

a) **Active Case Finding among Individuals with high vulnerability to TB & COVID**: Individuals who are vulnerable to develop TB (Contacts of TB, Past history of TB, Diabetes Mellitus, Chronic Smoker, Chronic alcoholic, Elderly, Bed ridden, Chronic Renal Diseases, those on immunosuppressive drugs) were mapped earlier through a door-to-door survey. List of Individuals with high vulnerability to develop TB in their field area were maintained at every primary health centre. Individuals with high vulnerability to develop TB were contacted directly/over telephone by the community health volunteer and screened for TB using the 4-symptom complex (4S = cough >2 weeks, fever >2 weeks, weight loss, night sweats). Since such individuals were also vulnerability for severe COVID-19, TB active case finding happened along with ensuring reverse quarantine for COVID-19. Reverse quarantine was ensured by the primary health care team by provide proactive care to the vulnerable individuals including counselling, education and delivering their essential medicines at door steps. All inmates of elderly homes and persons receiving palliative care were also screened for TB with the help of elderly care program and palliative care program.
b) **Bidirectional screening for COVID-19 and TB** - State had developed a COVID-19 surveillance system – universe being all individuals at risk for developing COVID-19. All such individuals have been contacted by the primary health care team daily for symptom surveillance. In addition to that, individuals presenting directly to a health facility with Influenza Like Illness/Severe Acute Respiratory Infection (ILI/SARI) symptoms were tested for COVID-19. Details of the tests were entered in a real time e-platform based management information system.

In the universe eligible for COVID-19 testing, the following actions for screening and testing for TB were taken[7]

a) ILI in a person with any vulnerability to develop TB were tested for TB.

Table 1 – Identified Problems and Potential solutions identified through key informant interviews.

Identified Problems	Potential Solutions
Difficult to access health facilities during lock-down. People are hesitant to come to hospitals.	Deliver diagnostic services at door steps. A field-based system for TB Case Finding
Decreased confidence in performing TB tests among peripheral health staff for fear of COVID-19	Enhancement of laboratory biosafety
Peripheral health staff oriented fully towards COVID-19.	Provider oriented Behaviour Change Communication
Stigma to 'Cough' among common man for fear of COVID-19	Community oriented Behaviour Change Communication
Health System preoccupied with COVID-19	Integrated approach pooling resources with minimal burden to the system.

b) Covid negative ILI were followed up after 10 days over telephone/direct visit to screen for 4S by the primary health care team. If the symptoms persisted for >14 days, TB test was done.
c) Individuals with SARI were screened for 4S. Those requiring hospital admissions were screened using Chest X ray.
d) Confirmed COVID-19 patients were screened for 4S and COVID-19 patients requiring hospital admissions were screened using Chest X rays.

All those screened positive with any one criterion in 4S or any abnormality in Chext Xray were tested for TB with an upfront molecular test with GeneXpert (Cephid, US)/Truenat (Molbio Diagnostics, Verna, India) closed nucleic acid amplification platforms.

4.2. *Biosafety enhancement of laboratories*

In addition to 43 molecular Nucleic Acid Amplification testing (GeneXpert/Truenat) machines provided by NTEP, 32 new machines were purchased. All molecular test machines were installed in laboratories equipped with Biosafety Class II A2/B2 cabinets. Seventy-five such public laboratories (1 per 400,000 population) were set up for performing bilateral tests for TB and COVID-19. Referral linkages were revised to improve testing turnaround times and result reporting. Human Resources were redistributed to run all the machines for an average of 12 hours a day.

4.3. *Strengthening specimen collection and transportation system*

Specimen collection and transportation system was established in a hub and spoke model from every village. The transportation mechanisms were locally customised to each setting-by human carriers or dedicated vehicles transporting to concerned hubs. Through the system, samples for COVID-19 and TB testing flowed to concerned hubs.

Standard operating procedure for collecting the sputum samples from home was prepared. 2-hour online training module was prepared with interactive videos and presentations. All category of staff including community health volunteers were trained.

Equipment for collection, packing and transporting were also locally developed with a prototype prepared at state level.

4.4. *Targeted advocacy and communication to find out missed cases*

A strong communication strategy was implemented focussing on all health providers and a separate one on general public. Successful advocacy was done with the managements of private hospitals, leaders of professional medical associations, doctors of other systems of medicines which resulted in bringing TB back to the minds of all health care providers. Innovative social media campaign, short messages by celebrities and opinion leaders, awards to local self-governments, non-financial incentives to health care providers, advocacy by the TB survivor's forum were the other key strategies deployed. Non-financial incentives including awards were declared to all category of staff for exceptional work.

4.5. *Ensuring partnership with private sector*

Through STEPS (System for TB Elimination in Private Sector),[8] the NTEP program was already in partnership with 380 major private hospitals in the state. The policies and ideas were disseminated timely to private sector also. Need for more molecular testing platforms in private sector was advocated for integrated testing of TB and COVID-19. This resulted in an enhanced capacity of molecular testing platforms in private sector with 62 additional GeneXpert/Truenat machines. While some of the major public hospitals were converted to exclusive COVID hospitals, majority of the private sector hospitals remained to provide exclusive non-COVID services. In some areas where public hospitals were offering COVID services, presumptive TB patients requiring detailed evaluation were referred to private hospitals by the field team where free X ray, free NAAT, free specimen collection transportation and free medicines were already being provided by the NTEP.

5. Implementation

Field based case finding efforts started in October 2020. Regular weekly review was done with program mangers at all level facilitating cross learning.

6. Relevant changes

TB notification figures started rising up reaching the estimated target in the month of December 2020. Approximately 87.4% of

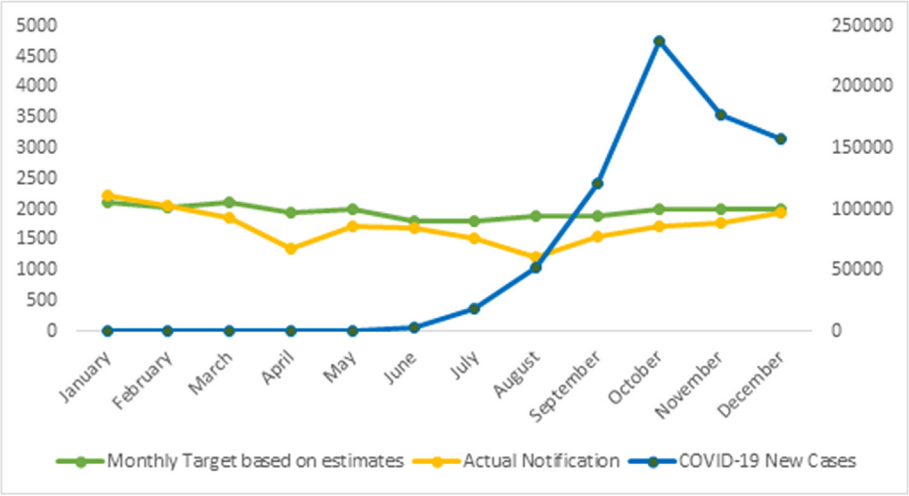

Fig. 1 – Monthly Trend in TB Notification, Kerala, 2020

the estimated cases were notified in 2020. Trend of month wise TB notifications against the estimated target is shown in Fig. 1. Private sector diagnosed 5795 TB cases in 2020 (4927 in 2019).

A total of 661,470 individuals were screened for TB as part of field-based case finding during October to December 2020, 37,685 presumptive TB cases were identified, 29,166 samples were tested and 802 individuals were diagnosed as TB. This constituted 15% of notified TB in last quarter 2020. Details of field-based case finding were shown in Table 2.

Other public health actions relevant to the diagnosed TB patients remain intact as evidenced by proportion of TB cases who knew their rifampicin resistance status at baseline (63% Vs 58%), proportion of TB patients with known HIV status (91% Vs 87%), household contact tracing visits (85% Vs 61%) and Lost to Follow Up rates (stable at 2%) during 2020 and 2019 respectively).

7. Discussion

Through (1) causal analysis (2) meticulous planning and establishment of systems (3) locally customised guidelines (4) better management of resources (5) integration with other programs and (6) good partnership with private sector, Kerala was able to catch up the TB notification to some extent and ensure that TB services remain intact even during the COVID-19 pandemic.

Screening for TB among those with COVID-19 symptoms and implementing integrated testing for TB and COVID-19 was a bold decision. High political and administrative commitment was the key to take such a bold decision and setting up the necessary systems. Effective leadership also tried to mobilise and coordinate all available resources.

NIKSHAY the case based real time surveillance system of NTEP ensured smooth flow of information without which it would have been difficult to detect the Problem and monitor the impact so meticulously.[6] The experience reiterates the need to have a strong surveillance system for every public health programs to pick up early warning signals and facilitate monitoring during a disaster.

Having not ensured the biosafety of laboratories and infection control systems, the health staff would not have gained confidence to collect and transport specimen and perform tests. Robust specimen collection and transportation system helped in preventing leaks in the cascade. Though the clinicians were apprehensive about the bilateral screening algorithm in the beginning, strong behaviour change communication with involvement of leaders of professional associations, celebrities, owners of private hospitals and social media campaign with clinical experiences by peers gained their trust.

Well-motivated and supported staff took on extra burdens. Such commitments have been witnessed previously also during the time of Kerala Floods.[9] However, current one was a long fight and it was difficult to maintain the motivation due to 'fatigue'. Building team spirit, highlighting health care

Table 2 – Summary of active TB case finding during October–December 2020.

Category	Mapped for Screening (Universe)	Actually screened	Presumptive TB identified	Tested for TB	Diagnosed as TB	Test Positivity Rate
Individuals with High Vulnerability to develop TB	976147	537371	30900	23585	610	2.6
Palliative Care/Bedridden	132800	66156	1753	1066	37	3.5
Elderly at old age homes	19494	15350	1743	1226	25	2
Influenza Like Illness	36417	34417	2437	2437	69	2.8
Severe Acute Respiratory Illness	6176	6176	852	852	61	7.2

workers and volunteers as heroes, non-financial incentives including appreciations and awards, payment of financial incentives on time, effective communication and supportive supervision were attempted. Integration of activities is another solution to minimise effort duplication by the health care staff.

Test positivity was unusually high among SARI. People with SARI are the most likely to seek care from a hospital and have a high probability to get a chest Xray done. This could be the reason for a higher positivity.

Experiences also show that bilateral screening for TB and COVID-19 is feasible in routine program setting. TB case finding could be improved and delay in diagnosis could be averted by integrating TB case finding into the screening and testing systems established for COVID-19. Process of bidirectional screening for COVID-19 and TB were documented in paper-based systems. Deliberate decision was made to collect only minimal reports to prevent system being overburdened. Incorporating integrated testing details to COVID-19 management information system can facilitate real time monitoring. Analysis of the cost would be essential to make further recommendations.

The experiences of ensuring TB services during pandemic in Kerala also affirms the importance of maintaining an integrated and strong TB control component in the public health sector and vesting ownership of the TB control programme with the primary health care team. Community-based and community-led responses that take diagnosis, care, and support to the doors of those affected have much potential in delivering TB services in the subsequent years of pandemic.

Conflicts of interest

The authors have none to declare.

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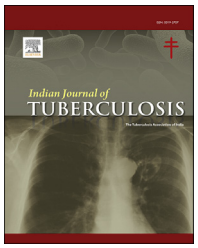

Review article

Predicting mortality in pulmonary tuberculosis: A systematic review of prognostic models

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ABSTRACT

Background: Pulmonary tuberculosis is a highly prevalent disease in low-income countries; clinical prediction tools allow healthcare personnel to catalog patients with a higher risk of death in order to prioritize medical attention.

Methodology: We conducted a literature search on prognostic models aimed to predict mortality in patients diagnosed with pulmonary tuberculosis. We included prospective and retrospective studies where prognostic models predicting mortality were either developed or validated in patients diagnosed with pulmonary tuberculosis. Three reviewers independently assessed the quality of the included studies using the PROBAST tool (Prediction model study Risk of Bias Assessment Tool). A narrative review of the characteristics of each model was conducted.

Results: Six articles (n = 3553 patients) containing six prediction models were included in the review. Most studies (5 out of 6) were retrospective cohorts, only one study was a prospective case-control study. All the studies had a high risk of bias according to the PROBAST tool in the overall assessment. Regarding the applicability of the prediction models, three studies had a low concern of applicability, two high concern and one unclear concern. Five studies developed new prediction rules. In general, the presented models had a good discriminatory ability, with areas under the curve fluctuating between 0.65 up to 0.91.

Conclusion: None of the prognostic models included in the review accurately predict mortality in patients with pulmonary tuberculosis, due to great heterogeneity in the population and a high risk of bias.

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1. Introduction

Tuberculosis is a global health problem especially in low-and-middle income countries, such as Peru. Tuberculosis is also an important cause of mortality in these countries. It is reported that in the year 2017 the estimated incidence of tuberculosis was 10 million cases worldwide, and there has been a 1.5% increase for each year since the year 2000.[1] However, the large number of deaths caused annually by this disease has been successfully decreasing over time.[1,2] In 2018 it was estimated that the incidence rate of tuberculosis in America was 28 per every 100.000 inhabitants.[3] The highest incidence rate was found in the Caribbean (61.2 per 100.00 habitants), followed by South America (46.2 per 100.000 habitants), Central America and Mexico (25.9 per 100.000 habitants) and finally North America (3.3 per 100.000 habitants).[4,5]

The Center for Disease Control and Prevention reports that 1.5 million deaths from tuberculosis occurred worldwide in 2018. The annual incidence of TB deaths varies greatly depending on the geographic location, the resistance of the strain of mycobacterium tuberculosis, and the resources available to each nation. In countries like Taiwan, the reported mortality incidence is 2.7 per 100,000; a figure that is similar to that of Peru with 3.7 deaths per 100,000 inhabitants in 2015. However, these data differs greatly when compared to countries like the United States, which reports an annual mortality of 0.2 cases per 100,000 inhabitants.[6–9]

Models have been created to predict mortality in multiple pathologies, such as COVID-19 infection or in patients with type 2 diabetes mellitus. Prognostic models help tackle mortality in low and middle income countries, allowing the treating physician to plan and prioritize the use of resources, which in many countries are scarce.[10] This is why the present work aims to gather and synthesize the evidence present in the literature regarding models for predicting mortality in patients with pulmonary tuberculosis.

2. Methods

2.1. Study design

The current study followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (The PRISMA Group, 2020) Statement. An abbreviated version of the protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) [CRD42020202778].[11]

2.2. Information sources and search strategy

A literature search on prognostic models aimed to predict mortality in patients diagnosed with pulmonary tuberculosis was conducted in the following databases: PubMed, Scopus, Ovid Medline, Ovid Embase, Web of Science, Scielo, Lilacs and Cochrane Library. The search strategy used both Mesh terms and free-text terms, as seen on Supplementary Material Appendix 1. The literature search was conducted in July 2020.

2.3. Study selection

We included prospective and retrospective studies where prognostic models predicting mortality were either developed or validated in patients diagnosed with pulmonary tuberculosis. For an article to be selected for this review it needed to have as part of their criteria for diagnosing pulmonary tuberculosis at least one of the following: prove the presence of mycobacterium tuberculosis either in microbiological culture in solid/liquid medium, a molecular test, positive bacilloscopy (sputum smear microscopy) or a histological sample.

We excluded case reports, scoping reviews, systemic reviews, conference abstracts and letters to the editor. We also excluded prognostic models that were purely based on imaging findings. No limits were placed regarding language, place of origin or publication date.

2.4. Screening and selection of studies

Three reviewers using the data management software "Rayyan QCRI" analyzed the titles and abstracts resulting from the search strategy independently. Once the potential literature to be included in the systematic review was identified, the full version of each of the articles was read. At this time, we applied the selection criteria to each of the studies. If an article did not comply with the totality of the selection criteria, said article was excluded from the review. In the event of missing information, the authors of the corresponding study were contacted. A secondary bibliographic search was carried out from the selected articles, the studies found by this method that met the selection criteria were included in the project. Once the previous processes were completed, the articles selected by the authors were grouped and duplicates were eliminated. In the event of disagreements regarding the inclusion of an article, a consensus was reached among the authors. Data extraction was carried out in a standardized file in Microsoft Excel, that followed the general guidelines of the "Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies" (CHARMS) Checklist.[12]

2.5. Methodological quality assessment

Three reviewers independently assessed the quality of the included studies using the PROBAST tool. (Prediction model study Risk of Bias Assessment Tool), which assesses both the risk of bias (RoB) and the applicability of each model. This evaluation is based in four domains: participants, predictors, outcome, and analysis. According to the characteristics of each study, these can be classified according to the risk of bias into high-risk, low-risk or unclear risk studies. The applicability of each study can be classified as: high concern, low concern, or unclear concern.[13]

2.6. Statistical analysis

A meta-analysis was not carried out because none of the prognostic models found in the present review had additional studies that validated this model in an external population with the same pathology. However, a descriptive analysis of each of the included articles was performed. The information

related to the characteristics of the population of each study, the development of the model and the performance of each rule were extracted and reported.

3. Results

3.1. Study identification

The initial search yielded 1822 articles, of which 707 were found to be duplicate studies and were therefore eliminated. The titles and abstracts of 1115 articles were analyzed, but only 23 articles met the inclusion/exclusion criteria. Of these, only 4 articles met the selection criteria when they were read in full text. In the secondary bibliographic search, we found 2 more articles that met the selection criteria and were therefore included in the review for a total of 6 studies. All this information can be seen on Fig. 1 (Appendix 1).

3.2. Study characteristics

The characteristics of each study are presented in greater detail in Table 1 (Appendix 2). There were a total of 6 prognostic rules, one in each article. Most studies (5 out of 6) were retrospective cohorts, only 1 study was a prospective case-control study. The geographic distribution was as follows: Japan (2 articles), Guinea-Bissau (2 articles), Portugal (1 article) and India (1 article). In 5 out of 6 studies, new prediction rules were developed, while in one, a prognostic score that had been initially created to predict mortality in patients with pneumonia (A-DROP Score) was validated for patients with pulmonary tuberculosis.[16] When adding the population of all the studies, there were a total of 3553 participants, with samples ranging from 103 participants to 1070 participants. Only the studies by Bastos et-al., Wejse et-al. and Rudolf et-al. included HIV positive population.

4. Methodological quality assessment

Table 2 (Appendix 3) presents the results of the methodological quality analysis. All the studies had a high risk of bias according to the PROBAST tool in the overall assessment. The origin of this bias comes mainly from the analysis and outcome domains, where most studies were rated as low quality. Furthermore, in the domain of predictors, most of the studies were classified as "unclear risk" because they did not make clear if outcome information was used when assessing predictors. The "Applicability" section refers to the applicability of a primary study to the review question; in this case, the overall assessment showed that 3 studies had a low concern of applicability, 2 high concern and 1 unclear concern.

4.1. Model development

The statistical analysis used in every study for the development of each model is enlisted on Table 3 (Appendix 4). As mentioned before, 5 studies developed new prediction rules, they used multiple modelling methods: Logistic Regression, Cox Regression and Polychoric Correlation. Only two studies explicitly declared the management of missing data, and in both cases, they decided to exclude every patient that did not count with the complete information of interest. Neither study reported making imputations for the handling of missing data.

4.2. Model performance

In general, the presented models had a good discriminatory ability, with areas under the curve fluctuating between 0.65 up to 0.91. The predictive model with the highest discriminatory power was the one reported by Horita et al with an AUC of 0.910 in the development cohort and 0.893 in the validation cohort. Three out of five studies that developed prediction rules carried out the validation of the developed model, where there were no marked differences regarding the AUC, since the variations between the development and validation cohorts of the same study were 0.019–0.1. A calibration model was only reported in one study, where the authors declared to have run a Hosmer–Lemeshow test, but they did not mention the results of the test. For more information, consult Table 3 (Appendix 4).

4.3. Predictors included in the models

In most studies, the description of the outcome measured was also used to define the predictors, like hypoxemic respiratory failure, that was defined by a saturation <90%. This predictor was the most used in the prediction models and was found in 3 studies in an inpatient setting.[14–16] On the other hand, there were two studies that measured predictors that could be measured in a prehospital setting such as the presence of cough, hemoptysis, dyspnea, chest pain, and night sweats.[17,18] Furthermore, there was only one study that used radiographic parameters as one of the predictors.[16] However, there was minimal overlap between scores definitions.

5. Discussion

To the best of our knowledge, this study is the first systematic review that evaluates mortality prediction scores in pulmonary tuberculosis. The six studies used for this review evaluated mortality in pulmonary tuberculosis according to different markers, conditions, and symptoms present in the patients at the moment of recruiting the data. We found that every score had a high risk of bias, due to different reasons for each specific case. The TBscore, TBscore II, and the score created by Singla et-al.,[16–18] had a low concern of applicability, probably due to the easy measurement of the predictors used in these scores. We consider that the prediction models TBscore and TBscore II are the easiest to apply in low-income countries, because they are mainly made up of clinical predictors, that can be measured by any health professional and in a pre-hospital scenario, without the need of a doctor. Nevertheless, the score created by Horita, et-al, obtained the best predictive value, even though it had a High RoB and a high concern of applicability.[19] This is probably because one of the four predictors is albumin levels, and as a laboratory marker it can be difficult to obtain, especially in rural areas of developing countries. However, we consider that it is a model that can be applied in countries of

medium to high socio-economic level, unlike the TBscore, TBscore II, and the score by Singla, et-al.

Although there is no common predictor that was used in the six prediction models, the most used were: age, systolic blood pressure and hypoxemic respiratory failure. The model created by Singla et al, was the only one that used radiological findings as a predictor, as 'advanced disease on chest radiography'. The score created by Horita et-al, includes oxygen requirement as a predictor. Oxygen requirement is not commonly seen in pulmonary tuberculosis, it is more frequent in miliary tuberculosis due to respiratory failure.[20] Only 3 of the 6 models used age as a predictor, and the model created by Bastos et-al, used comorbidities as a mortality predictor.[14] For our consideration, it would have been important to have these predictors in all the models, since people with comorbidities and advanced age are the ones associated with a higher mortality rate in pulmonary tuberculosis.[21] As there is no common predictor between all the models created, we cannot assume that there exists a specific predictor that predicts mortality in pulmonary tuberculosis. Therefore, it can be assumed that there is no prediction model that accurately predicts mortality in this disease. Despite this, we must mention that all these models are quite understandable and easy to apply and interpret.

Regarding the methodology used in each of the included studies. Four studies conducted retrospective cohorts for the development and validation of tools to predict mortality in pulmonary tuberculosis (Bastos et-al, Horita et-al, Wejse et-al and Rudolf et-al). One study validated a tool that was initially created to predict mortality in patients with pneumonia (A-DROP, Nagai et-al); and finally one study carried out the development of a tool, in a case and control study (Singla et-al). It would have been important for the developed models to have additional studies that externally validated the use of the tool, to have a better assessment regarding their quality and confidence. Regarding the discriminatory power, as already mentioned before, the model created by Horita et-al is the one with the highest power with an AUC of 0.919 for the development cohort and 0.893 for the validation cohort. The models that presented the lowest discriminatory power were the TBscore, with an AUC of 0.65 for the development cohort at 8 months, and the TBscore II with an AUC of 0.6817 for the validation cohort. Finally, in the TReaT model created by Bastos et-al, the predictors were chosen based on a univariate analysis, when according to the PROBAST recommendations, the predictors should be chosen after a multivariate analysis.[13] This being one of the reasons why this tool obtained a High ROB.

Tuberculosis is one of the first 10 causes of death worldwide. In 2018 it was attributed to approximately 1.5 million deaths. The WHO reported that for the year 2019, 10 million new cases of tuberculosis were registered.[22] Most deaths caused by tuberculosis are due to comorbidities like HIV, socio-economic status, malnutrition, inadequate prescriptions, bad control of the disease, among others. However, tuberculosis is a preventable and curable disease if treated correctly and on time.[23] Prediction models are used all around the world and for various diseases. A very known score used in ICUs worldwide is the SOFA score, which predicts mortality in ICU patients.[24,25] This score has helped in decision making, regarding treatment and care of patients, by classifying the damage or dysfunction of organs, to then predict morbidity and mortality. The use of this tool has helped save many lives worldwide. The use of scores to predict mortality in patients with tuberculosis around the world is uncertain, but the development and approval of one good score could help save over a million lives each year.[26]

During the past year, some studies have mentioned the importance of re-evaluating the worldwide TB situation, due to the COVID-19 pandemic. It is expected that this pandemic will worsen the panorama of tuberculosis globally.[27] Therefore, it is important to evaluate the use of a score system that can be easily used in countries with socio-economic limitations. This way, prompt decisions and measures can be taken to improve and control this public health problem.

One of the biggest limitations that most studies had was the poor quality of the reported data, since the moment in which the data was taken was not well specified. Another difficulty was the diagnostic criteria that was used to define the inclusion of patients, since several studies were carried out in low-resource countries, it was more complex to reach the gold standard for diagnosis of the disease in question. Lastly, the PROBAST tool is a very rigid tool, in which if one of the questions is answered as "no", the whole tool is considered as a high RoB.

6. Conclusions

There are no prediction models that accurately predict mortality in patients with pulmonary tuberculosis, due to great heterogeneity of the population and a high risk of bias. Therefore, we cannot conclude that there is a study that promises a score that can be used with acceptable confidence. Considering that pulmonary tuberculosis is a highly prevalent disease in low-income countries, it would be very useful to have high quality tools that allow healthcare personnel to catalog patients with a higher risk of death so that they can receive priority medical attention. The purpose of this study is to invite researchers to create scores that should preferably be guided with tools such as TRIPOD to develop high-quality instruments, and to externally validate the prediction models used in this study. To evaluate the quality of the prediction models we suggest the use of tools, like PROBAST.

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.2021.10.007.

Appendix 1

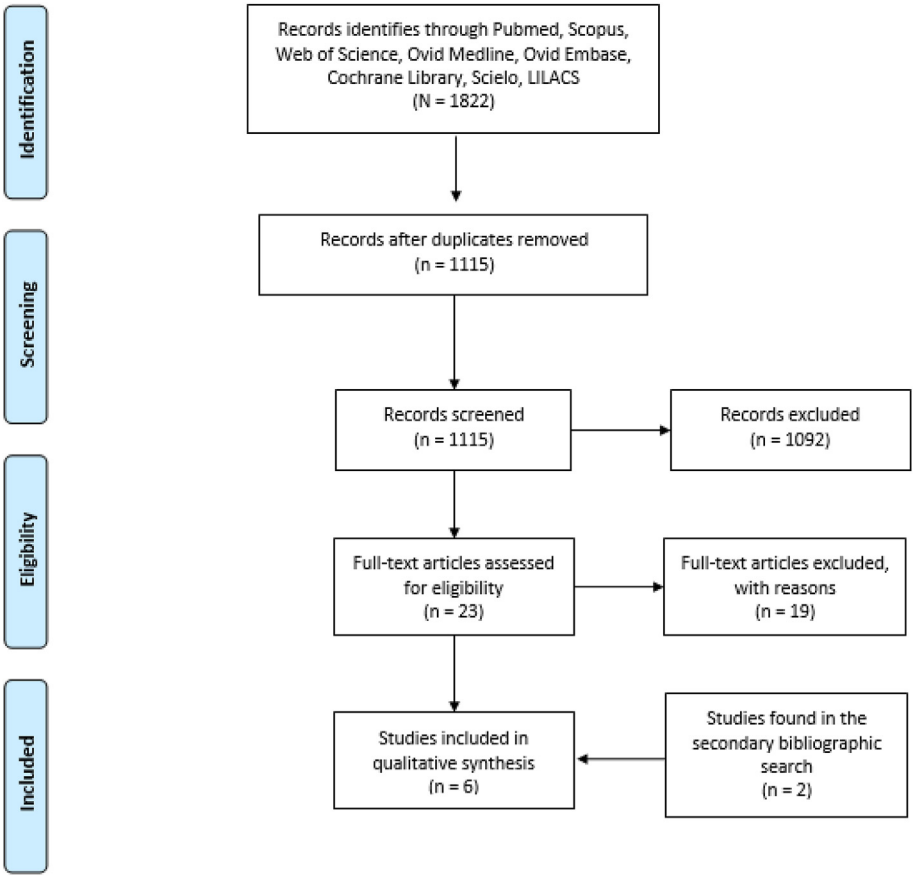

Fig. 1 – PRISMA Flow diagram.

Appendix 2

Table 1 – Table of Characteristics

Author, Reference	Name of the score validated	Validation/ Development population, Country	Ethnicity	Design	Sample size (in outcome/total) V; D	Age (years) V; D	TB Diagnosis	Outcome Definition/ Time of Measurement	TRIPOD
Bastos, et al 2016 [14]	TReaT	Chest Disease Center of Vila Nova de Gaia, Portugal	European, Portuguese	Retrospective Cohort	24/103; 121/681	35–64	WHO guidelines	Mortality/6 months	Development and Validation
Horita, et al 2013 [19]		Fukujuji Hospital, Japan	Japanese	Retrospective Cohort	196/244; 143/179	64.3 +- 20.1; 65.9 +- 18.8	Sputum microscopy and culture	Discharged alive	Development and Validation
Nagai, et al 2016 [15]	A-Drop	Yokohama City University Hospital, Japan	Japanese	Retrospective Cohort	73/345	54–82	Active smear-positive pulmonary TB diagnosed with sputum culture and smear	Mortality	Validation
Singla, et al 2020 [16]		National Institute of Tuberculosis Respiratory Diseases, India	Indian	Prospective Case-Control	49/250	>60 (53), <60 (197)	All consecutive bacteriological confirmed with direct sputum and XPERT MTB/RIF Assay	Mortality	Development
Wejse, et al 2008 [17]	TBscore	Epidemiological studies in Guinea-Bisáu	Fula, Balanta, Mandinka, mixed	Retrospective Cohort	172/698	39.42 (SD: 5.4)	WHO guidelines	Mortality/8 & 18 months	Development and Validation
Rudolf, et al 2013 [18]	TBscore II	Data base from Guinea-Bisáu	Fula, Balanta, Mandinka, mixed	Retrospective Cohort	64/1070	Female: 34 (33–36); Male: 36 (35–37)	WHO guidelines	Mortality/2 months	Development and Validation

Appendix 3

Table 2 – PROBAST quality analysis.

Study	ROB				Applicability			Overall	
	Participants	Predictors	Outcome	Analysis	Participants	Predictor	Outcome	ROB	Applicability
1	+	?	–	–	–	+	+	–	–
2	+	?	–	–	+	+	+	–	+
3	–	+	+	–	+	+	+	–	+
4	+	?	?	–	+	+	?	–	?
5	+	?	–	–	+	–	+	–	–
6	+	?	–	–	+	+	+	–	+

PROBAST = Prediction model Risk of Bias Assessment Tool; ROB = risk of bias. + indicates low ROB/low concern regarding applicability; – indicates high ROB/high concern regarding applicability; and ? indicates unclear ROB/unclear concern regarding applicability.

1 A prediction rule to stratify mortality risk of patients with pulmonary tuberculosis
2 TBscore II: Refining and validating a simple clinical score for treatment monitoring of patients with pulmonary tuberculosis
3 Risk factors for early mortality in patients with pulmonary tuberculosis admitted to the emergency room
4 Age, Dehydration, Respiratory failure, orientation disturbance, and blood pressure score predicts in-hospital mortality in HIV-negative Non-multi drug-resistant-smear-positive pulmonary tuberculosis in Japan
5 Development and validation of a tuberculosis prognostic score for smear-positive in-patients in Japan
6 TBscore: Signs and symptoms from tuberculosis patients in a low resource setting have predictive value and may be used to assess clinical course

Appendix 4

Table 3 – Performance and development of the prediction models.

First author, Year, Country	Model Development (Modelling Method, Method for selection of predictors included in the multivariable analysis and final model)	Model Performance	Final Model
Bastos et al 2016, Portugal[14]	Logistic Regression backward selection	AUC: Development = 0.82 (95%CI 0.78–0.87) Validation = 0.84 (95% CI 0.76–0.93) HL: Performed but not reported	Major Risk Factors: Hypoxemic respiratory failure (3 points) + Age ≥ 50 years old (2 points) Minor Risk Factors: Bilateral involvement (1 point) + At least one comorbidity (HIV, DM, Liver Failure, CHF, CRD) (1 point) + Hemoglobin <12 g/dL (1 point) ≤ 2, Low risk (mortality risk: 2.9%); ≥3 - ≤5, Moderate Risk (mortality risk: 22.9%); ≥6, high risk (mortality risk: 53.9%).
Horita et al 2013, Japan[19]	Logistic Regression Univariate analysis with P < 0.001 exclusion cutoff of P > 0.2 in MA	AUC: Development: 0.910 (raw score), 0.891 (risk group) Validation: 0.893 (raw score), 0.875 (risk group)	Age (years) + (oxygen requirement, 10 points) – 20 × albumin (g/dl) + (Activity of Daily Living: independent, 0 point; semi-dependent, 5 points; totally dependent, 10 points). Raw Score range: 83 and –61. Risk Group: Minimum = Raw score < –30 Low = –30 ⩽ raw score <0 Intermediate = 0 ⩽ raw score <30 High = 30 ⩽ raw score <60 Critical = 60 ⩽ raw score

Table 3 – (*continued*)

First author, Year, Country	Model Development (Modelling Method, Method for selection of predictors included in the multivariable analysis and final model)	Model Performance	Final Model
Nagai et al 2016, Japan[15]	External Validation of the A-DROP Score	AUC: Validation: 0.856 (in-hospital death), 0.870 (28-day death) Optimal Cut-off: >1.5 Sensitivity/Specificity for optimal cut-off: 85%/76% Youden Index for optimal cut-off: 0.61	Age (men ≥70 years, women ≥75 years) + Dehydration (blood urea nitrogen ≥21mg/dL) + Respiratory failure (arterial oxygen saturation ≤90% or arterial oxygen pressure ≤60 mm Hg) + Orientation disturbance (confusion) + blood Pressure (systolic blood pressure ≤90 mmHg) One point was assigned to each of A-DROP components. The total score ranged from 0 to 5, where five points suggested the poorest prognosis.
Singla et al 2020, India[16]	Logistic Regression UA and MA with P < 0.05	AUC: Development: 0.86 (early mortality 7 days) PPV: 94.88%, NPV: 19.90%	SpO2 ≤ 90% (4 points) + Respiratory rate >20 per minute (2 points) + Systolic blood pressure <90 mmHg (2 points) + Advanced disease on chest radiography (1 point) + Heart rate >100 per minute (1 point) The mortality rate associated with a risk score of 1–2, 3–5 and ≥6 was found to be 15%, 33% and 87.5% respectively.
Wejse et al 2008, Guinea Bissau[17]	Cox Regression P < 0.05 and Easy to measure in low resource settings.	AUC Development: 8-month follow-up: 0.65 (95% CI 0.6–0.7). 18-month follow-up: 0.75 (95% CI 0.66 –0.84).	Self-Report: Cough, Hemoptysis, Dyspnea, Chest pain, Night Sweat (1 point each if present) + Anemic conjunctivae (1 point) + Tachycardia (1 point) + Positive finding at lung auscultation (1 point) + Axillary temperature >37.0 °C (1 point) + BMI <18 (1 point) (if BMI lower than 16 add another point) + MUAC <220mm (1 point) (if MUAC lower than 200 mm add another point) Severity Class (SC) and mortality Risk: SC-I = TBscore 0–5, SC-II = TBscore 6–7, SC-III = TBscore ≥ 8. Total possible points: 13. 8 months: SC-III (21%), SC-II or SC-I (13%) 18 months: SC-III (71%), SC-I or SC-II (9%)
Rudolf et al 2013, Guinea Bissau[18]	Polychoric Correlation	AUC: Development: 0.7282 Validation: 0.6817	cough + dyspnea + chest pain + anemia + BMI <18 + BMI <16 + MUAC <220 mm + MUAC <200 mm. Severity Class (SC) and mortality Risk: SC-I = TBscore <2, SC-II = TBscore 2–3, SC-III = TBscore 4–7, SC-IV = TBscore >7. Total, possible points: 8.

MA: Multivariate Analysis; UA: Univariate Analysis; AUC: Area Under the Curve; HL: Hosmer-Lemeshow Statistic; PPV: Positive Predictive Value; NPV: Negative predictive value; DM: Diabetes Mellitus; CHF: Congestive Heart Failure; CRD: Chronic Respiratory Disease; BMI: Body Mass Index; MUAC: Middle Upper Arm Circumference

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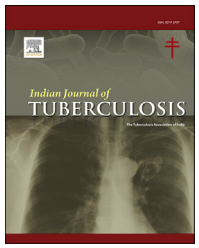

Original article

Unusual presentations of primary head and neck tuberculosis and review of literature

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ABSTRACT

Background/Purpose: The diagnosis of TB in the head & neck region is challenging due to diverse presentations and due to changing clinical pictures. The aim of this article is to report three unusual primary cases of head and neck tuberculosis in immunocompetent patients presenting to our hospital with description of their clinical presentation, appropriate diagnostic methods used and treatment response of these patients.

Methods: Three clinical cases were of primary tuberculosis of the lacrimal system, the thyroid gland and of the temporal space were clinically worked up. The aspirate from the swellings were sent for Cytology and Gene Xpert tests.

Results: The Gene Xpert tests were positive in these unusual cases and aided the Cytology in promptly confirming the diagnosis which otherwise would be missed if staining for AFB is negative. ATT was started and responded well to the treatment.

Conclusion: These cases demonstrate the importance of having a high index of suspicion for tuberculosis as a cause of head and neck swellings, especially in developing countries. It also illustrates the value of needle aspiration in such swellings and sending it for cytology and Gene Xpert for early diagnosis of tuberculosis.

1. Introduction

Tuberculosis (TB), a common granulomatous disease caused by Mycobacterium tuberculosis, remains a foremost cause of morbidity and mortality globally, especially in developing countries. Worldwide, 7.1 million people with TB were reported to have been newly diagnosed and notified in 2019, up from 7.0 million in 2018 and a huge rise from 6.4 million in 2017 and 5.7–5.8 million yearly in the period 2009–2012. In India, reports of people newly diagnosed with TB escalated from 1.2 million in 2013 to 2.2 million in 2019 (+74%).[1]

Tuberculosis has been a major health problem in terms of morbidity and mortality in developing countries like India. It is showing a dramatically increasing trend globally due to

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rampant use of steroids, increasing HIV cases and global migration. Furthermore, in developing countries poor socio-economic conditions, general debility, malnutrition, immunosuppression also leads to rise in TB cases.[2]

While pulmonary TB is the most common presentation, the incidence of extrapulmonary tuberculosis has also been showing a progressive increase in the recent years ranging between 5 and 15% approximately. In the head and neck region, TB can affect the lymph nodes, larynx, middle ear, sinonasal region, lacrimal system, Thyroid gland, oral cavity and pharynx.[3] The occurrence of TB in the head and neck region is quite common and its diagnosis is challenging due to diverse presentations and due to changing clinical presentations these days. Currently, slowly evolving patterns of TB that do not present with typical clinical symptoms are common, whereas acute fulminant rapidly progressive cases are infrequently found.[4]

The aim of this article is to report three unusual primary cases of head and neck tuberculosis in immunocompetent patients presenting to our hospital in year 2020 with description of their clinical presentation, appropriate diagnostic methods used and treatment response of these patients.

2. Clinical cases

2.1. *Case 1*

A female child, aged 11 years, presented with complaints of insidious onset of a painful swelling over left zygomaticotemporal region with 10 days duration (Fig. 1). The patient also had associated history of loss of appetite and generalised weakness. There is no history of toothache or extraction in recent past. No associated aural, nasal or dental symptoms and signs were present. Past medical history was unremarkable with no apparent immune-deficiencies. The swelling was diffuse with normal overlying skin. On palpation it was soft, fluctuant and tenderness was present. Rest of the ENT examination was within normal limits. USG imaging showed an anechoic collection with debris suggestive of abscess. CECT showed a multi-loculated, diffuse soft tissue density/

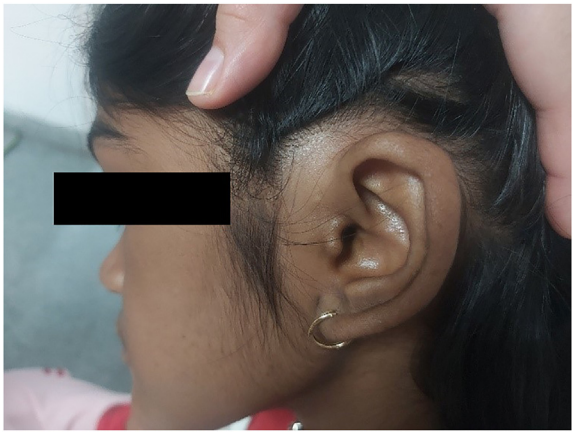

Fig. 1 – Patient presented with swelling in left zygomaticotemporal region.

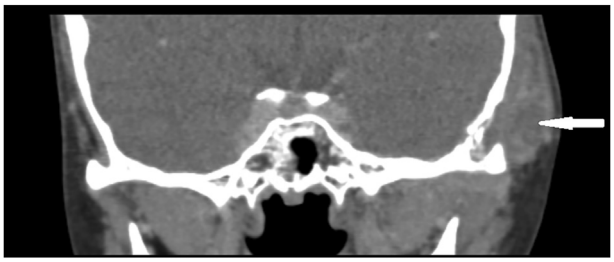

Fig. 2 – (Arrow) showing multiloculated, diffuse soft-tissue density with hypodense collections in left temporal region.

thickening with hypodense collections, in left temporal region in subcutaneous planes with thickening of underlying temporalis muscle, erosion of squamous part of temporal bone with no intracranial extension (Fig. 2). Around 12ml pus was aspirated using a wide bore needle and sent to Microbiology lab for Gene Xpert, AFB staining and culture. Gene Xpert confirmed MTB infection. Staining for AFB was negative but culture was positive for MTB. The patient was started on ATT and repeat aspirations were done twice in the follow up after which no collection occurred as the patient started responding to the medications.

2.2. *Case 2*

A 26 years old Male presented with watering from right eye and swelling at the right inner canthus of eye from past 2 years. He also had history of intermittent fever from the past 2 years. On examination there was a small (1 × 1cm) fluctuant tender swelling at the inner canthus of eye. Purulent discharge was seen at the punctum of right eye [Fig. 3(a)]. Rest of the ENT examination was within normal limit. Diagnosis of right side chronic dacryocystitis with lacrimal abscess was made and the swelling was aspirated using a syringe. Around 1 ml of pus was aspirated from the swelling which was sent for Gene Xpert, AFB staining and culture, Gram staining and pyogenic culture. Gene Xpert revealed mycobacterium tuberculosis sensitive to rifampicin while Gram stain was negative for microorganisms. Zeihl Neilson staining was positive for AFB while Chest x-ray was normal. The diagnosis of tubercular dacryocystitis was made and CECT PNS was advised which showed small peripherally enhancing hypodense lesion in inner canthus of right eye in the region of lacrimal sac with surrounding induration suggestive of abscess. The patient was started on ATT and the lacrimal swelling subsided after single aspiration. The patient responded well to ATT with minimal epiphora after one month of ATT [Fig. 3 (b)]. Patient is still under follow up and dacryocystorhinostomy is kept reserved if the patient is not completely cured of epiphora after completion of ATT (Fig. 4).

2.3. *Case 3*

A 24 years old female presented with complaint of painful swelling on the anterior midline of neck for the past 7 days. There was no history of difficulty in swallowing, difficulty in breathing or fever. On examination, the swelling, measuring 3 cm × 2 cm was present on anterior midline of neck at region

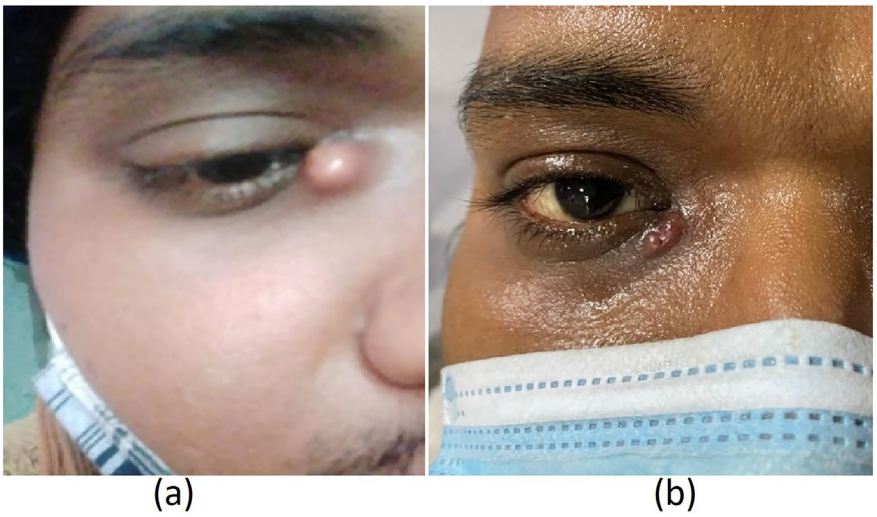

Fig. 3 – Before (a) and after (b) 2 months of ATT.

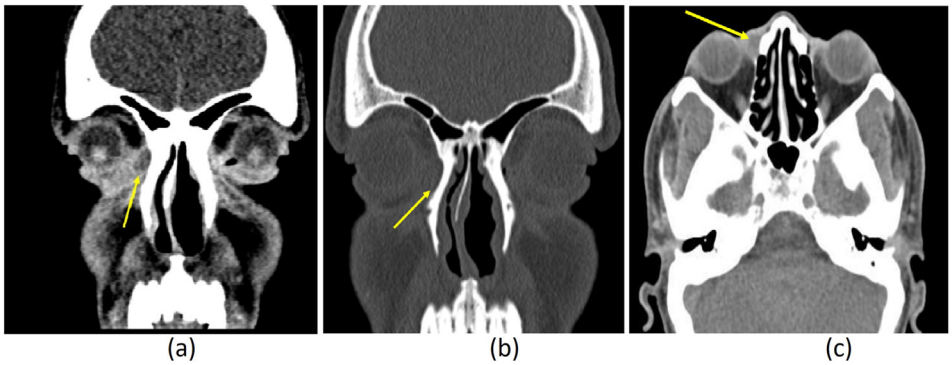

Fig. 4 – CT cuts (a), (b), (c) showing soft tissue window with enhanced hypodense lesion in right lacrimal region.

of thyroid which was fluctuant but non-tender and moving on deglutition (Fig. 5). Swelling was aspirated and around 5ml of pus was aspirated which was sent for Gene Xpert, AFB staining and culture, Gram staining and pyogenic culture. Gene Xpert revealed mycobacterium tuberculosis sensitive to rifampicin with no bacterium found in Gram stain. ZN stained smear revealed presence of AFB and Chest x-ray was within normal limits. Thyroid profile was normal and all the blood test was within normal limits except raised ESR. The diagnosis of primary tubercular thyroid abscess was established and patient was started on ATT. The patient responded well to ATT and is completely cured of thyroid swelling after completion of ATT.

3. Review of literature

Tuberculosis is one of the oldest diseases of mankind and is a leading cause of morbidity and mortality responsible for human suffering and loss of life. Tuberculosis can affect almost every part in the body except nail, hair and teeth. In a study done by Monga et al the most common site of EPTB in the head and neck region was in the cervical lymph nodes (81.25%) followed by sinonasal TB (6.2%), TB of oral cavity (4.1%), laryngeal TB, retropharyngeal region, buccal space and cutaneous TB of the head and neck region (one case each representing 2.05%.[3] Prompt and accurate diagnosis of head and neck tuberculosis especially of thyroid region, lacrimal system and temporal bone has always been a challenge for Otolaryngologists because of rarity and varied presentations. It is either missed or is often misdiagnosed as malignancy or acute infective condition leading to an unnecessary delay in diagnosis.

Thyroid tuberculosis is uncommon, with an incidence of around <0.4% probably because thyroid tissue is relatively resistant to tuberculous infection. Patient can present with a swelling of thyroid gland or can be completely asymptomatic.[5] The patients are usually euthyroid. Only three cases of hypothyroidism due to thyroid Tuberculosis have been documented till date.[6–8] It can also present as thyroid abscess as in our study. The most common presentation is a solitary thyroid nodule with cystic component.[9]

Prevalence of Ocular tuberculosis in India is around 5.6–10.13%. Ocular tuberculosis can involve uvea, sclera, cornea, choroid, lacrimal gland adnexa and periorbital cutaneous tissue. Tubercular uveitis is the most frequent form of ocular tuberculosis. Primary tuberculosis of lacrimal system is not very common with only a few cases reported.[10] In a study

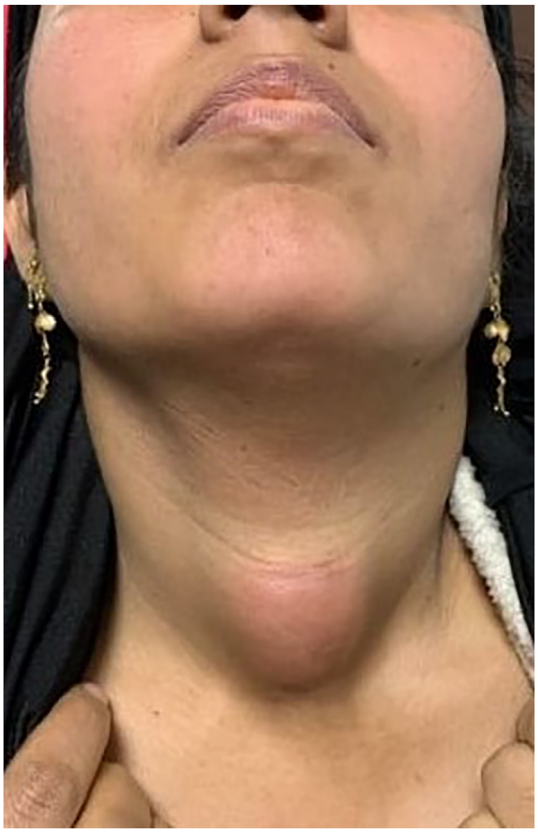

Fig. 5 – Midline neck swelling before the start of ATT.

done by Agrawal et al, only one patient had lacrimal gland TB out of 14 patients of orbital TB.[11] In another study by Donahue et al, out of 10,452 patients of Tuberculosis only 1.4% had orbital TB but none of them had Tubercular dacryocystitis.[12]

Tubercular dacryocystitis can present with epiphora and swelling in medial canthal area. Some authors have reported nasal or paranasal sinus involvement in tubercular dacryocystits. Hence nasal complaints and nasal examination should be evaluated in these patients. In a review of literature done by Sagar et al 8 out of 18 cases had nasal tuberculosis.[13] Many patients present with failed lacrimal surgeries or failed drainage procedures.

Head and neck tuberculosis can either be primary or secondary to tuberculous involvement in a distant site. Spread of the tuberculosis to the thyroid gland is by hematogenous or lymphatic spread or directly from laryngeal or tubercular cervical lymphadenopathy.[14] Similarly, Lacrimal system can be involved via haematogenous route, direct inoculation, and contiguous spread from nasal cavity, via skin or through tears in cases of conjunctival exposure. Tuberculosis of lacrimal system can either be involved in isolation or along with nasal/paranasal sinus tuberculosis, periorbital, lupus vulgaris or with cervical lymphadenopathy.[13] In our series all three cases were of primary Tuberculosis with no evidence of tuberculosis elsewhere in the body.

The temporal space can be divided into superficial and deep temporal spaces. The superficial temporal space extends superiorly to the pericranium, lateral to the temporalis muscle and medial to the temporoparietal fascia (galea). Inferiorly this space is continuous with the masseteric space. The deep temporal space extends superiorly to the attachment of the temporalis muscle to the inferior temporal crest, lateral to the temporal bone and deep to the temporalis muscle. Inferiorly this space is continuous with the infratemporal space.[15]

Temporal space tuberculosis is rare and only two cases have been reported in literature.[16,17] Bacterial or tubercular infections in this space have been reported secondary to paranasal sinus infection, maxillary sinus fracture, or are associated with the extraction of infected and non-infected teeth.[18]

Clinical features of temporal space abscess are facial pain and trismus with or without swelling in temporal space and masticator space region. These cases are often confused with diagnosis of temporomandibular joint dysfunction, parotid abscess, trigeminal neuralgia.[19–21] If not treated early superficial temporal space abscess can spread to deep temporal space and infratemporal space abscess. Infections involving the ITF can spread haematogenous into the cavernous sinus and orbit leading to serious complications.[22,23] Spread can also occur to the adjacent fascial spaces leading to mediastinitis, and even pericarditis.[23] Infact, Jiun-Lin Yan described a patient with a tubercular temporalis abscess with skull and dural invasion.[17] Therefore, early diagnosis and treatment is crucial to avoid morbidity and mortality. It is important to avoid a delay in diagnosis and commence treatment promptly.

Tuberculosis of the head and neck region is diagnosed on the basis of cytological or histological examination and identification of AFB. Histological demonstration of epithelioid cell granulomas with peripheral lymphocytic cuffing, Langhans giant cells, and central caseation necrosis proves the diagnosis. WHO has recommended molecular based assays which are rapid, sensitive and specific for diagnosis and early treatment of tuberculosis.[1] In our series all three cases were diagnosed within 48 hours as tuberculosis as on first visit aspiration of swelling was done and Gene Xpert sent. Results of Gene Xpert were itself diagnostic of mycobacterium tuberculosis and so unnecessary delay was avoided.

Imaging plays a vital role in head and neck tuberculosis in confirming the diagnosis and defining the extent of lesion. Ultrasound, CT scan or MRI can be done depending on the site of lesion and also considering patient's financial condition. In a case of tubercular dacryocystitis CT scan can detect a soft tissue density in lacrimal sac and evaluate involvement of nasal and paranasal sinus. Erosion or osteomyelitis of underlying bone should be ruled out .[13,14] In a case of Tubercular thyroid abscess heterogeneous hypoechoic mass is usually visualised on ultrasound and peripheral-enhancing low-density suppurative lesion with regional lymphadenitis might be seen on CT scan.

It is particularly vital to distinguish head and neck tuberculosis from cancer, acute infective condition, and other granulomatous disorders in these sites to avoid unnecessary surgery or improper treatment. In our case of tubercular dacryocystits if DCR was done instead of evaluating for presence of Mycobacterium tuberculosis in Lacrimal sac it would definitely have lead to failure of surgery. It is very important to differentiate tuberculosis from other granulomatous diseases such as De Quervain's thyroiditis and sarcoidosis in which

corticosteroids are used which can otherwise worsen the patients with tuberculosis.[5] The treatment of tuberculosis of the thyroid gland, lacrimal apparatus or temporal bone tuberculosis is not much different from the treatment of other forms of tuberculosis. At least 6 months of therapy is required using two or three of the following drugs – rifampicin, isoniazid, pyrazinamide, and ethambutol. Surgical drainage, resection or aspiration is required, along with the earlier described therapy only in selected cases. For instance, when the thyroid gland involvement leads to dysphagia, uncontrolled hyperthyroidism or is coinciding with malignancy of thyroid then surgical management is also done.

Similarly, in case of lacrimal apparatus tuberculosis, DCR should be reserved for case with continuous epiphora after completion of antitubercular treatment (ATT). One should always watch for possible sequelae like fistula formation or slow healing of wound after surgery. In all our three cases, only repeated aspiration was sufficient along with initiation of antitubercular treatment. Aspiration was done once in case of tubercular dacryocystitis and twice in cases of thyroid abscess and temporal bone abscess leading to complete disappearance of swelling. It is interesting that none of the cases had associated pulmonary tuberculosis in the present study.

These cases demonstrate the importance of having a high index of suspicion for tuberculosis as a cause of head and neck swellings, especially in developing countries It also illustrates the value of aspiration of these swellings by otorhinolaryngologist and promptly sending it for cytology and Gene Xpert, which leads to early diagnosis of tuberculosis.

In conclusion, thyroid, lacrimal apparatus and temporal bone tuberculosis are rare, but should be considered as differential diagnosis of thyroid masses especially in developing countries where there is a high prevalence of tuberculosis. Also, newer methods like GeneXpert can be combined with conventional culture and smear microscopy to increase diagnostic sensitivity in such cases for rapid diagnosis and timely management.

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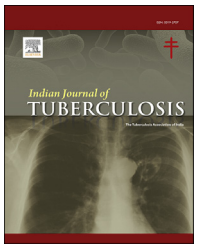

Original article

Pre-treatment delay and out of pocket expenses by notified new tuberculosis patients in an Indian mega city

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ABSTRACT

Background: Study was carried out to find out delay from onset of symptoms and out of pocket expenditure (OOPE) until initiation of anti-TB treatment (ATT) by new Tuberculosis (TB) patients registered in public health facilities in Bengaluru.

Methods: Notified patients (N = 228) selected purposively were interviewed at initiation of ATT regarding number and type of facilities visited and delay in initiating ATT. OOPE was elicited separately for in- and out-patient visits, towards consultation, purchase of medicines, diagnostic tests, transportation, hospitalization and food. Dissaving or money borrowed was ascertained.

Results: Two-thirds of participants were 15–44 years of age and 56% were males, mean annual household income was $4357.

About 75% first visited a private health facility; 68% and 87% respectively were diagnosed and started on ATT in public sector after visiting an average of three facilities and after a mean delay of 68 days; the median delay was 44 days.

Of mean OOPE of $402, 54% was direct medical expenditure, 5% non-medical direct and 41% indirect. OOPE was higher for Extra-pulmonary TB compared to PTB and when number of health facilities visited before initiating treatment was >3 compared to those who visited ≤3 and when the time interval between onset of symptoms and treatment initiation (total delay) was >28 days compared to when this interval was ≤28 days. About 20% suffered catastrophic expenditure; 34% borrowed money and 37% sold assets.

Conclusion: Concerted efforts are needed to reduce delay and OOPE in pre-treatment period and social protection to account for indirect expenditure.

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1. Background

Tuberculosis (TB) is a major health problem in India with 2.6 million incident cases and 0.44 million deaths annually.[1] 'End TB strategy' envisages that no TB affected family should incur catastrophic expenditure by 2020.[1] However, studies in recent past indicated that even patients registered for Anti-TB Treatment (ATT) in public health sector under the National TB Elimination Program (NTEP) incur high Out of Pocket expenditure (OOPE) as in other poorer income countries.[2–6] A study in south Indian Metropolis of Bengaluru during 2005 revealed that 75% TB patients first sought care at private facilities and consulted about 3.3 providers with a mean delay of 72 days while incurring OOPE of USD ($) 145 from onset of symptoms to initiating treatment under NTEP which constituted 87% of the total OOPE during TB care.[2] Thereafter, there have been concerted efforts at engaging private providers (PPs), access to online learning and creating public awareness.[7] Consequently, it was hypothesized that a reduction in delay before initiating ATT with consequent reduction in OOPE has happened over the years.

In view of the above, we undertook a study in Bengaluru city with the following
objectives:

1. To find out the mean delay from onset of symptoms until initiation of anti-TB treatment among new TB patients notified by NTEP in Bengaluru.
2. To find out the mean out of pocket expenditure incurred from the onset of symptoms until initiation of treatment, by new TB patients notified by NTEP in Bengaluru.

2. Methods

2.1. Study design

Descriptive study.

2.2. Study population

Study was carried out during September 2017–February 2018, among new adult TB patients initiated on ATT at public health sector facilities in the city. There are 92 public health centres/institutions (PHIs) - 54 Health Centres/Dispensaries, 35 hospitals and 3 medical colleges (MCs) in Bangalore Corporation limits. There are 14 Tuberculosis management Units (TUs) at sub-district levels.

2.3. Sampling

Sample size was estimated using the following formula[8]: -

$$n = \frac{2\sigma^2\left[Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right]^2}{(\mu_1 - \mu_2)^2}$$

where μ_1 represent the mean delay of 72 days during 2005, μ_2 the expected mean delay during 2017–2018 assumed at 58 days to detect 20% change in mean delay from 2005; α (level of significance) = 0.05, Z = 1.28 for 90% power, σ^2 represents the pooled variance calculated as under:

$$\text{Pooled variance}(\sigma^2) = \frac{s_1^2 + s_2^2}{2}$$

s_1 and s_2 represent the standard deviations at the two respective time points; for which a common value of 41 days was taken based on the 2005 study.

Thus, sample size of 180 was increased to 225 to account for 20% non-response.

Each month, PHIs were selected purposively while ensuring (a) enrolled patients were uniformly spread all over the municipality (b) number enrolled from MCs/hospitals and other PHIs was in ratio of notifications. In individual PHIs, either all notified patients were enrolled or a systematic sampling undertaken, depending on number initiated on ATT. Purposive sampling undertaken this way ensured that the study sample was well distributed across different parts of the city and different types of health facilities. Patients residing outside municipal area and those with drug resistant TB were excluded.

2.4. Data collection

Data collection was undertaken by personal interviews of each participant within a month of registration using a semi-structured interview schedule entailing collection of information regarding demographics, annual household income before onset of present illness, disease classification, number and type (public/private) of health facilities visited, delay in seeking treatment and delay in treatment initiation after first approach to a health facility. Reasons were elicited for patient delay of >7 days. OOPE was elicited in Indian Rupees (₹) for in-patient and out-patient visits, towards consultation, diagnostic tests, medicines, hospitalization, transportation, and food during visits. Information was obtained about change in occupation after onset of symptoms and additional workdays lost for taking rest or by attendees to look after them and loss of wages thereof. Any assets sold or money borrowed – returnable or non-returnable, with or without interest was ascertained. Participants were asked to grade the impact of their illness on family's financial status. Data obtain by personal interview was verified by cross checking with the records whenever available with the study participants.

Two Field Investigators from National Tuberculosis Institute, Bengaluru (NTI) interviewed each patient together and recorded the data after reconciling.

2.5. Definitions

Below Poverty Line (BPL) Family: Families having BPL cards which are issued to those living below poverty line based on a cut off for annual family income specified by state government every year.[9]

Patient delay: number of days between onset of TB suggestive symptoms to first visit to any health facility.

Health system delay: number of days between first visit to any health facility to date of initiating ATT.

Total delay: time interval from onset of symptoms to initiating ATT.

Health facility type: Public, Private-fully or less than fully qualified.

Direct medical expenditure: This included expenses towards consultation, investigations and medicines during outpatient and in-patient visits, and charges for special procedures and room/bed during hospitalization.

Direct non-medical expenditure: Patients and/or Attendees' expenditure for transportation and food.

Indirect expenditure: Loss of wages for patients and/or attendees, calculated separately for visits to health facilities and for additional work-days lost due to illness or caring for the sick.

Total expenditure: Sum of direct and indirect expenditure.

Catastrophic expenditure: OOPE at >20% of annual household income.

Current smoker: smoked any time from onset of symptoms till diagnosis.

Ex-smoker: smoking earlier but quit before onset of present illness.

Never smoked: never smoked.

Current Alcoholic: taken alcohol any time from onset of symptoms till diagnosis.

Ex-Alcoholic: taken alcohol earlier and quit before onset of present illness.

Never taken alcohol: never taken alcohol.

2.6. *Data management*

Data was entered in Epi-Info version 7.2.1.0 and analyzed in SPSS.

2.7. *Statistical methods*

Results were expressed as mean with standard deviation (SD) and median with inter-quartile range (IQR). Proportional distributions of direct medical, direct non-medical and indirect expenditure are presented. Proportion of annual household income spent on OOPE was estimated. Expenditure in Indian Rupees was converted to US Dollars, at a conversion ratio of 64 as in 2017. Means were compared using *t* test, P values <0.05 considered significant.

2.8. *Ethical considerations*

Prior approval was obtained from the Institutional Ethics Committee of NTI. Written informed consent was solicited from participants after explaining purpose and procedures of the study. Each patient was paid ₹200 ($3.1) as compensation for time spent for interview. Confidentiality was maintained by not entering personal identifiers to database.

3. Results

3.1. *Study population*

A total of 258 new adult TB patients initiated on ATT were line listed from 72 PHIs across 14 TUs - 45 HCs/Dispensaries, 26 hospitals and 1 medical college; 228 (88.4%) were interviewed as 30 refused consent or were not available.

Two-thirds of the participants had PTB, two-thirds were 15–44 years of age and 56% were males (Table 1). Most lived in urban areas, about 20% were illiterate. Before onset of present illness, 148 (65%) participants were salaried or self-employed, of the remaining were 42 housewives, 26 students, 4 had retired and 8 were unemployed. Average household size was 4. There was a mean of 2 earning members per family, 12 (5%) participants belonged to BPL families. Mean annual household income was ₹278 874 ($4357) with SD of ₹302 228 (USD 4723); median of ₹ 197 500 ($3086) with IQL ₹112 000–300 000 ($1875–4688). History of smoking and/or alcohol use was present in one-third. Sixty-one (27%) had diabetes and four (2%) were HIV reactive.

Table 1 – Socio-Demographic characteristics, personal habits, co-morbidities and household income of study participants (N = 228).

Type of TB	Pulmonary	153 (67.1)
	Extra Pulmonary	75 (32.9)
AGE	15-24	58 (25.4)
	25-34	47 (20.6)
	35-44	49 (21.5)
	45-54	40 (17.5)
	55-64	15 (6.6)
	≥65	19 (8.3)
Sex	Male	128 (56.1)
	Female	100 (43.9)
Type of residence	Urban	218 (95.6)
	Rural	10 (4.4)
Literacy	Literate[a]	181 (79.4)
	Illiterate	47 (20.6)
Employment	Employed	148 (64.9)
	Unemployed[b]	80 (35.1)
Household Size	≤4	135 (59.2)
	>4	93 (40.8)
Economic status	BPL	12 (5.3)
	APL	216 (94.7)
Annual household income in INR (before the onset of current illness)	13,000-120,000	58 (25.4)
	120,001-197,500	56 (24.6)
	197,501-300,000	60 (26.3)
	300,001-2760,000	54 (23.7)
Smoking status	Ex-smoker	53 (23.2)
	Current smoker	23 (10.1)
	Never smoked	152 (66.7)
Alcohol consumption	Ex Alcohol user	60 (26.3)
	Current alcohol user	21 (9.2)
	Never taken	147 (64.5)
Diabetes Mellitus	Yes	61 (26.8)
	No/Not-known	167 (73.2)
HIV	Reactive	4 (1.8)
	Non-reactive/ Status unknown	224 (98.2)

[a] Ability to read and write with understanding; Figures in parenthesis are percentages.

[b] *housewives-42, students-26, retired - 4 had, unemployed-8.*

Table 2 – Type of Health facility visited first and place of diagnosis and initiating treatment.

	N (%)
Type of Health facility visited first	
Public HF	56 (24.6)
Standalone private provider[a] (qualified)	116 (50.9)
Nursing Home (private)	26 (11.4)
Charitable trust/NGO	11 (4.8)
Pharmacy	8 (3.5)
Less than fully qualified provider (LTFQ)	11 (4.8)
Place of TB diagnosis	
Public	155 (68.0)
Private	73 (32.0)
Place of initiating ATT	
Public	199 (87.3)
Private	29 (12.7)

[a] With a qualified medical degree in allopathy/homeopathy/indigenous systems of medicine.

3.2. Health seeking behavior

Of the predominant presenting symptoms among PTB patients, 90% had cough and/or fever of any duration; 80% had complaints of loss of weight and/or appetite, 39% had night sweats. Among EPTB patients, fever (61%) followed by loss of appetite (59%), loss of weight (52%) and swelling in the neck (36%) were predominant presenting complaints.

One-hundred-seventy-two participants (75%) first visited a facility other than public health facility (Table 2); 155 (68.0%) and 199 (87%) respectively were diagnosed with TB and started ATT in public health facilities. They visited an average of 3 (range 1–7) facilities – out-patient and in-patient combined (Table 3) while the number of visits varied between 1 and 18, before initiating ATT. Two hundred and twenty-four (94%) had out-patient visits, 88 (39%) had history of hospitalization before ATT initiation - 70 for once and 18 on two occasions for symptoms related to present illness. Fifty-Five (24%) participants visited standalone labs.

Mean patient delay and health system delay was 15 days and 53 days respectively; total mean delay was 68 days and the median was 44 days (Table 4). Seventy-eight (34%) had patient delay of >7 days; predominant reasons being conscious ignorance of symptoms (N = 28), taking home remedies (N = 23), financial constraints (N = 13) and miscellaneous (N = 14). Health system delay was >2 weeks in about 75%. There was a positive correlation between number of health facilities visited and health system delay (r = 0.29, P = 0.01).

3.3. Out of pocket expenditure

The average OOPE incurred during out-patient visits, hospitalization and standalone labs by actual numbers of patients who visited these facilities are presented in Table 5 while average OOPE altogether by 228 patients is presented in Table 6 without considering reimbursement which was <1% of overall expenditure.

Mean OOPE incurred by 224 participants during out-patient visits was $66, with median of $27; 4 participants did not visit any out-patient facility. Of the mean, 83% was direct medical, 8% non-medical direct and 9% indirect expenditure. Transport expenses accounted for 80% of non-medical direct expenditure. Mean for EPTB was significantly higher compared to PTB. Number of participants that did not incur any direct medical, direct non-medical, indirect expenditure was 25, 67 and 156 respectively.

Mean OOPE incurred by 88 participants during hospitalization was $413, with median of $234. Of the mean, 83% was direct medical, 9% direct non-medical and 8% indirect expenditure. Average for EPTB was significantly higher than EPTB. One hundred and forty did not incur any in-patient cost; of participants who incurred, 5 had no direct medical expenditure and 46 incurred NIL indirect expenditure.

Fifty-five patients incurred OOPE at standalone labs i.e., other than health facilities visited as out-patients and in-patients, at an average of $66. Of them, 15 incurred no non-medical direct expenditure, 27 had NIL indirect expenditure and one incurred no cost.

Families of 113 patients (50%) incurred additional indirect expenditure at a mean of $288 due to additional work days lost by them at an average of 55 days for taking rest at home and by 60 of their family members at an average of 27 days. Seventeen students lost 22 additional school/college days on average.

Altogether, the average OOPE was $402, with the median of $192. Of the mean, 54% was direct medical expenditure, 5%

Table 3 – Number of health facilities visited (Excluding standalone labs).

No. of facilities visited	No. of participants visited as Out-patient	No. of participants visited as in-patient	Total OP + IP	Total OP + IP + Standalone Labs
1	50	72	26	23
2	81	15	83	72
3	51	1	53	47
4	36	–	42	40
5	3	–	18	32
6	3	–	4	9
7	–	–	2	5
Nil	4	140	–	–
Mean	2	1	3	3.1
Median with interquartile range	2 [2–3]	0 [0–1]	3 (2–4)	3 (2–4)

OP: out-patient, IP: In-patient.

Table 4 – Patient and health system delays.

Delay	Mean days [SD]	Median days [Interquartile range]
Patient delay	15 [37]	5 [2–12]
Health system delay	53 [63]	33 [13–71]
Total Delay	68 [69]	44 [25–85]

non-medical direct expenditure and 41% was indirect expenditure. Average expenditure incurred for EPTB was significantly higher at $521 compared to PTB at $344. The proportion of overall expenditure incurred on direct expenditure was higher for EPTB at 65% compared to 45% for PTB. Proportions of participants who incurred direct medical, non-medical direct and indirect expenditure were 93%, 86%, 42% respectively, as 16, 33 and 132 participants did not incur any direct medical, direct non-medical and indirect expenditure respectively.

Total OOPE was significantly higher for EPTB compared to PTB and when number of health facilities visited was >3, health system delay was >14 days and total delay >28 days (Table 7). It was higher when patient delay was <14 days and lesser for the un-employed.

Seventy-Seven (34%) borrowed money to meet illness related expenses – 42 to repay with interest, 28 without and 7 did not have to return. Eighty-four (37%) sold assets with majority selling (N = 29) jewelry.

Of the 148 employed before present illness, 9 had paid sick leave facility, 31 (21%) were now unable to work and three had to find alternate jobs.

Forty-two (18%) study participants were beneficiaries of any Government health scheme; 9 were enrolled for health insurance and 5 (2%) received monetary compensation.

Seventy-eight (34%) perceived no financial impact on family, of the remaining, one third each felt little/moderate/serious impact. Average OOPE accounted for 15% of annual household income and was >20% for 46 (20%) participants.

4. Discussion

The present study was undertaken to find the change in delay in TB diagnosis and to find OOPE until ATT initiation. New TB patients consulted 3 providers on average before initiating ATT, similar to earlier study in the same area during 2005 and as reported in a meta-analysis of Indian studies between 1996-2012.[2,10] Mean total delay was 68 days compared to 72 days in 2005. Median patient delay, health system delay and total delay in initiating ATT were 5, 33 and 44 days respectively. Corresponding figures during 2005 were 7, 34 and 53 days, and 19 days, 34 days and 58 days respectively in the meta-analysis. This suggests that while total delay in initiating treatment has reduced somewhat, there is no improvement in health system delay. About 75% patients as also in 2005 first approached a

Table 5 – Average Out of Pocket expenditure ($) incurred as out-patients, in patients and at standalone laboratories.

		Mean {SD}	Median (IQR)	Mean {SD}	Median	Mean {SD}	Median
Outpatient visits		PTB, N = 150		EPTB, N = 74		Total, N = 224	
	Direct medical	53{262}	16 (7–44)	59{96}	31 (10–72)	55{221}	21 (8–53)
	Direct non-medical	4{10}	2 (0–5)	7{11}	2 (0–1)	5{10}	2 (2–6)
	Indirect	5{32}	0 (0–0.3)	6{17}	0 (0–1)	6{28}	0 (0–2)
	Total	62{272}	22 (10–56)	72{103}	38 (16–88)	66{230}	27 (11–66)
In-patient visits		PTB, N = 54		EPTB, N = 34		Total, N = 88	
	Direct medical	269{474}	152 (55–283)	455{511}	316 (68–636)	341{494}	169 (59–466)
	Direct non-medical	39{34}	31 (19–48)	38{36}	27 (10–58)	38{34}	30 (16–51)
	Indirect	27{53}	0 (0–30)	47{65}	19 (0–76)	34{59}	0 (0–47)
	Total	334{489}	210 (117–345)	540{541}	426 (138–841)	413{516}	234 (122–515)
At standalone labs		PTB = 31		EPTB = 24		Total = 55	
	Direct medical	53{262}	16 (7–44)	59{96}	31 (10–72)	55{221}	21 (8–53)
	Direct non-medical	4{10}	2 (0–5)	7{11}	2 (0–1)	5{10}	2 (2–6)
	Indirect	5{32}	0 (0–0.3)	6{17}	0 (0–1)	6{28}	0 (0–2)
	Total	62{272}	22 (10–56)	72{103}	38 (16–88)	66{230}	27 (11–66)

4, 140 and 172 patients did not incur any out-of-pocket expenditure at out-patient, in-patient and standalone laboratory facilities respectively.
$ = 64 Indian Rupees at 2017 conversion rates.

Table 6 – Average total Out of Pocket expenditure ($).

	PTB, N = 153		EPTB, N = 75		Total, N = 228	
	Mean {SD}	Median (IQR)	Mean {SD}	Median	Mean {SD}	Median
Direct medical	155.58{540}	38 (13–143)	340{468}	116 (40–552)	216{524}	67 (16–198)
Direct non-medical	18.72{31}	6 (2–25)	29{39}	13 (2–43)	22{34}	8 (2–30)
Indirect	169.80{270}	39 (0–18)	152{307}	41 (0–170)	164{282}	39 (0–192)
Total OOP costs	344.11{637}	418 (56–213)	521{638}	234 (101–796)	402{641}	192 (65–517)

$ = 64 Indian Rupees at 2017 conversion rates.

Table 7 – Average Out of pocket expenditure ($) by disease classification, socio - economic characteristics, delay and number of health facilities visited.

		No	Median expenditure (IQR)	Mean expenditure [SD]	P value (means)
Disease classification	PTB	153	173 (58–418)	344 [637]	0.051
	EPTB	75	234 (101–796)	521 [638]	
Gender	Male	128	220 (87–646)	422 [502]	
	Female	100	157 (59–405)	377 [786]	0.602
Economic status	BPL	12	145 (29–557)	298 [394]	
	APL	216	200 (73–517)	408 [652]	0.565
Literacy	literate	181	183 (60–439)	398 [695]	
	Illiterate	47	285 (95–733)	420 [372]	0.832
Employment	Employed	148	234 (110–570)	481 [731]	
	Unemployed	80	112 (28–276)	256 [391]	0.003
Patient delay	≤14 days	179	216 (72–552)	445 [701]	
	>14 days	49	128 (42–313)	245 [300]	0.004
Health system delay	≤14 days	62	144 (30–236)	230 [327]	
	>14 days	166	234 (82–632)	467 [714]	0.001
Total delay	≤28 days	66	186 (55–409)	304 [373]	
	>28 days	162	202 (77–560)	442 [719]	0.058
Number of Health facility visited (including lab)	≤3	142	166 (50–438)	325 [400]	
	>3	86	274 (111–634)	530 [897]	0.048

$ = 64 Indian Rupees at 2017 conversion rates.

facility other than public facility, however 68% and 87% respectively were diagnosed and initiated ATT in public facilities. This suggests continued gaps in diagnostic process in private sector as also demonstrated in other studies in same area.[11,12] Consequently, patients continue to shop seeking alleviation of their suffering. This despite concerted efforts by NTEP at engagement of PPs. For facilitating diagnosis, there is provision under NTEP for field staff to pickup biological specimen from private facilities and transport to CBNAAT/Microscopy labs. However, this may be a minor support in entire diagnostic process and suggests a radical strengthening the engagement of private sector. In a study during 2016–17 in 18 districts from 17 states, active case finding (ACF) significantly reduced health system diagnostic delay.[13]

Shopping for diagnosis and associated delay result in considerable OOPE during diagnostic process. Average OOPE before initiating ATT in present study was $402, roughly 2.8 times the corresponding figure of $145 in 2005, attributable to inflation. In our study, 20% of participants' households incurred OOPE at >20% of annual income, proportion that adversely impacts outcome of ATT and quality of life hence termed as catastrophic expenditure.[14,15] In 2005, 86% patients reported costs >20% of annual income during diagnostic and treatment period; 87% being incurred during diagnostic process.[2] Reasons for drastic decrease in proportions suffering catastrophic expenditure towards TB diagnosis could only be speculative and beyond the scope of present study. Nevertheless, concerted efforts are required to achieve the target of no family suffering catastrophic costs towards TB care.

More than 50% of OOPE in present study was 'direct medical', which indicates scope for reducing proportions suffering catastrophic costs by skill up-gradation of providers and emphasizing the importance of diagnosing TB timely in a cost-effective manner. Provisions of extending diagnostic support to private sector as stated above has not benefited the vast majority of patients presenting there. This underlines the need for strengthening linkages to free/subsidized diagnostic facilities to enable cost-efficient diagnostic process which accounts for most of the expense during TB care pathway. Average cost of hospitalization was about six times higher than the average for out-patients, underlining need to convince providers to restrict hospitalization when necessary and need for social security mechanism to cover hospitalization cost.

About 40% of the OOPE incurred in our study was 'Indirect', primarily due to workdays lost by patients on account of sickness and their family members to take care of the sick. Of patients employed before initiating ATT, 20% were not able to work by one month of initiating ATT. Most were not linked to any health scheme or insurance and majority were not eligible for paid sick leave. About 34% borrowed money and 37% sold assets. It is generally the lower income households that are forced to dissaving and effects of taking loans last for a long time.[3,5,6] These highlights the need for social protection policies in addition to ACF and free diagnostics to all, to safeguard TB patients from further impoverishment and disease spiral as per the evidence generated earlier from three continents.[16]

Major limitations of the present study pertained to some degree of recall bias and non-availability of complete records of expenditure in case of majority of study participants. Nevertheless, since the interviews were conducted in-depth and within a month of treatment initiation, the study findings indicating continued diagnostic delay, considerable OOPE incurred by the patients leading to catastrophic expenses in a significant proportion can be considered to be reasonably reliable while emphasizing that further concerted efforts are required to be taken by the health system including the

private sector in order to reduce the pre-treatment delay and OOPE by the patients.

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Author contributions

Protocol development: VKC, PP; Data Collection: RS, BAS, NKH, RP, PS, GU, LN, VM, NN, GP; Data management: PP, RJ; Analysis: PP, VKC; Paper writing: VKC, PP, NS.

Conflicts of interest

The authors have none to declare.

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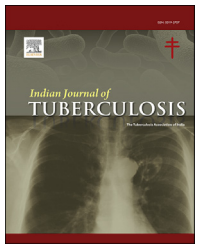

Original article

Evaluation of patient's experiences with daily DOTS

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ABSTRACT

Background/aims: Past few decades have seen major revisions in the Tuberculosis (TB) control programs time and again with a goal to strengthen the delivery of services and achieve elimination of the disease. Daily Directly Observed Treatment, Short-course (DOTS) Fixed dose combination (FDC) was one such major leap and aimed to simplify the treatment regimen, reduce pill burden, avoid drug monotherapy, improve compliance, reduce chances of drug resistance, decrease stigma and make the treatment more patient friendly.

We intended to study the impact and acceptance of this changed FDC daily DOTS at the grass root level. Clinical and microbiological parameters were also studied alongwith.

Methods: Prospective study was conducted in the Department of Pulmonary Medicine, Government Medical College and Hospital, Chandigarh from October, 2018 to October, 2020.138 sputum smear positive patients were enrolled at the time of initiation of treatment and studied till end of intensive phase (IP). Baseline socio-demographic and clinical details, any adverse drug reactions (ADR's), their subsequent management and sputum smear conversion at end IP were noted. Various patient and disease related factors were studied in relation to sputum smear conversion and ADR's. At end IP, experiences of the patients with the newly introduced daily regimen were assessed by using a structured questionnaire. The data was tabulated and statistically analyzed.

Results: Mean age of the patients was 39.31 ± 1.5 years. Majority were males, literate, married, employed, from urban background and moderately built.

During IP, 59 (42.8%) patients experienced ADR's. 31/59 patients needed admission while 28/59 patients were managed on outpatient basis. 31/59 patients improved with symptomatic management, while 28/59 patients required change in anti tubercular drugs for a short period of time. All the patients were shifted back to FDC daily DOTS after a few days. Though 59 patients reported ADR's, only 44/59 patients missed their doses. Rest 15/59 patients continued with the treatment despite mild ADR's and reported for management without missing any dose. Follow-up smear at end IP was negative in 130/138 patients (94.2%).

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93.5% patients preferred their family member as the DOTS provider. More than 90% of the patients were satisfied with basic provisions like treatment room privacy, cleanliness, safe drinking water and sign boards at DOTS centre. Satisfaction with the health care worker (HCW) (assessed by enquiring about the behavior of the HCW, explanation given about the disease and treatment, pre-treatment counseling, occurrence of ADR's, consequences of irregular treatment, warning signs for consultation, advise on nutrition requirement and follow-up information) was reported by 97.8% patients.

Sputum conversion rates were significantly higher in unemployed ($p = 0.043$). Non-adherence to treatment was significantly associated with ADR's ($p < 0.001$). Sputum conversion rates and ADR's were unaffected by education, rural/urban background, BMI, co-morbidities, addiction and previous history of anti-tubercular treatment.

Conclusion: Daily DOTS achieved appreciable sputum conversion rates at end IP. Non-adherence to treatment and ADR's were managed well with adequate psychosocial support, counseling, timely monitoring and treatment. FDC daily DOTS emerged as a highly acceptable regimen owing to various comprehensive measures adopted at the grass root level.

1. Introduction

Tuberculosis (TB) is a communicable disease caused by the bacillus *Mycobacterium tuberculosis*. It causes significant mortality and morbidity among the patients.[1] TB control programs have been frequently revised and strengthened based on the experiences of the past.[2–4] The Revised National Tuberculosis Control Programme (RNTCP) introduced in 1997,[3] focused on Directly Observed Treatment, Short-course (DOTS). It was revamped and renamed as National Tuberculosis Elimination Programme (NTEP) in 2020 with several newer strategies to augment the goal of ending TB ahead of the global timelines.[1,4]

Problems with the intermittent regimen practiced in India since decades were the high pill burden on alternate days, adverse effects because of the same causing discontinuation of one or more drugs and alternate day visits to the DOTS centre affecting day to day life and living.

To address these issues, DOTS Fixed dose combination (FDC) was launched.[5] It consisted of FDC's of four drugs namely isoniazid, rifampicin, pyrazinamide and ethambutol in the intensive phase (IP) and FDC's of three drugs namely isoniazid, rifampicin and ethambutol in the continuation phase (CP). It was introduced to simplify the treatment regimen, to reduce the pill burden, to avoid drug monotherapy, improve compliance and reduce the chances of development of drug resistance.[6] In addition, it aimed to make the treatment more patient friendly by allowing family members/friends to be the DOTS providers. Long standing issues of stigma associated with repeated visits to the DOTS centre were specifically considered. From the physician point of view, it aimed to reduce the errors in prescribing the medications with lesser chances of development of drug resistance.[7–11]

It was also noticed from the experiences of the past that non adherence and subsequent failures to treatment have remained a major problem in the Indian population because of lapses at the level of health care system and at the patient level. Poverty, lack of knowledge, co-morbidities, addictions, adverse drug reactions (ADR's), and social stigma are a few of the frequently quoted reasons. However, personal and family circumstances are also known to affect the success of any long duration treatment regimen. Impact of these psychosocial factors and individual patients' perception of TB and its treatment modalities have not been studied in the past in much detail.[12–16] Exploring these mentioned problems would give a holistic picture of the current status of TB management and is expected to aid in improving the health care services and refine the implementation of our TB elimination strategies for the future.

Adopting a new strategy in the second-largest country in the world needs a mighty support from the general population. Monitoring the strengths and weaknesses of this major radical shift from intermittent regimen to daily DOTS regimen in the field conditions hence has become a prerequisite to confidently continue practising the existing regimen and frame policies for the future. Very few studies have been carried out for assessing the execution of daily DOTS in field practice.[17] FDC daily DOTS was launched in our institute in October 2017. Our study hence intended to evaluate the acceptance of the daily DOTS at the grass root level, factors affecting the treatment adherence, adverse effect profile of the FDC's during the IP of the treatment, sputum smear conversion at end of IP and an overall impact of this changed regimen on the patients and their families.

2. Materials and methods

This was a prospective study which was conducted in the Department of Pulmonary Medicine at Government Medical College and Hospital (GMCH), Chandigarh over a span of two years from October 2018 to October 2020. After informed written consent, 177 sputum smear positive patients, irrespective of previous history of anti-tubercular treatment (ATT) were enrolled in our study.

2.1. Exclusion criteria

Patients less than 18 years of age, extra-pulmonary TB patients, patients with evidence of resistance to either isoniazid or rifampicin or any other TB drug, un co-operative, critically ill, those who lacked the capacity to give consent, and the patients who were referred outside the tricity of Chandigarh, Mohali and Panchkula for treatment were excluded from the study.

Clinico-demographic details, chest radiograph, routine blood investigations, baseline sputum smear microscopy, sputum cartridge based nucleic acid amplification test (CBNAAT) and line probe assay (LPA) were done as per the Technical Operational Guidelines (TOG).[18] Liquid culture and drug susceptibility testing was done if indicated.[18] Body mass index (BMI) was calculated according to the criteria of the World Health Organization. Patients were categorized as underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (≥30 kg/m^2).[19] Only those patients who reported back at the end of the IP were labeled as 'completing the study' and were finally analyzed. The patients were considered non-adherent to the treatment if they missed one or more doses of ATT.[7]

Patients were followed up for any ADR's to ATT. The type of ADR's and subsequent management for the same were also recorded. At the end of IP, the experiences of the patients with the newly introduced FDC's were assessed. The questionnaire for the same was accordingly constructed and duly validated before use in the study population. Those who had their sputum report as negative by sputum smear microscopy were considered to be sputum converted. Repeat CBNAAT, LPA, and liquid culture was done for patients who were found to be sputum smear-positive at the end of IP as per guidelines.[18]

Out of 177 patients, 33 patients turned out to be drug resistant and 6 patients died before the end of IP. These 39 patients were excluded from the study. 138 patients were hence finally analyzed.

The study was approved by the Institutional Research and Ethics Committee.

3. Statistical analysis

In this cohort study, opinions of patients regarding treatment were explored and qualitative analysis was done. ADR's were studied and compared using the normal test of proportions. Significance of association of ADR's with patients' characteristics were studied. The comparison was made using the chi-square test/Fisher exact test for categorical variables as appropriate and using Mann Whitney test for continuous variables. Sputum conversion rates at the end of the IP were studied. Sputum smear conversion rates were also studied in association with the characteristics of patients. All programmatic parameters were reported using proportion and oblique parameters. Data analysis was done using the latest version of SPSS software. The level of statistical significance was set at 5% ($p < 0.05$).

4. Results

Baseline socio-demographic characteristics of the patients are given in Table 1. The mean age of the patients was 39.31 ± 1.5 years. Male predominance in the study population was noted. Majority of the patients were literate, married, employed, and from urban background. Majority were moderately built with a BMI between 18.5 and 24.9 kg/m^2. Diabetes, hypertension, hypothyroidism, and seizure disorder were the most common co-morbidities. Substance abuse like smoking, alcohol intake, and afeem intake was seen in 34.1% of the patients.

One-fourth of the patients (25.4%, n = 35) had a previous history of ATT intake. Baseline bacillary load of the patients assessed by fluorescence smear microscopy showed an almost similar percentage of 1+ (n = 48, 34.8%), 2+ (n = 46, 33.3%) and 3+ (n = 41, 29.7%) grades.[20] During IP, 59 (42.8%) patients experienced ADR's in the form of abdominal symptoms, respiratory complaints, itching/skin rash, chest pain, etc. 31/59 patients (52.5%) were admitted to the hospital for the management of these ADRs. Rest 28/59 patients (47.5%) were managed on an outpatient basis. Out of the total of 59 patients, 31 patients improved with symptomatic management. Anti tubercular drugs were changed in 28 patients for a short period of time either during the hospital admission or on an out-patient basis. However, all of them were shifted back to FDC daily DOTS after a few days.

A total of 45 patients missed their doses. Though 59 patients reported ADR's, only 44 patients missed their doses because of the same. Rest of the 15 patients continued with the treatment despite mild ADR's and reported back to the health care facility without missing any dose. Predominant reasons for missing the doses in 44 patients were ADR's in the

Table 1 – Showing baseline characteristics of the patients (n = 138).

Parameter		N (Percentage)
Gender	**Male**	90 (65.2%)
	Female	48 (34.8%)
Age (in years)	**18–30**	58 (42.0%)
	31–40	25 (18.1%)
	41–50	15 (10.9%)
	>51	40 (29%)
Education	**Illiterate**	17 (12.3%)
	Literate	121 (87.7%)
Employment status	**Employed**	73 (52.8%)
	Unemployed	65 (47.2%)
Marital status	**Married**	79 (57.2%)
	Unmarried	59 (42.8%)
Residence	**Rural**	45 (32.6%)
	Urban	93 (67.4%)
BMI (kg/m^2)	**<18.5**	48 (34.8%)
	18.5–24.9	84 (60.9%)
	>25	6 (4.3%)
Presence of co-morbidities	**Yes**	35 (25.4%)
	No	103 (74.6%)
Addiction	**Yes**	47 (34.1%)
	No	91 (65.9%)

BMI- Body mass index.

Table 2 – Responses of the participants with respect to experiences with the DOTS centre and the HCW.

Parameter		N (Percentage)
Distance from the DOTS centre	<5 km	15 (10.9%)
	5–10 km	123 (89.1%)
Time taken to visit DOTS centre	<30 min	16 (11.6%)
	30–60 min	122 (88.4%)
First appointment easy	Yes	138 (100%)
	No	0 (0%)
Problem faced during first visit	Yes	2 (1.4%)
	No	136 (98.6%)
Waiting time in DOTS centre	<30 minutes	127 (92%)
	30–60 minutes	11 (8%)
Medical terms understandable	Yes	135 (97.8%)
	No	3 (2.2%)
Duration of treatment	Already knew	42 (30.4%)
	Explained by HCW	96 (69.6%)
Infectiousness of the disease explained by HCW	Yes	138 (100%)
	No	0 (0%)
Pre treatment counseling given by HCW	Yes	135 (97.8%)
	No	3 (2.2%)
Follow up information given by HCW	Yes	138 (100%)
	No	0 (0%)
Counseling regarding the repeat sputum examination given by HCW	Yes	138 (100%)
	No	0 (0%)
Consequences of the irregular treatment explained by HCW	Yes	134 (97.1%)
	No	4 (2.9%)
Nutritional advice given by HCW	Yes	132 (95.7%)
	No	6 (4.3%)
Warning signs for consulting a doctor explained by HCW	Yes	132 (95.7%)
	No	6 (4.3%)
Questions answered by HCW	Yes	138 (100%)
	No	0 (0%)
Treatment room privacy	Yes	138 (100%)
	No	0 (0%)
DOTS centre cleanliness	Yes	138 (100%)
	No	0 (0%)
Availability of sign boards	Yes	114 (82.6%)
	No	24 (17.4)
Availability of clean drinking water	Yes	41 (29.7%)
	No	97 (70.3%)
Patient friendly environment	Yes	136 (98.6%)
	No	2 (1.4%)
Flexibility in providing DOTS	Yes	138 (100%)
	No	0 (0%)
DOTS provider	Family member	129 (93.5%)
	HCW	9 (6.5%)
Daily wages affected	Yes	5 (3.6%)
	No	133 (96.4%)
Average number of visits/month for collecting medications	1–2	127 (92%)
	≥3	11 (8%)
Overall patient's satisfaction with services	Yes	135 (97.8%)
	No	3 (2.2%)
Overall family member's satisfaction	Yes	137 (99.3%)
	No	1 (0.7%)

DOTS- Directly Observed Treatment, Short-course.
HCW- Health care worker.

form of abdominal symptoms (n = 33/45, 73.4%), respiratory symptoms (n = 6/45, 13.3%) and skin rash (n = 5/45, 11.1%). Only one patient (n = 1/45, 2.2%) missed doses and stopped treatment on his own because of improvement in symptoms. Out of the 45 patients who missed their doses, majority skipped 2–4 days of treatment on an average (n = 38/45). Retrieval action was taken in all the patients who missed the doses. All of them were counseled and ADR's were managed as per need, as already stated.

Follow-up smear at the end of the IP was negative in 130/138 patients (94.2%).

The experiences with the DOTS centre and health care workers (HCW) are depicted in Table 2. More than 90% of the patients were satisfied with the basic provisions like treatment room privacy, cleanliness, clean and safe drinking water and sign boards at the DOTS centre. Majority of the patients had their DOTS centre within 10 km of their residence and were able to reach within 60 minutes duration. Flexibility was provided in collecting the drugs for all the patients. Patients had to personally visit the DOTS centre only once or twice because their family members were appointed as DOTS provider and were allowed to collect the medications on their behalf. Hence daily wages were not affected.

Satisfaction with the HCW and DOTS centre was seen in 135 patients (97.8%). Only less than 3% of the patients were not satisfied with the treatment, the main reasons reported were lack of nutritional advice, and inability in explaining the warning signs requiring consultation with a doctor, the consequences of irregular treatment, and the side effects of the drugs (Table 2).

Effect of various patient related factors on sputum conversion and ADR's is depicted in Tables 3 and 4 respectively. The sputum conversion rates were significantly higher in unemployed ($p = 0.043$) but were unaffected by education, rural/urban background, BMI, presence of co-morbidities, addiction and previous history of ATT. It was found that non adherence to treatment was significantly associated with ADR's ($p < 0.001$). ADR's were unaffected by education, rural/urban background, occupation, BMI, presence of co-morbidities, addiction and previous history of ATT.

5. Discussion

The Government of India is committed towards its goal to End TB by the year 2025.[21] Various changes are being incorporated from time to time, as per the latest available evidence to make this goal a reality. The introduction of daily FDC's in 2016 was a paradigm shift and aimed to address multiple issues related to acceptability, tolerance, adherence, drug resistance and ease of treatment. Our study explored the applicability,

Table 3 – Comparison of various patient and disease related factors with sputum smear conversion.

Patient and disease characteristics		Smear conversion		P value
		Yes N (Percentage)	No N (Percentage)	
Education	**Illiterate**	17 (100%)	0 (0.0%)	0.179
	Matric	71 (91%)	7 (9%)	
	Graduate	42 (97.7%)	1 (2.3%)	
Residence	**Rural**	41 (91.1%)	4 (8.9%)	0.280
	Urban	89 (95.7%)	4 (4.3%)	
Occupation	**Employed**	66 (90.4%)	7 (9.6%)	0.043
	Unemployed	64 (98.5%)	1 (1.5%)	
BMI (kg/m^2)	**<18.5**	48 (100%)	0 (0.0%)	0.065
	18.5–24.9	76 (90.5%)	8 (9.5%)	
	25.0–29.9	6 (100%)	0 (0.0%)	
Addiction	**Yes**	43 (91.5%)	4 (8.5%)	0.327
	No	87 (95.6%)	4 (4.4%)	
Co-morbidities	**Yes**	32 (91.4%)	3 (8.6%)	0.416
	No	98 (95.1%)	5 (4.9%)	
Previous ATT	**Yes**	31 (88.6%)	4 (11.4%)	0.099
	No	99 (96.1%)	4 (3.9%)	
Baseline sputum smear grade	**Scanty**	3 (100%)	0 (0.0%)	0.248
	1+	46 (95.8%)	2 (4.2%)	
	2+	44 (95.7%)	2 (4.3%)	
	3+	37 (90.2%)	4 (9.8%)	

BMI- Body mass index, ATT- Anti tubercular treatment.

Table 4 – Comparison of various patient and disease related factors with adverse drug reactions.

Patient characteristics		Adverse drug reactions		P value
		Yes N (Percentage)	No N (Percentage)	
Education	**Illiterate**	8 (47.1%)	9 (52.9%)	0.847
	Matric	34 (43.6%)	44 (56.4%)	
	Graduate	17 (39.5%)	26 (60.5%)	
Residence	**Rural**	22 (48.9%)	23 (51.1%)	0.311
	Urban	37 (39.8%)	56 (60.2%)	
Occupation	**Employed**	27 (37%)	46 (63%)	0.147
	Unemployed	32 (49.2%)	33 (50.8%)	
BMI(kg/m^2)	**<18.5**	24 (50%)	24 (50%)	0.436
	18.5–24.9	33 (39.3%)	51 (60.7%)	
	25.0–29.9	2 (33.3%)	4 (66.7%)	
Addiction	**Yes**	22 (46.8%)	25 (53.2%)	0.489
	No	37 (40.7%)	54 (59.3%)	
Co-morbidities	**Yes**	18 (51.4%)	17 (48.6%)	0.230
	No	41 (39.8%)	62 (60.2%)	
Previous ATT	**Yes**	15 (42.9%)	20 (57.1%)	0.989
	No	44 (42.7%)	59 (57.3%)	
Non adherence	**Yes**	44 (97.8%)	1 (2.2%)	<0.001
	No	15 (16.3%)	78 (83.7%)	

BMI- Body mass index, ATT- Anti tubercular treatment.

acceptability and success of this introduction of FDC's in real life conditions.

Baseline socio demographic and clinical details of the patients were collected and were found to be in accordance with the existing literature in the Indian background.[1] An important finding from our study was that though 42.8% patients experienced ADR's in one form or the other, all these patients could be shifted back/continued to FDC daily DOTS after symptomatic management. This finding proved the tolerability of this newly introduced regimen to a great extent. When compared with the data available for intermittent regimen, the daily FDC's fared better with lesser ADR's.[22–25]

Majority of the patients presented early during the course of treatment when they had symptoms suggestive of ADR's. They were very comfortable approaching the HCW because of a friendly approach of the professionals at the very start of the treatment. This facilitated in early identification and management of ADR's before the patient actually developed any severe adversity. Since the ADR's were managed comprehensively with assurance, counseling and addressal of the problems in a holistic manner, all the patients could be switched over to FDC's again in a few days leading to continuation of the treatment, ultimately resulting in better sputum smear conversions at the end of IP and no losses to follow-up.

Another important finding was that not even a single patient needed to be continued on an alternate regimen over long term. All the patients ultimately completed their IP's with FDC's only.

94.2% of the patients were sputum converted at the end of IP. Sputum conversion rate achieved with FDC daily DOTS is comparable to intermittent regimen.[26] Majority of the patients preferred their family members to be DOTS provider. This provision was really fruitful as it avoided the loss of work and daily wages of the bread winners of the families. Alternate day long distance travel during IP, as seen in the previous intermittent DOTS regimen was also addressed. Since medications were provided as per the availability and need of the patient with feedback from the family members who acted as the DOTS providers, a continuous supply of medicines to the patients was maintained as these family members were able to collect the medicines on the behalf of the patients. Both these provisions helped dramatically in dealing with the stigma which the patients earlier faced by visiting the DOTS centre again and again.

The non adherence to ATT in our patients was 32.6% only and it was much lesser than already reported in literature.[27,28] The major reason for non adherence for a short duration in our patients was ADR's. Only one patient stopped treatment because of improved symptoms but the HCW's were able to counsel him to start the treatment again. In the previous studies, non adherence was observed because of ADR's, pill burden, social stigma, loss of family and community support, addiction and health system related factors, etc.[29,30] However the ADR's being the almost only reason for non adherence in our patients again signals towards the success story of this newly introduced FDC DOTS regimen. Proper pre treatment counseling, friendly behavior, explanation of the warning signs and psychosocial support contributed significantly to this success so achieved.

Another important finding from our study was that though many patients knew about the infectiousness of the disease at the time of diagnosis, only 30.4% of the patients were aware of the perceived duration of the treatment, nutritional requirement and time of follow-up. Previous studies have shown that this knowledge amongst the general population is variable.[31–33] There is an urgent need for sensitization of the masses regarding various finer aspects of TB to prevent and manage this disease at the household level.

Overall, most of the patients and their relatives were satisfied with the daily DOTS regimen in our study. This was assessed by enquiring about the behavior of the HCW, the explanation given about the disease process and treatment regimen, pre-treatment counseling, occurrence of ADR's, consequences of irregular treatment and warning signs for consultation, advise on nutrition requirement and follow-up information. This was further assessed by indicators like the actual occurrence of ADR's and sputum smear conversion at the end of the IP. It is plausible here to comment that the addressal of social stigma through various measures incorporated in this regimen could also have added to the satisfaction levels of the patients as well as family members.

This interplay of different supportive services working in close cohesion was possibly responsible for the overall success of this changed regimen. When the impact of the patient and disease related factors on sputum conversion was studied, it was seen that education, rural/urban background, BMI, addiction, presence of co-morbidities, previous history of ATT or baseline sputum grading did not affect the sputum conversion rate. Higher sputum conversion rates were seen in patients who were unemployed, the reasons which could not be comprehensively explained. Though not definitive, however, better nutrition, lesser stress of the working environment, rest and care by the family members could possibly be contributing to the same. Similarly, when the impact of various patient and disease related factors on ADR's was studied, it was seen that education, rural/urban background, occupation, BMI, addiction, presence of co-morbidities or previous history of ATT did not affect the occurrence of ADR's. However, non adherence to treatment was significantly associated with ADR's. This is very expected since patients are deemed to stop the treatment because of intolerance to drugs and occurrence of ADR's. Another notable finding was that few patients who were not sputum converted at the end of IP reported slightly higher occurrence of ADR's when compared with patients who were sputum converted at the end of IP but it was statistically non significant ($p = 0.245$). This again highlights the fact that regular monitoring, early detection and timely management of ADR's in our study population contributed to the higher sputum conversion rates at the end of IP in a majority of our patients.

In a broader perspective, a single factor could not be held responsible for the success of the FDC daily DOTS. Different provisions as incorporated at the grass root level by taking care of the minutest of the things from the patient's point of view and working in an intertwined network with each other with one affecting the other in a positive manner can only explain the highly satisfactory results which we obtained with respect to the various indicators which we studied.

Our study is one small attempt to analyze the acceptance of the FDC daily DOTS by the patients to reveal the true picture from the patient's perspective. At the same time, we also analyzed the factors associated with the sputum conversion rates and occurrence of ADR's till the end of the IP, with a farsighted vision to utilize our findings for the betterment of the services in the future. Our study showed that the FDC daily DOTS achieved appreciable results with respect to the microbiological and clinical indicators. The services so provided are patient-friendly than ever before and are well appreciated by the patients and their family members.

5.1. *Limitations of the study*

Our study had the limitation of a small size with follow-up of the patients till end IP only. Since the intermittent regimen was immediately stopped and all the new smear positive patients were started on daily FDC's as per instructions, hence head to head comparisons between the two types of regimen could not be done.

6. Conclusion

The Government of India's daily DOTS initiative was thought to revolutionize TB treatment. The two main barriers

contributing to failure i.e. non adherence and ADR's have been addressed to quite an extent with the various changes incorporated in this daily DOTS regimen. Adequate psychosocial support, counseling, timely monitoring and management provided to the patients and their family members through this regimen helped in overcoming these obstacles and contributed to overall satisfaction as revealed from the results of our study.

Conflicts of interest

All authors have none to declare.

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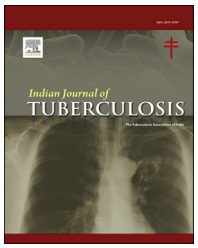

Original article

Midfoot tuberculosis: Clinical suspicion and early investigation is the key

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ABSTRACT

Background: Tuberculosis affecting midfoot is not common, leading to delay in diagnosis further leading to deformity and difficult management. Tissue diagnosis is always not possible at such sites. MRI is the better imaging modality to diagnose earlier than conventional radiographs. The aim of the study is to have a clinical suspicion of tuberculosis in midfoot pain and a low threshold to perform MRI in these patients.

Methods: The data of 7 patients were collected prospectively over 3 years. Inclusion criteria included midfoot pain for more than 4 weeks in a skeletally mature patient with no radiographic findings. MRI and laboratory investigations were done in all the patients. All the patients were given Anti-tubercular therapy and followed up for 12 months. The patients were assessed at 3, 6- and 12-months duration with ESR, CRP, MRI, VAS and AOFAS Midfoot scores.

Results: There were 3 males and 4 females included in the study with a mean age of 55.5 years. The mean duration of symptoms was 5.2 weeks. The mean ESR and CRP at presentation were 46 and 12 respectively which progressively decreased over 12 months. The mean VAS and AOFAS midfoot score at presentation were 4 and 70 respectively. None of the patients had any complication from ATT drugs. Residual pain was present in 4 patients with no functional limitation of the foot. The follow-up MRI showed healed tuberculosis in all the patients.

Conclusions: Tuberculosis can be a cause of vague midfoot pain in tuberculosis endemic countries. The MRI in such patients along with laboratory findings can lead to early diagnosis and the empirical institution of the ATT. The tissue diagnosis is not always possible in the early stages of the disease as there is no radiographic lesion or collection in the midfoot.

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1. Background

Tuberculosis is a chronic granulomatous infection that can potentially affect any organ or system of the body. Osteoarticular tuberculosis forms less than 3% of extrapulmonary tuberculosis.[1] The involvement of the foot is rare, and it represents around 0.1–0.3% of the cases.[2] The midfoot is essential for the proper functioning of the foot during the weight-bearing and gait cycle. The tuberculosis of the midfoot can present as vague or diffuse midfoot which is difficult to diagnose early. The consequences of later can lead to arthritis and deformity of the midfoot. In the early stage of the disease, the plain radiographs generally depict no significant osteoarticular changes in midfoot tuberculosis.[3] Magnetic resonance imaging (MRI) can demonstrate early changes which aid in diagnosis and help to commence the treatment early.[4] This study emphasizes the need for early MRI to diagnose vague midfoot pain, the aetiology of which can be tuberculosis. This not only leads to early clinical improvement but also may alleviate the need for midfoot surgery.

2. Methods

The data for the study were collected prospectively from 2017 to 2019 in a tertiary care centre. All the patients presented with the Outpatient department of Orthopaedics. Informed consent was taken from all the patients. The Institutional Ethical Committee approval was taken. The patients of either gender with midfoot pain for more than 4 weeks in skeletally matured individual and with complete follow up of at least 1 year were included in the study. All the patients were evaluated with radiographs (anteroposterior, lateral and oblique) of the foot, Rheumatoid factor, serum uric acid and haemogram. These aids, rule out inflammatory arthritis, gout and other pathologies. The patients with radiographic changes in midfoot, previously diagnosed systemic or localised aetiology of the midfoot pain apart from tuberculosis were excluded from the study. The pain was assessed by visual analogue score (VAS scores) at presentation and follow-ups. All the individuals were subjected to the radiographs of the foot, chest radiograph and MRI of the foot. The laboratory investigations include complete blood count, Erythrocyte sedimentation rate (ESR) (Westergren method), quantitative C reactive protein (CRP), serum uric acid, liver function test and rheumatoid factor. The MRI helps to rule out other bony or soft tissue pathologies.

The patients suspected of tuberculosis, suggestive of tuberculosis or non-specific pathology of midfoot based on MRI were included in the study. This forms the basis of commencement of Antitubercular treatment (ATT) empirically after the MRI findings. All the patients were followed for at least 1 year with MRI, ESR, quantitative CRP, AOFAS Midfoot score and complete blood count done in follow up done at 3, 6 and 12 months.

All the patients were given a standard daily dose of ATT (Rifampicin (R) 600 mg, isoniazid (G) 300 mg, pyrazinamide(Z) 1500 mg, ethambutol (E) 1200 mg) for 12 months (HRZE for 2 months + HRE for 10 months) in total.

3. Results

The duration of the study was 3 years. There were nine patients included in the study but two were lost in follow-ups. All 7 patients were included according to the inclusion criteria. There were 3 males and 4 females in the study with a mean age of 50.7 years (Range: 38–57 years). All were suffering from midfoot pain for an average of 5.5 weeks (Range: 4–8 weeks). Three patients (2 females and 1 male) had Diabetes Mellitus or Hypertension or both. Clinically no diagnosis could be made in these patients for vague foot pain (Fig. 1).

All the patient had normal serum uric acid level and rheumatoid factor. The average haemoglobin level in the study population was 10.6 g/dl with normal total leukocyte counts in all the patients. The differential count showed slightly raised leukocytes in three patients. The ESR was raised in all the patients with an average of 42 at the time of presentation which decreased in follow-ups. The CRP levels were raised with an average of 21.4 at presentation and decreased to normal level in follow-ups after 3months in all the patients. The liver function test was normal in all the patients. The Mean AOFAS midfoot score at presentation was 52 and progressively increased with treatment at 3, 6 and 12 months with values of 58, 82 and 92 respectively (Table 1).

The two views (AP and oblique) radiographs of the foot showed no radiological changes (Fig. 2).

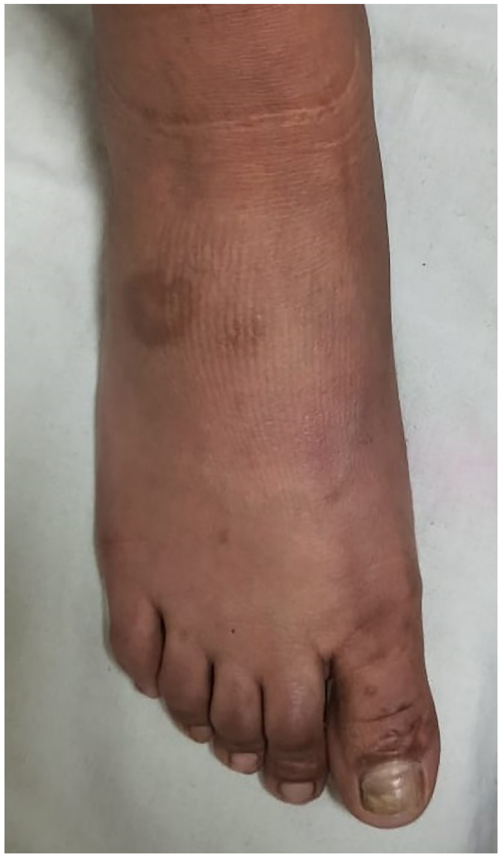

Fig. 1 – The clinical picture of the foot showing no significant swelling or deformity or sinus.

Table 1 – Descriptive and result data of the study.

S.No	Age/Sex	Duration (months)	Other Diseases	MRI	ESR (at months)				CRP (mg/dl) (at months) Normal level: <6mg/dl				VAS (at months)				AOFAS Midfoot (at months)				Complications
				Changes seen at presentation (Marrow signal changes: hypointense on T1W images, Hyperintense on T2W images)	0 day	3	6	12	0 day	3	6	12	0 day	3	6	12	0 day	3	6	12	
1	38/M	5	None	Middle and lateral cuneiform, with tenosynovitis of anterior group of tendons.	56	52	30	16	26	14	<6	<6	5	3	2	2	64	87	87	90	Residual pain
2	49/F	6	None	Medial cuneiform-1st tarsometatarsal joint, with synovial thickening	42	34	18	15	20	14	<6	<6	4	2	2	2	70	87	87	90	Residual pain
3	54/F	4	DM	Middle and lateral cuneiform with synovial thickening	48	42	30	22	28	16	10	8	6	3	2	2	64	84	87	90	Residual pain
4	56/M	4		Calcaneo-cuboid with tenosynovitis of later group of tendons	32	31	28	20	16	14	<6	<6	7	4	3	0	56	81	84	100	None
5	46/F	8		Medial cuneiform-1st tarsometatarsal joint with tenosynovitis of anterior and medial group of tendons.	38	36	30	18	15	12	6	<6	4	2	0	0	70	87	100	100	None
6	55/M	5	DM, HTN	Middle and lateral cuneiform with synovial thickening	44	41	32	24	21	14	10	8	6	4	3	2	61	81	84	87	Residual pain
7	57/F	7	DM, Pulmonary TB	Middle and lateral cuneiform-2nd and 3rd tarsometatarsal joint with synovial thickening	52	30	19	12	24	10	<6	<6	5	4	2	0	70	81	87	100	None

DM: Diabetes Mellitus; HTN: Hypertension; TB: Tuberculosis; MRI: Magnetic resonance imaging; ESR: Erythrocyte sedimentation rate; CRP: C- reactive protein; VAS: Visual analogue scale; AOFAS: American Orthopaedic Foot and Ankle Society score.

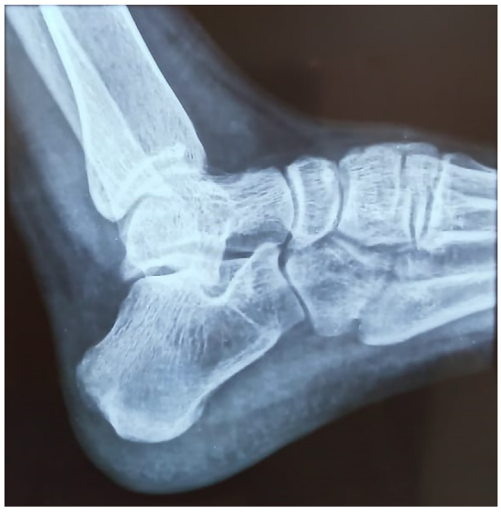

Fig. 2 – Radiograph oblique view showing no changes.

MRI of the foot was done in all the patients at presentation and follow-ups. There were changes in the midfoot of all the patients. Three patients showed hyperintense changes on T2W images on the middle and lateral cuneiform bones. One patient had similar changes at the calcaneocuboid joint. Two patients had MRI changes at medial cuneiform and 1st tarsometatarsal joint (Fig. 3) whereas one patient had MRI changes at 2nd and 3rd tarsometatarsal joint and middle and lateral cuneiforms. Synovial thickening was the most common associated lesion seen in 4 patients followed by tenosynovitis in 3 patients.

There was no soft tissue swelling seen in any of the patients. Joint effusions, localised cold abscess and sinus tract were absent in all the patients.

One patient had tubercular changes in the chest radiograph.

No Biopsy/FNAC or aspiration was done in any of the patients.

All the patients with suspected or suspicious tubercular lesion and non-specific pathologies on MRI were initiated treatment with ATT. All patients were started on Anti-tubercular chemotherapy (ATT) with an average of 7 days (Range:3–10days) from the presentation to the outpatient department of orthopaedics. All patients showed a clinical response to the treatment. There was a progressive reduction in VAS score, ESR and CRP levels at 3,6 and 12 months. MRI showed resolution of signal intensity changes at follow-ups. Residual pain was seen in four patients especially during activities. No patient required any surgery or orthosis.

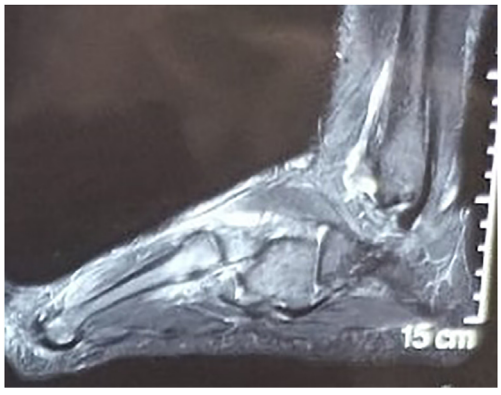

Fig. 3 – Sagittal section of MRI showing hyperintensity changes at medial cuneiform and 1st tarsometatarsal joint.

No complication was seen from ATT in any of the patients.

Due to the absence of any tissue or fluid sample, the definitive diagnosis of tuberculosis could not be made. But all the patients responded well to the empirical administration of ATT.

4. Discussion

The tuberculosis of the foot is an uncommon entity. The involvement of midfoot is even rare. There are few research articles published in English literature describing the tuberculosis of midfoot.[2–8] The description of tuberculosis of midfoot without any radiographic changes is not described in the literature.

Musculoskeletal tuberculosis forms only 5% of the tuberculosis spectrum. Out of which the foot tuberculosis constitutes 3%, mainly affecting the calcaneum and rarely the midfoot bones. The clinical presentation of musculoskeletal tuberculosis of the foot can vary from vague pain to active pus discharging sinuses and bone destruction. Tuberculosis is known to take on many disguises and thus diagnosis is delayed and difficult.[4] Due to such a rarity of midfoot tuberculosis, the diagnosis is often missed initially leading to bone destruction and delayed treatment.[5] Often the patients present with vague dull aching pain in the midfoot with or without the history of systemic features like weight loss, night cries, etc. The pain increases with activities of daily living. There can be subtle swelling over the dorsum of the midfoot in these patients. The presence of sinus over the dorsum of the midfoot is well described by a few authors.[1,2] The clinical diagnosis of midfoot tuberculosis requires a high index of suspicion and clinical experience. Musculoskeletal tuberculosis acts as a mimicker of other more common diseases like pyogenic or fungal infections, inflammatory disease, gout, traumatic or overuse injuries of the midfoot.[4]

Plain radiographs are the first line of investigation. There are four basic forms of tuberculosis foot involvement as described by Dhillon et al.[6] These forms are periarticular granuloma, most common, central granuloma, primary hematogenous synovitis and tenosynovitis. The pathology affecting only the bone is rare and is often misdiagnosed leading to the inevitable progression of the disease to adjacent joints. Thus, the initial presentation of the disease is generally suspected from the radiographic changes depicted in the midfoot bones. Mittal et al[7] described five radiographic types depicting lytic, a cystic lesion with sequestrate in smaller bones like cuboid. But for the radiological changes to appear there needs to be at least 50% bone loss.[8] Thus, the presence of radiological changes on the radiographs itself suggests delays in the diagnosis of tuberculosis of midfoot. As the disease commences in the periarticular area, then spread to the bone depicts the progression of tuberculosis. The presence of Phemister triad: juxta-articular osteoporosis, osseous erosion, narrowing of joint space is highly suspicious but not

diagnostic.[9] There were no radiological changes in the midfoot bones in any of the patients in this study. Thus, for early diagnosis of tuberculosis in such patients MRI is a useful modality.[10] The clinician should have a low threshold of acquiring MRI in suspicious patients.[11] The MRI performed very early in the stage of the disease as done in the study group leads to early diagnosis and treatment of the patients. Changes in MRI suggestive of tuberculosis in midfoot are typically hypointense on T2W images, maybe due to the presence of haemorrhage, inflammatory debris, fibrosis, and caseation necrosis. It is a very helpful sign for differentiating tuberculous arthritis from other proliferative synovial arthropathies.[12] After administration of intravenous gadolinium contrast, the thickened synovium enhances vividly. Chondral lesions and subchondral bone erosions may be visible at a stage when the joint space is still well preserved. Associated bone marrow edema, osteomyelitis, and soft tissue abnormalities such as myositis, cellulitis, para-articular abscess formation, tenosynovitis, bursitis, and skin ulceration/sinus tract formation may be seen. Sinus tracts are characterized by linear high signal intensity on T2W images with marginal 'tram-track enhancement' on gadolinium-enhanced images. Para-articular abscesses mostly show a thin and smooth enhancing wall.

The laboratory findings of slightly raised ESR and CRP corroborated the MRI impression of tuberculosis of the midfoot in this study. The diagnosis of paucibacillary musculoskeletal tuberculosis is via multimodal approach involving Fine needle aspiration cytology (FNAC), culture, histopathological examination, Polymerase chain reaction (PCR) and Enzyme-linked immunoassay (ELISA) test, Ziehl-Neelsen staining and Mantoux test.[6] Despite all these, there is no single diagnostic test with high sensitivity and specificity.[13] There was no lytic lesion in the bone or obvious swelling or fluid in the joint to aspirate, thus, the definitive diagnosis of tuberculosis was elusive. These results were contrary to the study by Dhillon et al,[14] in which they were able to definitively diagnose tuberculosis of the foot in 23 out of 24 patients. There were lesions in the bone or joint to aspirate and obtain the infected tubercular tissue. But in this study, there was no bony lesion to biopsy.

Anti-tubercular chemotherapy in such cases is often instituted on clinical suspicion in endemic areas.[14] The similar approach was followed with the patients in the study group. The clinical results were encouraging with dramatic improvement in all the patients. There are surgical interventions described in the literature for midfoot tuberculosis[15] but the early commencement of the treatment reduces the need for foot surgery. The clinical complaint of pain in the midfoot drastically improved over a few months although the treatment was continued for 12 months.

As there were no radiographic changes, follow-ups MRI was done showing resolution of signal intensity changes. None of the patients required any surgical debridement or had foot deformity or non-responder to the chemotherapy.

5. Conclusions

The early diagnosis of midfoot tuberculosis requires high clinical suspicion and a low threshold for acquiring MRI foot in such patients. Even in the absence of radiographic changes and definitive tissue diagnosis of tuberculosis, antitubercular chemotherapy can be institute empirically in endemic areas. Early diagnosis & prompt treatment is the key to prevent foot deformities and complications in patients with midfoot tuberculosis.

Contribution details

BH, DS contributed to the concept, design, Interviews, literature search, synthesis of information identified in the search, writing and editing of the manuscript, and data collection and analysis. All others contributed in the literature search, writing, and review of the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors have none to declare.

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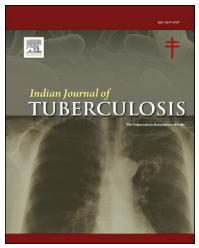

Original article

Evaluation of implantation markers and immune cell infiltration in endometrial biopsy of female genital tuberculosis

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ABSTRACT

Background: Female genital tuberculosis (FGTB) causes infertility in a significant number of females. The immunological impact of tuberculosis on endometrium in infertile females has not been studied before. The present study was designed to evaluate markers related to infiltrating immune cells and implantation in endometrial aspiration from infertile females and correlate with conventional tests and polymerase chain reaction (PCR) for tuberculosis (TB).

Methods: It was a prospective cohort study with 385 patients out of which IHC was done in 306 over a period of 3 years from 2013 to 2016 in a tertiary care hospital. Women with infertility, 20–35 years of age, without history of pulmonary TB or intake of antitubercular therapy were included. Endometrial samples were subjected to PCR for TB along with microbiological and histological examination for TB. Immunohistochemistry for CD45, CD3, CD20, CD4, CD8, CD68, CD138, Interferon gamma, Interleukin 10 (IL-10) and implantation markers MUC1 and Notch 1 were done on the endometrial samples along with 25 control subjects.

Results: Conventional tests for tuberculosis like staining for acid fast bacilli (AFB), granuloma on histology or culture positivity were seen in 2.61% (6/306; 1.96% had granulomas, 1/306; 0.32% was AFB positive, 2/306; 0.6% were liquid culture positive). PCR was positive in 190/306 (62.09%). CD3, CD20, CD45, CD68, CD4, CD8 and CD 138 expressing infiltrating cells were not significantly related to PCR positive cases. Interferon gamma expressing lymphocytes were significantly higher (38.94%) in PCR positive endometria compared to 26.72% in the PCR negative ($p = 0.04$). Notch −1 expression correlated significantly with the occurrence of pregnancy. A trend towards high intensity expression of Notch1 was seen in PCR negative cases. MUC-1 expression did not correlate with pregnancy although interferon gamma expression was significantly related to low intensity MUC1 expression.

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Conclusions: Immunohistochemical markers are not reliable tests in diagnosis of FGTB. Notch 1 expression though showing correlation with pregnancy has to be further evaluated with a panel of other implantation markers.
Study funding: Indian Council of Medical Research, New Delhi, India.

1. Introduction

Tuberculosis (TB) is an important health problem in developing countries. Female genital tuberculosis (FGTB) is one of the more subtle manifestations of this disease and primarily presents as infertility.[1] FGTB alone accounts for 9 per cent of all extra-pulmonary TB cases[2] and can affect the fallopian tubes (90–100%), endometrium (50%–80%), ovary (20%–30%), and less commonly the cervix, vagina, and vulva.[3] Tubal blockage as well as pelvic and abdominal adhesions are the main source of infertility.[3–5] It poses a diagnostic challenge and in the absence of clear guidelines, there is risk of over diagnosis and irrational administration of anti-tubercular therapy (ATT) with consequent development of drug resistance.[6]

The pathophysiology of FGTB is poorly understood and no study has evaluated disease pathogenesis and progression in patients positive for polymerase chain reaction test for TB (PCR-TB) on endometrial aspirate (EA). Endometrial histology of FGTB is based on identification of granulomas and is traditionally used as a gold standard for diagnosis, the other conventional gold standard tests being identification of acid fast bacilli (AFB) and mycobacterial culture by Lowenstein Jensen (LJ) medium or liquid culture. However PCR-TB tests identify a much larger number of cases in which the endometrium may not show presence of granulomas.

MUC1 is an antiadhesive protein with increased expression in implantation window in human studies.[7] NOTCH 1 has been associated with decidualization and reduced expression is seen in recurrent implantation failure and endometriosis.[8] A significant difference in the expression of endometrial receptivity markers expression have been observed in women with FGTB. The relationship of these implantation related markers with changes in the immune cell microenvironment of endometrium in tuberculosis have not been previously investigated. So the present study was designed to assess the impact of FGTB on implantation markers and infiltrating lymphocytes in patients with infertility.

2. Materials and methods

2.1. Patient profile

This study was conducted on 306 cases of infertile female patients suspected to have FGTB with adequate EA available. After institutional ethics clearance, patients in the 20–35 years age group with primary or secondary infertility for a period greater than 1 year and willing to follow protocol/consenting to participate, were included. Subjects who, on history, examination and investigation, were found to have any one or more of the following: male factor infertility, endometriosis, anovulatory cycles, polycystic ovarian syndrome, gonorrhoea or chlamydial infection on testing, active pulmonary or extra pulmonary tuberculosis, patients on ATT or received ATT in the past 5 years or not willing/able to follow protocol were excluded.

2.2. Microbiological examination

All patients were screened in the gynaecology outpatient of our institute between the years 2013 and 2016 and investigated for the exclusion criteria. After the patient was recruited, she underwent EA which was divided into three samples, one for routine microbiology, one for PCR and one for histopathological examination. The sample sent to microbiology was sent fresh and was evaluated for smear AFB using Ziehl Neelsen stain, a part was plated on LJ medium for AFB culture and liquid culture for AFB was done using the Mycobacterial Growth Indicator Tube (MGIT) technology. PCR-TB was done as described previously.[9,10]

2.3. Histopathological examination

Endometrial biopsy for histopathological evaluation was subjected to routine formalin fixed paraffin embedded tissue sections stained with Haematoxylin and Eosin. A total of 50 control cases which had been sent for conditions other than infertility were also included in which 25 were proliferative and 25 were secretory endometrium selected at random from routine specimens received in pathology and had not been sent for microbiology analysis. IHC slides were cut on Poly-L-Lysine coated slides. IHC for CD45, CD3, CD20, CD4, CD8, CD68, CD138, IFN gamma, MUC1 (Thermo scientific; 1:400 dilution with citric acid antigen retrieval at pH 6), Interleukin 10 and Notch 1 (IL-10; Thermo scientific; 1:200 dilution with citric acid antigen retrieval at pH 6), were done on both control and test samples. IHC was analysed as follows: LCA, CD68, CD3, CD4, and CD8 done by counting the number of positive cells per high power field (HPF), and at least 10 microscopic fields in the area of highest infiltration were examined, excluding lymphoid follicles. CD20 was evaluated as CD20 positive cells per HPF (from 3 highest HPF away from follicles). For CD138, IFN gamma and IL10, done as number of positive cells per HPF, and at least 10 microscopic fields in the area of highest infiltration were examined. The IHC staining score was based on number of positive cells as:

0 = no positive lymphoid cell
1+ = 1 to 3 positive lymphoid cells in the stroma
2+ = 4 to 10 positive lymphoid cells in the stroma
3+ = >10 positive lymphoid cells in the stroma

2.4. *Implantation markers*

Notch1 and MUC1 implantation markers show epithelial staining. They were graded according to intensity as follows: 0: no staining; 1+: faint low intensity staining; 2+: uniform moderate to high intensity; 3+: high intensity with deposits obscuring cells. Zero and 1+ were considered as low intensity; whereas 2+ and 3+ were considered as high intensity.

2.5. *Statistical analysis*

Statistical analysis was carried out using Stata 12.0 (college station, Texas, USA). A Chi-squared test was done to compare the expression of the various antigens on immunohistochemistry to gold standards and PCR-TB.

3. Results

3.1. *Patient profile and conventional tests*

A total of 385 patients were enrolled in the study after screening 732 patients. Of these 306 patients who had tissue adequate for IHC were kept for the present study. Fifty control endometrial tissues were also obtained for analysis. The 306 patients ranged in age from 20 to 35 years (mean 27.64 ± 3.98) with mean duration of infertility of 4.74 ± 3 years. On histopathology, 58 cases were of proliferative endometrium and 248 were secretory endometrium. Of the 306 cases, 8 had conventional tests positive including 6 (1.96%) granulomas (all of which were seen in the secretory phase) and 2 liquid cultures. One case with granuloma also showed AFB positivity. LJ culture was negative in all samples.

3.2. *Infiltrating immune cell markers*

The mean number of immune cells of different types infiltrating the endometrium were similar in the cases (CD3 30.38; CD20 4.43; CD45 81.01; CD68 13.86; CD4 6.52 and CD8 9.78) and in the 50 controls (CD3 27.93; CD20 4.39; CD45 66.36; CD68 14.52; CD4 5.74 and CD8 8.49). Plasma cells were seen in a total of 93/306 cases (30.39%) using CD138 at 1+ as positive. Normal control endometria (proliferative and secretory) also showed presence of CD138 positive plasma cells in 13/50 (26%) indicating high prevalence of nonspecific chronic endometritis in the general population. The different infiltrating immune cell markers (CD3, CD20, CD45, CD68, CD4, CD8) in the CD 138 positive and CD 138 negative EA were similar.

3.3. *PCR-TB, IFN gamma and IL10*

Of the 306 cases studied, more than half (190/306, i.e., 62%) were positive for PCR-TB. The PCR-TB positive and negative biopsies showed no difference in distribution of CD138 positive cells (p = 0.65) and the mean numbers of the other infiltrating immune cell markers (CD3, CD20, CD45, CD68, CD4, CD8) were also similar. IFN gamma positive lymphocytes were more frequently seen in the PCR-TB positive cases than in the PCR-TB negative cases. Using 3+ and above IHC score as cut off for IFN gamma positivity (Fig. 1A), 12.1% of the PCR-TB positive endometria had IFN gamma positive lymphocytes compared to 4.31% in the PCR-TB negative cases (p = 0.02). Using 3+ positivity as the criteria made it easier to interpret the IHC. However, keeping 1+ and above as positivity, IFN gamma was more frequently positive in the PCR-TB positive cases (38.94%) than in the PCR negative cases (26.72%; p = 0.04) which gives a better indication of the extent of positivity of IFN gamma and its relationship to PCR-TB positivity. IL10 expressing lymphocytes (1+ and above) were seen in 49/306 (16%). There was no difference in interleukin-10 positivity between PCR-TB positive and negative cases (p = 0.56).

3.4. *Implantation markers*

MUC 1 and Notch1 were done on 248/306 secretory endometria since proliferative endometrium is anyway not receptive to implantation. MUC1 was graded as 0 (20/248; 8.06%), 1+ (74/248; 29.83%), 2+ (147/248; 59.27%), and 3+ (7/248; 2.82%; Fig. 1B). For analysing MUC1 against different parameters, low intensity (grades 0 and 1) and high intensity (Grades 2 and 3) were grouped together. Cases with 3+ IFN gamma expressing lymphocytes in the endometrium had low intensity MUC expression in 68% whereas endometria without IFN gamma 3+ expressing lymphocytes showed low intensity MUC1 expression in 47% (p = 0.04). There was no difference in low and high intensity MUC 1 staining with regard to CD138 (p = 0.33), PCR-TB (p = 0.74) and IL-10 (p = 0.42).

Notch 1 was graded as 0 (20/248; 8.06%; Fig. 1C) and + (97/248; 39.11%), 2+ (127/248; 51.2%) and 3+ (4/248; 1.61%; Fig. 1D). For analysis against different parameters, low intensity

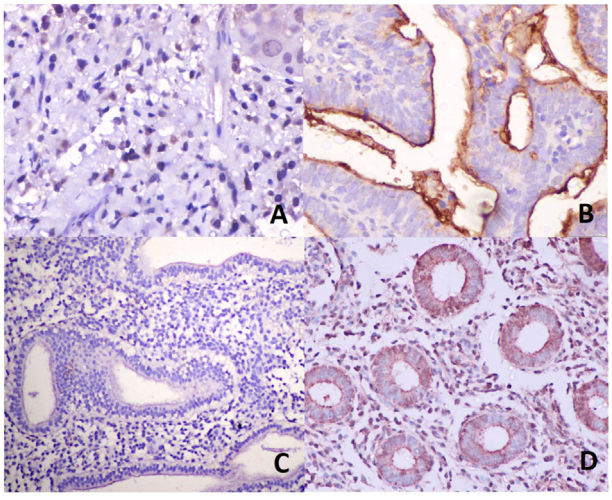

Fig. 1 – Endometrial aspirate tissue section stained using immunohistochemistry with Hematoxylin counterstain A: Interferon gamma showing many lymphocytes staining positive in the cytoplasm (3+). X400. B: MUC1 showing strong (3+) staining in the glandular epithelium. X400. C: Notch1 showing negative staining. X100. D: Notch1 showing strong (3+) staining in the glandular epithelium. X400.

(grades 0 and 1) and high intensity (Grades 2 and 3) were grouped together. PCR-TB negative endometrium showed a trend towards high intensity Notch1 expression (60.71%; p = 0.07; Table 1). There was no difference in low and high intensity Notch 1 with regard to CD138 (p = 0.82), IFN gamma 3+ (p = 0.7) and IL10 expression (p = 0.23).

3.5. *Pregnancy*

Conception occurred in 106/306 patients (35%). There was no difference in conception in the CD138 positive and negative cases (p = 0.95), between IFN gamma 3+ positive and negative cases (p = 0.72), IL10 (p = 0.32) and between MUC 1 low and high expression (p = 0.60). Conception was significantly related to Notch-1 expression since 66.66% of patients who conceived had high intensity Notch-1 staining compared to 44.93% of those who did not conceive (Table 2; p = 0.0009).

4. Discussion

The incidence of female genital tuberculosis (FGTB) in infertility varies from 3.2 to 40%.[11–14] Histopathology is characteristic and traditionally defined in terms of granulomatous inflammation.[15] Granulomas take at least one week to form and may take three weeks for full development. Only 6/306 cases (1.96%) in this highly selective group of latent FGTB showed granulomas, all of which were found in secretory endometrium. The present study demonstrates that EA is an essential procedure for evaluation of patients with infertility and it should be done only in the late secretory phase. Using conventional tests, the prevalence of FGTB was 8/306 (2.61%). This lower prevalence compared to other studies is likely to be consequent upon the exclusion criteria adopted.

CD138 was positive at 1+ level in 26% of normal controls. Although some authors give importance to even a single plasma cell being present, the American College of Pathologists prefers to use many plasma cells in typical setting of stromal spindling.[16,17] The presence of 3+ plasma cells would be the more appropriate finding to label a case as chronic endometritis. Since 3+ CD138 positive plasma cells are seen in 8% of normal endometrium, one should consider that chronic endometritis is a common condition in India as seen in other studies.[18] In the test subjects, chronic endometritis defined as 1+ positivity of CD138 was seen in 30.39% and at 3+ in 4.9%. This is not significantly different from the normal controls. It seems unrelated to the FGTB, being seen in PCR-TB positive and negative patients. It is possible that chronic endometritis has a role in contributing to the infertility in this selected group of infertility patients. Organisms other than Chlamydia and Gonorrhoea might be involved and is worth exploring in future studies.

Table 1 – Notch1 expression in PCR-TB positive and negative endometria.

Notch 1	PCR-TB + ve	PCR-TB -ve	Total	P-value
Low intensity	84 (51.21%)	33 (39.28%)	117	
High intensity	80 (48.78%)	51 (60.71%)	131	
Total	164	84	248	0.07

Table 2 – Relationship between conception and Notch1 expression in the endometrial sample.

Notch 1	Conceived	Not conceived	Total	p value
Low intensity	30	87	117	
High intensity	60	71	131	
Total	90	158	248	0.0009

PCR-TB in EA was positive in 62%. The detection rate of PCR for genital tuberculosis in the literature ranges from 48% to 60%.[9] PCR based tests suffer from false positivity. IFN gamma expression being higher in lymphocytes from endometria positive for PCR-TB suggests that true positives are being detected in PCR-TB test and that FGTB is causing subtle modifications in the milieu. Detection of IFN gamma positive lymphocytes in endometrium must not be confused with Interferon gamma release assay (IGRA) which is done in the blood using an in vitro assay.[19] The role of IFN gamma IHC in FGTB is not studied before though interferon gamma expression is related to endometrial receptivity in experimental animals using flow cytometric evaluation.[20] We found IFN gamma IHC stained lymphocytes surrounding the granuloma. Interpretation of low level Interferon gamma was difficult. It is better to select 3+ positivity as the better cut off for labelling a biopsy as having IFN gamma positive lymphocytes. Despite these limitations, IFN gamma IHC might be a potentially useful marker for studying endometrial responses in FGTB. Interleukin-10 was positive at 1+ level in 16% cases and at 3+ level in 1% cases and with difficult interpretation, this is not a good marker.

Pregnancy occurred in 35% of the enrolled patients. This is higher than in other studies.[21,22] FGTB might affect pregnancy inconstantly unless it is associated with full blown pulmonary or abdominal TB. Previous studies have mixed up two different situations and hence show more relationship to pregnancy failure than in this focussed study which excluded pulmonary and other types of active TB. Pregnancy was unrelated to the infiltrating lymphocytes and PCR findings. It was only significantly related to Notch1 expression with more pregnancies in those with high Notch expression. Notch 1 was a useful marker since high intensity staining with Notch-1 correlated with higher rates of pregnancy. 66.66% of conceived cases were high intensity for Notch 1 while 44.93% of non conceived patients had endometria which were high intensity for Notch1. A trend towards low intensity Notch1 staining in PCR positive cases (51.21%) versus 39.28% in PCR negative cases was seen, suggesting mild effects in modulation of pregnancy. High intensity Notch1 was unrelated to CD138, interferon gamma and IL10 staining.

Conception in subjects was not related to MUC-1 expression and hence it is not a very good marker to study potential for pregnancy. Implantation markers are very dynamic factors and might change from cycle to cycle. MUC-1 staining was not correlated with PCR-TB and is hence not a useful marker in the scenario of FGTB, although MUC-1 was correlating with 3+ positivity for IFN gamma. Cases with infiltration by high intensity interferon gamma expressing lymphocytes in the endometrium had low intensity MUC expression in 68% in comparison to cases without interferon gamma 3+ expressing lymphocytes (low intensity in 47%; p-value-0.04).

There has been no previous study focused on the immunohistochemical evaluation of epithelial implantation markers and their relationship to infiltrating immune cells in suspected cases of FGTB. The main strength of the study was that by excluding cases of pulmonary TB and other extrapulmonary TB that secondarily involve the endometrium, it was possible to focus on purely latent FGTB, the pathogenesis of which is poorly understood. Similarly, patients who had received ATT in the past five years were also excluded from the study. Most of the previous studies on FGTB have confounded the issue by not having such inclusion and exclusion criteria. The limitation of the study was only two implantation markers were investigated due to financial constraints. Despite the limitation, the present study lays strong foundation for future researchers to investigate the endometrium of infertile females with latent tuberculosis in more detail.

5. Conclusions

The immunohistochemical markers tested in this study cannot be used for diagnosis of FGTB either alone or in combination with PCR. However the findings suggest that FGTB and the latent bacilli are producing perturbations in a proportion of patients which might be affecting fertility. IFN gamma IHC is at best an investigational tool. Implantation markers need more precise timing of the biopsy and a wider panel of markers can be evaluated. Notch1 has emerged as a useful implantation marker from this study. The most important finding is that a good proportion of infertile patients conceived during investigation.

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Conflicts of interest

The authors have none to declare.

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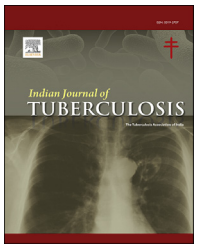

Original article

Spectrum of anti tubercular therapy induced cutaneous adverse drug reactions and its management through rechallenge: A prospective study at a Tertiary Care Centre

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ABSTRACT

Introduction: The use of multi-drug regimens including 1st and 2nd line anti tubercular drugs in management of tuberculosis (TB) has been associated with undesirable adverse drug reactions including cutaneous one. Re-challenge remains the only option to restart the safe therapy and combat the tuberculous infection simultaneously.

Materials and methodology: This cross-sectional study was conducted via prospective review of outpatients as well as indoor patients who presented with cutaneous adverse drug reactions to ATT between March 2020 and March 2021. Data were analysed regarding demographic profile, site of TB, ATT regimen, pattern of cutaneous lesions, offending drugs, past history of drug allergy, and reinstitution of ATT after re-challenge.

Results: Out of total 56 registered tubercular patients presented with cutaneous adverse drug reaction 30 were females (53.57%). The most common site of TB was pulmonary followed by cervical lymph node TB. The three most common adverse drug reaction detected were maculopapular rash 32 (57.1%) followed by lichenoid drug eruptions in 6 (10.7%) and urticaria in 2 (3.6%). Ethambutol was found to be common offending drug followed by other first line anti-tubercular drugs. 5 patients developed multiple drug hypersensitivity on re-challenging and have to introduce steroids along with ATT.

Conclusion: Adverse cutaneous drug reactions to ATT is like a double-edged sword as stopping ATT and starting treatment with systemic steroids can further flare up the infection with increased risk of disseminated and multidrug resistant tuberculosis. Re-challenge was found out to be safest way in identifying culprit drug and hence to restart a safer alternate ATT regimen for better management.

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1. Introduction

Tuberculosis (TB) is rightly compared to a nine headed dragon especially in developing countries like India, as The World Health Organization (WHO) estimates approximately 8.8 million new cases of tuberculosis (TB) and 1.6 million deaths from TB annually, among this 2.64 million cases were noted in India in 2019. TB is the ninth leading cause of death worldwide and effects for approximately 10 million people each year.[1,2]

The treatment of drug sensitive TB involves combinations of first line anti-TB drugs including isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA) while Drug resistant TB involves the use of second line anti-TB drugs like fluoroquinolones, second line injectable drugs and other newer drugs like Bedaquiline, Delamanid. Amongst all, pulmonary tuberculosis remains most common type of TB at time of presentation. Cutaneous adverse drug reactions (CADRs) are well known side effects of these drugs and can range from a mild pruritus to life threatening toxic epidermal necrolysis (TEN) which require discontinuation of the treatment, introduction of steroids and hence may complicate the tuberculosis management.[3]

Limitations in the use of *in vitro* and *in vivo* diagnostic tests in diagnosing the putative drug in TB drug allergy often necessitates the use of tailored Desensitization-Rechallenge (D-R) regimes to reintroduce appropriate TB treatment. Re-challenge- desensitization remains the only option for re-introducing ATT along with detailed history of drug allergy if already there. There are no clear guidelines regarding re-challenge and only limited studies in the literature.[4]

2. Materials and methodology

This was a prospective type of study conducted in the Department of Tuberculosis and Chest Diseases of our institution which included total 56 outdoor and indoor patients presented with cutaneous adverse drug reactions to Anti Tuberculous Treatment from March 2020 to March 2021. All the pertinent details were recorded including demographic profile, type and site of TB, initiation of Anti tubercular treatment, period of latency, previous drug allergies, medical history for risk factors, co-morbidities, and pattern of drug rash. All the routine investigations performed like complete hemogram, Liver Function Test, Renal Function Test, HIV, HbsAg, sputum AFB, Chest roentgenogram and CBNAAT (as and when required). Skin biopsy was done in few patients to rule out the differentials with other skin disorders and confirm the diagnosis. After finding the causative drug/drugs, patients were managed accordingly symptoms severity and were re-challenged with safe ATT as per the institution's protocol [Fig. 1] a modification of European Academy of Allergology and Clinical Immunology guidelines.[5]

3. Results

A total 56 patients with cutaneous adverse drug reactions to Anti Tuberculous Treatment presented to the institute were included in study over a period of 1 year. This study included 26 males and 30 females (male to female ratio 1:1.15) with age ranging from 19 to 75 years (mean age 35). Most common type of TB was Pulmonary TB seen in 36 (64.2%) patients and amongst extra-pulmonary TB, most common type was cervical tubercular lymphadenopathy in 8 (14.2%) followed by pleural effusion in 4 (7.1%), abdominal tuberculosis in 3 (5.4%), TB osteomyelitis, disseminated TB in 2 (3.5%)patients each, and tubercular meningitis in 1 (1.8%) patient. Majority of the patients, 48 (85.71%) were on category I, DOTS. The high-risk factor identified in our patients were elderly age 12 (21.42%), polypharmacy 18 (32.14%), pre-existing renal disease 7 (12.5%), diabetes mellitus 23 (41.07%), smoking 12 (21.42%) and alcohol intake 08 (14.28%). Out of 56, total 4 (7.1%) patients were found HIV positive but none of the patient was found to be having auto-immune disease which was ruled after all necessary investigations.

Latent period varied from 4 days to 136 days between the drug intake and onset of cutaneous rash (mean duration was 28 days). Amongst various clinical cutaneous patterns, maculopapular rash was the most common type of drug rash found in 32 (57.1%) followed by lichenoid drug eruptions in 6 (10.7%) and urticaria in 2 (3.6%). Severe CADR included DRESS in 11 (19.6%), Exfoliative dermatitis in 3 (5.4%) and AGEP (Acute Generalised Exanthematous Pustulosis) in 2 (3.5%) patients [Figs. 2 and 3].

ATT was withheld in all except six-patients who had lichenoid drug rash and had already completed intensive phase of treatment. These were managed with oral anti-histaminic, short course oral steroids along with continuation of their regular ATT. Other patients were initiated on oral steroids; clinical response appeared in 7–14 days with total duration of steroids therapy ranging from 10 to 110 days. 50 (89.28%) patients were then re-challenged with ATT as per protocol. The commonest implicated drug was found to be ethambutol (E) in 24 (42.85%) patients followed by pyrazinamide (Z) in 12 (21.42%), isoniazid (H) in 9 (16.07%), rifampicin (R) in 7 (12.5%) and levofloxacin in 2 (5%) patients [Fig. 4]. Eight (14.28%) patients developed drug rash to more than one ATT drug on re-challenging. 5 patients developed rash to 2 ATT drugs out of which 3 patients developed rash to HE and 1 patient each developed rash to RE, ZE. Two patients developed rash to 3 ATT drugs (HZE) and one patient developed rash to four drugs including three 1st line ATT drugs (except R) and levofloxacin.

4. Discussion

As defined by WHO, Adverse drug reaction is "a response to a drug that is noxious and unintended and occurs at doses normally used in human for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function."[6] The majority (75–80%) of them are caused by predictable, no immunologic pharmacodynamics of drugs. The rest 20–25% events are unpredictable, that may or may not be immune-mediated. Immune-mediated reactions account for 5–10% of all drug reactions.[7] The incidence of cutaneous adverse drug reactions (CADRs) reported in patients on antitubercular therapy is 5.7%.[8] Various type of

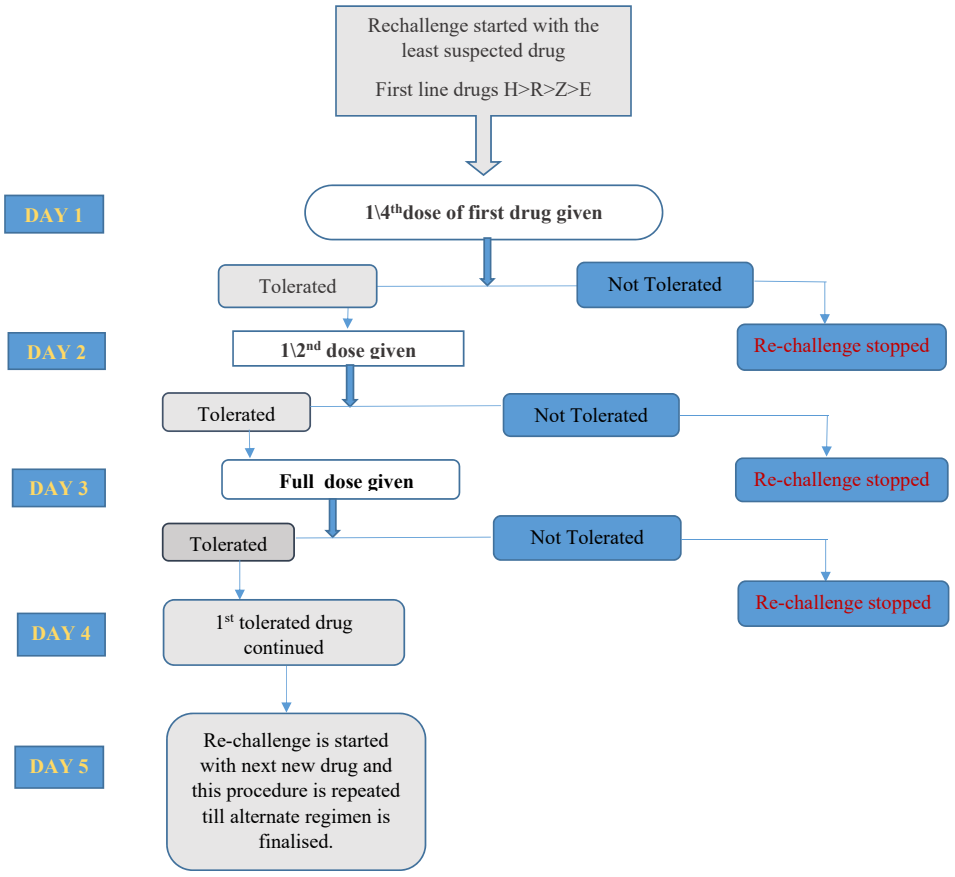

Fig. 1 – Flow chart showing Re-challenge protocol.

cutaneous rash reported in literature are urticarial drug rash, fixed drug eruptions, maculopapular rash, lichenoid drug rash, Acute Generalized Exanthematous Pustulosis (AGEP), Exfoliative dermatitis, drug reaction with eosinophilia and systemic symptoms (DRESS), Systemic Drug related Intertriginous and Flexural Exanthema (SDRIFE) and toxic epidermal necrolysis/Stevens Johnson Syndrome (TEN/SJS).

Various risk factors for development of CADR are genetic susceptibility, elder age group, female gender, diabetes, organ failure, poly-pharmacy, infections such as HIV, EBV, autoimmune diseases (rheumatoid arthritis, Sjogren's disease, SLE), malignancy especially haematological and probably fixed dose combinations of ATT.[9]

With increasing age, CADR is more prone to develop. This can be explained by a pharmacokinetics of the drugs. In our

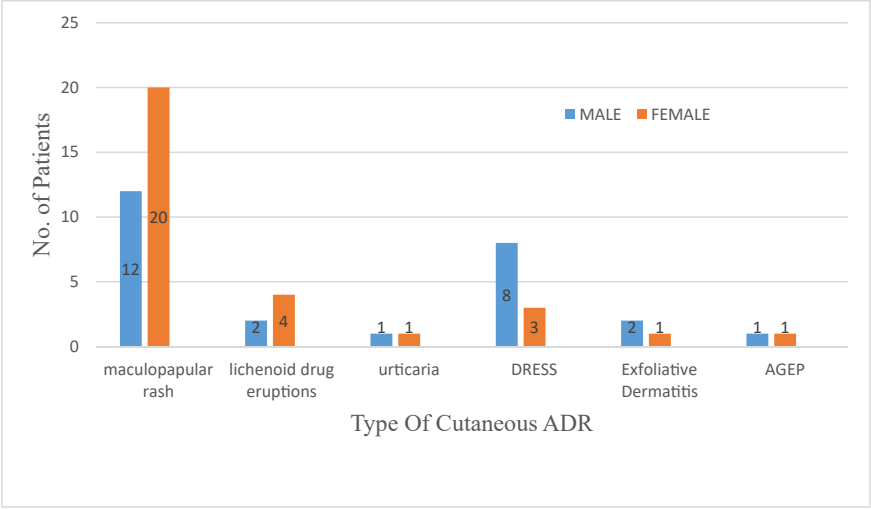

Fig. 2 – Demographic profile of patients with adverse drug reactions.

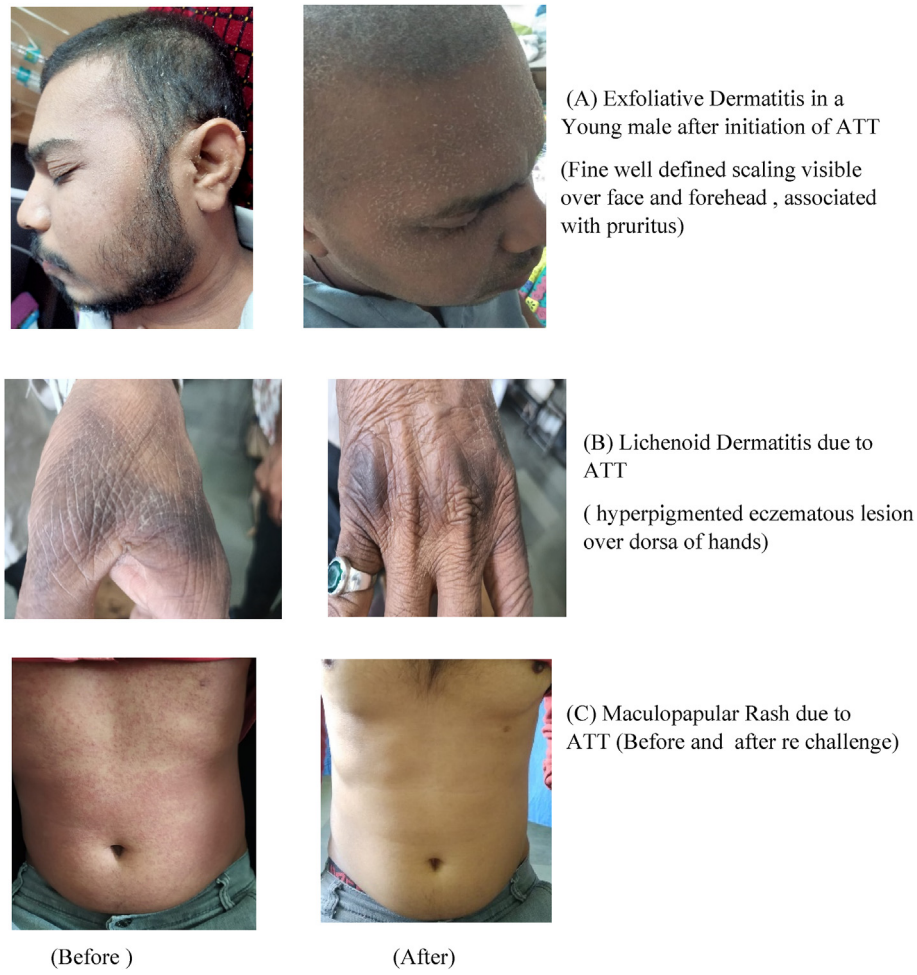

Fig. 3 – Clinical Photographs of Adverse Drug Reactions Due to AKT. (A) Exfoliative Dermatitis in a Young male after initiation of ATT (Fine well defined scaling visible over face and forehead, associated with pruritus). (B) Lichenoid Dermatitis due to ATT (hyperpigmented eczematous lesion over dorsa of hands) (C) Maculopapular Rash due to ATT (Before and after re challenge).

study 24 (42.9%) patients were in the age group of 55–75 years. The mean age in our study was 47.7 years which was comparable to the study by Sood A et al.[10]

Total 41.07% patients had diabetes mellitus and most of them presented with multiple drug hypersensitivity. Patients with diabetes are more prone to CADRs due to oxidative stress and polypharmacy.[11] A total of 12.5% patients had deranged renal functions test. Chronic renal failure affects both renally excreted drugs and also drugs metabolized by the liver through the effect of uraemia caused by renal failure.[8] History of alcohol consumption was present in 14.28% patients. Chronic alcoholism activates enzymes which convert some drugs into toxic metabolites which damage liver and affect metabolism of drugs.[8] Smoking affects the metabolic process by acting as liver enzyme inducer of hepatic cytochrome P-450.[8] A total 21.42% percent of our patients were smokers.

FDC was started in India in 2016, our study reported a slightly increased rate of drug reaction after this, however this may be due to increased rate of TB detection due to better National Programme implementation, adherence to treatment due to better tracking system, early detection of CADR or probably due to increased dosage of drugs given in daily than thrice weekly regimen.

Latent period between intake of drug and onset of rash varied between 4 days andto 136 days however the mean duration was 28 days. The onset of urticarial rash was seen within days to weeks while lichenoid rash was seen after months of taking ATT. But majority of the patients developed rash within 2 months of treatment that is before the completion of intensive phase of ATT. Our results are in agreement with other studies who have documented this aspect.[12,13]

The most common type of rash seen with ATT was maculopapular rash followed in frequency by lichenoid, urticarial, DRESS, Exfoliative dermatitis and AGEP. Our results were comparable to other studies by Thong et al.[14] [Table 1].

The patients in our study were managed with oral steroids till the rash and systemic symptoms subsided. Stopping ATT and treatment with steroids increases the risk of disseminated disease and multidrug resistant tuberculosis. Therefore, we believe that re-challenge should be initiated as early as possible considering the relative safety of re-challenging.

Table 1 – Comparison of our study data with other studies.

	Thong et al. (2014)	Tan WC et al (2007)	Lehloenya et al. (2011)	Our Study
Maculopapular rashes	8 (72%)	34 (72.3%)	2	32 (57.1%)
Urticaria	1	4 (8.5%)	–	2 (3.2%)
AGEP	–	–	–	2 (3.5%)
Erythema multiforme	–	2 (4.2%)	–	–
SJS/TEN	–	–	13/17 (20/26%)	–
DRESS	2	–	25 ()	11 (19.6%)
Erythroderma	–	1	–	–
Lichenoid rash	–	1	3	6 (10.7%)
Others	–	–	–	3
Total	11	42	60	56

There are no specific re-challenge guidelines, we re-challenged with each ATT drug as per our institution's protocol till culprit drug was found and final regimen established.

Re-challenge is defined as a controlled administration of a drug in order to diagnose drug hypersensitivity reactions. Tuberculosis outcomes are better if re-challenge is undertaken and only the offending drug is removed from the treatment regimen. Re-challenge is of utmost importance because of increased burden of TB in India/World, limited number of first line ATT drugs, increased toxicity of second line drugs and keeping second line drugs reserve for resistant cases. It helps in avoiding treatment interruption due to ADR thereby decreasing morbidity, mortality and transmission rate. Interruption of therapy during the intensive phase is associated with a three times higher risk of death. The sequence of re-challenge is still a matter of debate whether most effective drugs, rifampicin, and isoniazid should be re-challenged first or the drugs least likely to cause a reaction. However, all first line drugs cause CADR and no good studies quantify the contribution of each drug. So, it is suggested to re-challenge with rifampicin and isoniazid to decrease the chances of resistance as use of isoniazid and rifampicin in the treatment regimen of tuberculosis is associated with superior outcome. More than 90% of re-challenge reactions occur within 72 hours. So, re-challenging with a new first line drug every 96 hours is recommended, while monitoring closely for features of a re-challenge reaction.

Among the ATT drugs, Tan et al. reported pyrazinamide as the commonest drug causing CADR (38%); however, most common drug implicated in our study was ethambutol (45%) as depicted in [Fig. 4].

Ten (25%) patients in our study showed multiple drug sensitivity, 1 of them to 3 drugs and one to 4 ATT drugs. Lehloenya et al. reported multiple drug hypersensitivity (MDH) in 12.5% cases co-infected with HIV.

Multiple drug hypersensitivity is a predilection to react to different chemically and structurally unrelated drugs which are metabolized by different pathways with no evidence of cross reactivity. Specific types of drugs, higher concentration, fixed dose combination and long lasting treatment predisposes to MDH. MDH develops as a consequence of massive T cell activation due to long lasting hypersensitivity to different drugs. Reactions may be simultaneous, sequential, or distant (after long interval). Fixed dose combination and

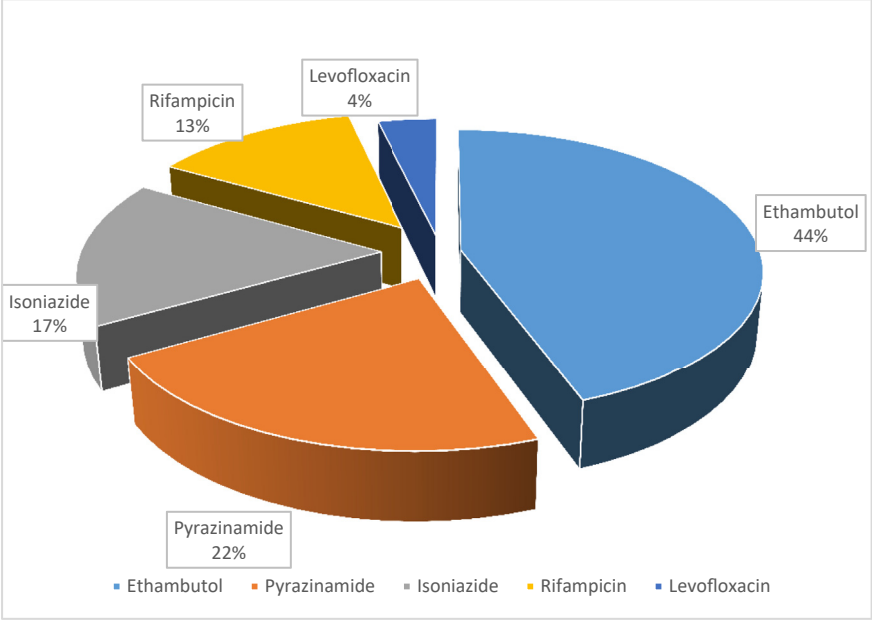

Fig. 4 – Individual drugs causing Cutaneous ADRS in OUR Study.

higher doses of INH, pyrazinamide, ethambutol, and streptomycin can lead to MDH with ATT.

5. Conclusion

Adverse drug reaction to anti-tubercular drugs is like a double-edged sword as complete stoppage of ATT and simultaneous treatment of CADR with systemic steroid further flares up the infection and increases the risk of developing multidrug resistant tuberculosis, a biggest threat to community. Re-challenge is a ray of hope as it decreases the risk of ATT interruption/default. The offending drug can be found out by re-challenge and safer ATT regimen can be implemented. Any type of cutaneous drug reaction may develop from any of the first line drugs. Hence, the patient should be counselled regarding ADRs before initiation of ATT and physician should have high suspicion of ADR/CADR for early detection and reinstitution of alternate regime. However, multidrug hypersensitivity is another challenge in reinstitution of safe ATT regime.

Conflicts of interest

The authors have none to declare.

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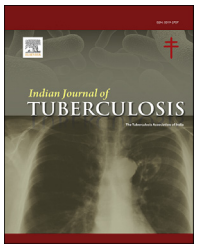

Original article

Accuracy of Timika X-ray scoring system to predict the treatment outcomes among tuberculosis patients in India

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ABSTRACT

Background: Timika scoring system is a radiographic grading tool, widely employed for grading the severity of tuberculosis (TB). We evaluated the predictive accuracy of this tool for adverse treatment outcomes among TB patients in Indian setting.
Methods: We undertook a longitudinal analysis of cohort data under the RePORT-India consortium. Cohort having participants with active pulmonary TB were included. CXRs were independently scored by chest physicians. Timika scoring system had a total score of 140. The predictive nature of the tool was assessed using the ROC analysis.
Results: Around 364 laboratory confirmed TB patients were enrolled. The mean (SD) of overall Timika score was 62.3 (24.9). Sputum conversion was achieved among 218/260 (83.8%) patients available at end of intensive phase. AUC for Timika score was 0.53 (95% CI: 0.43–0.63) and for percent lung affected, was 0.56 (95% CI: 0.46–0.65). Unfavorable treatment outcome was observed among 67/287 (23.3%) at the end of continuation phase. AUC for percent lung affected was 0.62 (95% CI: 0.54–0.70) and for Timika score was 0.59 (95% CI: 0.51–0.67).
Conclusion: Both Timika scoring system and percent lung affected had poor predictive accuracy, highlighting the inability of a single CXR scoring system to predict the treatment outcome in Indian setting.

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1. Introduction

Globally, tuberculosis (TB) still remains one among the top ten causes of mortality. TB has caused an estimated 10 million incident TB cases and an estimated 1.45 million deaths in the year 2018.[1] India accounts for one-fourth of this global incidence and mortality.[1] However, India has made tremendous progress over the past few decades in reducing TB incidence and mortality due to the advent of latest diagnostic and management modalities. India has also effectively decentralized highly sensitive molecular assays for resistance testing and increased access to advanced treatment regimens at peripheral level facilities.[2] India has a well-established and standardized TB programme ensuring planning, implementation and evaluation of TB prevention and treatment services. In spite of this progress, various factors influence the response and outcomes to TB treatment.[2] Early identification of the disease, severity and prediction of treatment response or outcome can aid us in providing adequate care to this target group thereby improving treatment success.

Chest radiographs (CXR) is used for evaluating suspected TB patients regarding diagnosis, assessing the severity of condition and predicting treatment response. Previous studies have shown that CXR findings such as cavitation, bilateral involvement of lungs indicate severe forms of disease leading to poor prognosis.[3–6] There is no single validated, standardized or reproducible scale encompassing CXR findings. The "Timika scoring system" has been proposed by Ralph et al for grading the disease severity.[7] It was developed as a pragmatic tool that can be used in clinics and field or sentinel sites. It consists of two components, i.e. cavitation and percentage of lungs affected in both sides due to any lesions related to TB.[7] However, studies around the world have shown wide variations in the validity and reliability of this scoring system for predicting treatment outcomes.[8–10] Hence, there is a need to evaluate this system in the Indian setting, to determine its generalizability as a predictor of treatment outcome as we have done in this study.

2. Methods

2.1. Study setting and study population

This longitudinal analysis of data was a part of large-scale ongoing cohort study under Regional Prospective Observational Research for Tuberculosis (RePORT)-International.[11] RePORT International embodies a consortium of regional cohorts such as RePORT India, RePORT China, RePORT Brazil, RePORT Philippines, RePORT South Africa and RePORT Indonesia.[11] Data and specimen collection of all these cohorts are linked through implementation of a common protocol. Under RePORT India consortium, five teams are operating in data and specimen collection. Among these, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) in collaboration with the Boston Medical Center and New Jersey Medical University - Rutgers University has developed two prospective observational cohorts: one with the participants having active pulmonary TB and second with the household contacts of an active pulmonary TB. Cohort having participants with active pulmonary TB were included for this study.

This study encompassed TB patients from 3 districts in South India, Puducherry from the Union territory of Puducherry (population ~1.3 million), and two adjacent districts from Tamil Nadu: Villupuram (population ~3.5 million) and Cuddalore (population ~2.6 million). Enrollment started in May 2014 in Puducherry, August 2014 in Cuddalore and November 2015 in Villupuram. The details of the study protocol have been reported previously.[12–15]

One Tuberculosis unit (TU) in Puducherry and two TUs each in Villupuram and Cuddalore were selected for recruitment of study participants. Service delivery at the sub district level is operational through the Designated microscopy centres (DMCs) and the Peripheral Health Institutions (PHIs) with TUs as the nodal point. Under the program, diagnosis and treatment are provided free of cost. Persons diagnosed with TB at the DMC's were referred to their nearby Primary Health Centre (PHC) for initiation of treatment after screening for comorbidities like diabetes mellitus, HIV etc. The sociodemographic details, clinical course, medication adherence and comorbidity profile are maintained at the PHC and followed up using treatment cards.

We enrolled only newly diagnosed sputum smear positive TB patients (at least 1+ acid fast bacilli [AFB]) $\geq$ 6 years that were diagnosed at the National Tuberculosis Elimination Programme (NTEP) DMCs and peripheral health centers from the selected districts. We excluded patients with prior history of TB disease or treatment, multi-drug resistant TB and patients on TB treatment for $\geq$1 week.

2.2. Ethics and consent

Written consent or assent in addition to parents' consent (in case of participant <18 years) was obtained from all participants enrolled in the study. The study protocol was approved by the Institute Ethics Committee and Scientific Advisory Committee of JIPMER, and the Institutional Review Boards at Boston University Medical Campus and Rutgers-New Jersey Medical School.

2.3. Study procedure

At the time of enrollment, all of the people living with TB (PLWTB) were assessed with a sputum smear and liquid culture and basic demographic details were obtained. CXR was performed for all the participants enrolled from January 2015. Each CXR was independently scored with a standardized form by two well-trained CXR readers (chest physicians). The form consisted of the following sections: the first section contained the details on quality of the three views (antero-posterior, postero-anterior and lateral) and described it as follows: acceptable, poor but readable, not readable/acceptable. The second section contained the details of evaluation of abnormal CXRs; CXRs were checked for the presence or absence of cavitation and opacity in the upper (apex to anterior end of second rib), mid (2nd to 4th rib), and lower zone (anterior end of 4th rib to diaphragm) independently. Pleural

effusion, mediastinal adenopathy, bronchiectasis, collapsed lung and hilar adenopathy were also evaluated and interpreted as absent or present. Abnormal CXRs based on the above-mentioned findings were interpreted based on a two-component scoring measure called as Timika scoring system.[7]

For the first component of scoring system, CXR was stratified into six zones (upper, middle and lower zone in right and left lungs) of almost similar size with two horizontal lines. In each of the zone, the percentage of area showing active disease and the involvement was estimated by visually estimating the extent of opacification. The percentage area affected in all the six zones were added together and divided by 600 to determine the total percentage of lung affected (0–100 points). Second component is presence of any cavitation (given a fixed score of 40 points if cavitation is present). The sum of these two scores was used for the final interpretation of severity of TB (0–140 points).[7]

After the baseline demographic and radiological assessment, we explain the participants how to produce the sputum and give time for half an hour. We recorded the sputum collection as not done, if they fail to produce sample. Sputum smear examination was done at the end of intensive phase (2 months after treatment initiation) to evaluate for sputum conversion (positive to negative) and at the end of the continuation phase (6 months after treatment initiation) to check for adverse treatment outcomes such as bacteriological failure (assessed by performing both smear and culture), clinical treatment failure, lost to follow up, death). The accuracy of Timika scoring system in predicting the above-mentioned adverse treatment outcomes was assessed.

2.4. *Study definitions*

2.4.1. *TB diagnosis*
Diagnosis of active TB was made using sputum culture - positive for MTB either by solid or liquid culture.

2.4.2. *Timika scoring system*
Score ranging from 0 to 140 – higher the score, severe the condition of patient.[7]

2.4.3. *TB treatment outcomes*
At the end of intensive phase, sputum smear examination was done to check the sputum conversion rate. At the end of continuation phase (six months after treatment initiation), final treatment outcomes were assessed. Patients who have completed treatment and/or cured (bacteriological/clinical) were considered to have favorable outcome and patients who have lost to follow-up/treatment not complete/bacteriological or clinical failure/dead were considered to have unfavorable outcome.

2.4.4. *Bacteriological cure*
Participant has completed the first-line, standard multidrug therapy and has a documented negative culture results at the last month of treatment and at least one previous occasion.

2.4.5. *Treatment completed*
Participant has received at least 90% of the number of doses recommended under the RNTCP standard multidrug therapy.

2.4.6. *Lost to follow-up*
Participant no longer participates in the study visits follow-up or the outcome status cannot be determined.

2.4.7. *Treatment not completed*
Participants who drop out or interrupt the treatment for two or more consecutive months, but continues to be in the study follow-up.

2.4.8. *Bacteriological failure*
Participant with sputum culture positive at five months or later during the treatment and the culture was not determined to be false positive.

2.4.9. *Clinical failure*
Participant who completes at least four full months of TB treatment but has persistent illness, progression, recurrence of symptoms or signs of TB that are found to be because of TB and not because of any underlying causes.

2.4.10. *Death*
Participant died of any reason after enrolled into the study and before the completion of study period.

2.5. *Statistical analysis*

Filled questionnaires were scanned and transferred to Boston Medical Center using the Verity TeleForm Information Capture System software V10.8 (Sunnyvale, CA, USA), and it was read into a Microsoft Access (Seattle, WA, USA) database. Errors in the data entry process were reviewed and duly corrected by the on-site team in India. Data were extracted from the RePORT India consortium project database for the JIPMER site and analyzed using Stata v.14.2 software. Continuous variables such as age was summarized as mean and standard deviation (SD). Categorical variables were summarized as proportions. Accuracy of the Timika scoring system in predicting the adverse TB treatment outcomes was assessed using the sensitivity and specificity. Outcome data at end of intensive and continuation phase (sputum conversion and favorable/unfavorable outcome at end of follow-up) was taken as gold standard. We performed a non-parametric estimation of Receiver Operator Characteristic (ROC) curve to obtain the optimal cut-off at which the Timika score accurately predicts the adverse treatment outcomes. Area under the curve (AUC) for Timika score and percentage of lungs affected were compared to see which has better predictive accuracy. P value less than 0.05 indicates there was statistically significant difference between the two-scoring system.

3. Results

In total, 364 laboratory confirmed TB patients were assessed for radiological abnormalities at the baseline. However, data was available for only 260 patients (28.5% missing data) at the end of intensive phase and 287 patients (21.1% missing data) at the end of continuation phase as they were not able to produce the sputum during the time of data collection. Since this missing data might introduce a bias into the study, we

compared the baseline characteristics that can have influence on the outcome between the patients with and without missing data (Table 1). Baseline characteristics such as age, gender and baseline weight were almost similar between these groups at the end of intensive and continuation phase, while behavioral habits such as smoking and alcohol use were slightly different between the two groups at the end of intensive phase only.

The mean (SD) age of the participants was 43.4 (13.9) years. Among the radiological abnormalities, cavitation was present in 81.9% of the study participants. The most common location of cavitation was right upper lobe (35.6%) followed by the right middle lobe (33.2%). Opacification (shadows other than cavitation) was found in 93.7% of the patients, most commonly in the right lower lobe (54.5%). Other radiological abnormalities found were bronchiectasis (9.6% in right lung and 9.4% in left lung), hilar adenopathy (6.4% in right lung and 3.9% in left lung), pleural effusion (3.6% in right lung and 3.3% in left lung) and mediastinal adenopathy (3.6% in both right and left lung). Collapsed lung was found in only 5 study participants (1.4%) and all of them were in the right lung.

Based on the above-mentioned radiological abnormalities, the mean (SD) percentage of lung affected was 29.5 (16.8). Summing the scores obtained from presence of cavitation and percentage of lung affected, the mean (SD) of overall Timika score was 62.3 (24.9).

All these patients were followed up and assessed at the end of intensive phase (for sputum conversion) and continuation phase (for adverse treatment outcomes). Sputum conversion data at end of intensive phase is available for only 260 patients. Sputum conversion was achieved among 218 (83.8%) of these patients. ROC was performed to find the predictive accuracy of Timika score and percentage of lung affected for sputum conversion at two months (Figs. 1 and 2). AUC for Timika score was 0.53 (95% CI: 0.43–0.63) and for percentage of lung affected, it was 0.56 (95% CI: 0.46–0.65). Since both the ROCs crosses the diagonal line which represents the perfect chance, both the scoring system has no better odds of predicting the sputum conversion at end of intensive phase than a random chance.

Adverse treatment outcomes at the end of continuation phase is available for 287 patients. Out of these, 67 (23.3%) had

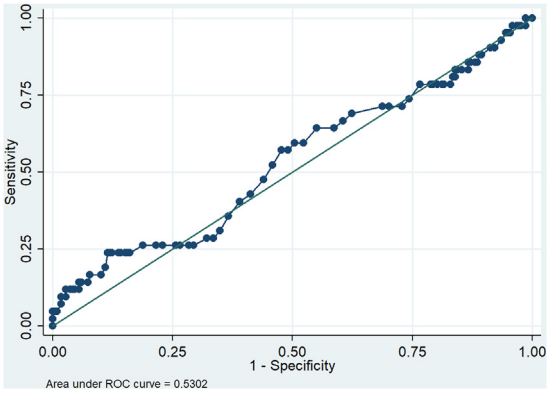

Fig. 1 – Receiver-operator characteristic curve for sputum smear conversion at the end of intensive phase for a range of possible Timika score cut-off points.

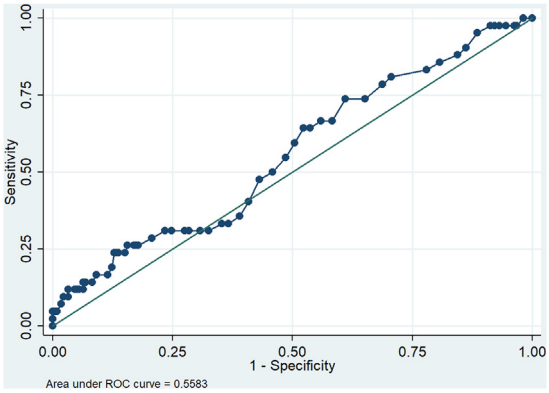

Fig. 2 – Receiver-operator characteristic curve for sputum smear conversion at the end of intensive phase for a range of possible percentage of lung affected cut-off points.

unfavorable treatment outcome at end of follow-up period. ROC was performed to find the predictive accuracy of percentage of lung affected and Timika score for unfavorable outcomes at end of follow-up (Fig. 3). AUC for percentage of lung affected was 0.62 (95% CI: 0.54–0.70) and for Timika score, it was 0.59 (95% CI: 0.51–0.67). Comparison of both the

Table 1 – Baseline characteristics of the study participants (N = 364).

Baseline characteristics	Participants without missing data at end of intensive phase (N = 260) n (%)	Participants without missing data at end of continuation phase (N = 287) n (%)	Participants with missing data (N = 104) n (%)	Participants with missing data at end of continuation phase (N = 77) n (%)
Age (Mean ± SD) in years	42.4 (13.6)	43.4 (13.9)	44.9 (13.1)	42.1 (12.1)
Weight (Mean ± SD) in kg	49.7 (11.1)	49.3 (10.3)	49.8 (11.0)	51.5 (13.4)
Gender				
Male	195 (74.3)	225 (78.4)	87 (84.5)	57 (76)
Females	64 (24.7)	62 (21.6)	16 (15.5)	18 (24)
Smoking				
Yes	70 (27.0)	89 (31.0)	38 (36.9)	19 (25.3)
No	189 (73.0)	198 (69.0)	65 (63.1)	56 (74.7)
Alcohol use				
Yes	141 (54.5)	170 (59.2)	69 (67.0)	40 (53.3)
No	118 (45.6)	117 (40.8)	34 (33.0)	35 (46.7)

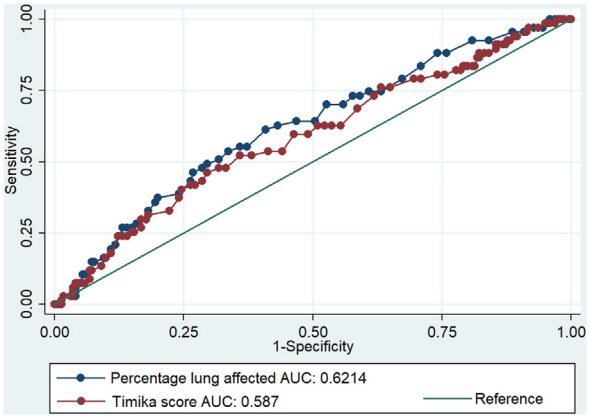

Fig. 3 – Comparison of Receiver-operator characteristic curve for unfavorable outcomes at the end of continuation phase for a range of possible Timika score and percentage of lung affected cut-off points.

ROCs did not show statistically significant difference indicating that one scoring system is not significantly better than the other ($p = 0.16$). The optimal cut-off for percentage of lung affected for predicting adverse treatment outcomes was 30 with sensitivity 61.8% and specificity 59.1% and for Timika score, the optimal cut-off was 70 with sensitivity 52.2% and specificity 64.1%.

4. Discussion

This study was performed among the newly diagnosed TB patients in Puducherry and Tamil Nadu recruited under JIPMER RePORT cohort. We applied the Timika scoring system and sub-component of this scoring system on these subjects to assess the accuracy in predicting the adverse TB treatment outcomes at end of intensive and continuation phase.

Both Timika scoring system (AUC = 0.53; 95% CI: 0.43–0.63) and percent lung affected (0.56; 95% CI: 0.46–0.65) had poor predictive accuracy for sputum conversion at two months. The accuracy obtained in our study is far less than the previous studies assessing the accuracy of CXR findings for prediction of sputum conversion at two months. Study conducted in Indonesia[7] showed the highest accuracy (AUC = 0.74 for Timika score and AUC = 0.68 for percent lung affected), followed by Uganda[8] (AUC = 0.72 for Timika score and South Africa[10] (AUC = 0.68 for Timika score), all of which are statistically significant and higher than the current study findings. This suggest that application of Timika scoring system may not be generalizable to the TB population for prediction of failure in sputum conversion (end of intensive phase) in an Indian setting.

For predicting the adverse treatment outcomes at end of treatment, scores obtained by percent lung affected (AUC = 0.62; 95% CI: 0.54–0.70) has better accuracy compared to scores obtained by Timika scoring system (AUC = 0.59; 95% CI: 0.51–0.67). Though the ROC does not cross the line of chance, both the scoring systems are still not accurate enough to predict the adverse treatment outcomes. The predictive accuracy of Timika scoring system for final adverse treatment outcome was almost similar to the previous study conducted in South Africa (AUC = 0.61).[10] However, the optimal cut-off for prediction in the previous study was 63.5, while in the current study, it was 70. This finding suggests that though the Timika scoring system showed statistically significant predictive accuracy with final TB treatment outcomes across different studies, predicting based on a single optimal cut-off value did not provide consistent or reliable results across the different study populations.

This study has certain strengths. This study is one of the largest to assess the predictive accuracy of adverse TB treatment outcomes using a standardized CXR scoring system. Use of both 2-month and 6-month treatment outcome to assess the accuracy is an added advantage to the study. Well-trained CXR readers, close monitoring of treatment with adequate adherence; sputum smear performed in a quality-controlled laboratory adds to the strengths of the study.

This study has certain limitations. The TB patients in our study were selected from three districts in South India, and hence the generalizability of the results may be limited. Another limitation of the study could be the inclusion of smear positive patients only. It might also be responsible for poorer performance of Timika scoring system in our study. We did not perform the CXR scoring measure at the end of intensive and continuation phase. This would have helped in identifying the change in the CXR score with respect to the progress of disease. None of our patients were HIV-infected. Immunocompromised HIV patients have atypical presentations and also reduced rate of cavitation. This can influence the predictive accuracy of the scoring system. The lesser number of patients with unfavorable or adverse treatment outcomes and 20–30% patients that didn't have their outcomes (either at end of intensive or end of continuation phase) assessed in our study limiting the power to explore the correlation between the scoring system and TB treatment outcomes.

Timika scoring system, a simple and standardized CXR scoring system including two components i.e. the absence or presence of cavitation and extent of overall radiological involvement in both sides of lungs can help in early identification of severe cases and provide appropriate care. However, we found that the CXR scoring system alone cannot accurately predict the sputum conversion at two months or final treatment outcome at six months. However, it can still be a useful tool in a prediction model consisting of multiple clinical, laboratory and radiological parameters. Hence, there is a need to do a large-scale study on evaluation of prediction model with multiple parameters for accurately identifying the high-risk patients thereby improving treatment outcomes. Though the current drugs and programme are effective in curing tb independent of severity of disease at baseline, this prediction model in turn will further help in reducing the resistance and mortality among pulmonary TB patients.

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Conflicts of interest

The authors have none to declare.

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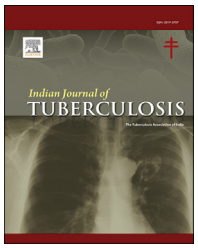

Original article

A peptide-based vaccine ACP derived from antigens of *Mycobacterium tuberculosis* induced Th1 response but failed to enhance the protective efficacy of BCG in mice

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ABSTRACT

Background: Tuberculosis (TB) is a global infectious disease, but there is no ideal vaccine against TB except the Bacille Calmette-Guérin (BCG) vaccine.

Methods: Herein, 25 candidate peptides were predicted from four antigens of *Mycobacterium tuberculosis* based on their high-affinity binding capacity for the human leukocyte antigen (HLA) DRB1*0101. Three T-helper 1 (Th1) immunodominant peptides ($Ag85B_{12\text{-}26}$, $CFP21_{12\text{-}26}$, and $PPE18_{149\text{-}163}$) were identified by ELISPOT assays in the humanized C57BL/6 mice. They resulted in a novel Th1 peptide-based vaccine ACP named by the first letter of the three peptides. In addition, the protective efficacy was evaluated in humanized or wild-type C57BL/6 mice and the humoral and cellular immune responses were confirmed in *vitro*.

Results: Compared with the PBS group, the ACP vaccinated mice showed slight decreases in colony-forming units (CFUs) and pathological lesions. However, when using it as a booster, the ACP vaccine did not significantly enhance the protective efficacy of BCG in humanized or wild-type mice. Interestingly, we found that ACP vaccination significantly increased the number of interferon-γ positive (IFN-γ^+) T lymphocytes and the levels of IFN-γ cytokines as well as antibodies. Furthermore, the IL-2 level was significantly higher in humanized mice prime-boosted with BCG and ACP.

Conclusions: Our results suggested that ACP vaccination could stimulate higher levels of cytokines and antibodies but failed to improve the protective efficacy of BCG in mice, indicating that the secretion level of IFN-γ may not be positively correlated with the

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protection efficiency of the vaccine. These findings provided important information on the feasibility of a peptide vaccine as a booster for enhancing the protective efficacy of BCG.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* complex (MTBC) and has been the leading cause of death from a single infectious agent. According to the latest global tuberculosis report released by the World Health Organization (WHO), there were more than 10.0 million new cases of TB and 1.45 million TB deaths in 2018.[1] Geographically, most new cases of TB came from South-East Asia, Africa, and the Western Pacific. Two-thirds of TB cases worldwide were accounted by the following eight countries, including India (27%), China (9%), Indonesia (8%), Pakistan (6%), Philippines (6%), Bangladesh (4%), Nigeria (4%) and South Africa (3%).[1] It is worrisome that these countries are all developing countries. The local economic level and medical and health conditions are far behind those of developed countries, making the prevention and treatment of tuberculosis very difficult.

As the only licensed and widely used TB vaccine, Bacille Calmette-Guérin (BCG) has been recommended as part of national childhood immunization programs among 153 countries, including above high TB burden countries. Vaccination of children with the BCG is a major category of health care interventions for TB prevention. A growing number of studies have indicated that BCG reduced the mortality of extrapulmonary TB in infants, but it failed to protect against pulmonary TB in adults.[2] Furthermore, BCG has variable efficacy (0%–80%) against pulmonary TB, and the possible reasons have been discussed in our previous study.[3] Among these reasons, the most important one is that the protective efficacy of BCG only maintains for 10–15 years, which is why BCG vaccination in childhood loses its protective effect on adulthood. Therefore, it is urgent to change the immune strategy to improve the efficiency of BCG protection. Recently, two major immunization strategies have been used to enhance the efficacy of BCG or make up for its deficiencies, including the development of recombinant live mycobacterial vaccines and the utilization of subunit vaccines containing *M. tuberculosis* antigens to enhance BCG vaccine.[4]

Previous studies have shown that BCG vaccination could produce an observable T-helper 1 (Th1) -type cellular immune response characterized by the secretion of a significantly high level of Th1 cytokines.[5–7] In addition, class II major histocompatibility complex (MHC II) plays an essential role in bridging the presentation of peptides and the activation of adaptive immune responses.[5,8] Therefore, screening immunodominant epitopes to construct a peptide-based vaccine that can induce Th1 immune response is a new way to enhance the immune protection efficiency of the BCG vaccine.

Herein, the candidate epitopes were predicted by bioinformatics technology, the Th1-immunodominant peptides were identified with an enzyme-linked immunospot (ELISPOT) array. They resulted in a novel peptide-based vaccine ACP named by the first letter of the three peptides $Ag85B_{12\text{-}26}$, $CFP21_{12\text{-}26}$, and $PPE18_{149\text{-}163}$. The efficacy of the new vaccine was evaluated in humanized or wild-type C57BL/6 mice, and its potential immune mechanism was explored in splenocytes *in vitro*.

2. Materials and methods

2.1. *Mice and ethics*

The wild-type C57BL/6 mice (7–8 weeks of age, female) were purchased from Vital River Laboratories (Beijing, China). The female HLA A11/DR1 humanized mice (HLA-A11$^{+/+}$DR1$^{+/+}$H-2-β2m$^{-/-}$/IAβ$^{-/-}$ [9]) at 6–8 weeks of age were presented as a gift from the Beijing Institute of Microbiology and Epidemiology (Beijing, China). This study was carried out under the principles of the Experimental Animal Regulation Ordinances of the China National Science and Technology Commission. In addition, the protocol was approved by the Animal Ethical Committee of the 8th Medical Center of PLA General Hospital.

2.2. Mycobacterium tuberculosis *strain*

The culture and purification of the *M. tuberculosis* H37Rv strain were performed following our previous studies.[10,11]

2.3. *Prediction and synthesis of human leukocyte antigen DRB1*0101 epitopes*

The candidate epitopes (15-mer) binding affinities to the HLA-DRB1*0101 (DR1) allele were predicted using the IEDB as previously described.[12] For each epitope, a percentile rank for each of the three methods (combinatorial library, SMM_align, and Sturniolo) was generated by comparing the epitope's score against five million random 15 mers selected from the IEDB database. The smaller the number of percentile ranks, the higher the affinity. The median percentile rank of the three methods was then used to generate the rank for the consensus method. The top ten epitopes of Ag85A and the top five epitopes of Ag85B, CFP21, and PPE18 were synthesized (90% purity) by SBS Genetech Co. LTD (Beijing, China). These synthesized peptides were lyophilized to store at −80 °C.

2.4. *ELISPOT for screening immunodominant peptides*

The candidate peptides were screened following our previous study.[13] Briefly, twenty humanized mice were randomly divided into four independent experiments (5 mice per test). Examination 1: each mouse was immunized with 100 μl

complete Freund's adjuvant (CFA) and 100 μl inactivated *M. tuberculosis* (5×10^6 CFUs); Examination 2: each mouse was inoculated with 100 μl inactivated *M. tuberculosis* (5×10^6 CFUs); Examination 3: each mouse was inoculated with 100 μl CFA and 100 μl lysate of *M. tuberculosis* (5 mg/ml); Examination: each mouse was inoculated with 100 μl incomplete Freund's adjuvant (IFA) and 100 μl lysate of *M. tuberculosis* (5 mg/ml). The mice were killed on the 15th day after immunization, and their spleens were collected to isolate splenocytes. The red blood cells were lysed using a Red Blood Cell Lysis Buffer (Solarbio, Beijing, China). The isolated splenocytes (3×10^5) in 100 μl of Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco, Shanghai, China) were added into 96-well ELISPOT plate pre-coated with IFN-γ (Mabtech AB, Nacka Strand, Sweden). Splenocytes in each well were incubated with 10 μl of each peptide (2 μg) at 37 °C for 20 h. Each peptide in Examinations 1 and 2 was repeated twice, and each peptide in Examinations 3 and 4 was repeated three times. Then, the number of spots forming cells (SFCs) was counted by a CTL-S5 Versa ELISPOT Reader (CTL, Cleveland, OH, USA). The stimulation index (SI) value of peptide greater than two will be considered as an immunodominant peptide according to our previous study,[13] and the SI value was obtained by the ratio of SFCs in cells stimulated with peptide and that in cells stimulated with RPMI 1640 medium.

2.5. Preparation and synthesis of a novel polypeptide

The nucleotide sequences of immunodominant peptides identified with ELISPOT experiments were linked in a linear series. The optimal arrangement of these peptides was determined by using DNASTAR Lasergene (Madison, WI, USA) according to their amphipathic regions, antigenic index, and hydrophilicity. The three-dimensional structure of linked immunodominant peptides was predicted by the SWISS-MODEL database (https://swissmodel.expasy.org/interactive). The novel vaccine was synthesized by SBS Genetech (Beijing, China).

2.6. Immunization and challenge

Female humanized mice or wild-type mice were randomly divided into four groups: PBS (negative control), BCG (positive control), ACP, and BCG + ACP (primary immunization with BCG and booster immunization with ACP) groups. Briefly, the mouse in PBS, BCG, ACP, or BCG + ACP group was injected subcutaneously with 30 μg CpG-ODN2395 adjuvant (Sangon, Shanghai, China) in 100 μl PBS, 30 μg BCG (Chengdu Institute of Biological Products Co., Ltd., Chengdu, China) in 100 μl PBS, 30 μg ACP and 30 μg CpG-ODN2395 adjuvant in 100 μl PBS, or 30 μg BCG in 100 μl PBS, respectively. On days 28 and 42 after primary immunization, mouse in the PBS group was immunized subcutaneously with 20 μg CpG-ODN2395 adjuvant in 100 μl PBS, mouse in ACP or BCG + ACP group was immunized subcutaneously with 20 μg ACP and 20 μg CpG-ODN2395 adjuvant in 100 μl PBS, and mouse in BCG group was not immunized. Fourteen days later, the mouse in each group was challenged with 1.75×10^5 CFUs of *M. tuberculosis* H37Rv strain *via* tail vein injection. The body weight of each mouse was detected weekly.

2.7. CFUs counting

On day 28 post challenge, mice were sacrificed, and their organs were collected for CFUs counting. In detail, half of the liver and the left lobe of the lung were homogenized in 3 ml of physiological saline and diluted at 1:10, 1:100, and 1:1000 with normal saline, respectively. A volume of 0.1 ml dilution from each sample was inoculated on modified Lowenstein–Jensen medium plates (Baso Biotechnology Co., Ltd., Zhuhai, China) in duplicate. Then, the plates were incubated at 37 °C. Twenty-eight days later, the mycobacterial CFUs on each plate were counted.

2.8. Histopathology

The remaining part of the liver and the lung's right lobe were collected to observe the histopathological lesions in the tissue section. Each tissue sample was fixed in 4% (vol/vol) formalin overnight, embedded in paraffin, and cut into 5-μm thickness slices. The tissue sections were stained with hematoxylin and eosin (H&E) according to previous studies.[14–17] Five tissue sections of each mouse were observed under microscopy (Olympus Corporation, Tokyo, Japan).

2.9. ELISPOT for counting the number of lymphocytes secreting IFN-γ

Twenty-eight days post challenge, mice were sacrificed and their spleens were collected for isolating splenocytes according to the method above. The isolated splenocytes were equally divided into two parts: one for the ELISPOT experiment and the other for the Cytometric bead array (CBA). The splenocytes (3×10^5) were added into the wall of 96-well ELISPOT plate and incubated with 10 μl of PBS, 2 μg of ACP peptide, or phytohemagglutinin (PHA) at 37 °C, respectively. Twenty-four hours later, the plate was incubated with biotinylated detection mAb R4-6A2 and streptavidin-ALP following the operation manual of the Mouse IFN-γ ELISPOT Kit (Mabtech AB, Nacka Strand, Sweden). The SFCs and SI were determined according to the methods mentioned above.

2.10. Cytokine analysis

The rest of the isolated splenocytes was used for Th1/Th2/Th17 cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17A) analysis. Approximately 3×10^5 splenocytes in 100 μl 1640 medium were added into each well of 96-well cell culture plate. First, the ACP polypeptide was adjusted to 60 μg/ml with 1640 medium containing 10% heat-inactivated fetal bovine serum (Gibco, Shanghai, China). Then, the ACP polypeptide was added into the well of 96-well cell culture plate in 50 μl/well and incubated in a 37 °C humidified incubator with 5% CO_2. Forty-eight hours later, the culture solution was centrifugated for 5 min at 500 g. Finally, the supernatant was gently transferred into a new 1.5 ml microcentrifuge tube, and the level of Th1/Th2/Th17 cytokines was detected by a Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA).

2.11. Enzyme-linked immune sorbent assay (ELISA)

On days 14, 28, 42, 56, and 74 after primary immunization, the blood sample of each mouse in the same group was collected and pooled together, respectively. The pooled blood sample of each group was centrifuged at 4000 rpm for 10 min. The separated serum of each group (in triplicate) was used to detect the ACP-specific IgG, IgG1, and IgG2a with a Mouse ELISA Kit (Solarbio, Beijing, China) according to the manufacturer's instructions. In addition, the OD_{450} value of each sample was read with a Multiskan Ascent (Thermo Fisher Scientific, Shanghai, China).

2.12. Statistical analysis

The GraphPad Prism 8 software (San Diego, CA, USA) was used to computed all statistics. The data were shown as mean ± standard error of the mean (SEM). The results of CFUs and pathological lesions were analyzed with one-way analysis of variance (ANOVA) or Kruskal–Wallis test according to the data normality and homogeneity of variances. According to the data normality, the results of ELISA and ELISPOT experiments were analyzed with an Unpaired *t*-test or nonparametric test (Mann Whitney test). The significant difference was defined as a *P*-value <0.05.

3. Results

3.1. Th1 immunodominant peptides

Previous studies have reported that four antigens of *M. tuberculosis* (Table 1, Ag85A, Ag85B, CFP21, and PPE18) have the potential to be used as epitope vaccines.[18–23] Herein, we selected these four antigens to predict the candidate epitopes. The amino acid sequences were obtained from the National Center for Biotechnology Information database (NCBI, https://www.ncbi.nlm.nih.gov/). Then the human leukocyte antigen (HLA) -DRB1*0101 restrictive epitopes were predicted by the Immune Epitope Database (IEDB, http://www.iedb.org/). The results indicated that 25 epitopes had a high-affinity binding ability to HLA-DRB1*0101 (Table 2). The binding capacity of each peptide was verified by using ELISPOT assays. It was found that the number of splenocytes secreting interferon-gamma (IFN-γ) stimulated with four peptides ($Ag85B_{12\text{-}26}$, $CFP21_{12\text{-}26}$, $CFP21_{14\text{-}28}$, and $PPE18_{149\text{-}163}$) were significantly higher in at least eight independent experiments (Fig. 1A). Further analysis revealed that eleven amino acids overlapped between $CFP21_{12\text{-}26}$ and $CFP21_{14\text{-}28}$ peptides, and $CFP21_{12\text{-}26}$ peptides had a higher stimulation index (SI) value than $CFP21_{14\text{-}28}$ peptide. Therefore, the $CFP21_{12\text{-}26}$ peptide was selected for further study. The genes of the $Ag85B_{12\text{-}26}$ (GRRLMIGTAAAVVLP), $CFP21_{12\text{-}26}$ (VVVATTLALVSAPAG), and $PPE18_{149\text{-}163}$ (AAAMFGYAAATATAT) peptides were linked in linear to construct a novel vaccine named ACP (Fig. 1B).

Furthermore, the domain plays a vital role in the functioning of the protein. The spatial structure of the protein has a positive influence on its immunological function. Therefore, the SWISS-MODEL database was used to construct the three-dimensions (3D) structure of ACP polypeptide to determine its spatial arrangement and the number and location of structural domains. Our results showed that ACP polypeptide was mainly composed of α-helix (Fig. 1C). The dihedral angles ψ and φ of amino acid residues in the polypeptide structure were in the maximum allowable region (Fig. 1D), indicating that the spatial conformation of ACP was reasonable.

3.2. ACP vaccination induced slight decreases in colony-forming units (CFUs) but failed to enhance the protective efficacy of the BCG vaccine

The flow chart of immunization and challenge was shown in Fig. 2. In humanized mice, our results indicated that the CFUs in the livers collected from mice immunized with BCG ($P = 0.0032$ or $P = 0.0194$) and BCG + ACP ($P = 0.0124$) were significantly lower than that from mice vaccinated with ACP

Table 1 – The basic information about vaccine candidate proteins of *M. tuberculosis*.

Protein Name	Accession No.[a]	Locus_tag	Gene Name[b]	Length (aa)	Annotation[b]	Group[c]	Summary Information[b]
Ag85A	CCP46633	Rv3804c	*fbpA/85A/mpt44*	338	Secreted antigen 85-A FbpA	II	Predicted possible vaccine candidate
Ag85B	CCP44652	Rv1886c	*fbpB/85B/mpt59*	325	Secreted antigen 85-B FbpB	I	Predicted possible vaccine candidate
CFP21	CCP44754	Rv1984c	*cfp21/clp1/culp1*	217	Probable cutinase precursor CFP21	NA	Probable cutinase precursor CFP21.
PPE18	CCP43952	Rv1196	*PPE18*	391	PPE family protein PPE18	NA	Member of the *Mycobacterium tuberculosis* PPE family

NA, not available.

[a] The National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Data were retrieved on 3 Mar 2017.

[b] The Gene name, annotation, and summary information are based on the data deposited at the NCBI. Data were retrieved on 3 Mar 2017.

[c] The group is based on a previous study (See Zvi et al., 2008). The antigens are sorted by the qualitative score (Qual Total) and subsequently by the quantitative score (Quant Total). Group I includes all antigens with a qualitative score eight and above, provided that the quantitative score is not lower than 12. The rest of the antigens having a qualitative score of 8 and those having a qualitative score of 7 and a quantitative score not lower than nine were clustered into Group II. Group III included antigens with qualitative scores of 7 (and a quantitative score of 8) and 6 (with a quantitative score nine and up).

Table 2 – Overview of *M. tuberculosis* peptide predicted binding affinities to DRB1*0101 alleles[a].

Protein	Candidates	Start	End	Peptide	Percentile rank[b]
Ag85A	$Ag85A_{49-63}$	49	63	LPVEYLQVPSPSMGR	0.96
Ag85A	$Ag85A_{50-64}$	50	64	PVEYLQVPSPSMGRD	0.96
Ag85A	$Ag85A_{51-65}$	51	65	VEYLQVPSPSMGRDI	0.96
Ag85A	$Ag85A_{48-62}$	48	62	GLPVEYLQVPSPSMG	1.24
Ag85A	$Ag85A_{16-30}$	16	30	RRLVVGAVGAALVSG	1.53
Ag85A	$Ag85A_{165-179}$	165	179	VVGLSMAASSALTLA	1.71
Ag85A	$Ag85A_{183-197}$	183	197	PQQFVYAGAMSGLLD	1.81
Ag85A	$Ag85A_{47-61}$	47	61	PGLPVEYLQVPSPSM	1.9
Ag85A	$Ag85A_{164-178}$	164	178	AVVGLSMAASSALTL	1.9
Ag85A	$Ag85A_{182-196}$	182	196	HPQQFVYAGAMSGLL	2.05
Ag85B	$Ag85B_{11-25}$	11	25	WGRRLMIGTAAAVVL	0.39
Ag85B	$Ag85B_{12-26}$	12	26	GRRLMIGTAAAVVLP	0.47
Ag85B	$Ag85B_{179-193}$	179	193	HPQQFIYAGSLSALL	0.6
Ag85B	$Ag85B_{180-194}$	180	194	PQQFIYAGSLSALLD	0.6
Ag85B	$Ag85B_{181-195}$	181	195	QQFIYAGSLSALLDP	0.62
CFP21	$CFP21_{12-26}$	12	26	VVVATTLALVSAPAG	4.22
CFP21	$CFP21_{14-28}$	14	28	VATTLALVSAPAGGR	4.36
CFP21	$CFP21_{15-29}$	15	29	ATTLALVSAPAGGRA	4.36
CFP21	$CFP21_{13-27}$	13	27	VVATTLALVSAPAGG	4.77
CFP21	$CFP21_{16-30}$	16	30	TTLALVSAPAGGRAA	5.79
PPE18	$PPE18_{113-127}$	113	127	ENRAELMILIATNLL	0.77
PPE18	$PPE18_{114-128}$	114	128	NRAELMILIATNLLG	0.77
PPE18	$PPE18_{115-129}$	115	129	RAELMILIATNLLGQ	0.77
PPE18	$PPE18_{116-130}$	116	130	AELMILIATNLLGQN	0.77
PPE18	$PPE18_{149-163}$	149	163	AAAMFGYAAATATAT	1.06

[a] The MHC II binding predictions were made on 4 Mar 2017 using the IEDB analysis resource Consensus tool.
[b] For each peptide, a percentile rank for each of the three methods (combinatorial library, SMM_align, and Sturniolo) is generated by comparing the peptide's score against the scores of five million random 15 mers selected from SWISSPROT database. A small numbered percentile rank indicates high affinity. The median percentile rank of the three methods was then used to generate the rank for consensus method.

or phosphate buffer solution (PBS) (Fig. 3A). The CFUs in the lungs collected from mice immunized with BCG ($P = 0.0379$ or $P = 0.0441$) and BCG + ACP ($P = 0.0092$ or $P = 0.0110$) were significantly lower than that from mice immunized with PBS or ACP (Fig. 3B). Surprisingly, although the CFUs in the liver or lung of the ACP vaccinated mice were lower than these of the PBS immunized mice, no significant differences were observed between the two groups. Besides, ACP boosted immunity did not significantly enhance the immune protective effect of BCG. In wild-type mice, the CFUs mean values of BCG, ACP, and BCG + ACP vaccinated mice were lower than those of PBS immunized mice, but the CFUs in the livers (Fig. 3C) or lungs (Fig. 3D) had no significant difference among the groups.

3.3. *Single ACP vaccination elicited slightly reduced pathological lesions*

The histopathological assay was used to evaluate the protective efficacy of single ACP vaccination and its boost vaccination (Fig. 4A). In humanized mice, the lymphocytic inflammation severity score in the lungs collected from mice immunized with BCG + ACP was significantly lower than that of mice immunized with PBS (Fig. 4B, $P = 0.0442$), the alveolar wall severity score in the lungs collected from mice immunized with ACP ($P = 0.0346$) or BCG + ACP ($P = 0.0165$) was significantly lower than that of mice immunized with PBS (Fig. 4C), the granuloma number in the livers collected from mice immunized with BCG ($P = 0.0008$), ACP ($P = 0.0317$), or BCG+ACP ($P = 0.0029$) was significantly lower than that of mice immunized with PBS (Fig. 4D). On the contrary, in wild-type mice, there was no significant differences in the lymphocytic inflammation severity score (Fig. 4E) or the alveolar wall severity score (Fig. 4F) among the groups, and the granuloma number of the livers collected from mice immunized with BCG + ACP was significantly higher than that of mice immunized with PBS (Fig. 4G, $P = 0.0390$).

3.4. *ACP vaccine induced a high level of IFN-γ^+ T lymphocytes*

Although each peptide that constitutes ACP vaccine induced splenocytes secreting high levels of IFN-γ, it was unclear whether ACP composed of these peptides in series had the same ability. Therefore, in this experiment, we evaluated the ability of inducing APC specific–IFN–γ production in humanized and wild-type mice that immunized with different antigens (PBS, BCG, ACP, BCG+ACP). The results showed that the ACP vaccine induced more IFN-γ^+ T lymphocytes in the spleens of humanized (Fig. 5A, $P = 0.0043$) or wild-type mice (Fig. 5B, $P = 0.0066$).

3.5. *ACP or BCG + ACP vaccination stimulated high levels of Th1-type cytokines*

In humanized mice, the level of interleukin −2 (IL-2) secreted by splenocytes of BCG + ACP immunized mice was

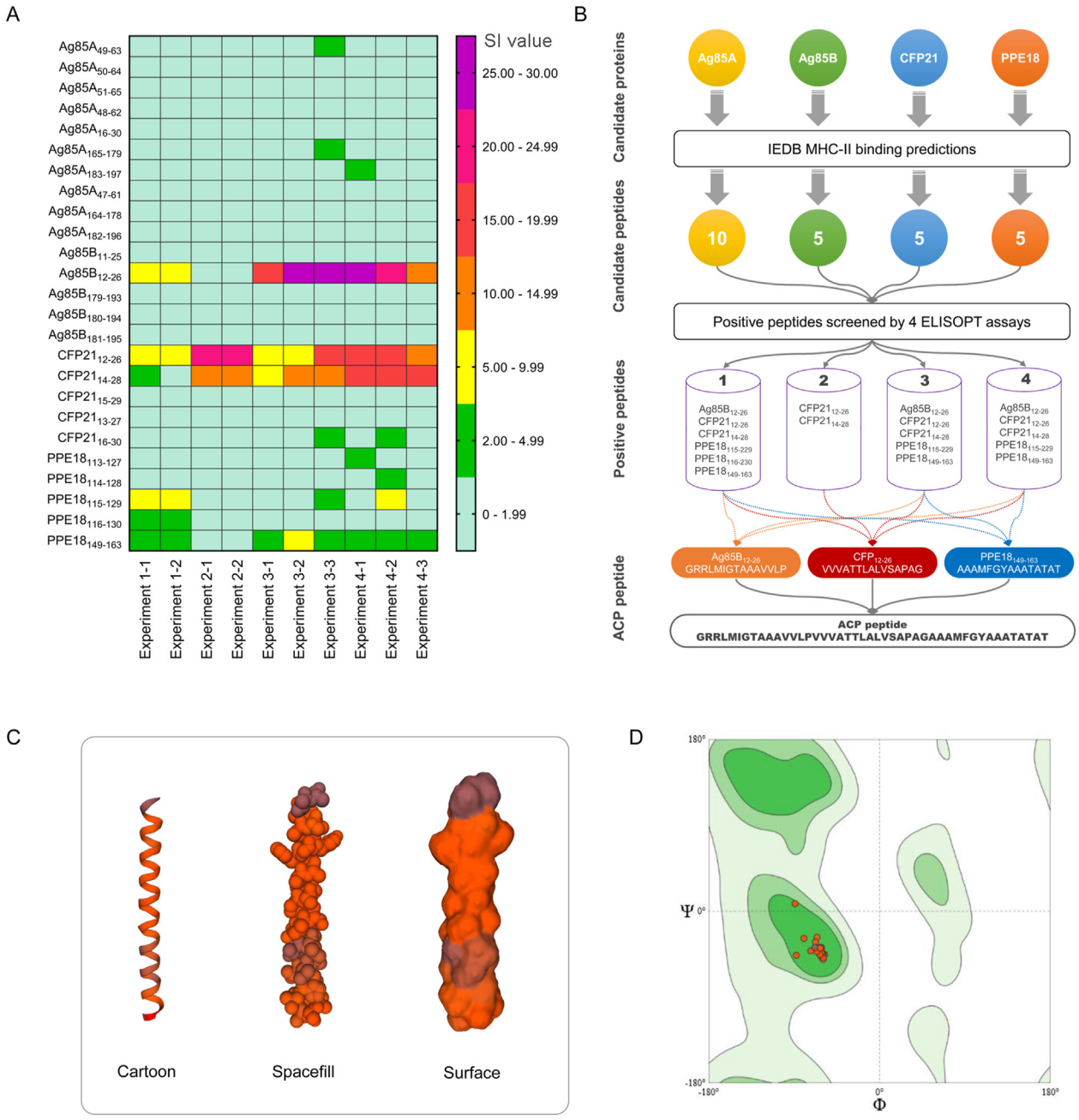

Fig. 1 – Immunodominant peptides screening, ACP polypeptide construction, and its spatial structure prediction. (A) The predicted epitopes were artificially synthesized, and the immunodominant peptides were screened with ten independent experiments. A peptide with a SI greater than 2 was an immunodominant peptide. (B) Schematic diagram of immunodominant peptides screening and ACP construction. (C) The optimal template was selected to complete the ACP model construction according to amino acid sequence coverage, inhibition rate, and QMEAN score. Its spatial structure is presented in three ways, including Cartoon, Spacefill, and Surface. QMEAN is a composite estimator based on different geometrical properties and provides both global and local absolute quality estimates based on one single model. QMEAN Z-scores around zero indicates good agreement between the model structure and experimental structures of similar size. Scores of −4.0 or below are an indication of models with low quality. (D) The Ramachandran Plots were used to evaluate the rationality of ACP space conception. The greater the number of dihedral angles ψ and φ of amino acid residues contained in the maximum allowable region, the more reasonable the spatial conformation of the protein.

significantly higher than that of PBS ($P < 0.0001$), BCG ($P < 0.0001$) or ACP ($P < 0.0001$) immunized mice (Fig. 6A), but the levels of IL-6 (Fig. 6B, $P = 0.0002$), IFN-γ (Fig. 6C, $P = 0.0286$), and tumor necrosis factor -α (TNF-α, Fig. 6D, $P = 0.0001$) in BCG + ACP immunized mice were lower than these in PBS immunized mice. Furthermore, the level of IL-6 in ACP immunized mice was higher than that in BCG or BCG + ACP immunized mice (Fig. 6B, $P = 0.0082$ or $P < 0.0001$).

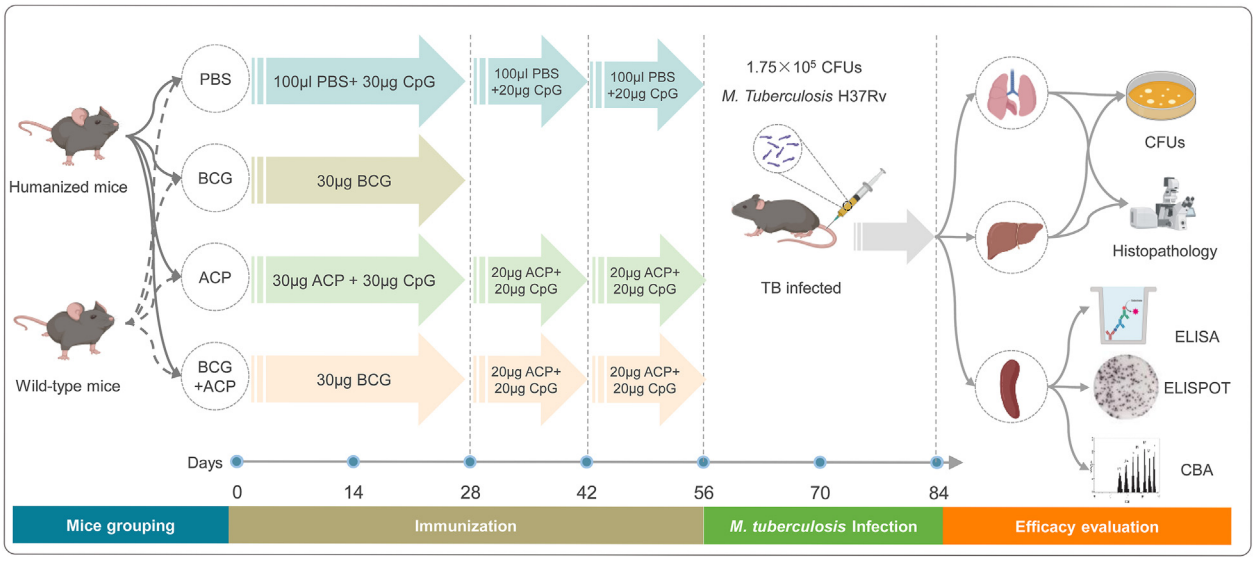

Fig. 2 – The flow chart of immunization and challenge.

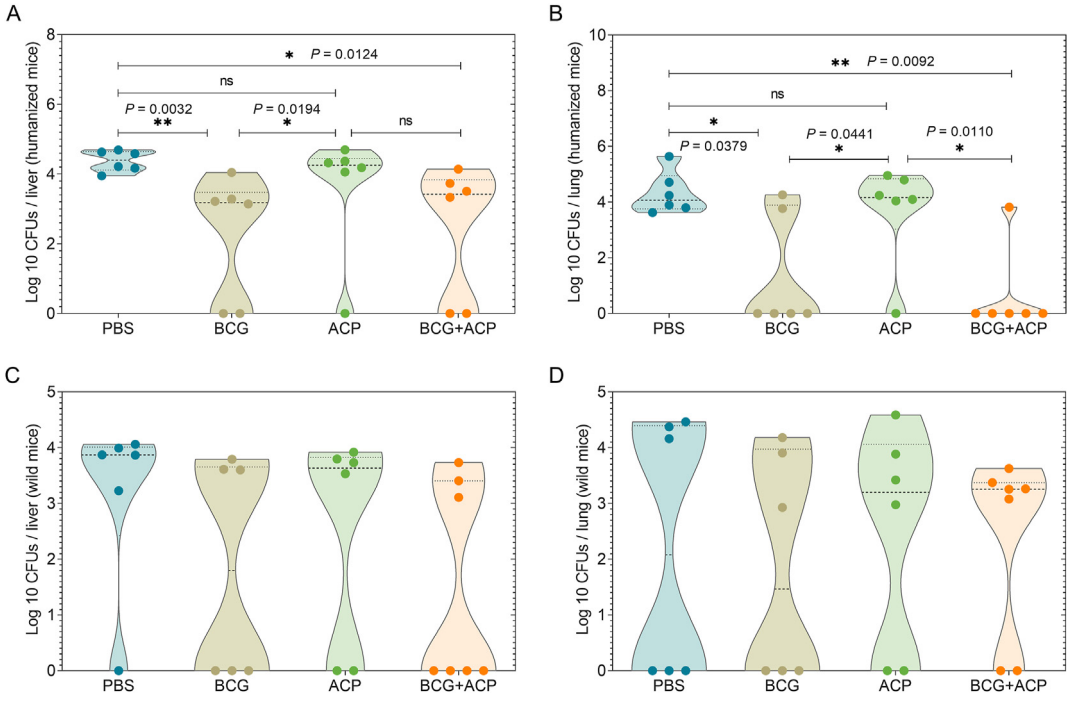

Fig. 3 – Evaluation of protective efficacy of ACP. Humanized or wild-type C57BL/6 mice were immunized with PBS, BCG, ACP, and BCG + ACP, respectively. Fourteen days post last immunization, the mouse in each group was challenged with *M. tuberculosis* H37Rv strain. Twenty-eight days later, the mouse was sacrificed, and the half part of the liver (A and C) and the left lobe of the lung (B and D) were collected for CFUs counting. The data expressed as CFUs were compared with one-way analysis of variance (ANOVA) or Kruskal–Wallis test according to the data normality and homogeneity of variances. All data were shown as mean + SEM ($n = 6$ or 7). $P < 0.05$ was considered significantly different. *$P < 0.05$; **$P < 0.01$.

Interestingly, the level of IFN-γ in ACP immunized mice was higher than that in PBS (Fig. 6C, $P = 0.0361$) or BCG + ACP immunized mice (Fig. 6C, $P = 0.0286$). The level of TNF-α in ACP immunized mice was higher than that in BCG or BCG + ACP immunized mice (Fig. 6D, $P = 0.0014$ or $P < 0.0001$).

In wild-type mice, the levels of IL-2 (Fig. 6E, $P = 0.0241$), IL-6 (Fig. 6F, $P = 0.0058$), IFN-γ (Fig. 6G, $P = 0.0269$), and TNF-α (Fig. 6H, $P = 0.0286$) in BCG + ACP immunized mice were visibly lower than these in PBS immunized mice. In contrast, the levels of IL-2 (Fig. 6E, $P = 0.0263$ or 0.0063), IL-6 (Fig. 6F, $P = 0.0036$ or 0.0009), IFN-γ (Fig. 6G, $P = 0.0164$ or 0.0106), and TNF-α (Fig. 6H, $P = 0.0286$) in ACP immunized mice were higher than these in BCG or BCG + ACP immunized mice, respectively. In addition, there were no significant difference

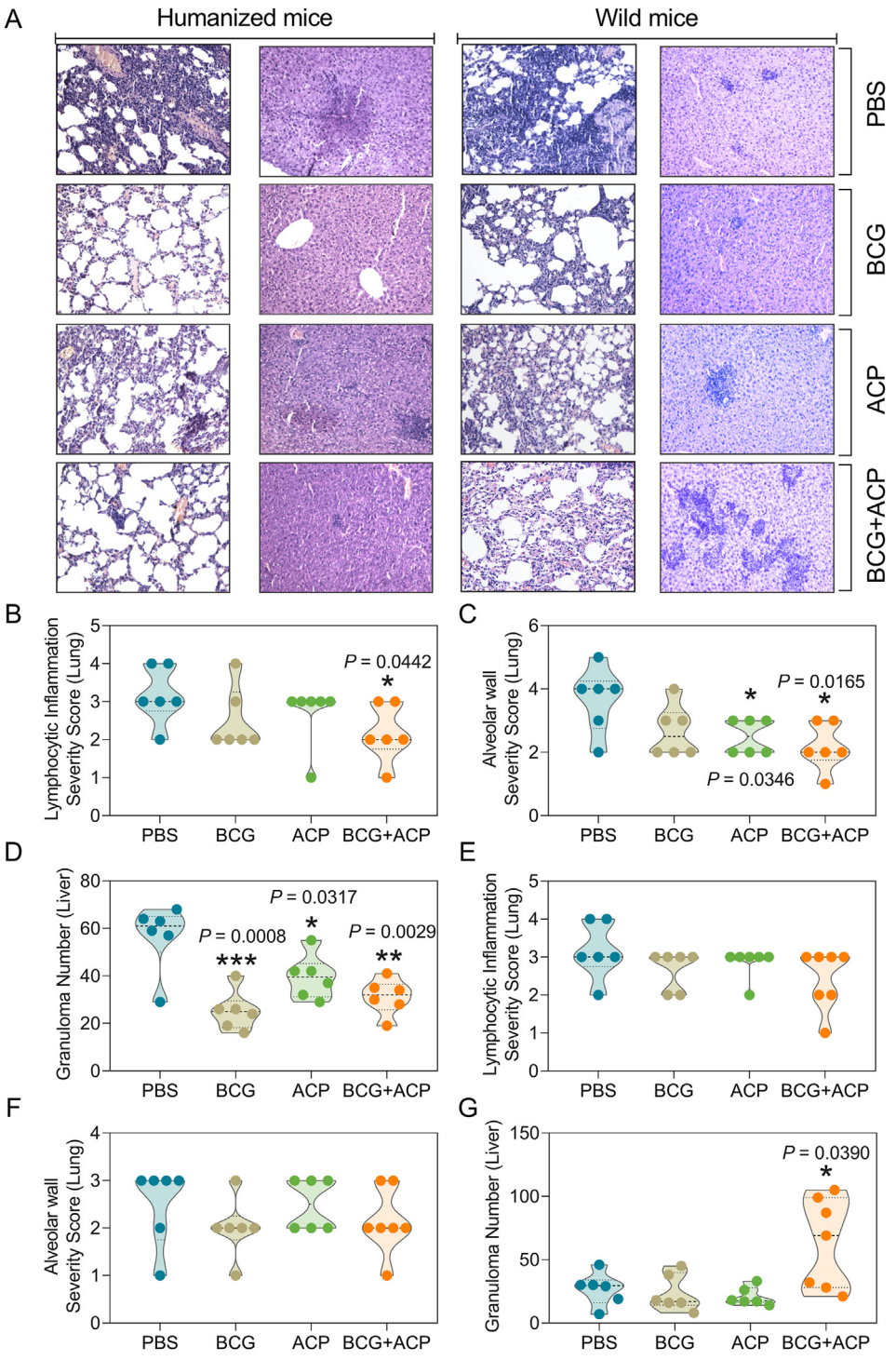

Fig. 4 – Histopathological characteristics of mice treated with polypeptide. Five tissue sections of each organ were randomly selected to evaluate the histopathological lesions (A). The selected tissue section of each mouse was observed using a microscope with original magnification times of 400×. Larger granulomas and more inflammatory cells in livers and severe thickened interstitial alveolar walls in lungs were observed in humanized or wild-type mice vaccinated PBS, but not the mice vaccinated with BCG, ACP, and BCG + ACP. Furthermore, the lymphocytic inflammation severity score and alveolar wall severity score in lung, and granuloma number in liver collected from humanized mice (B–D) or wild type mice (E–G) were compared between four groups with one-way analysis of variance (ANOVA) or Kruskal–Wallis test according to the data normality and homogeneity of variances. All data were shown as mean + SEM (n = 6 or 7). $P < 0.05$ was considered significantly different. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

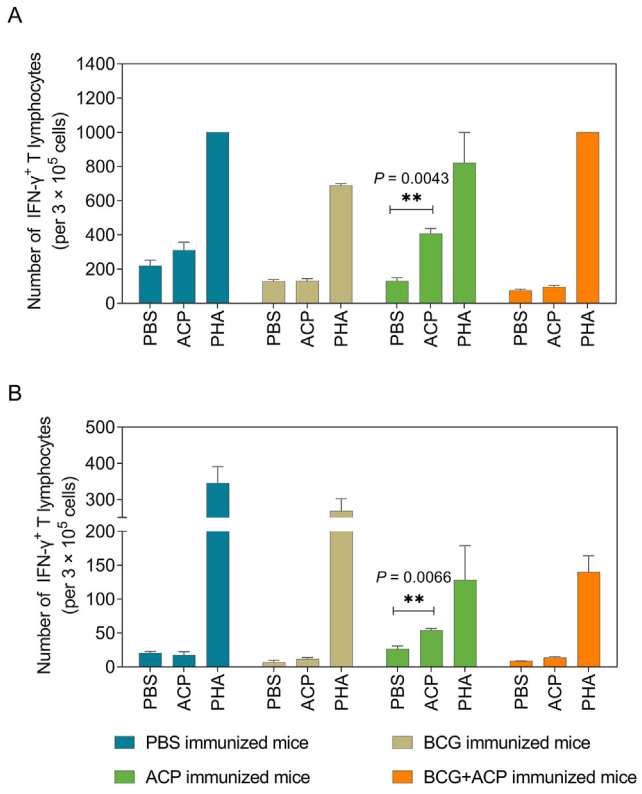

Fig. 5 – IFN-γ^+ T lymphocytes detection with ELISPOT. The splenocytes obtained from humanized mice (A) or wild-type mice (B) immunized with PBS, BCG, ACP, or BCG + ACP were stimulated with PBS (as a negative control), ACP, or PHA (as a positive control) *in vitro*. The spots number of IFN-γ^+ T lymphocytes in each group were determined with ELISPOT assay. The data were analyzed with the Unpaired *t*-test or Mann Whitney test according to the data normality. All data were shown as mean + SEM ($n = 2$ in negative or positive control groups, $n = 4$ in the ACP group). $P < 0.05$ was considered significantly different. **$P < 0.01$.

in the levels of other cytokines (IL-4, IL-10, and IL-17A) between the four groups of humanized or wild-type mice (data was not showed).

3.6. *ACP vaccine induced high levels of antibodies in humanized mice but not wild-type mice*

ELISA was performed to determine the levels of ACP-specific IgG, IgG1, and IgG2a antibodies in serum samples collected from mice immunized with PBS, ACP, or BCG + ACP. In humanized mice, the levels of IgG (Fig. 7A, $P < 0.001$), IgG1 (Fig. 7B, $P < 0.01$), and IgG2a (Fig. 7C, $P < 0.05$) antibodies in ACP or BCG + ACP immunized mice were significantly higher than these in PBS immunized mice on the 14th day after the first immunization. After that, the levels of IgG, IgG1, and IgG2a antibodies began to decline gradually, reaching a trough on the 28th day, rebounded to a peak on the 56th day, and then decreased. However, there was no remarkable difference in antibody levels among wild-type mice groups, and the trend of antibody levels similar to that of humanized mice was not observed in wild-type mice (Fig. 7D–F). Interestingly, the ratio of IgG2a to IgG1 was more significant than one in both humanized (Fig. 7G) and wild-type mice (Fig. 7H), indicating that the immune response induced by ACP tended to a Th1 type immunity.

4. Discussion

This study prepared a peptide-based vaccine derived from four protective antigens Ag85A, Ag85B, CFP21, and PPE18. Ag85 protein complex is an essential secretory protein of *M. tuberculosis*. It consists of three components, Ag85A (Rv3804c), Ag85B (Rv1886c), and Ag85C (Rv0129c). Although the role of the Ag85 complex protein in the physiology and pathogenesis of *M. tuberculosis* is unclear, a large number of studies have shown that both Ag85A and Ag85B proteins can activate protective immune responses in animal models and human beings.[24,25] CFP21 (Rv1984c) is a secretory protein of *M. tuberculosis*, which has carboxylesterase and acylglycerol lipase activities and involves in fatty acid hydrolysis and lipid metabolism.[26] A recent study identified CFP21-derived HLA-A*0201 restricted epitopes and found that a native peptide p134 (AVADHVAAV) could be an excellent candidate to develop peptide vaccines against *M. tuberculosis*.[22] Chun Wang et al demonstrated that a DNA vaccine expressing the fusion protein of CFP21 and MPT64 could enhance the protective immunity of the BCG vaccine against *M. tuberculosis* infection in the mouse model.[27] PPE18 (Rv1196) is one of the members of a promising vaccine candidate M72/AS01E, which has been investigated in several phases I/II clinical trials.[3,28,29] Two recent studies revealed that the efficacy of the M72/AS01E vaccine was 57.0%, and it induced a significantly higher level of memory Th1-type CD4 T cells.[30,31]

MHC restriction of an epitope plays a crucial role in the recognition of T lymphocytes and antigen-presenting cells. Therefore, the influence of MHC restriction on epitope processing and immune response should be fully considered in the design of a peptide-based vaccine. According to the statistics of the IEDB database, as many as 63% of epitopes are known to be related to MHC class II molecules, of which nearly 90% are related to HLA-DR molecules. Our previous study found that the most frequent HLA-DR alleles in the Chinese population are DR01, DR09, and DR15[9,32,33] Hence, we used the HLA-A11/DR1 transgenic mouse model to identify and evaluate new peptide vaccines for the Chinese people. As a result, twenty-five peptide candidates were identified by the IEDB database for their high-affinity binding ability to HLA-DRB1*0101 molecules. $Ag85B_{12\text{-}26}$, $CFP21_{12\text{-}26}$, $CFP21_{14\text{-}28}$, and $PPE18_{149\text{-}163}$ peptides induced significantly higher levels of IFN-γ in splenocytes obtained from humanized mice, which indicated that these immunodominant peptides might have a potential ability to activate NK cells, T cells, dendritic cells, and macrophage to defend the host against intracellular bacteria such as *M. tuberculosis*.[13,34] On this basis, we designed a novel vaccine named ACP, and its protective efficacy was evaluated in humanized and wild-type mice. Our results revealed that the single ACP vaccination resulted in a slight decrease in the CFUs and pathological lesions compared to the

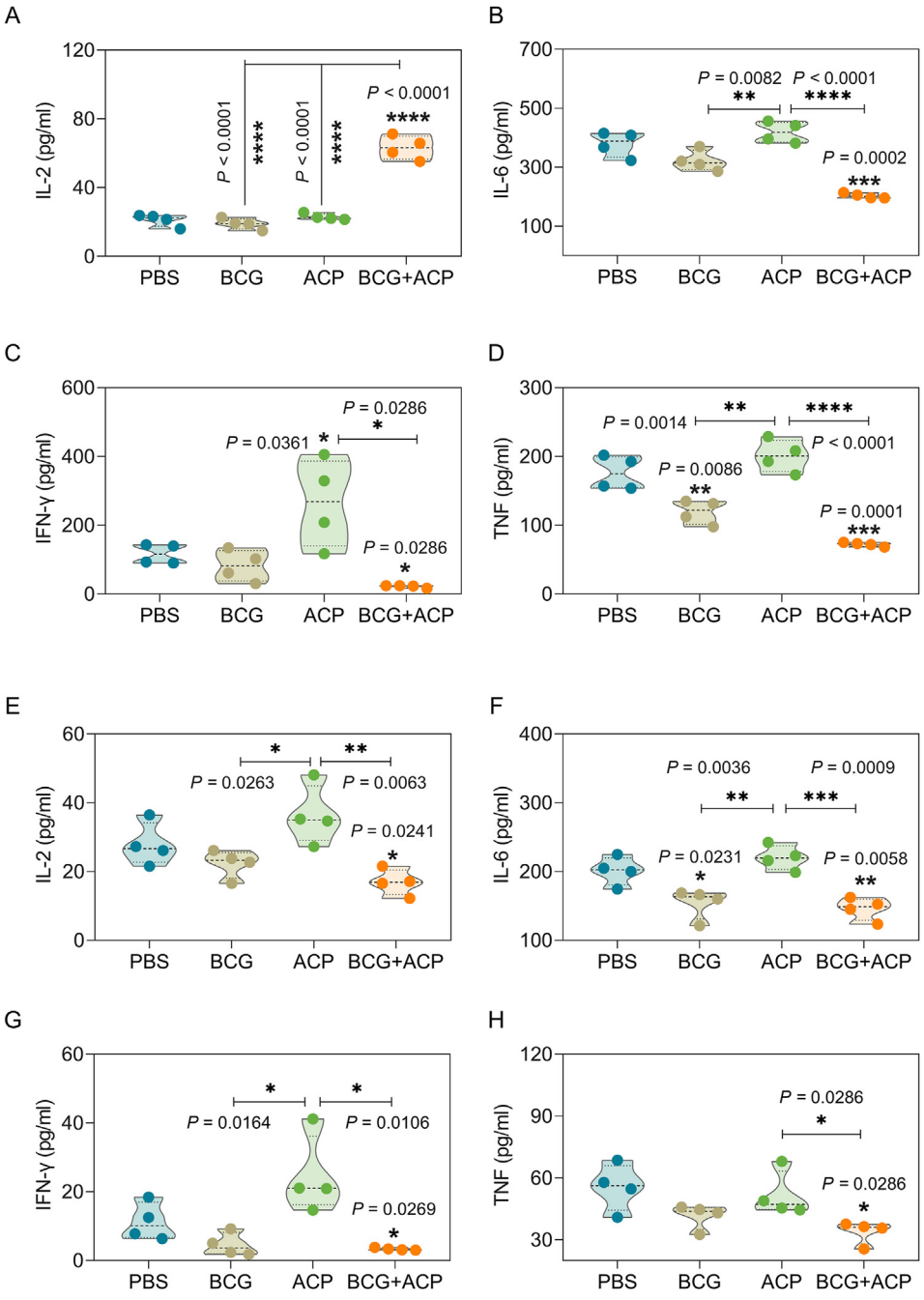

Fig. 6 – Th1, Th2, and Th17 Cytokines. The splenocytes isolated from humanized mice (A to D) or wild-type mice (E to H) immunized with PBS, BCG, ACP, or BCG + ACP were incubated with ACP polypeptide for 48 hours. The levels of IL-2, IL-6, IFN-γ, and TNF-αcytokines in the supernatant were detected with a Mouse Th1/Th2/Th17 Cytokine Kit. Significant differences among four groups of humanized or wild-type mice were analyzed with one-way analysis of variance (ANOVA) or Kruskal–Wallis test according to the data normality and homogeneity of variances. All data were shown as mean + SEM ($n = 4$). $P < 0.05$ was considered significantly different. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

PBS group. Still, no significant difference was observed between the two groups. Unfortunately, when using it as a booster, the ACP vaccine did not significantly enhance the protective efficacy of BCG in humanized or wild-type mice. However, we found that the ACP vaccine could dramatically stimulate higher levels of cytokines (such as IFN-γ, IL-6, and TNF-α rather than IL-4) and antibodies (especially IgG2a) in humanized and wild-type mice, which indicated that the ACP vaccine was more likely to induce a Th1 type immune response. Interestingly, although the peptide-based vaccine was designed based on the HLA-DRB1*0101 alleles, it also caused cellular immune responses in wild-type mice. We compared the concentration of the cytokines stimulated by the ACP vaccine and found that the concentrations of IL-6, IFN-γ, and TNF-α were remarkably higher in humanized mice than those in wild-type mice, which is consistent with

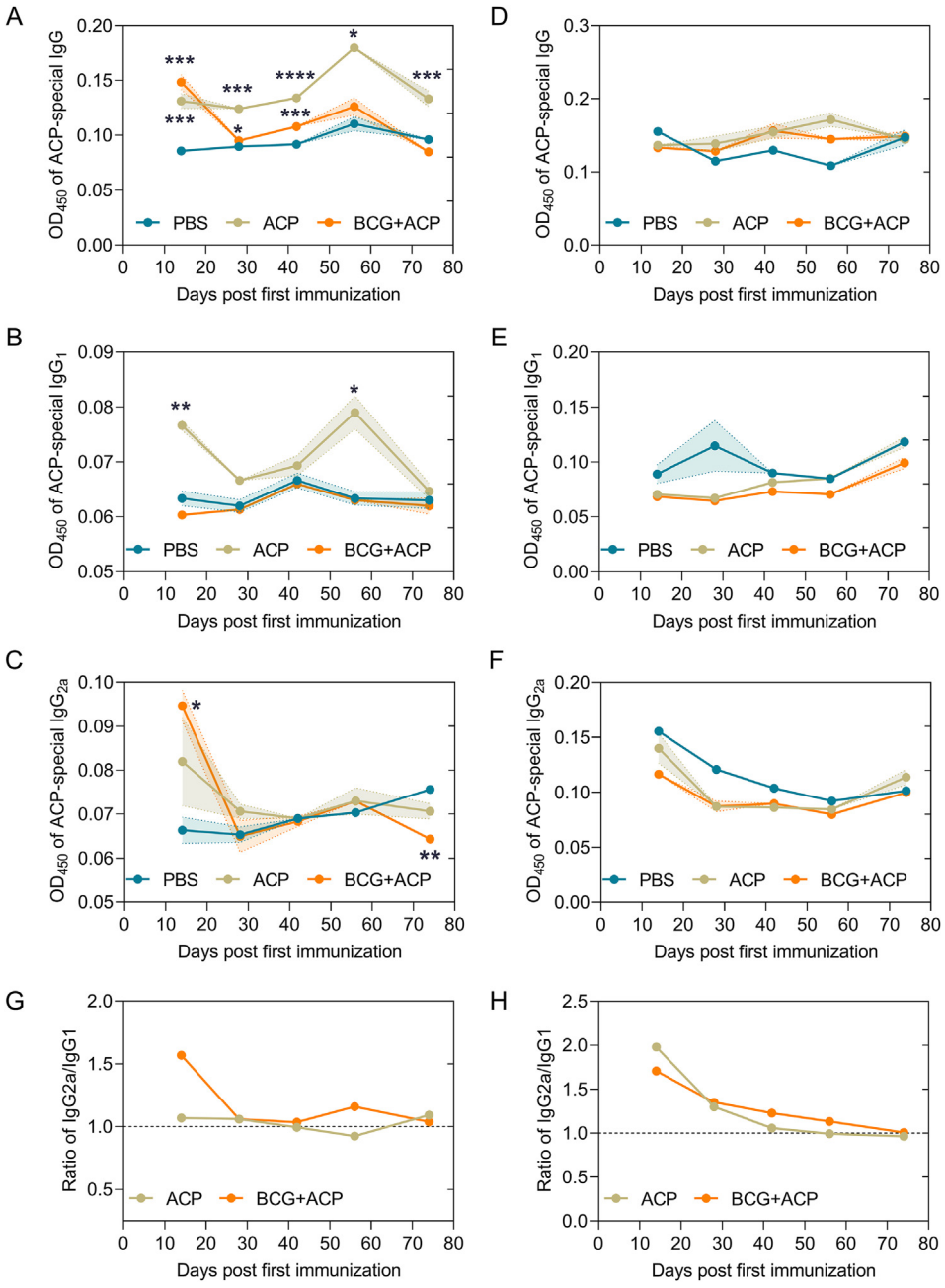

Fig. 7 – ACP-specific IgG, IgG1, and IgG2a antibodies. Serum samples were collected from humanized or wild-type mice immunized with PBS, BCG, ACP, and BCG + ACP, respectively. ELISA was used to detect ACP-specific IgG (A or D), IgG1 (B or E), and IgG2a (C or F) in sera of humanized or wild-type mice in triplicate. The ratio of IgG2a/IgG1 in humanized (G) or wild-type mice (H) was also presented. The significant differences of OD_{450} of IgG, IgG1, and IgG2a were analyzed using the Unpaired *t*-test or Mann Whitney test according to the data normality. Results were expressed as mean ± SEM ($n = 3$), and dotted lines represented the error bar. $P < 0.05$ was considered significantly different. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

our previous study.[9] The above data shows that the peptide-based vaccine can stimulate a strong cellular immune response in the humanized mouse model and induce a weaker specific cellular response in wild-type mice. The difference between the two animal models lies in the strength of the cellular immune response induced by ACP.

Why did this peptide-based vaccine ACP successfully induce a significantly high level of Th1 type immune response but have insufficient protection on humanized or wild-type mice and fail to improve the protective efficacy of BCG? We have analyzed this from the following aspects, hoping to provide some new references for the design and research of peptide-based vaccines in the future.

Firstly, the secretion level of IFN-γ may not positively correlate with the vaccine's protection efficiency. As an intracellular bacterium, the elimination of *M. tuberculosis* in the body mainly depends on $CD4^+$ T cells such as Th1, Th2, and Th17 cells. It was reported that Th1 cells were critical

for killing and eliminating *M. tuberculosis* by secreting IFN-γ.[35,36] Furthermore, innate immune cells work together to eradicate the mycobacterial invaders and generate IL-12, IL-18, and IL-23, which stimulate T lymphocytes to secret enhanced IFN-γ, TNF-α and activate macrophage to killing intracellular bacteria.[37] Herein, the number of IFN-γ^+ T lymphocytes was determined by using an ELISPOT assay, and the data suggested that the ACP vaccine could stimulate a significantly higher level of IFN-γ^+ T lymphocytes in humanized or wild-type mice. Furthermore, we found that the ACP vaccine could stimulate splenocytes producing more IFN-γ, IL-6, and TNF-α rather than IL-4 in humanized and wild-type mice, which indicated that the ACP vaccine was more likely to induce a Th1 type immune response. Although cellular immune responses such as IFN-γ cytokine has been widely used to evaluate the immunogenicity of vaccines, our data indicated that there was no positive correlation between the level of IFN-γ and the protective efficiency of the vaccine. Recent studies demonstrated that IFN-γ might play a minimal role in controlling pulmonary *M. tuberculosis* infection,[38] and central memory T cells and locally secreted IgA correlated with protection against TB disease.[39,40]

Secondly, IL-2 might play a decisive role in host responses to intracellular bacteria such as *M. tuberculosis*. In this study, prime-booster with BCG and ACP induced significantly higher levels of IL-2 and lower levels of IFN-γ and TNF-α. IL-2 regulates the differentiation of T cells and promotes the formation of effector cytolytic T cells.[41] Previous studies indicated that IL-2 could activate T cells *in vitro*, improve T cell anergy, repair T cell proliferation, and treat multiple drug-resistant tuberculosis.[42–44] In recent years, the theory of T cell exhaustion has been put forward, which suggested that continuous antigen stimulation would cause T cells to gradually lose their functions, reduce proliferation, and decrease the secretion of IFN-γ and TNF-α.[45] A study found that persistent stimulation from *M. tuberculosis* antigen resulted in T cell dysfunction and exhaustion, but the treatment of IL-2 restored antigen-specific T cell responses and protective efficacy.[44] Therefore, the role of IL-2 in *M. tuberculosis* infection and vaccine immune response needs to be further clarified.

Thirdly, B cells and antibodies should be given more attention when designing a peptide vaccine. Although the cellular immune response, especially the role of T cells, has been widely concerned in the prevention of *M. tuberculosis* infection and TB vaccine design, increasing data indicated that B cells and antibodies had a variety of potential protective roles,[3,46] and could enhance both cellular and humoral immunity at the stage of *M. tuberculosis* infection.[47] In this study, the ACP vaccine induced significantly higher IgG, IgG1, and IgG2a in humanized mice than wild-type mice, especially in the early immunisation stage. Furthermore, the ratio of IgG2a to IgG1 was greater than one, indicating that ACP mainly depends on the Th1 type cellular immune response, which was consistent with the results of the cellular immune response. Unfortunately, as an exploratory study, the ACP vaccine prepared in this study was based on Th1 epitopes, not B cell epitopes. It may be one of the reasons for the inadequate protection of the ACP vaccine. Nevertheless, it reminded us that not only Th1 epitopes but also B cell epitopes and even CTL epitopes should be considered in the design of peptide-based vaccines in the future.

5. Conclusions

In summary, the current study identified three immunodominant peptides that could stimulate splenocytes secreting a high level of IFN-γ, and a novel peptide-based vaccine ACP was prepared by linearly linking these immunodominant peptides. These results demonstrated that the ACP vaccine could induce a significantly high Th1-type immune response and slightly reduce the CFUs and pathological lesions in the lung of mice. However, when using it as a booster, the ACP vaccine did not significantly enhance the protective efficacy of BCG in humanized or wild-type mice. These data remind us that when designing a tuberculosis vaccine, the role of $CD4^+$ T lymphocytes and the role of $CD8^+$ T lymphocytes and B lymphocytes should be fully considered in the future.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Data availability

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

Author contributions

Conceptualization: XQW and WPG; Project administration: XQW; Methodology: WPG, JM, YL, YX, LW, JW, YSZ, and SHS; Data curation: WPG, YL, and JM; Software: WPG; Writing-original draft: WPG; Writing-review and editing: WPG, and XQW; Funding acquisition: WPG, and YSZ. All authors reviewed and approved the final manuscript.

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Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or

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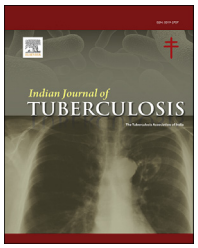

Original article

Clinical presentation and mortality risk factors for COVID-19 among diabetic patients in a tertiary care center in South India

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ABSTRACT

Background: Non-communicable diseases (NCD) like hypertension, diabetes, cardiovascular and cerebrovascular diseases are the most common comorbidities among COVID-19 patients. The clinical presentation and mortality pattern of COVID-19 are different for patients with comorbidities and without comorbidities.

Objective: To determine the clinical presentation of COVID-19 and risk factors for COVID-19 mortality among diabetic patients in a tertiary care hospital in South India.

Methods: A record-based cross-sectional study was conducted by reviewing the case records of COVID-19 patients admitted for treatment from June 2020 to September 2020 in a tertiary care centre in South India. Potential risk factors for COVID-19 mortality were analysed using univariate binomial logistic regression, generalized linear models (GLM) with the Poisson distribution. Survival curves were made using the Kaplan–Meier method.

Results: Out of 200 COVID-19 patients with diabetes with a mean (SD) age of 56.1 (11.8) years, 61% were men. The median survival time was slightly lesser in male COVID-19 patients (15 days) as compared to female patients (16 days). The risk of mortality among

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COVID-19 patients with diabetes is increased for patients who presented with breathlessness (aRR = 4.5 (95% CI: 2.3–8.8)), had positive history of smoking (aRR = 1.9 (95% CI: 1.1–3.8)), who had CKD (aRR = 1.8 (95% CI: 1.1–2.8)) and who had cardiac illness (aRR = 1.6 (95% CI: 0.9–2.7)).

Conclusion: Diabetes patients with COVID-19 need to be given additional care and monitoring especially if they present with breathlessness, positive history of smoking, cardiac illness and, CKD. Public health campaigns and health education activities to control smoking is needed to reduce the COVID-19 mortality in diabetes patients.

1. Introduction

The emerging infection of the SARS CoV-2 virus causing COVID-19 disease resulted in the global pandemic with 119,218,587 cases and 2,642,673 deaths all over the world.[1] India has contributed to 10% of the global caseload with 11,646,081 cases and 159,967 deaths.[2] The morbidity and mortality pattern of COVID-19 is different for patients with comorbidities and without comorbidities.[3] Non-communicable diseases (NCD) like hypertension, diabetes, cardiovascular and cerebrovascular diseases are the most common comorbidities among COVID-19 patients.[3] A systematic review and meta-analysis showed that 9% of the COVID-19 patients were comorbid with diabetes.[4]

India is undergoing the epidemiological transition with a dual burden of diseases, both communicable and non-communicable diseases contributing to morbidity.[5] Diabetes is one of the common NCDs contributing to the dual burden of diseases with 65·0 million Indians having diabetes in 2016.[6] With the increasing prevalence of diabetes in India among adults aged 20 years or older from 5·5% in 1990 to 7·7% in 2016[6] and the worse prognosis of COVID-19 among diabetic patients,[7] the impact of the COVID-19 pandemic in India will be abominable.

Patients with diabetes who have compromised immunity and a poorly regulated inflammatory cytokine storm might be the possible mechanisms for severe COVID-19 disease among diabetes patients.[8] Increased glycation of ACE2 and reduced ACE2 in diabetic patients with poor control of glycemic status explains the increased susceptibility to severe ARDS and lung damage in COVID-19 patients.[9] Diabetic- COVID-19 patients with other comorbid health conditions like hypertension, ischemic heart diseases, obesity were more likely to have severe disease, hospitalization, and death.[7]

A study in Wuhan has shown that diabetic patients with COVID-19 had higher odds of Intensive care unit (ICU) admission and death.[10] An Italian study showed that 8.9% of the COVID-19 admitted had diabetes. Another British study has shown that the risk of mortality was more among COVID-19 patients with uncontrolled diabetes with a hazard ratio of 2·36 [95% CI 2·18–2·56].[11] The literature related to the clinical presentation of COVID-19 among diabetes patients in India is very limited. With this background, this study was conducted to assess the clinical presentation of COVID-19 and risk factors for COVID-19 mortality among diabetic patients in a tertiary care hospital in South India.

2. Methodology

A record-based cross-sectional study was conducted by reviewing the case sheets of COVID-19 patients admitted for treatment in the tertiary care center in Bangalore, India. Since June 2020, this center was designated as Dedicated COVID Hospital (DCH) by the Government of Karnataka (GoK) and standard care is being free of cost to the COVID-19 patients.

The clinical and socio-demographic details were collected from the case records maintained in the hospital. The data collected were entered in MS Excel. The anonymity and confidentiality of the information collected were maintained throughout the study. During the period from June 2020 till September 2020, 854 records of confirmed COVID-19 cases were analyzed. Out of them, there were 200 COVID-19 patients with diabetes comorbidity which were included in the current study. Patients were followed from the date of admission till the date of discharge/death from the hospital.

The variables included in the study are age, sex, the presence of symptoms like fever, cough, breathlessness, sore throat, loose stools chest pain, headache, myalgia. The details like the history of travel, contact, smoking, alcohol consumption were also included in the study. The presence of other comorbidities like hypertension, chronic kidney disease (CKD), respiratory diseases, malignancies, tuberculosis, and hypothyroidism was also collected from the case records. After the period of stay in the hospital, the outcome of the patients was categorized as discharge and death.

2.1. Operational definitions

2.1.1. *History of contact*

A person who is involved in any works related to providing direct care without proper personal protective equipment for COVID-19 patients or staying in the same close environment as a COVID-19 patient in the last 14 days before the onset of symptoms.[12]

2.1.2. *History of travel*

A person who has travelled to any foreign countries in the last 14 days before the onset of symptoms.

2.1.3. History of smoking

A person who is a current smoker or former smoker is considered as having a positive history of smoking.[13]

2.1.4. History of alcohol consumption

Use of alcoholic beverages either on individual occasions (binge drinking) or as a regular practice is considered a positive history of alcohol consumption.[14]

2.2. Statistical analysis

The data collected were entered in MS Excel and analyzed using STATA statistical software version 14 (StataCorp LCC, Lakeway Drive College Station, Texas, USA).[15] The continuous variables were summarized using mean (SD) or median (interquartile range (IQR)) based on the distribution of data. Other categorical variables were summarized using frequencies and proportions. Chi-square test and Fischer's exact tests are used to assess the statistical significance of association between the categorical variables. Binomial logistic regression was used to do the univariate analysis to determine the factors associated with mortality among diabetic patients with COVID-19. Relative risk (RR) with a 95% confidence interval (95% CI) was used to express the strength of association. A generalized linear model (GLM) with Poisson distribution was used for multivariate analysis and adjusted relative risk (aRR) with 95% CI was calculated. The variables which were significantly associated with the outcome (p-value <0.05) in the univariate analysis were included in the multivariate analysis. A p-value less than 0.05 was considered statistically significant. Kaplan–Meier method was used to plot the survival curves and the survival distributions between the groups were tested using the Log-rank test.

2.3. Ethical considerations

The ethical approval to conduct the study was taken from the Institutional Ethics Committee and due permissions were taken from the authorities. The anonymity and confidentiality of the data were maintained throughout the study.

3. Results

In total 200 COVID-19 patients with diabetes were included in the study whose mean (SD) age was 56.1 (11.8) years. Out of 200 study participants, 78 (39%) were women and 122 (61%) were men. The symptoms with which COVID-19 patients with diabetes presented were fever (59.5%), cough (48%), difficulty in breathing (42%), myalgia (26.5%), sore throat (3%), headache (11%), loose stools (3.5%), loss of taste/smell (5%) and chest pain (5%). The median (IQR) survival time in the hospital among COVID-19 patients with diabetes was 15 (12–20) days. The median survival time slightly lesser for male patients (15 (95% CI: 10–17) days) and female COVID-19 patients (16 (95% CI: 12–20) days) with diabetes but the difference was not statistically significant (p-value 0.490) [Fig. 1].

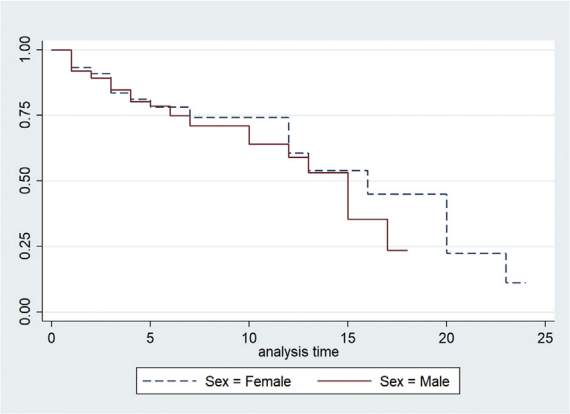

Fig. 1 – Kaplan–Meier survival estimates.

History of contact with a positive case of COVID-19 was present in 38 (19%) and history of travel to a foreign country was present in 23 (11.5%) of the study participants. The comorbidities present in the COVID-19 with diabetes were hypertension (56.5%), ischemic heart disease (9.5%), hypothyroidism (7%), CKD (3.5%), respiratory diseases like chronic obstructive pulmonary disease (COPD), and asthma (3%), tuberculosis (3.0%) and malignancy (1.5%).

Our results showed that the risk of death due to COVID-19 among diabetes patients increased by RR = 5.2 (95% CI: 2.7–9.9) times among those who presented with breathlessness as compared to those who did not have breathlessness [Table 1]. Diabetic COVID-19 patients with a history of smoking had a 3.3 (RR = 3.3, 95% CI: 1.7–6.1) times increased risk of mortality as compared to non-smokers and this association was statistically significant [Fig. 2]. COVID-19 Patients with diabetes and CKD had 3.2 times (RR = 3.2, 95% CI: 1.8–5.4) increased risk of death as compared to those without CKD [Table 2]. Similarly, COVID-19 patients with diabetes and cardiac illness had 2.1 times (RR = 2.1, 95% CI: 1.2–3.8) increased risk of mortality and the association was statistically significant.

In the multivariate analysis, the variables which were significantly associated with mortality in the univariate analysis like presenting with breathlessness, history of smoking, CKD, cardiac illness were included in the model. After adjusting for other variables included in the model, the risk of mortality among COVID-19 patients with diabetes is increased for patients who presented with breathlessness (aRR = 4.5 (95% CI: 2.3–8.8)), had a positive history of smoking (aRR = 1.9 (95% CI: 1.1–3.8)), who had CKD (aRR = 1.8 (95% CI: 1.1–2.8)) and who had a cardiac illness (aRR = 1.6 (95% CI: 0.9–2.7)). These associations were also statistically significant in multivariate analysis [Table 3].

4. Discussion

Our analysis has identified that patients presenting with breathlessness, patients with comorbidities CKD and cardiac illness, positive history of smoking were significantly

Table 1 – Association of demographic and clinical characteristics with mortality among diabetes patients with COVID-19 admitted to the tertiary care center, N = 200.

Characteristics	Categories	Discharged n = 152 Frequency (%)	Died n = 48 Frequency (%)	RR (95% CI)[a]	p-value
Age	0–30	2 (100)	0	–	
	31–60	97 (79.5)	25 (20.5)	1	0.104
	61–90	53 (69.7)	23 (30.3)	1.5 (0.9–2.4)	
Sex	Female	60 (76.9)	18 (23.1)	1	0.807
	Male	92 (75.4)	30 (24.6)	1.1 (0.6–1.7)	
Fever	No	62 (76.5)	19 (23.5)	1	0.882
	Yes	90 (75.6)	29 (24.4)	1.0 (0.6–1.7)	
Cough	No	75 (72.1)	29 (27.8)	1	0.186
	Yes	77 (80.2)	19 (19.8)	0.7 (0.4–1.1)	
Breathlessness	No	106 (91.4)	10 (8.6)	1	**<0.001**
	Yes	46 (54.8)	38 (45.2)	5.2 (2.7–9.9)	
Loose stools	No	148 (76.7)	45 (23.3)	1	0.181
	Yes	4 (57.1)	3 (42.9)	1.8 (0.7–4.4)	
Chest pain	No	144 (75.8)	46 (24.2)	1	0.767
	Yes	8 (80)	2 (20)	0.8 (0.2–2.9)	
Headache	No	133 (74.7)	45 (25.3)	1	0.263
	Yes	19 (86.4)	3 (13.6)	0.6 (0.2–1.5)	
Myalgia	No	109 (74.2)	38 (25.8)	1	0.321
	Yes	43 (81.2)	10 (18.8)	0.7 (0.4–1.3)	
Sore throat	No	146 (75.2)	48 (24.7)	–	–
	Yes	6 (100)	0		

The bold values are indicates that the p-values are statistically significant.
[a] RR-relative risk.

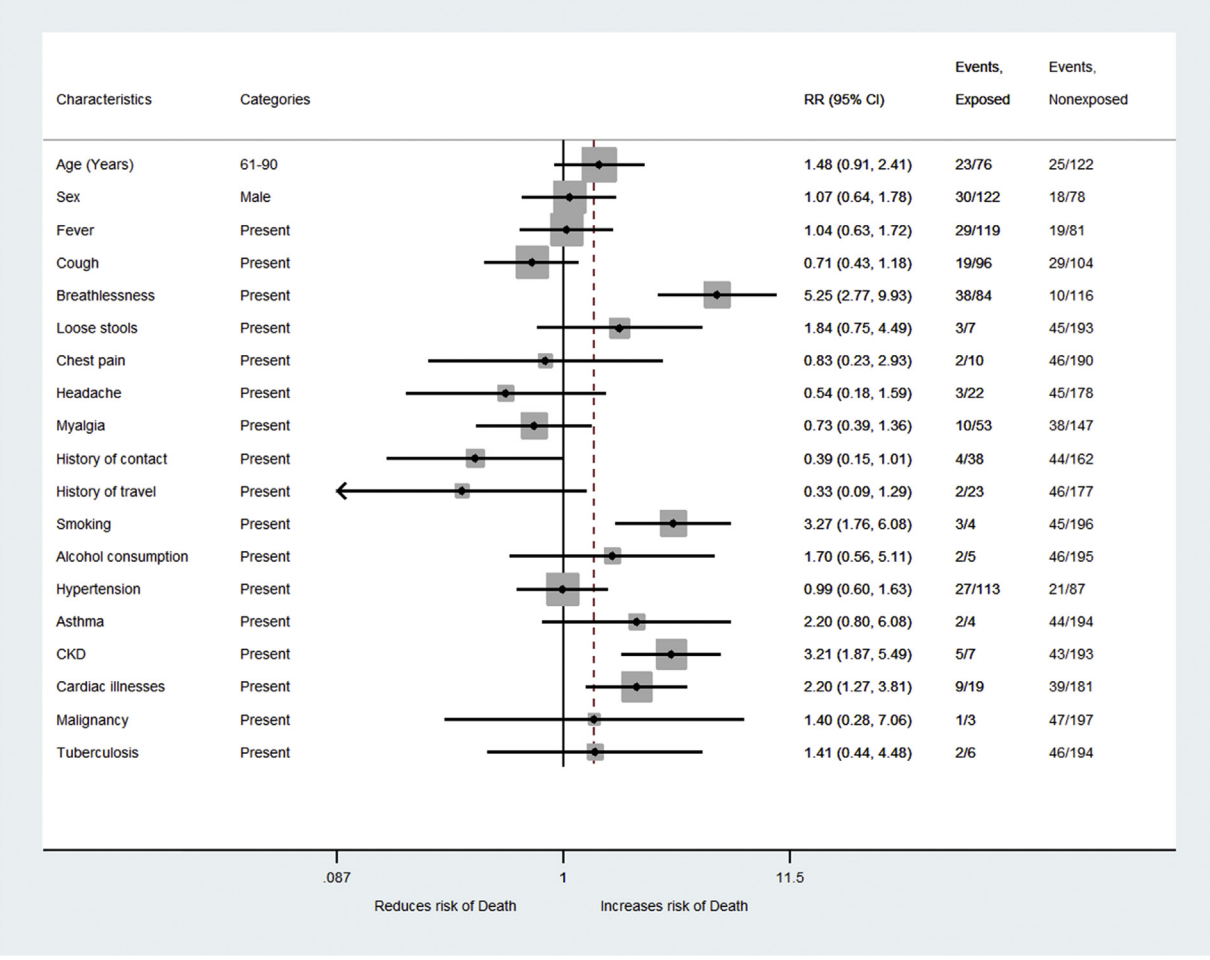

Fig. 2 – Forest plot of univariate analysis of risk factors for mortality among COVID-19 patients with diabetes.

Table 2 – Association of demographic and clinical characteristics with mortality among diabetes patients with COVID-19 admitted to the tertiary care center, N = 200.

Factor	Categories	Discharged n = 152 Frequency (%)	Died n = 48 Frequency (%)	RR (95% CI)[a]	p-value
History of contact	No	118 (72.9)	44 (27.1)	1	0.053
	Yes	34 (89.5)	4 (10.5)	0.4 (0.1–1.0)	
History of travel	No	131 (74)	46 (26)	1	0.111
	Yes	21 (91.3)	2 (8.7)	0.3 (0.1–1.3)	
History of smoking	No	151 (77)	45 (23)	1	**<0.001**
	Yes	1 (25)	3 (75)	3.3 (1.7–6.1)	
History of alcohol consumption	No	149 (76.4)	46 (2.6)	1	0.348
	Yes	3 (60)	2 (40)	1.7 (0.5–5.1)	
Pregnancy	No	149 (75.6)	48 (24.4)	–	–
	Yes	3 (100)	0		
Hypertension	No	66 (75.9)	21 (24.1)	1	0.968
	Yes	86 (76.1)	27 (23.9)	0.9 (0.6–1.6)	
Respiratory disease	No	150 (77.3)	44 (22.7)	1	0.126
	COPD[b]	0	2 (100)	–	
	Asthma	2 (50)	2 (50)	2.2 (0.8–6.0)	
Tuberculosis	No	148 (76.3)	46 (23.7)	1	0.565
	Yes	4 (66.6)	2 (33.4)	1.4 (0.4–4.4)	
CKD[c]	No	150 (77.7)	43 (22.3)	1	**<0.001**
	Yes	2 (28.6)	5 (71.4)	3.2 (1.8–5.4)	
Cardiac illnesses	No	142 (78.5)	39 (21.5)	1	**0.005**
	Yes	10 (52.6)	9 (47.4)	2.1 (1.2–3.8)	
Malignancy	No	150 (76.2)	47 (23.8)	1	0.686
	Yes	2 (66.6)	1 (33.4)	1.4 (0.3–7.0)	
Hypothyroidism	No	138 (74.2)	48 (25.8)	–	–
	Yes	14 (100)	0		

The bold values are indicates that the p-values are statistically significant.
[a] RR-relative risk.
[b] COPD-Chronic Obstructive Pulmonary Disease.
[c] Chronic Kidney Disease.

Table 3 – Multivariate analysis of clinical characteristics with mortality among diabetes patients with COVID-19 admitted to the tertiary care center, N = 200.

Characteristics	Categories	RR (95% CI)[a]	aRR (95% CI)[b]	p-value
Breathlessness	No	1	1	**<0.001**
	Yes	5.2 (2.7–9.9)	4.5 (2.3–8.8)	
History of smoking	No	1	1	**<0.001**
	Yes	3.3 (1.7–6.1)	1.9 (1.1–3.8)	
CKD[c]	No	1	1	**<0.001**
	Yes	3.2 (1.8–5.4)	1.8 (1.1–2.8)	
Cardiac illnesses	No	1	1	**0.005**
	Yes	2.1 (1.2–3.8)	1.6 (0.9–2.7)	

The bold values are indicates that the p-values are statistically significant.
[a] RR-relative risk.
[b] aRR-adjusted relative risk.
[c] Chronic Kidney Disease.

associated with mortality due to COVID among diabetes patients. A systematic review and meta-analysis by Galbadage T et al showed that gender was significantly associated with mortality with the male sex having a high risk of death due to COVID-19.[16] But the current study depicted that among COVID-19 patients with diabetes as comorbidity, gender is not significantly associated with mortality [Fig. 1]. This result is similar to another study done in the UK which also showed that gender was not significantly associated with mortality among diabetes patients with COVID-19.[17]

Our study showed that patients presenting with breathlessness had 4 times increased risk of mortality even after adjusting for other confounding variables which similar to findings among non-diabetic COVID-19 patients.[18–20] Smoking increased the risk of death by 1.9 times among COVID-19 patients with diabetes which is similar to other studies as evinced from a systematic review and meta-analysis by Salah HM et al.[17] Smoking increases the expression of Angiotensin-Converting Enzyme-2 (ACE2) which is also linked to the effects of COVID-19.[21] Smoking and COVID-19 cause endothelial

injury, hypercoagulable state, and disturbed immune system which explain the increased risk of mortality among COVID-19 patients.[22,23]

The current study showed that having the additional co-morbidities like cardiac diseases and CKD along with diabetes increased the risk of mortality among COVID-19 patients by 1.6 times and 1.8 times respectively. ACE2 dependent pathway in the kidney is affected by the SARS-CoV-2 virus leading to acute kidney injury and death which might be the reason for the increased mortality among COVID-19 patients with CKD.[24] The effects of the SARS-CoV-2 virus on the cardiovascular system can be explained in many aspects. It anchors on the transmembrane ACE2 to enter the host cells including type 2 pneumocytes, macrophages, endothelial cells, pericytes, and cardiac myocytes.[25] The virus can also destabilize atherosclerotic plaques which lead to the development of acute coronary syndromes.[25] The above mechanisms lead to inflammation, multi-organ failure, and death.

4.1. *Strengths and limitations*

Our study has a few strengths. We analyzed the data from a large number of COVID-19 patients with diabetes with appropriate statistical analysis. Our study has few limitations. This is a record-based study from a tertiary hospital, so the generalizability of the study results needs to be done with caution. The duration of symptoms at the time of presenting in the hospital was not studied which describes the health-seeking behavior. The control blood glucose among diabetes patients which might affect mortality was not included in the study. Nevertheless, published literature based on original studies on diabetes and COVID-19 is very limited in India and our study provides valuable evidence on the clinical profile and risk factors for COVID-19 mortality among diabetes patients from India.

5. Conclusion

Almost 1/5th of COVID-19 patients admitted to the hospital had comorbidity diabetes. Among COVID-19 patients with diabetes, those who presented with breathlessness, comorbidities like CKD and cardiac illness, positive history of smoking were significantly associated with mortality. Early identification of these risk factors and their appropriate management is crucial to prevent mortality among COVID-19 patients. Diabetes patients with COVID-19 can be given additional care with prompt monitoring of the symptoms especially breathlessness. Public health campaigns and health education activities to control smoking is needed to reduce the COVID-19 mortality in diabetes patients. The post-COVID-19 sequelae among diabetes patients need to be assessed with cohort studies with a longer follow-up period.

Conflicts of interest

The authors have none to declare.

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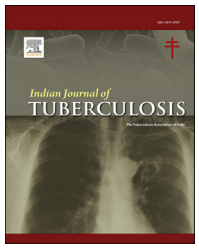

Original article

Identification of novel anti-tuberculosis agent: An in silico investigation

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ABSTRACT

Background: Multi-drug resistance tuberculosis is chronic and highly affected to mankind. Millions of people are affected by tuberculosis and lost their lives every year. *Mycobacterium tuberculosis* is resistant to the most commonly used anti-TB drugs, hence new drugs need to be developed in a short time. In this direction, many chemical compounds including benzimidazole derivatives have been identified as potent anti-tb agents.

Method: Various benzimidazole derivatives were subjected to in-silico computational screening to identify the potent anti-tubercular analogues. The ADME pharmacokinetics evaluation was performed to identify the drug-like molecules. Molecular docking investigation of selected compounds was performed against *Mycobacterium Tuberculosis* Enoyl Reductase (Inha) with PDB ID: 2B37, 1QG6, 4TZK, and 4TZK. The common pharmacophore hypothesis was generated using the molecular docking post-processing module.

Result: The result of ADME pharmacokinetics of some compounds is very close to the drug-like properties and can be developed as good inhibitors. Molecular docking study suggests that the proposed benzimidazole and 4*H*-pyran derivative have better binding affinity than standard and triclosan derivatives. Results from the pharmacophore hypothesis development study also support and suggest our prediction regarding the minimum pharmacophore features required in ligands to behave as a *Mycobacterium Tuberculosis* inhibitor.

Conclusion: Coumarin, phenylurea clubbed benzimidazole moiety and pyrano[2,3-c]pyrazole derivatives have shown greater selectivity and potency towards *Mycobacterium Tuberculosis*. By employing a combination of ADME, docking, and pharmacophore study calculations, novel potent hits to inhibit enoyl-acp reductase were identified with the points for consideration for designing of enoyl-acp reductase inhibitor.

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1. Introduction

Tuberculosis is one of the most dangerous death-threatening infectious diseases in the world for mankind. In the last few decades, the emergence of Drug-resistant tuberculosis has threatened all the improvements that have been made in TB control worldwide. According to the 2020 World Health Organization report, there were 10.4 million new TB cases in 2019 and 1.8 million deaths which include 0.3 million were co-infected with HIV.[1] Existing treatment for tuberculosis decrease drug compliance and carries significant side effects which leads to the emergence of multi-drug resistant (MDR) TB globally.[2] Hence, there is an urgent need for exploring other new therapeutic agents that carry better compliance, shorten the treatment, and are equally effective against TB. Despite this challenge, it is necessary to understand that numerous candidate molecules show potent anti-mycobacterial activity. The molecular studies of these compounds can pave milestones evaluating their potency which can be further explored using *in-vitro* and *in-vivo* studies.[3]

In this direction, a variety of new heterocyclic molecules are being synthesized to check the probable effectiveness of the molecules in fighting against TB. Researchers found that INH derivatives, or derivatives of other families of active compounds such as benzimidazole show potent activity against TB.[4,5] Benzimidazole enhances the good antitubercular activity comparable with INH and that would retain their activity against a panel of INH resistant strains.[5–7] Due to the priceless significance of benzimidazole as a lead compound, making it a promising starting point for the discovery of new anti-TB drugs.[8,9]

However, in the past, it is not easy to discover new drugs in a short time that act as an existing drug. The process of drug discovery may take 10–20 years, which include the synthesis of a number of a chemical entity which screened for several pharmacological and pharmacokinetic studies. Nowadays, computational methods played an important role in the drug discovery process during the last 10 years. Docking study is a part of rational drug design and used in the prediction of the binding of ligands or drug candidates with the receptor, as well as in the prediction of the activity and affinity of the molecules.[10] In recent decades, the pharmaceutical industry has preferred more traditional target advances in ligand- and receptor-based computational methods to improve ligand-binding affinity at a substrate-competitive site. This structure-based approach is thought to significantly reduce the time and cost of hit-to-lead and lead-to-drug development by reducing the number of compounds that need to be synthesized.[11,12]

The antibiotics isoniazid, rifampicin, Pyrazinamide, and ethambutol is the milestone agent against TB, have been used for decades as frontline drugs to treat tuberculosis infections. However, existing treatment strategies are not acceptable due to the rise of multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB bacteria and it poses a serious threat in the world.[13,14] Both Isoniazid and pyrazinamide inhibit the mycolic acid biosynthesis, rifampicin inhibits DNA-dependent RNA polymerase and ethambutol inhibits arabinosyltransferase in the cell wall of bacteria. There are numerous potential anti-tuberculosis drug targets which of them, and evidence has proven that the enoyl reductase (InhA) in the type II fatty acid biosynthesis pathway is a target for INH.[15,16] EmbC is a target for the front-line antibiotic EMB, the enzyme responsible for arabinan chain elongation in LAM synthesis.[17] Pyrazinamide is highly active against the persisting tubercle bacilli at an acidic pH due to this activity it plays a key role in shortening the duration of anti-tuberculosis treatment.[18] The prodrug PZA is metabolized into its active form, pyrazinoic acid (POA) by the amidase activity of each target of the *Mycobacterium tuberculosis* nicotinamidase/pyrazinamidase.[19] Moreover, the common mechanism for all antibacterially active rifamycins includes Rifampicin, the inhibition of DNA-dependent RNA polymerase, leading to an elimination of RNA synthesis and cell death by targeting the protein in bacteria.[20]

With regards to the above-mentioned molecular targets of drugs, we have carryout the docking study of designed compounds using the frontline standard drug as reference ligand to find out the binding affinity as well as a novel mode of action through their interactions. The possibility of inhibition activity shown by PDB bound with existing first-line inhibitors leads us to design the novel derivatives as probable anti-tuberculosis moieties by selecting the target for a small group of designed inhibitors. The objective of the present work was to design novel anti-TB derivatives by generating key interaction sites, ADMET properties, receptor-based pharmacophore, and molecular dynamics to generate its analogues leading to better inhibitors for the deadly disease Tuberculosis.

2. Materials and methods

2.1. Materials

For the computation of designed compounds, Chem3DUltra 12.0, Marvin suite, and Schrodinger software (Schrodinger, LLC, New York, NY, 2018) were used.

2.2. Target and ligand selection

The ligand selection was based on the potent activity of benzimidazole moiety against bacteria of TB. Many researchers found that benzimidazole has an affinity to kill tuberculosis bacteria.[5–7] With this knowledge, we have designed a number of benzimidazole derivatives and sketched them in the Marvin suite. The receptor was selected based on the target of existing first-line anti-tuberculosis agents. There are four different anti-tuberculosis drugs in the first-line and each drug has a different target in TB bacteria. We have differentiated receptors for each drug target based on the ligand which already available in the form of the crystal structure of the protein. The 3-D structures of *Mycobacterium Tuberculosis* Enoyl Reductase (Inha) were retrieved with PDB ID: 2B37, 1QG6, 4TZK, and 4TZK with resolution 2.6 Å, 1.9 Å, 1.62 Å, and 2.0 Å respectively from the RCSB protein data bank. The downloaded protein 2B37 having 5-octyl-2-phenoxyphenol as an inhibitor for each chain and NAD co-factor for all the proteins. Protein 1QG6 having NAD and TCL as an inhibitor. Protein 4TZK having one chain (A), complexes with 1-cyclohexyl-n-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide Whereas, protein 4TZT has an

n-(3-chloro-2-methylphenyl)-1-cyclohexyl- 5-oxopyrrolidine-3-carboxamide as inhibitor.

2.3. ADME study

Most of the new drug candidates reject in clinical studies because of poor ADMET properties. Therefore, good ADMET property is an important aspect of drug discovery to avoid the elimination of compounds in clinical studies. So in this direction, we have performed the predicted absorption, distribution, metabolism, excretion (ADME) properties of all the compounds using discovery studio 2.0. ADME properties include aqueous solubility, blood–brain barrier (BBB), plasma protein binding (PPB), absorption, hepatotoxicity, and cytochrome P450 CYP2D 6_Probability enzyme inhibition study.

2.4. Docking methodology

Molecular docking is a structure-based drug design approach that has become an integral part of drug discovery imparting knowledge on thermodynamic interactions, binding affinities, and binding modes of the enzyme-inhibitor complex. In the other words, molecular docking refers to the complex process of using the information accommodate in the three-dimensional structure of a macromolecular target and of correlated ligand–target complexes to design novel drugs for significant human diseases.

2.4.1. *Ligand preparation*

The 3D structures of the main moiety (ligands) were sketched with the chem draw and Marvin *suite* and saved as an *SDF* file. The main moiety was subjected to develop a library of four different series in the enumeration module in Schrodinger software. Developed a library of compounds that contain more than 20,000 compounds in each series. Subsequently, the ligand was optimized using the *LigPrep* module in Maestro, which performs the addition of missed hydrogens, correction of chirality's and ionization states, adjusting realistic bond lengths and angles by generating different possible structural conformation of ligands and it creates a library of 32 conformations for each ligand set. The OPLS-2005 force-field was used to assign the partial atomic charges. Finally, each of these structures was subjected to energy minimization until their average RMSD reached 0.001 Å and the resulting structures were then used for carrying out a docking study. (Structure and Lipinski rule of five of designed compounds are shown in Supplementary material).

2.4.2. *Glide*

The *Glide* (Grid-Based Ligand Docking with Energetics) program of Schrödinger molecular modeling suite to gauge the binding affinity of the. Glide performs the exhaustive search of enzyme-inhibitor interactions and identification of the possible binding site of the macromolecular targets. Receptor-grid files were generated after preparing correct forms of

Table 1 – ADME prediction of Set 1.

Compound	MW (130 –725)	Percent human oral absorption (>80% – high & <25% – poor)	QPlog BB (–3.0 –1.5)	QPlog HERG (below –5)	QPPCaco (<25 poor, >500 great)	QPlog Khsa (–1.5 –1.5)	PSA (70 –200 Å)	QPlog S (–6.5 –5)
1a	490.4032	41.778	–2.431	–4.041	118.434	–0.348	168.927	–3.514
1b	440.848	41.778	–2.431	–4.041	118.434	–0.348	168.927	–3.514
1c	459.291	41.778	–2.431	–4.041	118.434	–0.348	168.927	–3.514
1d	424.3964	41.778	–2.431	–4.041	118.434	–0.348	168.927	–3.514
1e	503.745	38.251	–3.591	–4.317	62.456	–0.768	224.524	–4.074
1f	435.404	45.839	–3.205	–4.99	35.259	–0.364	201.929	–5.055
1g	465.43	45.839	–3.205	–4.99	35.259	–0.364	201.929	–5.055
1h	450.419	31.286	–2.33	–4.174	68.434	–0.288	168.927	–4.078
1i	440.848	31.286	–2.33	–4.174	68.434	–0.288	168.927	–4.078
1j	450.459	51.035	–3.759	–4.878	33.259	–0.441	218.404	–4.365
1k	408.3974	26.255	–2.926	–4.428	45.137	–0.721	195.708	–4.52
1l	420.433	26.255	–2.926	–4.428	45.137	–0.721	195.708	–4.52
1m	420.433	32.501	–4.087	–4.748	102.001	–1.02	244.215	–3.284
1n	514.3	37.863	–3.268	–4.684	56.148	–0.212	201.929	–4.63
1o	449.431	37.863	–3.268	–4.684	56.148	–0.212	201.929	–4.63
1p	435.404	57.662	–4.296	–5.088	31.209	–0.649	229.044	–5.113
1q	404.434	34.018	–2.152	–4.156	119.469	–0.232	167.542	–4.472
1r	406.406	29.072	–2.17	–4.388	215.769	–0.451	168.927	–4.371
1s	469.846	29.072	–2.17	–4.388	215.769	–0.451	168.927	–4.371
1t	450.459	61.011	–3.869	–4.68	211.701	–0.95	229.285	–2.978
1u	434.46	33.209	–2.491	–4.344	318.737	–0.045	168.927	–4.622
1v	480.401	33.209	–2.491	–4.344	318.737	–0.045	168.927	–4.622
1w	469.303	26.864	–2.734	–4.439	77.761	–0.729	189.138	–3.467
1x	439.864	58.301	–3.774	–4.53	111.673	–0.981	217.622	–3.575
1y	435.448	34.927	–3.223	–4.455	342.984	–0.933	211.539	–3.047

Table 2 – ADME prediction of Set 2.

Compound	MW (130 –725)	Percent human oral absorption (>80% – high & <25% – poor)	QPlog BB (–3.0 –1.5)	QPlog HERG (below –5)	QPPCaco (<25 poor, >500 great)	QPlog Khsa (–1.5 –1.5)	PSA (70 –200 Å)	QPlog S (–6.5 –5)
2a	712.5324	29.921	–3.742	–5.513	221.327	0.602	217.582	–8.556
2b	423.27	66.514	–1.415	–4.93	219.391	0.179	135.114	–6.968
2c	438.241	82.118	–0.693	–5.311	584.372	0.601	107.29	–8.672
2d	389.371	61.973	–1.518	–4.886	114.698	0.305	149.901	–7.261
2e	413.258	72.213	–1.088	–5.025	160.674	0.948	119.938	–8.772
2f	457.712	52.137	–1.468	–5.231	175.896	0.923	152.175	–9.013
2g	411.2344	29.159	–3.449	–5.448	32.415	0.718	218.422	–8.785
2h	437.297	55.051	–1.996	–5.363	138.609	0.609	161.244	–8.663
2i	419.397	46.448	–2.58	–5.434	99.212	1.015	191.821	–9.65
2j	409.243	83.933	–0.574	–5.307	722.345	0.579	107.156	–8.566
2k	408.259	35.937	–2.919	–5.218	85.342	0.748	199.273	–8.541
2l	448.479	37.321	–2.743	–5.432	76.988	0.692	190.957	–8.473
2m	389.371	68.218	–1.424	–4.768	143.417	0.668	147.049	–7.731
2n	427.285	54.944	–1.646	–5.618	92.395	0.947	136.49	–9.622
2o	457.712	35.089	–4.486	–5.46	80.275	0.549	255.106	–8.409
2p	390.399	64.083	–1.485	–5.05	102.061	0.663	146.245	–8.173
2q	419.397	73.147	–0.551	–4.582	924.108	0.674	113.108	–7.495
2r	380.788	51.667	–2.008	–4.826	47.709	0.122	169.885	–6.805
2s	376.3914	67.656	–1.427	–4.844	194.392	0.341	154.072	–7.278
2t	359.389	25.711	–3.335	–5.366	61.655	0.778	227.896	–8.951
2u	364.789	28.972	–3.186	–5.283	53.843	0.29	224.848	–7.787
2v	423.27	21.353	–3.688	–5.331	111.218	0.695	234.936	–8.656
2w	359.389	40.573	–3.021	–5.499	86.471	0.891	199.658	–9.126
2x	360.373	36.367	–2.902	–5.362	96.388	0.66	198.567	–8.404
2y	468.267	35.874	–3.162	–5.459	97.252	0.392	187.863	–7.865

Table 3 – ADME prediction of Set 3.

Compound	MW (130 –725)	Percent human oral absorption (>80% – high & <25% – poor)	QPlog BB (–3.0 –1.5)	QPlog HERG (below –5)	QPPCaco (<25 poor, >500 great)	QPlog Khsa (–1.5 –1.5)	PSA (70 –200 Å)	QPlog S (–6.5 –5)
3a	435.3272	100	–1.012	–6.524	521.376	0.572	97.957	–6.752
3b	351.33	100	–1.012	–6.524	521.376	0.572	97.957	–6.752
3c	380.328	100	–0.502	–5.689	613.731	0.286	97.752	–5.852
3d	472.3842	100	–0.502	–5.689	613.731	0.286	97.752	–5.852
3e	406.83	100	–1.625	–7.896	277.013	0.721	124.237	–8.128
3f	432.44	100	–1.625	–7.896	277.013	0.721	124.237	–8.128
3g	466.295	100	–0.6	–4.291	547.847	0.564	108.337	–6.211
3h	421.841	100	–0.6	–4.291	547.847	0.564	108.337	–6.211
3i	448.395	100	–0.6	–4.291	547.847	0.564	108.337	–6.211
3j	463.3372	100	–0.6	–4.291	547.847	0.564	108.337	–6.211
3k	481.2372	100	–0.714	–4.159	414.53	0.555	108.328	–6.152
3l	386.7754	100	–0.714	–4.159	414.53	0.555	108.328	–6.152
3m	434.3874	100	–0.714	–4.159	414.53	0.555	108.328	–6.152
3n	415.453	100	–0.714	–4.159	414.53	0.555	108.328	–6.152
3o	485.281	100	–0.648	–4.271	522.509	0.557	108.382	–6.108
3p	401.426	100	–0.648	–4.271	522.509	0.557	108.382	–6.108
3q	455.3972	100	–0.648	–4.271	522.509	0.557	108.382	–6.108
3r	415.453	100	–0.648	–4.271	522.509	0.557	108.382	–6.108
3s	431.412	100	–0.749	–4.128	407.131	0.544	108.348	–6.042
3t	480.322	100	–0.749	–4.128	407.131	0.544	108.348	–6.042
3u	417.425	100	–0.749	–4.128	407.131	0.544	108.348	–6.042
3v	440.284	100	–0.749	–4.128	407.131	0.544	108.348	–6.042
3w	386.7754	100	–0.85	–4.072	230.913	0.832	110.805	–5.645
3x	394.355	100	–0.85	–4.072	230.913	0.832	110.805	–5.645
3y	365.357	100	–0.85	–4.072	230.913	0.832	110.805	–5.645

Table 4 – ADME prediction of Set 4.

Compound	MW (130 –725)	Percent human oral absorption (>80% – high & <25% – poor)	QPlog BB (–3.0 –1.5)	QPlog HERG (below –5)	QPPCaco (<25 poor, >500 great)	QPlog Khsa (–1.5 –1.5)	PSA (70 –200 Å)	QPlog S (–6.5 –5)
4a	818.089	80.265	–0.859	–5.691	885.881	0.373	128.918	–6.49
4b	543.51	80.453	–0.275	–6.117	3286.834	0.163	103.345	–7.104
4c	498.513	80.568	–0.32	–5.973	3272.554	0.239	97.89	–6.783
4d	500.957	80.309	–0.478	–5.403	2052.94	–0.472	100.633	–4.754
4e	500.5044	80.256	–0.974	–5.447	262.377	0.274	118.242	–6.466
4f	527.511	80.592	–0.935	–6.145	1226.374	–0.26	130.649	–7.271
4g	595.852	80.67	–0.375	–5.834	3281.308	0.436	96.944	–6.73
4h	733.998	80.366	–0.859	–5.436	285.289	0.041	118.174	–6.296
4i	531.927	80.397	–0.458	–5.25	2242.489	0.466	103.382	–6.568
4j	512.54	80.272	–0.856	–5.791	1330.355	–0.309	128.398	–6.556
4k	531.927	80.403	–1.081	–6.26	1124.337	–0.015	123.646	–7.534
4l	527.511	80.338	–0.886	–5.935	1454.948	–0.336	126.882	–6.61
4m	485.4934	80.447	–0.727	–6.211	831.948	0.226	125.031	–6.56
4n	484.5054	80.223	–1.022	–5.622	834.026	0.178	130.523	–6.207
4o	545.411	80.326	–0.881	–5.755	757.33	0.441	126.607	–6.801
4p	673.4934	80.579	–0.806	–5.403	256.898	0.308	115.958	–6.617
4q	542.482	80.662	–1.343	–5.788	665.654	0.384	152.421	–6.885
4r	555.609	80.322	–0.555	–6.155	2652.861	0.349	108.197	–7.305
4s	515.4754	80.611	–1.033	–5.532	687.551	0.304	122.471	–7.567
4t	494.569	80.348	–0.784	–5.988	1082.842	–0.199	120.827	–7.311
4u	576.381	80.327	–0.963	–5.868	693.715	0.502	127.726	–7.224
4v	527.511	80.343	–1.167	–5.986	746.597	0.257	134.332	–6.969
4w	536.387	80.376	–0.883	–6.008	819.35	0.232	131.223	–7.018
4x	496.541	80.623	–0.591	–5.592	782.421	1.244	119.966	–8.369
4y	484.486	80.264	–0.897	–6.047	1284.463	–0.309	119.71	–7.003

proteins and ligands using a receptor-grid generation program (maestro by Schrödinger). For grid generation potential of non-polar parts of the receptor was softened by scaling van der Waals radii of ligand atoms by 1.00 Å with a partial charge cut-off of 0.25. It is having all types of the option of speed vs accuracy. It is having three mode of docking, high-throughput

Table 5 – Docking energies of compounds of Set 1.

PDB Code	2B37		1QG6		4TZK		4TZT	
Compound	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*
1a	–8.873	–43.404	–10.317	–50.361	–11.239	–50.607	–9.917	–47.332
1b	–8.871	–51.63	–9.582	–48.885	–11.027	–52.369	–9.524	–45.376
1c	–8.400	–53.764	–9.518	–50.697	–10.975	–53.04	–9.514	–41.358
1d	–8.215	–52.997	–9.518	–50.697	–10.748	–45.533	–8.924	–50.815
1e	–8.215	–52.997	–9.272	–49.146	–10.748	–45.533	–8.921	–48.17
1f	–8.163	–40.581	–9.272	–49.146	–10.68	–52.561	–8.866	–53.282
1g	–8.163	–40.581	–8.832	–55.725	–10.408	–52.254	–8.695	–42.753
1h	–8.111	–46.872	–8.708	–53.951	–10.116	–53.177	–8.686	–53.856
1i	–8.058	–51.305	–8.428	–54.665	–10.116	–53.177	–8.636	–51.279
1j	–8.058	–51.305	–8.428	–54.665	–10.116	–53.177	–8.521	–53.832
1k	–8.04	–58.443	–8.097	–50.671	–10.116	–53.177	–8.521	–53.832
1l	–8.04	–58.443	–8.097	–50.671	–10.105	–42.3	–8.382	–46.732
1m	–7.84	–45.975	–8.069	–48.282	–10.095	–48.426	–8.343	–44.814
1n	–7.84	–45.975	–8.004	–54.457	–10.023	–42.651	–8.343	–44.814
1o	–7.835	–58.709	–7.927	–52.083	–9.89	–55.091	–8.269	–40.643
1p	–7.8	–50.958	–7.881	–48.595	–9.635	–59.34	–8.232	–38.552
1q	–7.799	–58.258	–7.881	–48.595	–9.542	–49.631	–8.232	–38.552
1r	–7.737	–50.411	–7.851	–52.624	–9.542	–49.631	–8.224	–54.121
1s	–7.736	–47.213	–7.851	–52.624	–9.473	–50.045	–8.088	–47.234
1t	–7.718	–50.701	–7.786	–46.452	–9.451	–47.578	–8.088	–47.234
1u	–7.718	–50.701	–7.739	–52.711	–9.368	–57.464	–8.013	–49.655
1v	–7.699	–59.148	–7.707	–46.607	–9.272	–49.335	–8.013	–49.655
1w	–7.592	–49.782	–7.677	–48.793	–9.238	–61.115	–7.984	–36.339
1x	–7.481	–48.244	–7.677	–48.793	–9.158	–42.096	–7.984	–36.339
1y	–7.423	–50.246	–7.645	–49.977	–9.095	–51.256	–7.953	–38.901

Table 6 – Docking energies of compounds of Set 2.

PDB Code	2B37		1QG6		4TZK		4TZT	
Compounds	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*
2a	−6.918	−49.323	−7.744	−53.927	−10.232	−52.803	−9.495	−53.195
2b	−6.659	−46.788	−7.687	−54.691	−9.999	−51.471	−9.31	−37.477
2c	−6.4	−45.118	−7.375	−50.266	−9.465	−54.447	−9.139	−41.58
2d	−6.317	−51.328	−7.365	−52.976	−9.443	−45.911	−8.877	−39.462
2e	−6.284	−46.628	−7.361	−53.37	−9.42	−42.033	−8.742	−32.202
2f	−6.219	−49.578	−7.171	−47.627	−9.335	−52.67	−8.639	−53.411
2g	−6.191	−42.324	−7.128	−49.522	−9.29	−48.338	−8.618	−49.237
2h	−6.142	−45.827	−7.12	−48.674	−9.174	−47.05	−8.45	−49.581
2i	−6.11	−47.03	−7.083	−51.113	−9.125	−36.981	−8.418	−42.077
2j	−6.098	−47.795	−7.052	−47.956	−9.124	−55.873	−8.349	−53.669
2k	−6.057	−43.315	−7.048	−50.368	−9.099	−52.735	−8.34	−50.533
2l	−6.043	−47.099	−7.046	−50.338	−8.909	−39.804	−8.335	−49.871
2m	−6.026	−47.448	−7.022	−51.425	−8.896	−54.768	−8.329	−42.909
2n	−6.013	−48.84	−6.971	−49.424	−8.864	−51.048	−8.329	−37.406
2o	−6.006	−48.121	−6.97	−50.131	−8.846	−49.402	−8.225	−42.672
2p	−6.002	−46.793	−6.937	−50.096	−8.835	−52.428	−8.112	−35.401
2q	−5.996	−50.188	−6.93	−48.464	−8.822	−54.387	−8.059	−41.529
2r	−5.981	−47.422	−6.914	−50.044	−8.815	−54.844	−8.015	−43.202
2s	−5.979	−49.398	−6.901	−50.292	−8.736	−51.694	−7.998	−37.431
2t	−5.976	−45.652	−6.847	−46.952	−8.736	−45.932	−7.967	−54.918
2u	−5.972	−48.088	−6.798	−50.466	−8.722	−56.607	−7.961	−40.257
2v	−5.967	−47.224	−6.796	−51.005	−8.66	−51.212	−7.919	−39.942
2w	−5.963	−46.905	−6.79	−49.867	−8.655	−49.296	−7.865	−52.436
2x	−5.945	−48.456	−6.784	−49.954	−8.635	−53.996	−7.77	−39.685
2y	−5.917	−49.959	−6.752	−51.287	−8.632	−47.362	−7.74	−38.218

Table 7 – Docking energies of compounds of set 3.

Code	2B37		1QG6		4TZK		4TZT	
Compounds	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*
3a	−8.97	−49.642	−9.011	−40.007	−11.795	−49.495	−11.549	−41.382
3b	−8.97	−49.642	−9.011	−40.007	−11.795	−49.495	−11.549	−41.382
3c	−8.97	−49.642	−8.396	−41.472	−11.795	−49.495	−11.313	−42.915
3d	−8.97	−49.642	−8.396	−41.472	−11.795	−49.495	−11.313	−42.915
3e	−8.933	−58.525	−8.396	−41.472	−11.549	−46.703	−11.089	−45.376
3f	−8.933	−58.525	−8.396	−41.472	−11.549	−46.703	−11.089	−45.376
3g	−8.933	−58.525	−7.609	−50.392	−11.533	−56.329	−10.976	−44.812
3h	−8.933	−58.525	−7.609	−50.392	−11.533	−56.329	−10.976	−44.812
3i	−8.884	−57.499	−7.609	−50.392	−11.239	−55.311	−10.976	−44.812
3j	−8.884	−57.499	−7.609	−50.392	−11.239	−55.311	−10.976	−44.812
3k	−8.884	−57.499	−7.573	−42.139	−11.239	−55.311	−10.322	−40.597
3l	−8.884	−57.499	−7.573	−42.139	−11.239	−55.311	−10.322	−40.597
3m	−8.65	−45.493	−7.548	−38.429	−10.866	−37.915	−10.322	−40.597
3n	−8.65	−45.493	−7.548	−38.429	−10.866	−37.915	−10.322	−40.597
3o	−8.646	−41.668	−7.548	−38.429	−10.865	−46.454	−10.25	−52.126
3p	−8.646	−41.668	−7.548	−38.429	−10.865	−46.454	−10.25	−52.126
3q	−8.646	−41.668	−7.393	−41.248	−10.865	−46.454	−10.25	−52.126
3r	−8.646	−41.668	−7.393	−41.248	−10.865	−46.454	−10.25	−52.126
3s	−8.505	−51.012	−7.358	−39.696	−10.865	−46.454	−9.834	−48.674
3t	−8.505	−51.012	−7.358	−39.696	−10.865	−46.454	−9.834	−48.674
3u	−8.505	−51.012	−7.358	−39.696	−10.865	−46.454	−9.834	−48.674
3v	−8.505	−51.012	−7.358	−39.696	−10.865	−46.454	−9.834	−48.674
3w	−8.505	−51.012	−7.333	−51.805	−10.776	−44.61	−9.808	−40.528
3x	−8.505	−51.012	−7.333	−51.805	−10.776	−44.61	−9.808	−40.528
3y	−8.505	−51.012	−7.333	−51.805	−10.776	−44.61	−9.808	−40.528

virtual screening (HTVS), standard precision (SP), and extra precision (XP) mode. The XP mode is used for exhaustive sampling and advanced scoring, resulting in even higher enhancement.

2.4.3. *Target and ligand preparation*

Each set of ligands were optimized using the LigPrep module in Schrodinger software by generating different possible structural conformation of ligands set and it creates a library

Table 8 – Docking energies of compounds of Set 4.

PDB Code	2B37		1QG6		4TZK		4TZT	
Compounds	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*	DS*	Gevdw*
4a	−9.222	−60.562	−8.857	−40.743	−9.026	−59.457	−8.116	−37.991
4b	−9.219	−59.537	−8.643	−52.496	−8.857	−52.616	−7.936	−51.989
4c	−8.963	−57.353	−8.443	−59.173	−8.594	−57.394	−7.914	−62.853
4d	−8.61	−51.523	−8.267	−62.666	−8.478	−51.812	−7.793	−59.241
4e	−8.59	−58.405	−7.975	−53.713	−8.411	−61.323	−7.765	−56.353
4f	−8.506	−62.948	−7.835	−32.359	−8.319	−60.166	−7.724	−53.471
4g	−8.446	−58.63	−7.701	−68.914	−8.099	−66.014	−7.654	−53.064
4h	−8.334	−61.783	−7.646	−58.077	−8.039	−46.218	−7.647	−58.353
4i	−8.317	−56.293	−7.598	−69.069	−8.006	−48.313	−7.563	−51.718
4j	−8.311	−55.75	−7.537	−51.579	−7.99	−59.531	−7.46	−48.056
4k	−8.281	−58.35	−7.48	−42.229	−7.937	−42.07	−7.409	−48.03
4l	−8.171	−61.308	−7.474	−56.149	−7.913	−41.423	−7.405	−53.51
4m	−8.111	−65.013	−7.376	−68.713	−7.855	−49.417	−7.34	−61.478
4n	−8.054	−56.3	−7.296	−51.443	−7.805	−67.5	−7.118	−56.633
4o	−8.019	−52.772	−7.145	−57.387	−7.802	−63.939	−7.045	−52.979
4p	−7.95	−58.207	−7.053	−54.721	−7.762	−55.189	−7.034	−50.503
4q	−7.872	−60.68	−7.039	−69.785	−7.705	−40.958	−7.018	−49.104
4r	−7.847	−55.492	−7.035	−67.037	−7.677	−53.365	−6.978	−45.449
4s	−7.825	−56.846	−7.034	−65.781	−7.672	−48.962	−6.942	−43.929
4t	−7.812	−60.968	−6.974	−59.066	−7.645	−54.195	−6.893	−50.922
4u	−7.785	−58.092	−6.968	−60.601	−7.412	−54.112	−6.87	−52.686
4v	−7.713	−55.296	−6.918	−57.178	−7.398	−38.485	−6.668	−54.501
4w	−7.71	−57.462	−6.894	−57.52	−7.397	−48.732	−6.613	−44.593
4x	−7.668	−57.767	−6.89	−62.75	−7.393	−55.433	−6.61	−43.671
4y	−7.666	−46.419	−6.878	−27.967	−7.353	−54.043	−6.553	−41.485

of more than 20,000 ligand set. The ligands were first screened for ADME study using QuikPro then subjected to docking study. The target protein was prepared by deleting surrounding water molecules, bond cofactor, inhibitors together with the addition of side-chain atoms followed by energy minimization and optimization protein.

2.4.4. *Protein preparation and receptor grid generation*

The protein complex was refined for docking, using the *Protein Preparation Wizard in Glide* which involved assigning the correct bond orders, the addition of missing hydrogens corresponding to pH 7.0 (considering the ionization states for the acidic as well as basic amino acid residues), removal of the crystallographically observed water and assigning the correct charge and protonation state of the protein structure. Finally, the Optimized Potentials for Liquid Simulations-2005 (OPLS-2005) force field were used for the energy minimization of protein until the average RMSD of the non-hydrogen atoms reached 0.3 Å in order to relieve the steric mismatch between the residues due to the addition of hydrogen atoms. The receptor grid file was generated using prepared protein as an input file, the grid file was used as an input file for docking study.

2.4.5. *Docking study*

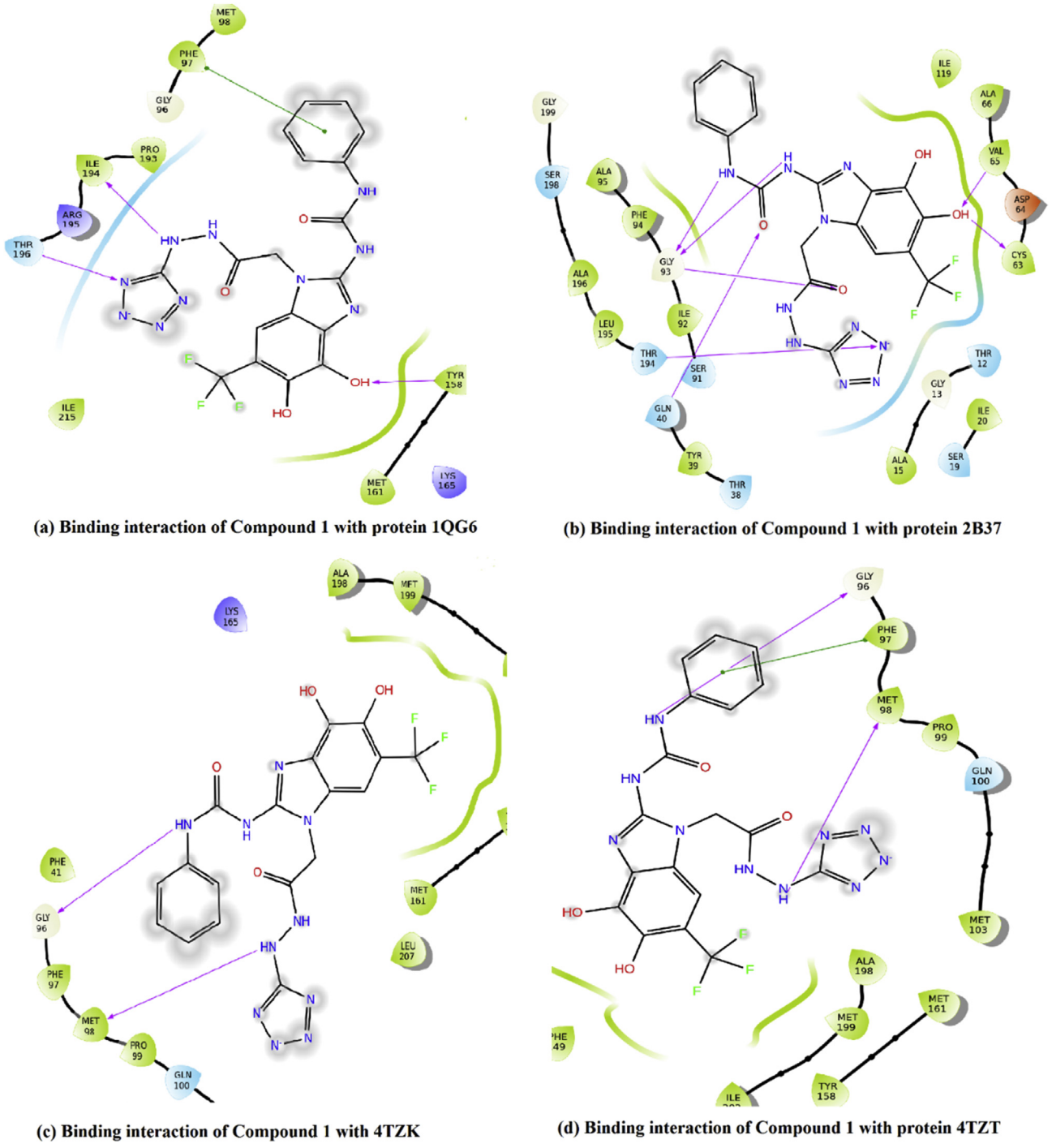
The molecular docking study was performed by using *Ligdock* in glide, Maestro (Schrödinger suite) to find out the binding affinity of the designed compounds towards selected protein to expand the knowledge of their activity against mycobacterium tuberculosis. The XP mode was used for allowing flexible torsions in ligands for performing docking studies, highly robust and very accurate. The parameter selected for the docking run was default and a model energy function named Glide score (Gscore) is used which combines force field and empirical terms for selecting the best docking pose, generated as output. The output files were visualized and analyzed using the Pose Viewer function in *Maestro* to lining the active site of the enzyme by the key thermodynamic elements of interaction with the residues. The validation of the docking protocol and the parameters set for calculation were done by re-docking the native ligands into the active site of proteins.

$$\text{Gscore} = a^{*}\,\text{vdW} + b^{*}\,\text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

Where, vdW is van der Wall energy; Coul is Coulomb energy; Lipo is lipophilic interaction; H-bond signifies hydrogen-bonding; Metal is metal-binding term; BuryP is forgotten polar groups; RotB represents freezing rotatable bonds; Site is polar interactions at the active site; and a, b is the coefficients of vdW and Coul.

2.5. *Pharmacophore hypothesis generation*

Pharmacophore is the hybrid approach of ligand and structure-based technique which uses docking energy score for finding the biologically active part of ligands against the enzyme. We have generated a ligand-based pharmacophore by superposing existing drugs as reference active molecules and extracting common chemical features that are significant for their biological activity.



(a) Binding interaction of Compound 1 with protein 1QG6

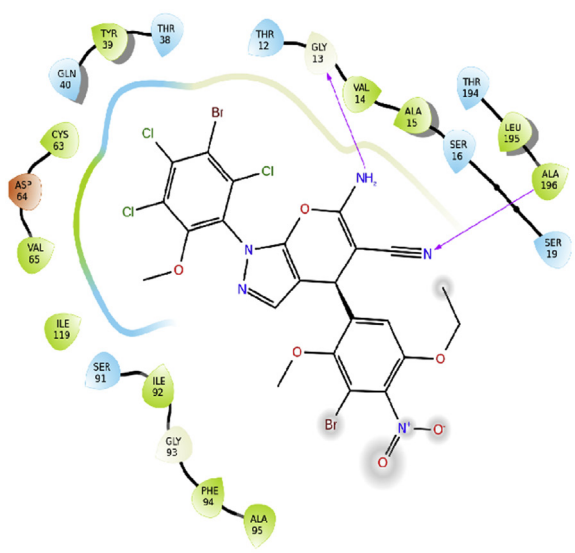
(b) Binding interaction of Compound 1 with protein 2B37

(c) Binding interaction of Compound 1 with 4TZK

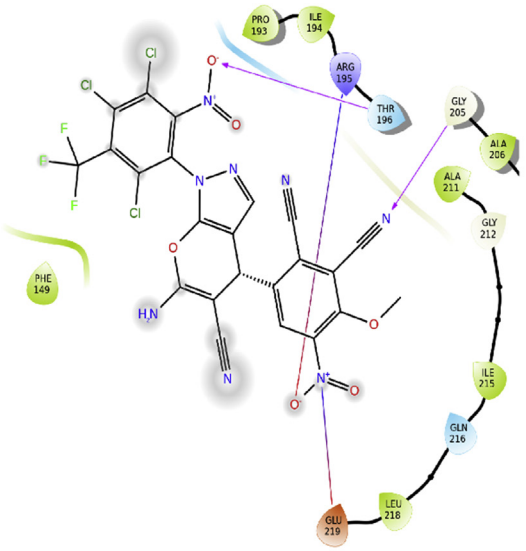
(d) Binding interaction of Compound 1 with protein 4TZT

Fig. 1 – Biding interactions of compounds 1a of set 1 with the receptors.

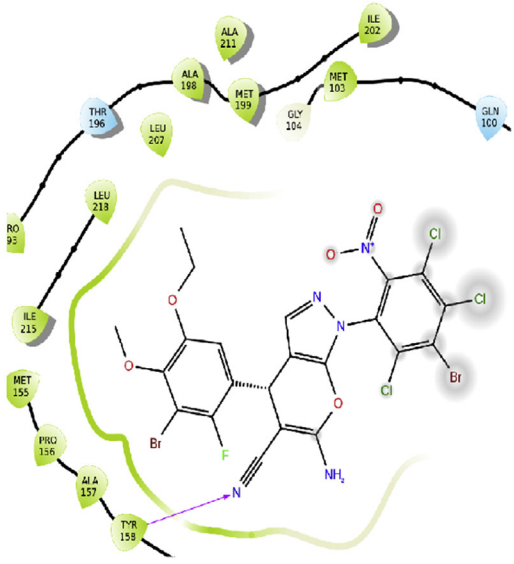
The pharmacophore hypothesis was generated by docking post-processing module of script option was selected and the input file is given in.xpdes format. Pharmacophore was generated by using all the default chemical features (hydrogen bond acceptor (A), hydrogen bond donor (D), aromatic ring (R), hydrophobe (H), positive ionizable (P), and negative ionizable (N)).[21] Common pharmacophore hypotheses were considered, which indicated five common sites to the selected molecule. Moreover, the best common pharmacophore hypothesis was selected based on the survival score, until at least one hypothesis was found and scored successfully. The common pharmacophore hypothesis was scored using default parameters for vector, site, selectivity, volume, energy terms, and a number of matches. The pharmacophore hypothesis was generated using the *PHASE* module in maestro (Schrodinger software) and all the parameters were set as default.



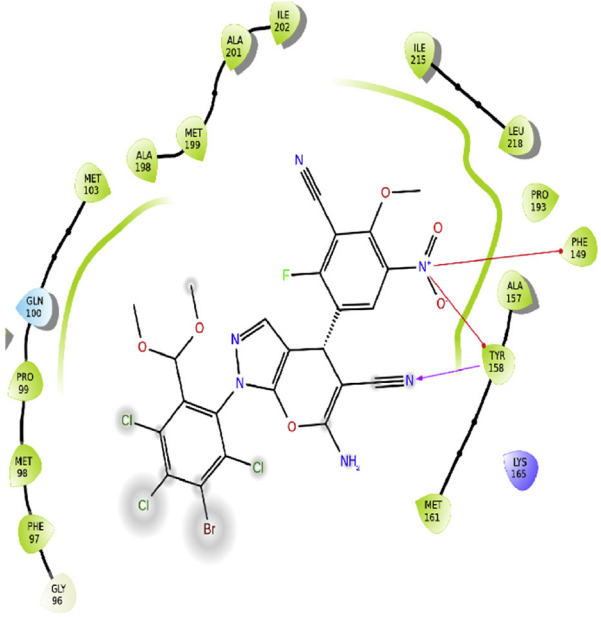
(a) Binding interaction of Compound 1 with protein 1QG6



(b) Binding interaction of Compound 1 with protein 2B37



(c) Binding interaction of Compound 1 with protein 4TZK



(d) Binding interaction of Compound 1 with protein 4TZT

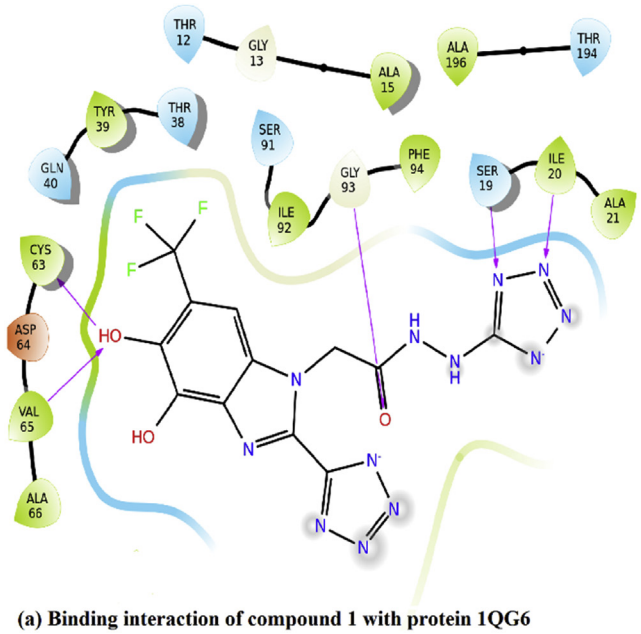
Fig. 2 – Biding interactions of compounds 2a of set 2 with the receptors.

3. Result and discussion

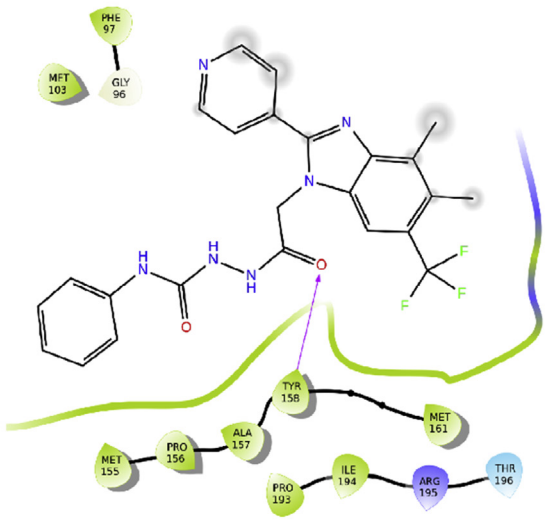
3.1. *ADME study*

The absorption, distribution, metabolism, and excretion study were predicted primarily for four different libraries of compounds. The predicted result of descriptor along with their range is given in Tables 1–4 for each set. Following principal descriptors are included in the study. Molecular weight (MW), Percent human oral absorption in GI, Brain/Blood QPlogBB, HERG K+ channel blockage: log IC_{50} (QPlog HERG), apparent Caco-2 permeability in nm/sec (QPPCaco), log Khsa serum protein binding (QPlogKhsa), van der Waals polar surface area (PSA) and aqueous solubility (QPlogS) were calculated.

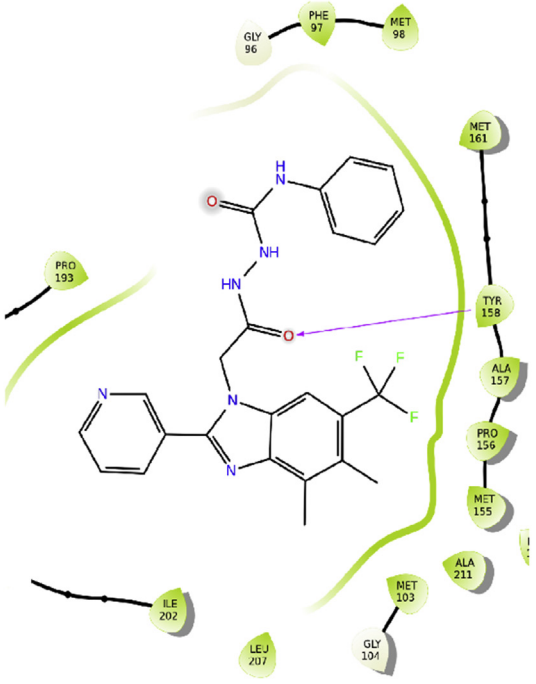
We have designed more than 50,000 derivatives for each set with the help of the enumeration module in maestro using 40 different inbuilt fragments. Here, we have selected only 25 compounds from each set that have excellent ADME properties or better than 95% of drugs property. However, the selected 25 derivatives of each set are based on the primary ADME prediction of the analogues and these compounds are further used for docking study to investigate the binding interaction between proteins and compounds. The molecular weight of compounds from set 1 (Table 1) shows that most of the compounds have acceptable molecular weight.



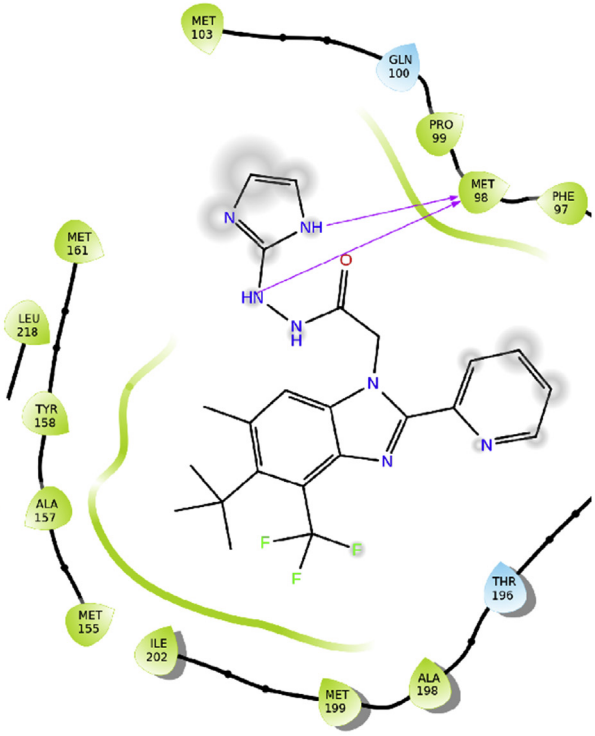
(a) Binding interaction of compound 1 with protein 1QG6



(b) Binding interaction of compound 1 with protein 2B37



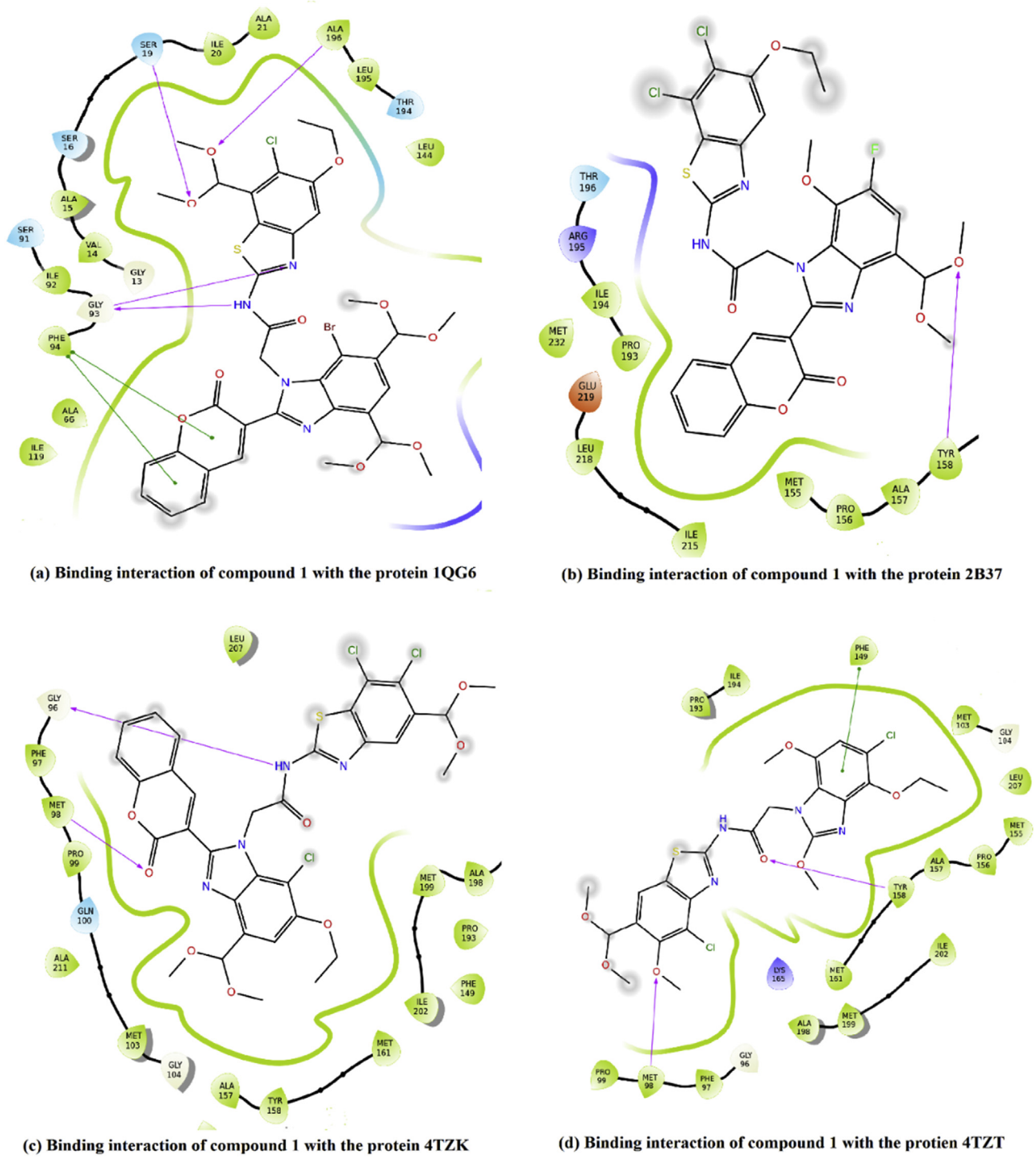
(c) Binding interaction of compound 1 with protein 4TZK



(d) Binding interaction of compound 1 with protein 4TZT

Fig. 3 – Biding interactions of compounds 3a of set 3 with the receptors.

The human oral absorption prediction is based on a quantitative linear progression model. Designed compounds from set 1 have good oral absorption in the gastro intestine. So it can be stated from the prediction of the oral absorption results that the compounds that have oral absorption between 25 and 80% can serve as a good qualitative model for human oral absorption. The log BB for blood/brain is found good in most of the compounds. However, few compounds have lower log BB values than the standard value. The log HERG (log IC_{50}) values of all the selected compounds are below the standard value (−5) which shows that the lower is HERG value, the laser is the blockage of K+ ion channels. For the



(a) Binding interaction of compound 1 with the protein 1QG6

(b) Binding interaction of compound 1 with the protein 2B37

(c) Binding interaction of compound 1 with the protein 4TZK

(d) Binding interaction of compound 1 with the protien 4TZT

Fig. 4 – Binding interactions of compounds from set 4 with the receptors.

prediction of non-active transport, the PCaco descriptor is used which has a value of more than 25. Here, the selected 25 compounds have an acceptable PCaco value of more than 50. The predicted log Khsa of the compounds is in the range which shows that the compounds have good protein binding affinity. Moreover, the van der Waals polar surface area and aqueous solubility of selected compounds are very good which shows that the compounds have good water solubility which is the primary need for oral medication. The polar surface area of few compounds is higher which indicated that these compounds can be poor at permeating cell membranes.

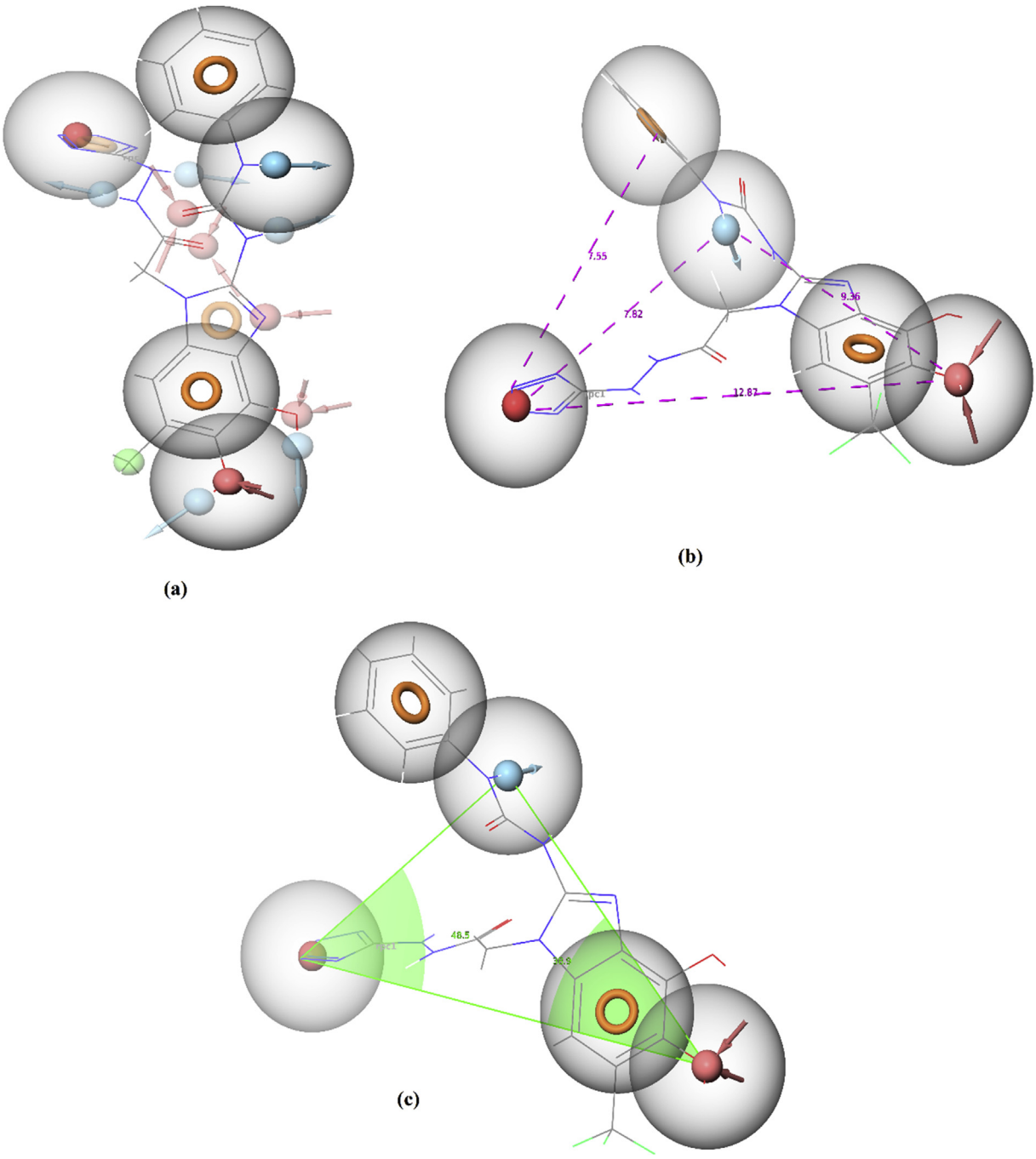


Fig. 5 – (a) Selected pharmacophore and generated pharmacophore of ligand 1a from set 1. (b) Pharmacophore hypothesis and distance between sites. (c) Generated angle between pharmacophore sites. All the distance and angle are in °A unit.

The molecular weight of compounds from the set has an acceptable range accept few compounds which have higher molecular weight but these compounds have good value in other descriptors. The percentage of human oral absorption of the selected compounds has value in the range. Compound 22 from set 2 (Table 2) has less than 25% of human oral absorption. All the compounds have good log BB values which are found active in the CNS system. Most of the compounds have an acceptable log HERG value. Most of the compounds have a higher than 500 PCaco value. All the selected compounds from set 2 have acceptable log Khsa, PSA, and log S values which indicate that these compounds which consider excellent drug-like compounds. The compounds from set 3 (Table 3) have favorable molecular weight and human oral absorption. The log BB value of set 3 is excellent than the standard value which is high in active CNS. The HERG drug binding of compounds is found in the batter. The PCaco value of compounds is found potent with an acceptable range and has good cell permeability. The polar surface area of the compounds is higher than 100 and the solubility of these compounds is less

Table 9 – Predicted pharmacophore result of ligand 1a from set 1 with different pharmacophore hypothesis.

Hypothesis	Survival score	Site score	Vector score	Volume Score	Selectivity score	Matched Ligand Sites	Fitness score
ADNRR	5.146	0.903	0.998	0.857	2.088	A(4) D(10) N(13) R(16) R(17)	2.757
ADNRR	5.134	0.887	0.999	0.857	2.091	A(4) D(10) N(13) R(14) R(17)	2.742
DDRRR	5.13	0.902	0.977	0.857	2.094	D(6) D(7) R(15) R(16) R(17)	3
AAANR	5.127	0.864	0.999	0.857	2.107	A(3) A(4) A(5) N(13) R(17)	2.719
DHNRR	5.124	0.798	0.998	0.857	2.171	D(10) H(12) N(13) R(16) R(17)	2.652
AHNRR	5.124	0.783	0.998	0.857	2.186	A(3) H(12) N(13) R(16) R(17)	2.638
AAHNR	5.123	0.786	0.999	0.857	2.18	A(3) A(4) H(12) N(13) R(17)	3
AAHNR	5.123	0.777	0.998	0.857	2.19	A(3) A(5) H(12) N(13) R(17)	3
AADNR	5.122	0.889	0.998	0.857	2.077	A(4) A(5) D(10) N(13) R(17)	3
AAHNR	5.122	0.776	0.997	0.857	2.192	A(3) A(5) H(12) N(13) R(16)	3
AANR	4.648	0.866	0.999	0.857	1.626	A(3) A(4) N(13) R(17)	2.722
ADNR	4.641	0.899	0.999	0.857	1.586	A(4) D(10) N(13) R(17)	3
AAAN	4.627	0.852	0.998	0.857	1.62	A(3) A(4) A(5) N(13)	2.706
AANR	4.624	0.863	0.998	0.857	1.605	A(1) A(4) N(13) R(17)	2.718
AANR	4.623	0.883	0.998	0.857	1.584	A(4) A(5) N(13) R(17)	2.738
AAAN	4.621	0.835	0.998	0.857	1.63	A(1) A(3) A(4) N(13)	2.69
AANR	4.612	0.848	0.998	0.857	1.608	A(3) A(5) N(13) R(17)	2.703
AAHN	4.608	0.753	0.998	0.857	1.7	A(3) A(5) H(12) N(13)	3
AAHN	4.603	0.763	0.999	0.857	1.683	A(3) A(4) H(12) N(13)	3
AAHN	4.601	0.743	0.998	0.857	1.703	A(1) A(3) H(12) N(13)	3

than −6 which can be stated as a significant range for drug-like compounds. Moreover, The PKhsa value of set 3 is less than 1.5 which is acceptable.

The selected compounds of set 4 (Table 4) also have good predicted ADME properties. The molecular weight of the compounds does not exceed the standard range. The human oral absorption in the gastro intestine of the compounds higher than 80% which indicates that the compounds will finely absorb in the intestine. The blood/brain barrier range of set 4 is below 1.5. The compounds have an acceptable range of log HERG prediction. The PCaco permeability of the compounds of set 4 is higher than 500 which is excellent for drug-like compounds. The aqueous solubility and polar surface area value of the compounds is in the range accept the aqueous solubility of the compound 2, 6, 11, 18, 19, 20, 21, 23, 24, and 25 is lower. The ADME or pharmacokinetic properties of the compounds are very important to serve as a drug or medicine. The observed result of principal descriptors and ADME from set 1, set 2, set 3, and set 4 are very close to the drug-like compounds and can be developed as good inhibitors. The compounds which have good ADME properties are further subjected to docking study to investigate the binding affinity of the compounds against enoyl acp reductase.

3.2. *Docking study*

In order to validate the novel function of benzimidazole and 4*H*-pyran derivatives, it was thought important to study the interaction between 25 selected designed molecules with the protein of *Mycobacterium Tuberculosis* enoyl acp reductase (Oxidoreductase) using computational docking methodology. The docking study was done on the known active site of four different proteins 2B37, 1QG6 4TZK, and 4TZT. This protein belongs to the H_{37}Rv strain of MTB which will help to identify the best molecule which may have good inhibitory action against Mycobacterium Tuberculosis. For this purpose, the Schrodinger Glide program was used and the designed molecules were docked the crystal structure of MTB enoyl acp reductase (PDB: 2B37, 1QG6, 4TZK, 4TZY). To validate docking results, know native ligands of the protein and standard drug of MTB were also docked in the binding site of the receptor. The docking analysis of the designed compounds was also done as four different sets and each set was docked into the four different proteins to identify the potent ligand. The docking results of the designed compounds are given in Tables 5–8 for each set with all the protein.

The docking score represents the ligand binding free energy with the receptor while glide van der Waals energy represents the attraction between molecule and receptor with either covalent or ionic bonding.

The docking result of set 1 suggests that these compounds have a potent binding affinity with all the proteins. Compound 1a have a significant docking score with the proteins 2B37, 1QG6, 4TZK, and 4TZT at −8.873, −10.317, −11.239, and −9.917, respectively. Moreover, the docking score is increased in the case of 2B37 and 4TZK. The rest of the compounds have also displayed potency against Mycobacterium Tuberculosis enoyl-acp reductase with interesting docking energies. The interaction of compound 1a with the proteins is given in Fig. 1. It was observed that the compound has bonded with the 1QG6 receptor with three hydrogens and one aromatic bond. The side chain (N'-(2H-tetrazol-5-yl)propionohydrazide) of the compound have two hydrogen bond with residue ILE 194 and THR 196 (Fig. 1a) while the substituted hydroxyl group on benzimidazole have bonded with one hydrogen bond with TYR 158 (Fig. 1a). The aromatic bond of the ligand is bonded with receptor PHE 97 (Fig. 1a). In the case of protein 2B37, we observed that there are seven hydrogen bonding interactions between ligand and receptor at −8.873 docking score. The sidechain of the ligand has two hydrogen bonds with residue GLY 93 (Fig. 1b) and THR 194 (Fig. 1b). The main benzimidazole moiety has five hydrogen bonds with the residue VAL 65, CYS 63, GLN 40 (Fig. 1b), and two hydrogen bonds with GLY 93 (Fig. 1b). Furthermore, the hydrogen bonding in 4TZK was

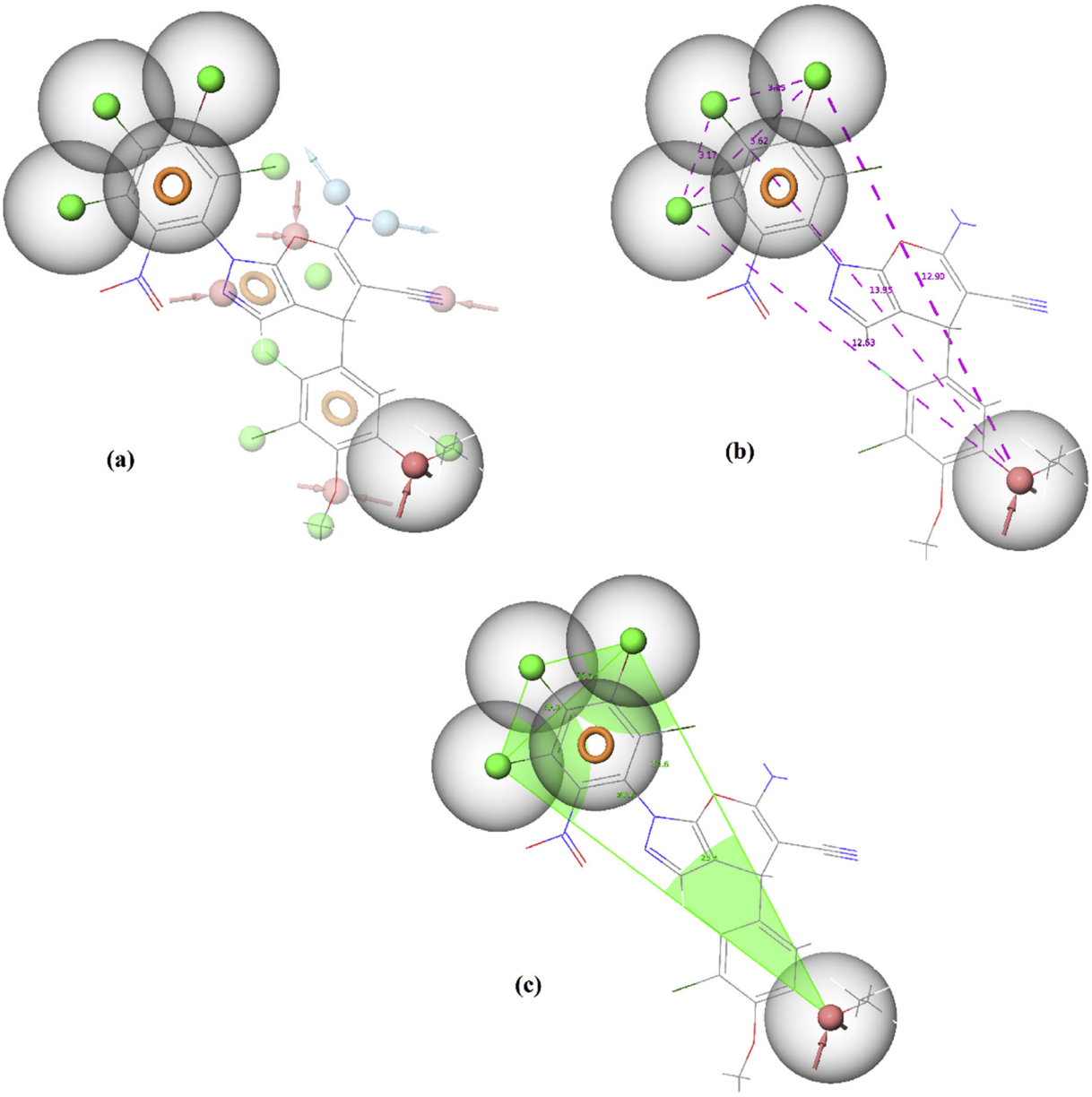


Fig. 6 – (a) Generated pharmacophore feature and selected pharmacophore feature of ligand 2a from set 2. (b) Pharmacophore hypothesis and distance between sites (c) angle between pharmacophore sites.

found at residue MET 98 (Fig. 1c) with side chain and GLY 96 (Fig. 1c) with benzimidazole. However, there are only two hydrogens and one aromatic bond was seen in the protein 4TZT but the docking score was very impressive. The side chain of the ligand has one hydrogen bond with the residue MET 98 (Fig. 1d) and benzimidazole moiety has a hydrogen bond with GLY 96 (Fig. 1d) and aromatic interaction with the residue PHE 97.

Here, the series of the compounds from set 2 is based on 4H-pyran moiety and the designed derivatives have different substituted aldehyde and phenylhydrazine. Moreover, the docking result against each protein has a different binding score due to the different binding affinity of the compounds against the receptor. The study revealed that the result from the docking study of set 2 supports the significance of the compounds and maybe get a good biological activity against Mycobacterium Tuberculosis. In the series of set 2, compound 2a has a 2-bromo, 1,3,4-trichloro 5-methoxyethane substitution while aromatic aldehyde has a 1-bromo, 4-ethoxyethane, 1-methoxyethane 4-nitro substitutions. This compound has formed two hydrogen bond between free NH2 of pyran and GLY 13 (Fig. 2a) residue of protein 2B37 at −6.918 docking score. The compound 1 with 1,3,4-trichloro,2-triflоromethane, 5-nitro substation on phenylhydrazine and 1,2-dinitrile, 4-methoxyethane, 5-nitro substations on aromatic hydrogen can bind strongly with the protein 1QG6. The docking result of

Table 10 – Predicted pharmacophore result of ligand 2a from set 2 with different pharmacophore hypothesis.

Hypothesis	Survival score	Site score	Vector score	Volume score	Selectivity score	Matched Ligand Sites	Fitness score
AHHHR	5.581	1	1	0.998	2.282	A(5) H(10) H(11) H(13) R(19)	2.998
AHHHR	5.581	1	1	0.998	2.282	A(5) H(10) H(11) H(13) R(19)	2.998
AAHHR	5.57	1	1	0.998	2.271	A(4) A(5) H(11) H(13) R(19)	2.997
AHHHR	5.563	1	1	0.998	2.264	A(5) H(9) H(11) H(13) R(19)	2.998
AAHHH	5.559	1	1	0.998	2.261	A(4) A(5) H(10) H(11) H(13)	2.997
AAHHH	5.559	1	1	0.998	2.26	A(4) A(5) H(10) H(11) H(13)	2.997
AAHHR	5.558	1	1	0.998	2.259	A(4) A(5) H(11) H(13) R(19)	2.997
AHHHR	5.556	1	1	0.998	2.257	A(5) H(10) H(11) H(13) R(19)	2.998
AAHHH	5.552	1	1	0.998	2.253	A(4) A(5) H(10) H(11) H(13)	2.997
AAHHH	5.551	1	1	0.998	2.253	A(4) A(5) H(10) H(11) H(13)	2.997
AHHR	4.978	1	1	0.998	1.679	A(5) H(11) H(13) R(19)	2.998
AHHR	4.97	1	1	0.998	1.671	A(5) H(11) H(13) R(19)	2.998
AHHR	4.968	1	1	0.998	1.669	A(5) H(11) H(13) R(19)	2.998
AHHR	4.96	1	1	0.998	1.661	A(5) H(10) H(11) R(19)	2.998
AAHH	4.952	1	1	0.998	1.654	A(4) A(5) H(11) H(13)	2.997
AHHR	4.946	1	1	0.998	1.647	A(4) H(10) H(11) R(19)	3
AHHR	4.943	1	1	0.998	1.644	A(5) H(11) H(13) R(19)	3
AHHR	4.94	1	1	0.998	1.642	A(5) H(10) H(13) R(19)	2.998
AHHR	4.94	1	1	0.998	1.642	A(5) H(10) H(13) R(19)	2.998
AAHH	4.94	1	1	0.998	1.641	A(4) A(5) H(11) H(13)	2.997

Compound 2a with docking score −7.744 against the protein 1QG6 undergoes hydrogen bonding between the interaction between the nitrile substitutions on aromatic aldehyde with GLY 205 (Fig. 2b) and between nitro substitution on phenylhydrazine with THR 193 (Fig. 2b) residue. Interestingly, compound 1a which has 3-bromo, 1-fluoro, 4-methoxyethane, 5-ethoxyethane substitutions on aromatic aldehyde, and 2-bromo, 1,2,3-trichloro, 5-nitro substitutions on phenyl hydrazine have increased its docking score against protein 4TZK. However, the compound formed only one hydrogen bond between nitrile substitution and TYR 158 (Fig. 2c) residue with a docking score of −10.232. Furthermore, in the case of protein 4TZT, compound 1a with substitution of 2-bromo, 1,3,4-trichloro, 5-diethoxyethane on phenylhydrazine and substitution of 1-fluoro, 3-methoxymethane, 2-nitrile, 4-nitro on aromatic aldehyde has a docking score −9.495 and found to form a hydrogen bond between the nitrogen of nitrile and TYR 158 (Fig. 2d) residue of protein 4TZT.

Compound 3a from a series of set 3 forms a five hydrogen bond with protein 2B37 with moderate binding energy (−8.970). The docking energies of set 3 with the receptors are given in Table 3 and the binding interaction of the compound with receptors is given in Fig. 3. Hydroxy substitution on compound 3a shows two hydrogen bonding with the residue CYS 63 and VAL 65 (Fig. 3a). The nitrogen on tetrazole ring has a formed two nitrogen bond with the residue SER 19 and ILE 20 (Fig. 3a). Moreover, the complex of compound 3a with receptor 1QG6 has good binding energy and formed one hydrogen bond between oxygen and residue TYR 158 (Fig. 3b). We have found that docked complex between protein 4TZK and compound 3a have forms high binding energy (−11.795) and hydrogen bonding between oxygen and residue TYR 158 (Fig. 3c). Additionally, the complex of compound 3a and protein 4TZT have also good binding energy (−11.549) and found form two hydrogen bond between the nitrogen of imidazole and nitrogen of amide with residue MET 98 (Fig. 3d).

The obtained result from the docking study of set 4 suggests that the compounds of set 4 have a different binding affinity with each protein. The docking energies of the selected compounds from series set 4 against receptors are given in Table 3 and the hydrogen bonding of compounds with amino acids is visualized in Fig. 4. In the series of set 4, benzimidazole moiety is attached with the substituted benzothiazole and 4-coumarin derivatives. Here, benzothiazole and benzimidazole are substituted with different electron-withdrawing and electron-donating functional groups. Compound 4a have a good docking score −9.222 against 2B37 receptor, the substitution of 1-bromo, 2–4 (1,1 dimethoxyethne) on benzimidazole moiety while benzothiazole has 2-chloro, 1-ethoxyethene, 3-methoxyethane. Compound 4a is found to form a four hydrogen bond with the 2B37 receptor. The two hydrogen bond is formed between the oxygen of benzothiazole with residue ALA 196 and SER 19 (Fig. 4a). Furthermore, the receptor GLY 93 (Fig. 4a) form two hydrogen bonds with the nitrogen of benzothiazole ring, and receptor PHE 94 forms aromatic interaction with the 4-coumarin ring. In the case of receptor 1QG6, compound 4a with −8.857 docking score is able to form a one hydrogen bond with the residue TYR 158 (Fig. 4b).

Moreover, compound 4a which has 1-chloro, 2-ethoxyethane, 4-dimethoxyethane substitution on benzimidazole and 1,2-dichloro, 3-dimethoxyethane on benzothiazole have a good docking score in the case of protein 4TZK. It was observed that the compound increased the docking score −9.026 against 4TZK and formed two hydrogen bonds where one hydrogen bond between residue MET 98 (Fig. 4c)with oxygen atom on 4-coumarin and other hydrogen bonds between GLY 96 (Fig. 4c) with a nitrogen atom in the linkage. Compound 1a which has the highest docking score (−8.116) against the protein 4TZT has a 1-chloro, 2-methoxyethan, 3-dimethxyethane substitution on benzothiazole, and 2-chloro, 4-methoxymethane, 1-methoxyethan substitution on benzimidazole and 4-coumarin were replaced with a methoxy group. This compound is found to form one hydrogen bond between the atom oxygen with

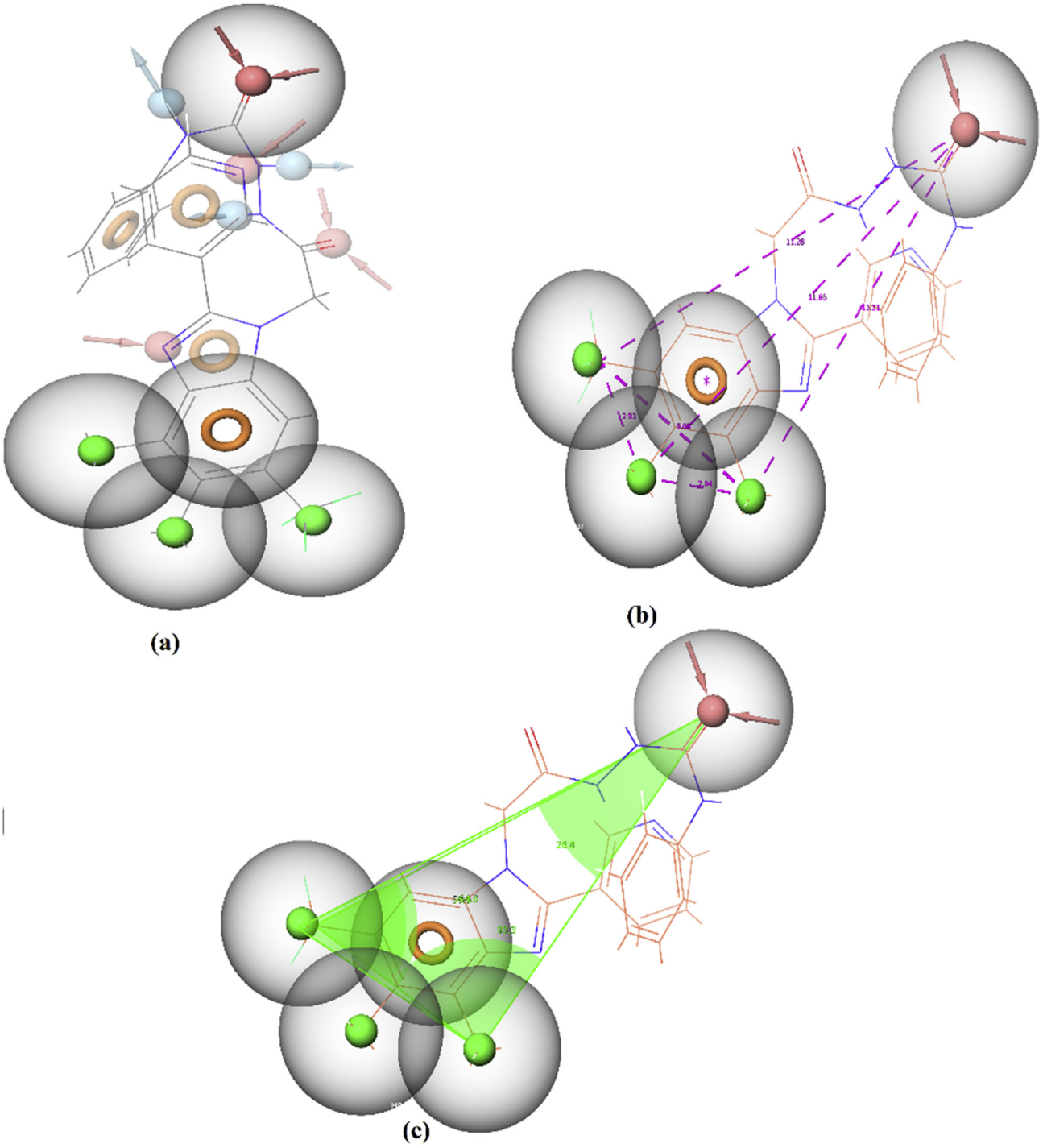


Fig. 7 – (a) Generated pharmacophore feature and selected pharmacophore feature of ligand 3a from set 3. (b) Pharmacophore hypothesis and distance between sites (c) angle between pharmacophore sites.

residue MET 98 (Fig. 4d) and an aromatic bond with the residue PHE 349 (Fig. 4d).

From the docking result, we observed that the selected designed analogues showing excellent *In silico* inhibition activity against four different proteins from mycobacterium tuberculosis enoyl acp reductase. However, each derivative has different binding energy with different proteins but the basic moiety is benzimidazole and 4H-pyran. The best derivative could be differentiated with the help of given figures. The observed result suggests that the proposed benzimidazole and 4*H*-pyran derivative having better binding affinity than standard and triclosan derivatives. These analogues could be further developed as an anti-bacterial drug after the synthesis. The docking validation was performed with relocking of selected proteins with native ligands.

Table 11 – Predicted pharmacophore result of ligand 3a from set 3 with different pharmacophore hypothesis.

Hypothesis	Survival score	Site score	Vector score	Volume score	Selectivity score	Matched Ligand Sites	Fitness score
AHHHR	5.507	1	1	1	2.206	A(4) H(8) H(9) H(10) R(12)	3
DHHRR	5.497	1	1	1	2.196	D(7) H(8) H(9) R(11) R(12)	3
DDHHR	5.491	1	1	1	2.19	D(5) D(7) H(8) H(10) R(12)	3
DDHHR	5.487	1	1	1	2.186	D(5) D(7) H(8) H(9) R(12)	3
DHHRR	5.482	1	1	1	2.181	D(5) H(8) H(9) R(11) R(12)	3
AHHRR	5.473	1	1	1	2.172	A(4) H(8) H(10) R(11) R(12)	3
DDHHR	5.472	1	1	1	2.171	D(5) D(7) H(9) H(10) R(12)	3
DHHRR	5.471	1	1	1	2.17	D(7) H(8) H(9) R(12) R(13)	3
AHHR	4.923	1	1	1	1.622	A(4) H(8) H(10) R(12)	3
AHHR	4.909	1	1	1	1.608	A(4) H(8) H(9) R(12)	3
DHHR	4.902	1	1	1	1.601	D(7) H(8) H(10) R(12)	3
DHHR	4.9	1	1	1	1.599	D(7) H(8) H(9) R(12)	3
AHHR	4.89	1	1	1	1.589	A(4) H(9) H(10) R(12)	3
DHHR	4.89	1	1	1	1.588	D(5) H(8) H(9) R(12)	3
DHHR	4.886	1	1	1	1.585	D(7) H(9) H(10) R(12)	3
AHHR	4.885	1	1	1	1.584	A(4) H(8) H(10) R(11)	3
AHHR	4.881	1	1	1	1.58	A(4) H(8) H(9) R(11)	3
DHHRR	5.48	1	1	1	2.179	D(7) H(9) H(10) R(11) R(12)	3
DHHR	4.887	1	1	1	1.586	D(5) H(8) H(10) R(12)	3
DHHRR	5.487	1	1	1	2.186	D(7) H(8) H(10) R(11) R(12)	3

3.3. *Pharmacophore hypothesis development*

The common pharmacophore hypothesis was generated using the molecular docking post-processing module. The pharmacophore was developed for a single ligand in each set (Series of set 1, 2, 3, 4) which has the highest docking score against a particular receptor in a different set of ligands. Here, from the docking study, we have selected four different ligands for pharmacophore generation from each set. Ligand 1a from set 1, which have the highest docking score (−11.239) against protein 4TZK, ligand 2a from set 2 which have the highest docking score (−10.232) against protein 4TZK, ligand 3a from set 3 which have the highest docking score (−11.795) against protein 4TZK and the ligand 4a from set 4 which have highest docking score (−9.222) against protein 2B37 were selected for pharmacophore generation.

The generated pharmacophore of the ligand from set 1 is shown in Fig. 5 and the result is displayed in Table 9. Here, a different hypothesis was generated but the selected hypothesis which is shown in the figure was selected based on the highest survival score. The generated pharmacophore has five features with the highest survival score (5.146) to bind with the protein 4TZK. The two aromatic rings required for the hydrophobic interaction within the pocket namely R16 and R17 together with one acceptor (A4), one donor (D10), and one negative (N13) site. In the case of ligand 2a from set 2 with the highest docking score against receptor 4TZK, the generated pharmacophore is shown in Fig. 6 and the predicted pharmacophore results are presented in Table 10. The selected hypothesis has five features with the highest survival and fitness score 5.581 and 2.998 respectively. The generated hypothesis has three hydrogen bonding features namely H10, H11, and H13 along with one aromatic ring (R19) and one hydrogen acceptor (A5) feature.

Furthermore, ligand 3a from set 3 which also have the highest docking score against the receptor 4TZK has generated five feature AHHHR hypothesis with the highest survival (5.507) and fitness score (3). The generated pharmacophore are shown in Fig. 7 and the predicted pharmacophore are shown in Table 11. Ligand 3a from set 3 has been found to generated five feature pharmacophores, where three hydrogen bonding namely H8, H9, and H10 together with one hydrogen acceptor (A4) and one aromatic ring (R12). This pharmacophore feature has supported ligand 3a to bound strongly within the pocket of protein 4TZK and able to generate the highest docking score. Additionally, in the case of ligand 4a from set 4, the generated pharmacophore is presented in Fig. 8 and the predicted pharmacophore results are given in Table 12. The generated pharmacophore hypothesis has five features with the highest fitness (2.657) and survival score (5.24), where two aromatic reigns which required for the hydrophobic interaction with the binding site of protein 2B37 namely R18 and R20. The rest of the feature involving two hydrogen bonding (H14, H16) and one hydrogen acceptor (A4) feature. This comparative pharmacophore hypothesis development study also supports s and suggests our prediction regarding the minimum pharmacophore features required in ligands to behave as a mycobacterium tuberculosis inhibitor and it will help in screening large databases as the development of new antibacterial agents in the future.

4. Conclusion

The focal point of the current work was the development of a new biologically active scaffold based on benzimidazole and 4H-pyran clubbed with coumarin, isoniazid, and phenylhydrazine, and aromatic aldehyde derivatives. This compact system was developed with the hope of generating new bioactive chemical entities that could be useful as potent antibacterial and anti-tuberculosis agents. Enoyl ACP reductase of the type II fatty acid synthase (FAS-II) is a major component of the mycobacterial cell wall which involved the biosynthesis of mycolic acid. Mycolic acid is the main component of mycobacteria and due to this enoyl acp reductase enzyme is an interesting target for novel Anti-tuberculosis drug

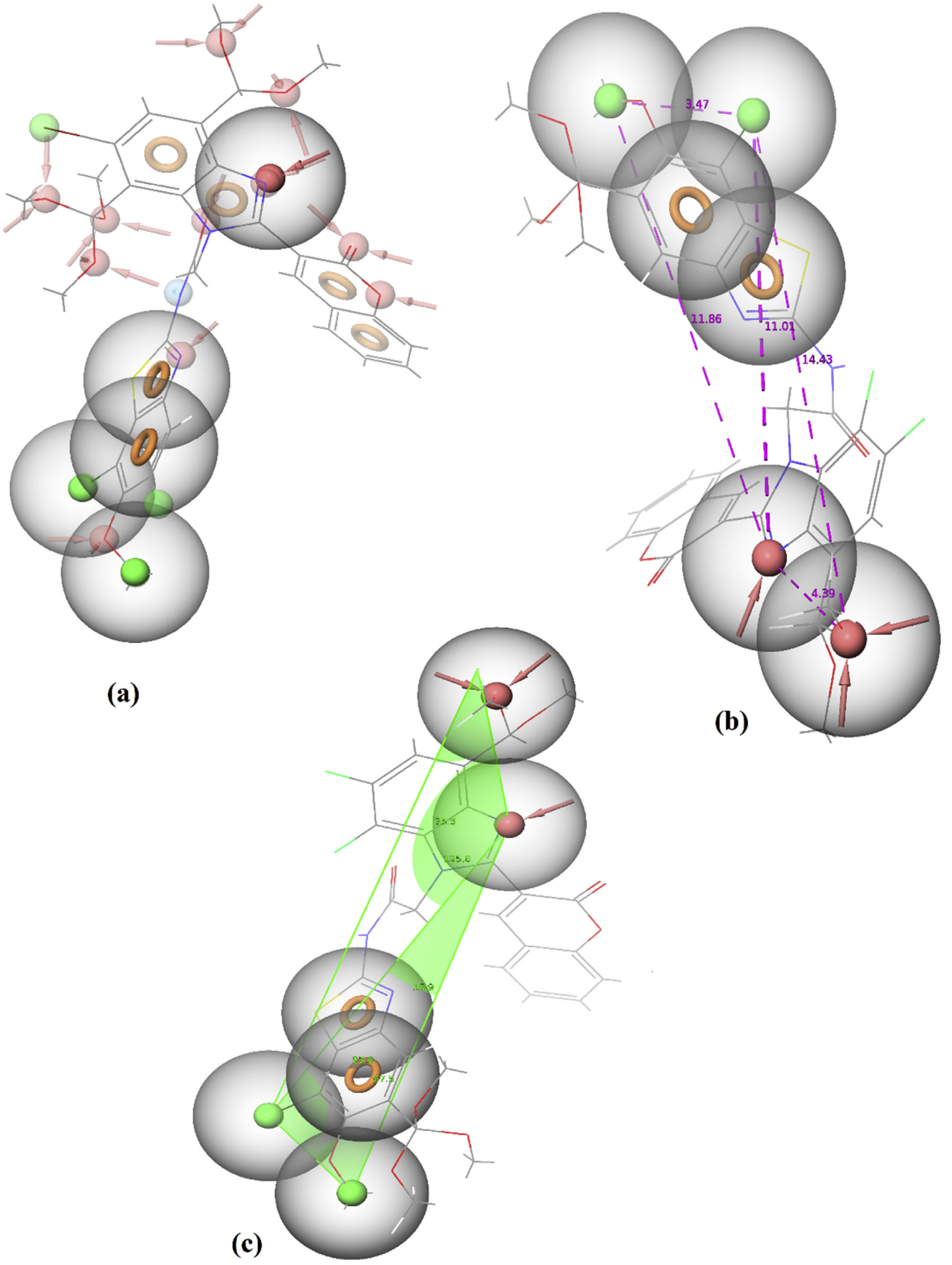


Fig. 8 – (a) Selected pharmacophore and generated pharmacophore of ligand 4a from set 4. (b) Pharmacophore hypothesis and distance between sites. (c) Generated angle between pharmacophore sites. All the distance and angle are in °A unit.

Table 12 – Predicted pharmacophore result of ligand 4a from set 4 with different pharmacophore hypothesis.

Hypothesis	Survival score	Site score	Vector score	Volume score	Selectivity score	Matched Ligand Sites	Fitness score
AHHRR	5.24	0.945	0.972	0.741	2.282	A(4) H(14) H(16) R(18) R(20)	2.657
AHHRR	5.223	0.968	1	0.793	2.161	A(1) H(14) H(16) R(18) R(20)	2.761
AAHHR	5.216	0.927	0.975	0.746	2.267	A(4) A(11) H(14) H(16) R(20)	2.648
AHHRR	5.215	0.958	1	0.793	2.163	A(10) H(14) H(16) R(18) R(20)	2.751
AAHHR	5.209	0.915	0.975	0.74	2.278	A(4) A(10) H(14) H(16) R(20)	2.63
AHHRR	5.207	0.927	0.973	0.749	2.256	A(4) H(14) H(16) R(20) R(22)	2.65
AHHRR	5.201	0.965	1	0.667	2.268	A(4) H(14) H(16) R(18) R(20)	3
AAHHR	5.198	0.962	1	0.793	2.142	A(1) A(10) H(14) H(16) R(20)	2.755
ADHHR	5.221	0.918	0.971	0.744	2.286	A(4) D(12) H(14) H(16) R(20)	2.633
AHHRR	5.196	0.97	1	0.793	2.132	A(1) H(14) H(16) R(20) R(22)	2.764
AHHR	4.639	0.965	1	0.794	1.58	A(1) H(14) H(16) R(18)	2.758
AHHR	4.627	0.988	1	0.792	1.546	A(9) H(14) H(16) R(20)	3
AHHR	4.626	0.945	0.957	0.737	1.686	A(4) H(14) H(16) R(18)	2.639
AHHR	4.638	0.965	1	0.793	1.579	A(11) H(14) H(16) R(20)	2.758
AHHR	4.659	0.96	1	0.792	1.606	A(10) H(14) H(16) R(20)	2.752
AHHR	4.631	0.955	1	0.793	1.582	A(10) H(14) H(16) R(18)	2.748
AHHR	4.673	0.974	1	0.792	1.606	A(1) H(14) H(16) R(20)	2.766
AHHR	4.642	0.975	1	0.666	1.7	A(4) H(14) H(16) R(20)	3
HHRR	4.628	0.983	1	0.79	1.555	H(14) H(16) R(18) R(20)	2.772
AHHR	4.664	0.954	0.958	0.738	1.712	A(4) H(14) H(16) R(20)	2.651

development. In this present study, a library of more than 50,000 compounds from each set was developed with help of the enumeration module in maestro. Compounds that are clubbed with isoniazid, coumarin, and derivatives of 4H-pyran have shown greater selectivity and potency towards Mycobacterium Tuberculosis enoyl acp reductase enzyme. By employing a combination of ADME, docking, and pharmacophore study calculations, novel potent hits to inhibit enoyl acp reductase were identified with the points for consideration for designing of enoyl acp reductase inhibitor. The ADME study revealed that the designed analogues showed better pharmacokinetic properties than 95% of drug molecules. It is clear from the molecular docking study that, most of the compounds exhibited excellent binding energy with all the four proteins and showed strong binding affinity against receptors. Additionally, selected compounds exhibited greater binding energies against the protein 4TZK. The comparative pharmacophore study was done with the highest binding energy scoring compounds from each set. The pharmacophore study has shown that the predicted hypothesis has the minimum pharmacophore features required in the ligand molecules to behave as an anti-tuberculosis inhibitor. Furthermore, the similarity of selected target structures also suggests that there is the possibility of being targeted by a different group of compounds. Here, our proposed inhibitors in the future could be either used in combination with a standard drug or alone which can together act interactively more effective and have a different mode of action to treat Tuberculosis.

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.2021.08.014.

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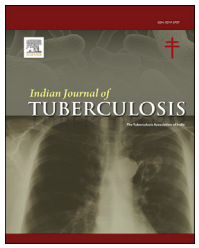

Original article

Spectrum of pulmonary aspergillus diseases in post TB lung diseases

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ABSTRACT

Introduction: Post-Pulmonary TB structural lung disease with cavitation and bronchiectasis favours the growth of Aspergillus. It leads to progressive lung destruction and the persistence of symptoms after successful ATT and can mimic smear-negative PTB. There is lack of prevalence study of this disease from India. Antifungal therapy is very beneficial, as it reduces both morbidity and mortality. The present study is being undertaken to study the occurrence of spectrum of PA in PTBLD.

Methods: This is a prospective observational study, conducted at one of the tertiary chest institute of India over a period of one year, after approval from institutional human ethics committee. A total of 60 patients with history of treatment for PTB were recruited. Active PTB were excluded. Diagnosis of PA in were established on the basis of clinical, radiological, microbiological and serological parameters. Based on this, the spectrum of PA viz. CPA, ABPA and IPA were established.

Results: The mean age was 47.88 ± 12.89 years with males being 60%. Mean duration of illness was 6.57 ± 5.11 years with mean asymptomatic period of 4.97 ± 7.41 year. Cough and breathlessness (100%) being the most common symptom followed by wheezing (58%). PA was diagnosed in 48% of cases out of which 43% cases were of CPA. The most common subtype of CPA was simple aspergilloma 14 (54%) followed by CCPA 10 (38%), 2CFPA (8%). ABPA was diagnosed in two cases of PA and one case of aspergillus sensitization. None of the case diagnosed as IPA.

Conclusion: We found high prevalence of PA among PTBLD, especially CPA. Early recognition and treatment with antifungal has the potential to reduce the morbidity and mortality. There is a need of prospective community-based larger multicentric studies to precisely define the prevalence of these disorders.

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1. Introduction

*Aspergillus*is a ubiquitous saprophytic mold that plays an essential role in recycling carbon and nitrogen.[1] The lung is the most frequent site of aspergillus infection.[2] While humans constantly inhale *Aspergillus* conidia, such conidia are effectively eliminated in immunocompetent individuals.[3] *Aspergillus* species cause a wide spectrum of illnesses in humans depending on the immune status of the host, which determines the nature of the host-Aspergillus interaction. Hence, in immunocompetent hosts, isolation of *Aspergillus* spp. in respiratory secretions typically reflects colonization, not infection.[1,3,4]

The spectrum of disease that results from the Aspergilla becoming a resident in the lung is known as 'Pulmonary Aspergillosis' (PA). In atopic individuals, the fungus triggers robust immune reactions leads to Allergic Bronchopulmonary Aspergillosis (ABPA).[5] In patients with pre-existing cavitary pulmonary lesions growth of *Aspergillus* spp. can lead to aspergilloma formation. A less severe, more insidious locally invasive form known as Chronic Pulmonary Aspergillosis (CPA).[6] Finally, the invasive form of the fungus in immunocompromised individuals causing frequently fatal angioinvasive infection called invasive pulmonary aspergillosis (IPA).[7–9] It is associated with a nearly 100% mortality rate in the absence of prompt and effective antifungal treatment.[2]

CPA is characterized by an indolent clinical course evolving over months to years, depending on host immune status. There are various predisposing condition for development of CPA. Globally, previous pulmonary tuberculosis far outstrips the other causes as the leading predisposing factor. The prevalence of CPA complicating tuberculosis (TB) is variable and depends on local incidence rates of TB within the population. Denning et al estimated, around 1.2 million people worldwide have CPA as a sequel to pulmonary TB (PTB).[10] Even when treated, CPA has a case fatality rate of 20–33% in the short-term and of 50% over a span of 5 years.[11] ABPA has also been diagnosed in patients without asthma. After the first such description in 1981, by Glancy et al many case reports have been documented.[12] Dhooria et al also bring to light about high prevalence of aspergillus sensitisation (32%) in Post PTB patients.[13] After aspergillus colonization in the airways and healed cavities in lungs, sensitization may develop if the subject is atopic leads to development of ABPA.[14]

Post-PTB structural lung disease favours the growth of aspergillus, leads to progressive lung destruction and persistence of symptoms after successful anti-tuberculosis treatment and can mimic smear-negative PTB. Pulmonary aspergillosis as a sequel of PTB, occurs more commonly than generally appreciated. Most of the aspergilllus lung diseases occur in the country with higher endemicity of PTB. Nearly 50% of these cases occurred in India and China.[10] Most of the studies on prevalence of PA are from other countries, and the data from India is scarce. This study was done to study the occurrence of spectrum of PA in treated cases of PTB and it awareness among threating physician.

2. Material and methods

This is a prospective observational, cross-sectional study, conducted at the Viswanathan Chest hospital, of Vallabhbhai Patel Chest Institute, University of Delhi over a period of one year between 2017 and 2018, after approval from institutional human ethics committee. Patients of 18–60 years age, irrespective of gender, with history of treatment for PTB and radiological evidence of post tuberculosis sequelae with complaint of breathlessness, cough, sputum production, wheezing, malaise, weight loss and decreased appetite and no definitive pulmonary diagnosis, who are stable, ambulatory and cooperative, giving voluntary informed consent to take part in the study were included. Patients with active TB, pregnancy, lactating females and who are not giving consent were excluded.

These patients were subjected to detailed clinical examination and underwent for chest X-ray (PA-view), Sputum examination direct smear via Ziehl-Neelsen stain for Acid fast bacillus (AFB), genexpert, pyogenic and fungus culture, skin prick test (SPT) against *Aspergillus* species, serum total IgE levels, serum precipitin, specific IgG and IgE against *Aspergillus*, spirometry with reversibility, high-resolution computer tomography (HRCT) chest. The other relevant investigations like fiberoptic bronchoscopy, serum and BAL galactomannan was done as per requirement.

Based on radiological evaluation, the various documented sequelae of PTB were fibrosis, fibro-cavitation, bronchiectasis, calcification, aspergilloma, pleural thickening and mediastinal lesion. In addition, the diagnosis of PA was established on the basis of clinical, radiological, microbiological evidences and serological parameters. Based on this, the spectrum of Pulmonary Aspergillosis was diagnosed viz. CPA as per Denning et al 2015.[15] ABPA as per Rosenberg–Patterson criteria[16] and IPA as per EORTC/MSG 2008 updated in 2014 criteria.[17]

2.1. *Skin prick test*

The Skin Prick test was conducted with all the enrolled patients. Allergen extract (1:10w/v, 50% glycerinated) was procured from All Cure Pharma Pvt. Ltd. (New Delhi, India). Buffered saline and histamine have been used as negative and positive controls, respectively. SPT was undertaken by applying a drop of antigen on the healthy skin of volar surfaces of the forearm and pinching it with 26.5-gauge sterile needles. The skin test reactions were graded by calculating the mean diameter as (D + d)/2; D = the largest diameter and d = orthogonal or perpendicular diameter at the largest width of D after 15–20 minutes compared to the positive control wheal size, i.e. histamine diphosphate (10 mg/mL). A positive reaction to a particular allergen is stated by a mean wheal diameter of 3 mm or more, greater than the negative control (buffered saline). Before performing SPT, oral drugs including

antihistamines, and any other drugs considered to affect SPT were stopped 1 week before the tests[18]

2.2. Specific IgE and IgG

Specific immunoglobulins against aspergillus in the patient's serum were determined by Enzyme Linked Immunosorbent Assay (ELISA) with standardized kits according to the manufacturer's protocol.[19]

2.3. Serum precipitins

It was detected using Ouchterlony's immunodiffusion test. An agar plate with a central well was filled with 100 μL of the patient's serum and the other wells around the central wells was filled with the antigen extracts of the fungi and incubated at room temperature for 48 hours. A positive test to a particular fungus was defined as the development of a band of precipitation between the well containing the patient's serum and a fungal antigen.

2.4. Statistical analysis

The data was tabulated on MS Excel and analysis was performed using SPSS version 16.0 software. The data was summarized through frequency tables for qualitative variables and mean ± SD for quantitative variables. Comparison of quantitative variables were done by using Mann–Whitney test while qualitative variables were compared using Fisher's Exact test. A p-value < 0.05 was considered statistically significant.

3. Results

We enrolled 60 patients with history of treatment for PTB for evaluation of various pulmonary aspergillosis. The mean age of patients was 47.88 ± 12.89 years. The majority 36 (60%) were males and remaining 24 (40%) female. Thirty one (52%) patients had history of smoking whereas 29 (48%) were never smoker. Mean duration of illness was 6.57 years with mean duration of asymptomatic period after ATT was 4.97 ± 7.41 years. The most common symptom was Cough and exertional dyspnoea in all cases, followed by wheezing 35 (58.33%) and hemoptysis 22 (36.67%). The details demographic characteristics of enrolled patients is shown in Table 1.

Spirometry was done in all the patients and showed abnormal in all. The spirometry abnormality was mixed pattern defect in 30 (50%) cases, followed by obstructive pattern 19 (31%) and restrictive pattern in 11 (18%). Chest Xray abnormalities was unilateral in 40 (67%) with right sided in 23 (38.33%), left sided involvement in 17 (28.33%) patients. While the lesion were bilateral involvement in 20 (33.33%) patients. The lesion was single in 13 (21.6%) and multiple in 47 (78.3%) cases. The common Xray abnormalities are cavitation, fibrosis, calcification and bronchiectasis (Fig. 1). The specific IgE against *aspergillus* was positive in 6 (10%) and negative in 54 (90%) patients, while the specific IgG against aspergillus was positive in 26 (43.3%) and negative in 34 (56.6%) cases. Serum total IgE was elevated in 6 (10%) and normal in remaining 54 (90%) patients. Similarly SPT against *aspergillus* was positive in 17 (28.3%) and negative in 43 (71.6%) cases.

The HRCT chest abnormal lesion was seen in all the patients with the most common radiological lesion was fibrocavitation in 34 (56.67%) followed by fibrocavitation with fungal ball (aspergilloma) in 27 (45%), fibrosis 22 (36.67%), pleural thickening 19 (31.67%),calcification (calcified nodules and pleural calcification) 14 (23.33%). The other less common lesion were bronchiectasis 7 (11.67%) and mediastinal (calcification) in one (1.67%). The details of HRCT findings with some radiological image are shown in Table 2 and Figs. 2 and 3.

Out of total 60 post-TB lung disease (PTBLD) patients, 29 (48%) cases were diagnosed with one of the Pulmonary Aspergillus associated disease on basis of various mentioned criteria. The most common type of pulmonary aspergillus associated disease was chronic pulmonary aspergillosis in 26 (43%) patients. Among various CPA, the most common form was Simple Aspergilloma 14 (23.33%) followed by Chronic Cavitary Pulmonary Aspergillosis (CCPA) 10 (16.67%) and Chronic Fibrosing Pulmonary Aspergillosis (CFPA) 2 (3.33%). There are two (3.33%) cases of Allergic Bronchopulmonary Aspergillosis (ABPA) while one case has diagnosed as aspergillus sensitization (1.67%). Invasive pulmonary aspergillosis was not found in any case. The pulmonary aspergillosis was most commonly found in fibrocavitory form of radiological disease with 27 (73%) cases in 34 FCD patients (Table 3).

4. Discussion

After treatment despite microbiological cure of active PTB, it leads to extensive structural lung changes in more than two-thirds of the patients.[20–22] The estimated prevalence of lung impairment after PTB varied from 18% to 87%.[23] There is also 2 to 5 times higher risk of mortality among them compared to control.[24] The residual changes of TB in form of parenchymal, airway, pleural, chest wall, vascular and mediastinal pathologies, collectively term as post-TB lung disease or post-TB sequelae.[20,25] The PTBLD are either structural complications in form of bronchiectasis, residual cavitation, lung/pleural fibrosis, bronchial stenosis, pneumothorax and tuberculoma

Table 1 – Demographic details of all enrolled patients.

Parameter	n (%)
Male	36 (60)
Female	24 (40)
Smoker	31 (51.67)
Non-smoker	29 (48.33)
Mean Age (years)	47.88 ± 12.89
Mean duration of Illness (years)	6.57 ± 5.11
Mean Asymptomatic period after ATT	4.97 ± 7.41
Symptoms	
Cough	60 (100)
Breathlessness	60 (100)
Wheezing	35 (58.33)
Hemoptysis	22 (36.67)
Loss of Weight	17 (28.33)
Loss of Appetite	17 (28.33)
Repeated Cold	10 (16.67)
Fever	9 (15)

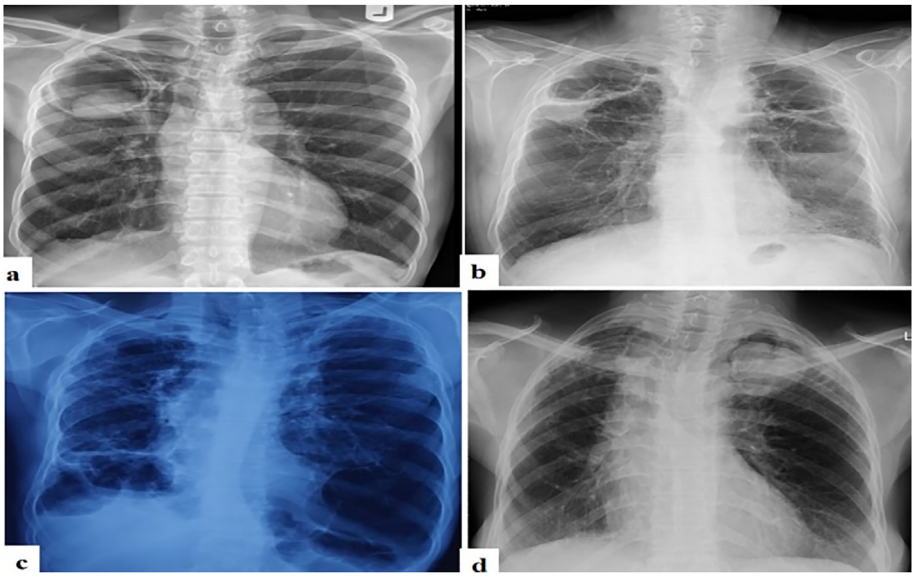

Fig. 1 – Chest X ray showing different radiological pattern of aspergillosis. a) Right upper lobe aspergilloma seen in a case of simple aspergilloma. b) Bilateral upper lobe fibrocavitory lesion seen in case of CPA. c) bilateral parenchymal lesion with predominant right side fibrosis with multiple cavities in case of ABPA. d) Left upper lobe cavitory lesion with air crescent seen in case of aspergillosis.

etc or infectious complications like exacerbations of COPD, pneumonia, empyema, recurrence of TB and colonisation/infection with Aspergillus/non-tuberculous mycobacteria. The PTBLD may be one of the important causes of chronic lung disease worldwide, however it is hardly recorded in developing counties due to lack of research.[20,26] The residual cavities of post TB patients is the one of the important risk factor for pulmonary aspergillosis. The residual cavities are seen in 20–40% of patients after PTB treatment.[22,27,28] We also found high prevalence of cavitory diseases in nearly 57% post-TB patients. Post TB cavity is considered as the central of pathogenesis for pulmonary aspergillosis as it is an area of the lung with poor host defences and pulmonary drainage, leads to growth of aspergillus.

Pulmonary aspergillosis refers to a spectrum of diseases that include aspergilloma, chronic pulmonary aspergillosis, invasive aspergillosis and ABPA.[29] In recent years, infections caused by aspergillus species have become an emerging focus, as the number of patients infected with aspergillus species has increased dramatically. This is associated with significant morbidity in a wide range of susceptible hosts in terms of respiratory symptoms and abnormal lung function.[2] CPA is a progressive disease that commonly occurs in immunocompetent individuals with underlying post-TB structural lung diseases. The prevalence is varied from 6.3% to 15.3% among PTBLD from other part of world.[20,30,31] In TB endemic countries, the proportion of CPA in PTBLD is higher than non-TB endemic countries. Based on a model, the global burden of post-TB CPA was estimated as 1.2 million cases annually with <1/100,000 case in non-TB endemic to 42.9/100,000 in TB endemic countries.[10] There is no direct prevalence study of pulmonary aspergillosis from India. However the as per estimated burden of CPA report, the annual incidence of CPA varied 0.027–0.17 million cases.[32]

We found high prevalence of PA in post PTB with 29/60 (48%) patients. Similar to our study Akbari JG et al reported that the 45% of pulmonary aspergillosis was seen in underlying post-TB patients.[28] Jain S et al in another study from India found that nearly 64% of their post-TB has pulmonary aspergiollosis with aspergilloma was the most common in 57% cases.[33] The proportion of patients with PA with previous TB varies from 15.3% in United kingdom to 93% in Korea.[34,35] This high difference in the prevalence of pulmonary aspergillosis and PTBLD as the underlying cause of aspergillosis was due to different study protocol, prevalence of TB in different areas and not recognizing the post-TB sequelae as one of the major causes of chronic lung disease especially in underdeveloped and developing countries.

In the present study, out of 29 cases of pulmonary aspergillosis we found 26 (43%) cases as CPA. This finding showed the high prevalence of CPA over other entities of pulmonary aspergillosis in post PTB patients. All CPA cases were present in tuberculous cavities which constitute 76% (26/34) post TB fibrocavitory disease patients. Among the 26 cases of CPA,

Table 2 – Details of various post TB HRCT finding.

Post TB HRCT finding	n (%)
Fibrocavitation	34 (56.67)
Aspergilloma	27 (45)
Fibrosis	22 (36.67)
Pleural thickening	19 (31.67)
Calcification	14 (23.33)
Bronchiectasis	7 (11.67)
Mediastinal lesion	1 (1.67)

Table 3 – The various type of Pulmonary Aspergillus diseases.

Diagnosis (Pulmonary Aspergillus)	n (%)
Pulmonary Aspergillosis	29 (48)
CPA (Total)	26 (43)
Aspergilloma	14 (23.33)
CCPA	10 (16.67)
CFPA	2 (3.33)
ABPA	2 (3.33)
Aspergillus sentization	1 (1.67)

aspergilloma was most common in 14 (53.8%) followed by CCPA in 10 (38.4%) and CFPA in two (7.6%) patients. Similar to our study Jain S et al also found aspergilloma is the most common type of CPA in 57% followed up chronic necrotizing pulmonary aspergillosis (CNPA) in 36% and ABPA in 7% cases of their 110 post TB sequelae patients.[33] Another study by *Aydoğdu K et al* also showed the similar higher prevalence of aspergilloma (52%) over CCPA (48%) in 77 pulmonary resection cases of aspergillosis.[36] While Smith NL et al in a study of 126 pulmonary aspergillos is found the CCPA was most common form in 89% followed by aspergilloma in 8% and CFPA in 3% patients.[34] Page ID et al in a study of 285 PTBLD patients found CCPA in (10) 3.5%, CFPA in 3 (1.1) and one cases of simple aspergilloma. They also found that the pulmonary aspergillosis was more common in HIV negative and cavitory form PTBLD patients.[31] Hedayati MT et al in a study from Iran also found CCPA is more common type seen in 11.3 compared to aspergilloma in 2.4% of their 124 TB patients.[30] The difference in the prevalence and types of pulmonary aspergillosis in different studies is due to difference in prevalence of TB, study design, facility for diagnosis of aspergillosis and similarity of symptoms of TB. The sputum negative pulmonary TB is difficult to differentiate from pulmonary aspergillosis, because of similar symptoms and radiology findings. Treatment of sputum negative TB on clinicoradiological basis, less awareness of post-TB sequelae symptoms, inadequate facilities for testing for specific IgG/IgE and precipitins against aspergillus, especially in TB endemic countries like India probably results in the delayed and underdiagnosis of CPA. Thus, it is imperative to exclude CPA in patients with suspected sputum smear-negative PTB, given the high prevalence of CPA in India.

We found two cases of ABPA (3.33%) and only one case of aspergillus sensitization (1.67%). Jain S et al also found 5 (7%) cases of ABPA in their 110 PTBLD patients.[33] Although ABPA also has been diagnosed in patients without asthma. Our ABPA diagnosed patients did not have history of asthma but they had atopy as shown by a history of allergic rhinitis and raised total IgE. The relationship of tuberculosis to manifestations of atopy is complex. It has been suggested that the CD4+/Th 1 type of immune response in TB may protect against the development of asthma that is associated with a Th 2 type of response.[37] Mycobacteria culture supernatants have been shown to effectively prevent the development of asthma associated with altered Th1/Th2 cytokines in sensitized mice.[37] Therefore, it is possible that protection against atopic conditions may wane and allergic conditions may manifest after tuberculosis is treated. As aspergillus colonization in the airways and healed cavities is common in

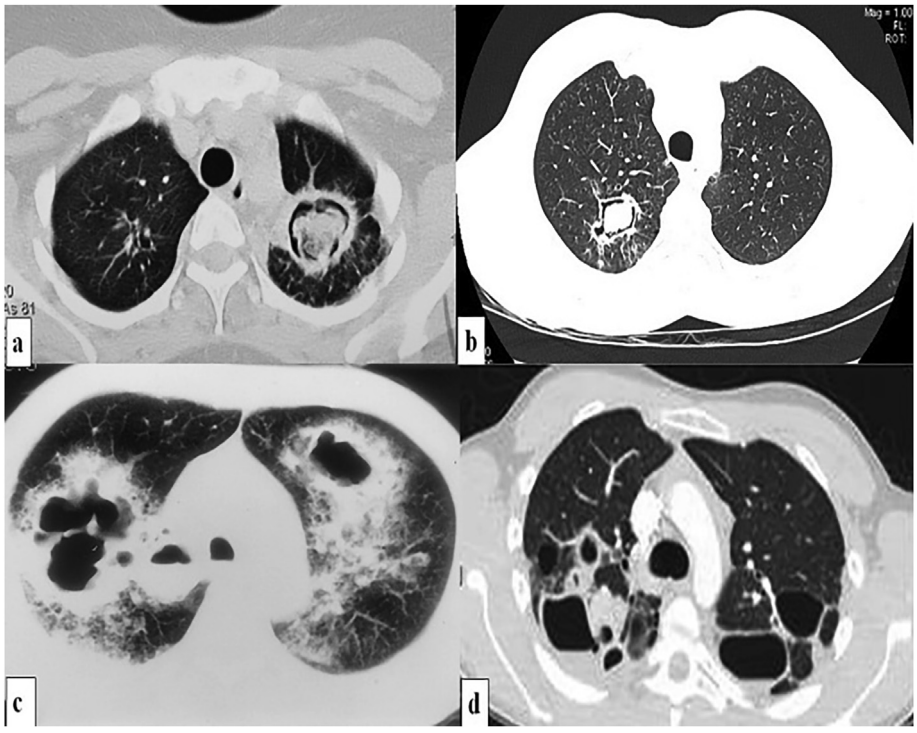

Fig. 2 – HRCT chest showing different pattern of aspergillosis radiology findings. a) Left upper lobe thick wall cavitory in a case of aspergilloma. b) Right upper lobe thin wall cavitory lesion in a case of CCPA. c) Bilateral thick wall cavitory lesion with pericavitary infiltrate in a case of CFPA. d) Multiple cavitory lesions of bilateral lung in a case of CCPA.

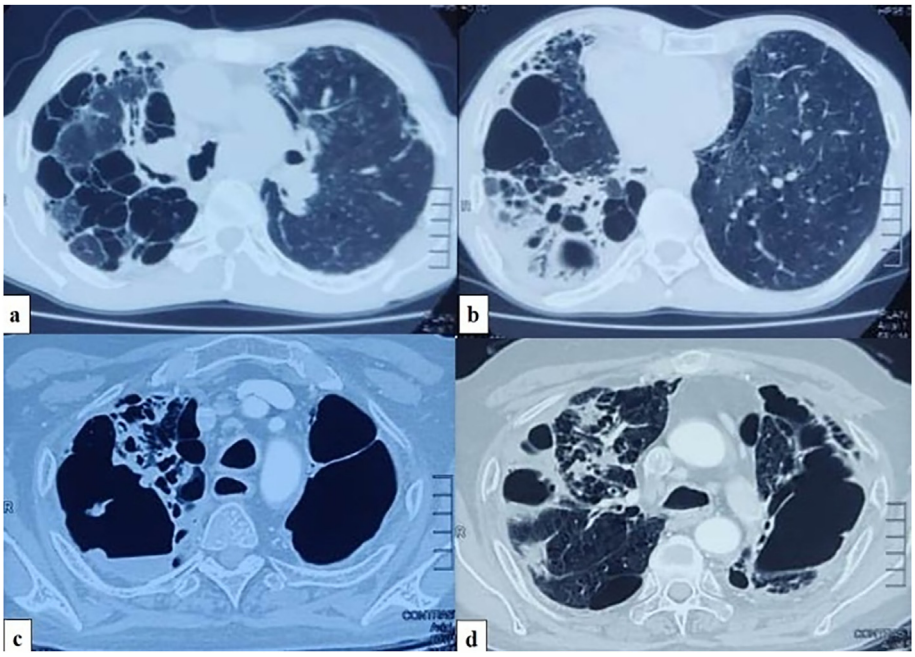

Fig. 3 – HRCT chest showing different pattern of aspergillosis radiology findings. a and b) Multiple cavitory lesion with fibrosis and pericavitary infiltrate predominantly on right side all lobes involvement in a case of CCPA. c and d) Bilateral multiple cavitory lesion with fibrosis and air fluid level in right upper lobe cavity in a case of CFPA.

previously treated tuberculosis; sensitization may develop if the subject is atopic followed by development of ABPA.[38] Our study and Jain et al[33] highlights the curious link between tuberculosis and atopy and also suggests that presence of atopy without asthma is sufficient for develop ABPA. Therefore patients with post-tubercular cough and dyspnoea should be investigated for ABPA if they are atopic.

5. Conclusion

In TB endemic countries, pulmonary symptoms and persistent or progressive pulmonary shadow is generally attributed to relapse of PTB and empirically treated with ATT. It is imperative to exclude pulmonary aspergillosis in suspected sputum smear-negative PTB. Early recognition and treatment has the potential to reduce the morbidity and mortality. This study is to increase the physician awareness and need for widespread availability of pulmonary aspergillosis testing facility. There is a need for prospective community-based larger multicentric studies to precisely define the prevalence of these disorders.

Presentation at meeting

NAPCON 2018.

Conflicts of interest

The authors have none to declare.

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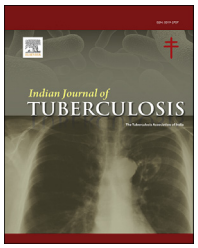

Original article

Effectiveness of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis at various stand-alone laboratories in Delhi

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ABSTRACT

Background: Globally, EPTB accounts for 15% of the notified incident TB cases. Laboratory confirmation of EPTB is challenging and majority of the cases remain undetected for a longer time. A major breakthrough in the diagnosis of EPTB was the introduction of nucleic acid amplification tests (NAAT). One such test-the Xpert MTB/RIF assay also known as Cartridge based nucleic acid amplification test (CBNAAT) was endorsed by the Scientific and Technical Advisory Board of the WHO for the diagnosis of Tuberculosis. The present study was conduct to evaluate the outcome of various extrapulmonary samples tested in the year 2019 at different standalone NAAT laboratories in Delhi.

Materials and methods: A total of 20,238 samples consisting mainly of Pus (21.77%), Cerebrospinal fluid (CSF) (14.96%), Biopsies (13.87%), Pleural fluid (10.49%), Lymph node aspirations (FNAC aspirates) (6.75%), synovial fluid (0.54%) and gastric aspirates (26.4%) tested at 22 standalone NAAT laboratories were included in this study.

Results: *Mycobacterium tuberculosis* was detected in 3496 samples and resistance to rifampicin was detected in 329 of the samples. The overall yield of all the specimens combined was 17.2%. Highest yield was seen in Lymph nodes aspirates (FNAC) (36.0%), followed by pus (35.4%), tissues (15.7%), synovial fluid (13.5%), Endometrial tissues (10.7%), Pleural fluid (9.5%), Gastric aspirates (9.4%) and CSF (6.5%). The lowest yield was seen in Cavitary fluids (6.2%).

Conclusion: The results of this study highlight the usefulness of Xpert MTB/RIF assay in the diagnosis of EPTB. In particular, this assay proved to be of great utility while testing pus samples, tissue samples and lymph node FNACs.

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1. Background

Over the past two decades, tuberculosis (TB) has been reported from both developing and developed countries and it continues to be the number one killer infectious disease worldwide. In 2018, there were 10.0 million incident cases of TB and 1.2 million deaths worldwide. India alone contributed 26.9% of these incident cases. 8.6% of the incident TB cases globally in 2018 were among people living with HIV.[1] Coincidence with human immunodeficiency virus (HIV) epidemics has led to the change in disease pattern with a higher incidence of extrapulmonary tuberculosis (EPTB). Globally, EPTB accounts for 15% of the notified incident TB cases.[1]

Laboratory confirmation of EPTB is challenging and majority of the cases remain undetected for a longer time. Conventional tests for the diagnosis of EPTB are limited in accuracy and often require invasive procedures and special expertise. Smear microscopy -the most widely used test has shown variable sensitivity values (0–40%) for the diagnosis of EPTB.[2–4] Similarly, culture in addition of having longer turnaround time has shown sensitivity values ranging from 0 to 80% for various extrapulmonary samples.[5–8] A major breakthrough in the diagnosis of EPTB was the introduction of nucleic acid amplification (NAA) tests. These tests detect nucleotide sequences unique to *Mycobacterium tuberculosis* directly in extrapulmonary specimens and give results within few hours, offering better accuracy than smear microscopy and shorter turnaround time than culture.[2,8–10]

One such test-the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) also known as Cartridge based nucleic acid amplification test (CBNAAT) a fully automated real time hemi nested PCR system implementing molecular beacon technology for the diagnosis of pulmonary TB infection, was endorsed by the Scientific and Technical Advisory Board of the WHO as the most sensitive fast test for TB diagnosis in paucibacillary respiratory samples (WHO 2010). While CBNAAT has been approved for TB detection in sputum by regulatory agencies, CBNAAT for TB detection in EPTB specimens is considered ''off-label'' use and has shown promising results with good accuracy (81.3% sensitivity and 99.8% specificity).[11–13]

In India upfront CBNAAT is offered for the diagnosis of EP-TB cases and over the past three years there has been a significant scale up of CBNAAT facilities. So far, 1180 CBNAAT machines have been deployed, across all parts of the country. In 2018, 9.3% 0f all the EP-TB cases were tested using CBNAAT at various standalone CBNAAT facilities that have been established at District levels.[14] The union territory of Delhi with a population of 1.8 million has 33 such standalone CBNAAT sites. The present study was conduct to evaluate the outcome of various extrapulmonary samples tested at these sites in the year 2019.

2. Materials and methods

Twenty-two standalone CBNAAT sites contributed in this study by providing retrospective results of extrapulmonary samples accepted and tested for the diagnosis of EP-TB from January to December 2019. All the technicians involved in the testing and reporting had received initially training at the New Delhi Tuberculosis centre (NDTC), New Delhi as per standard National Tuberculosis Elimination program (NTEP) guidelines. NDTC is the State Tuberculosis training and demonstration centre (STDC) and an Intermediate reference laboratory (IRL) providing diagnostic and follow-up services for drug-resistant tuberculosis (DR-TB) to NTEP Delhi. All the contributing sites have participated and successfully cleared first round of proficiency testing for Xpert MTB/RIF conducted by National Reference laboratory- National Institute of Tuberculosis, Bengaluru India. All the CBNAAT instruments are covered under Annual Maintenance Contract (AMC) and are calibrated periodically by authorized personnel from Cepheid India.

All the samples for testing were collected inside sterile 50mL screw capped centrifuge tubes (Falcon tubes) and processed as per the WHO endorsed standard operation procedures for non-respiratory specimen.[15]

The number of samples processed in each laboratory ranged from 14 to 3618, for a total of 20,238 samples. The specimen consisted mainly of Pus (4406/20238 {21.77%}), Cerebrospinal fluid (CSF) (3028/20238 {14.96%}), Biopsies (2809/20238 {13.87%}), Pleural fluid (2123/20238 {10.49%}), Lymph node aspirations (FNAC aspirates) (1367/20238 {6.75%}), synovial fluid (111/20238 {0.54%}) and gastric aspirates (5350/20238 {26.4%}) (Table 1). Body fluids except Pleural fluid, CSF and synovial fluid were grouped as cavitary fluids. Since, the most preferred specimen for diagnosis of female genital tuberculosis (FGTB) is endometrial biopsy; these specimens were also grouped separately. Although normally used to diagnose pulmonary tuberculosis (PTB), Gastric aspirates (GA) differ substantially from sputum. Therefore Gastric Aspirates were also included in the study.

3. Results

Of the 20238 samples tested, valid results were obtained for 19837 (98.0%) samples while, the remaining 401 (2.0%) specimen showed error, no result and indeterminate results. *M. tuberculosis* was detected in 3496 (17.2%) while 16341 (80.7%) specimen were negative for *M. tuberculosis*. Resistance to

Table 1 – Different extrapulmonary samples tested.

S. No.	Sample type	NO. Tested	Frequency (%)
1	Gastric Aspirate	5350	26.44
2	Pus	4406	21.77
3	C.S.F	3208	15.85
4	Tissue	2809	13.88
5	Pleural fluid	2123	10.49
6	FNAC	1367	6.75
7	Endometrial tissue	439	2.17
8	Cavitary fluids	388	1.92
9	Synovial fluid	111	0.55
10	Urine	33	0.16
11	Menstrual blood	4	0.02
	Total	**20238**	

CSF- Cerebrospinal Fluid; FNAC- Fine Needle Aspiration Cytology; Cavitary fluids include Ascetic Fluid-332, pericardial fluid- 10, Peritoneal fluid-45 and abdominal fluid-1.

Table 2 – Yield of different Extra-pulmonary samples tested.

S. No	Sample type	Number Tested	Total MTB Not Detected	MTB Detected, Rif Resistance Detected	MTB Detected, Rif Resistance Not Detected	Total MTB detected	Positivity rate	Rif resistance detection rate
1	Pus	4406	2745	131	1431	1562	35.45	8.39
2	Menstrual blood	4	2	0	2	2	50.00	0.00
3	Endometrial tissue	439	388	4	43	47	10.71	8.51
4	Cavitary fluids	388	357	1	20	21	5.41	4.76
5	Pleural fluid	2123	1882	19	184	203	9.56	9.36
6	Synovial fluid	111	95	3	12	15	13.51	20.00
7	C.S.F	3208	2914	20	188	208	6.48	9.62
8	FNAC aspirate	1367	859	58	435	493	36.06	11.76
9	Tissue	2809	2315	55	386	441	15.70	12.47
10	Gastric Aspirate	5350	4751	38	466	504	9.42	7.54
11	Urine	33	33	0	0	0	0.00	0.00
	Total	**20238**	**16341**	**329**	**3167**	3496	17.27	9.41

rifampicin was detected in 329 (329/3496 [9.41%]) specimen while no rifampicin resistance was seen in 3167 (3167/3496 [90.6%]).

The overall yield of all the specimens combined was 17.2%. Highest yield was seen in Lymph nodes aspirates (FNAC) (36.0%), followed by pus (35.4%), tissues (15.7%), synovial fluid (13.5%), Endometrial tissues (10.7%), Pleural fluid (9.5%), Gastric aspirates (9.4%) and CSF (6.5%). The lowest yield was seen in Cavitary fluids (6.2%) while none of the Urine samples tested positive for presence of *M. tuberculosis* (Table 2).

Rifampicin resistance was detected in 329 (9.41%) of the total 3496 samples testing positive for MTB. The highest rifampicin resistance rate was observed in synovial fluid fluids (20.0%) followed by tissue samples (12.47%) and lymph node FNAC samples (11.76%) while cavitary fluids had the lowest Rifampicin resistance rate (4.76%) (Table 2).

4. Discussion

To the best of our knowledge this is first study to report yield of over 20,000 different extrapulmonary samples tested by GeneXpert MTB/RIF. The aim of this study was not to access the performance of GeneXpert for the diagnosis of extrapulmonary tuberculosis as this has already been established in a number of studies across the world.[16–23] The present study is more focused on evaluating the yield of different extrapulmonary samples tested by GeneXpert.

A remarkable yield of 36.0% and 35.4% was seen in fine needle aspiration cytology/biopsy and Pus samples respectively. Various studies across the world have reported good performance of Xpert MTB/RIF for diagnosis of tuberculosis among FNAC and Pus samples. A recent study conducted by Fantahun et al, reported a sensitivity and specificity of 92% and 74% respectively of Xpert MTB/RIF for the diagnosis of tuberculosis lymphadenitis using FNAC samples.[24] Another study by Louis J et al, also demonstrated excellent diagnostic accuracy of Xpert MTB/RIF assay in patients with tuberculous lymphadenitis using FNAB samples. They reported an overall assay sensitivity and specificity of 96.7% and 89.9% and were correctly able to identify MTB in 100% of cytology smear-negative samples.[10] Habous et al. in their study reported 100% sensitivity and specificity for 29 pus samples tested by Xpert MTB/RIF taking culture as the reference standard.[25] The results of our study are thus consistent with the findings of previous studies. In addition rifampicin resistance was detected in 11.7% and 8.4% of samples testing positive for MTB among FNAC aspirates and Pus samples respectively. This property makes this assay even more useful in guiding the treatment regimen for better management of patients.

Third highest yield of 15.7% and rifampicin resistance detection rate of 12.4%in this study was seen among various tissue samples tested. Diagnosis of tuberculosis from sample collected from deep seated tissues is always challenging. This is a reasonable yield considering the paucibacillary nature of these samples. A sensitivity of 88% of Xpert MTB/RIF assay for diagnosis of TB among tissue samples was reported in a recent review of 27 published studies.[26] The yield obtained in this study is marginally lower than that reported in previous studies.[27,28] This can be explained by the fact that these studies were conducted on a small number of tissue samples and there was a possible sampling bias.

In the absence of a single reference standard, the treatment of Tuberculous pleural effusion (TPE)- the second most common site of extrapulmonary tuberculosis in most circumstances is empirical (nonmicrobiological). The sensitivities of smear microscopy and culture for TPE are about 10% and 20% respectively.[29,30] Sehgal et al in their recent review on diagnostic performance of Xpert MTB/RIF assay in TPE reported a pooled sensitivity and specificity of 51.4% and 98.6% respectively.[31] Contrary to this review, a considerably fair yield of 9.5% was obtained among 2123 pleural effusion samples tested in this study. Resistance to rifampicin was detected in 9.3% of MTB positive samples. For the sake of argument based on low reported sensitivity even if Pleural effusion is considered as a suboptimal sample, exceptional specificity of Xpert MTB/RIF assay for pleural fluids still makes it an excellent 'rule in' test for TPE diagnosis and the ability to detect rifampicin resistance will always be an added advantage.

10.7% of all the endometrial tissues tested in our study were found to be positive for MTB. With 50–60% of all the females with genital tuberculosis have been reported to have endometrial involvement, the results of our study are encouraging.[32] In addition, the assay was also able to detect

rifampicin resistance in 8.5% of the cases. A study by Sharma et al. found the assay to be highly specific but with a low sensitivity.[33] They reported a yield of 2.9% among 240 samples tested which is significantly lower than the present study. Another study by Farhana et al. reported an additional yield of 55.5% cases with the use of this assay.[34] Two menstrual blood samples were also tested in this study. Although a high yield of 50% was seen, the sample size is too small to comment.

Microbiological confirmation of Tuberculous Meningitis (TBM) - the most severe form of tuberculosis is rare. This often leads to delayed treatment initiation and hence increased mortality and morbidity. A moderate yield of 6.5% was observed among 3208 CSF samples tested in this study. These results are consistent with previous studies that have reported lower sensitivities of Xpert MTB/RIF for diagnosis of TBM.[20,23,35] The lower yield observed in this study can be explained by the fact that owing to the difficulty/complexity of obtaining CSF samples, generally lower volumes (as low as 0.1mL) of CSF are tested as compared to other extrapulmonary samples. Xpert MTB/RIF assay depends upon capture and lysis of bacilli, therefore, low volumes of sample tested may decrease its yield and hence the sensitivity.[36,37] The sensitivity and yield of the assay can be improved if high volumes (>7mL) of CSF are tested. An additional processing step of concentrating the CSF samples by centrifugation at high speed and then using the pallet for testing may also be helpful in increasing the yield.[20]

A suboptimal yield of 6.2% was obtained for 388 cavitary fluids tested in this study. The results are consistent with several previous studies.[13,16,20,23] The paucibacillary nature of these fluids and possible presence of PCR inhibitory may be responsible for this low yield. Synovial fluid on the other hand demonstrated an excellent yield of 13.5% with a very high rifampicin resistance detection rate of 20.0%. None of the 33 urine samples in this study were found to be positive for MTB. Similar results have been reported in previous studies.[18,20]

Gastric Aspirate is the specimen of choice for diagnosing tuberculosis among smear negative TB suspects and people unable to expectorate sputum particularly among paediatric age group.5530 gastric aspirates tested in this study gave a considerable yield of 5.4% and in addition rifampicin resistance was also detected among 7.5% of the samples. Excellent sensitivity and specificity of Xpert MTB/RIF assay for diagnosis of TB using gastric aspirate samples has been reported in various studies. Lyu et al in there study reported 80% sensitivity and 94% specificity, 89.3% sensitivity and 82.7% specificity was reported by Tortoli E. et al.[13,38] The results of our study recorded a good yield and are thus in line with the previous reports and support the WHO's recommendation of using Xpert MTB/RIF assay as the initial test in children with suspected tuberculosis instead of attempting to identify/detect Mycobacteria through smear and culture.[39]

5. Conclusion

The results of this study highlight the usefulness of Xpert MTB/RIF assay in the diagnosis of EPTB. In particular, this assay proved to be of great utility while testing pus samples, tissue samples and lymph node FNACs. The yield obtained from CSF samples was not very encouraging but can be possibly improved if larger volume of sample is used for testing or by using concentrated samples. Contrary to previous published reports, pleural effusion showed a promising yield while, the diagnostic utility of Xpert MTB/RIF assay using cavitary fluids and Urine samples remains to be limited. In addition, the results of this study suggests a clear role of testing gastric aspirate samples in diagnosing PTB particularly among the sputum-scares population.

Another important aspect as evident in this study is the ability of this assay to rapidly determine patient's Multi-drug resistance status. This is of prime importance as this allows faster initiation of appropriate therapy resulting in increased treatment success rates and decreased mortality rates. Last but not the least, the findings of this study also highlights the success of decentralized MTB/RIF assay implementation.

Conflicts of interest

The authors have none to declare.

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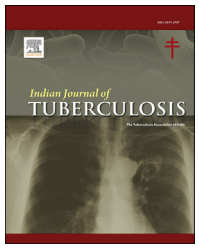

Original article

Pulmonary function in cured pulmonary tuberculosis cases

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ABSTRACT

Background: In clinical practice it has been observed that several patients of cured pulmonary tuberculosis (PTB) suffer with lung dysfunction and these problems are less documented routinely. Prevalence of these abnormalities remains unknown. Aim of this study is to estimate the lung function abnormality and exercise capacity including diffusion capacity of lung for carbon monoxide (DLCO) in cured PTB cases.

Methods: A hospital based observational descriptive study was carried out among 100 patients with PTB, who had been declared cured. These patients were evaluated by spirometry and DLCO to assess their lung function and were classified as normal or abnormal. Modified medical research council (mMRC) dyspnea scale for symptom assessment and 6-minute walk test (6MWT) to determine the exercise capacity was also done. Borg's scale was used for dyspnea assessment in 6MWT.

Results: 83 (83%) patients having abnormal spirometry, 17 (17%) had obstructive pattern, 32 (32%) had restrictive pattern and 34 (34%) had mixed pattern. 22 (22%) patients had mild decrease in DLCO, 43 (43%) patients had moderate decrease in DLCO, while only 4 (4%) had severe decrease in DLCO. More than half of the patients having normal spirometry had reduced in DLCO.

Conclusion: The prevalence of abnormal lung functions is high even after complete antitubercular treatment. DLCO could be a better tool for evaluation of lung function in these patients. There is need to strengthen the National Programme to detect and treat TB patient earlier, also there is need to formulate guidelines for pulmonary rehabilitation of cured PTB patient.

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1. Introduction

Tuberculosis (TB) is the ninth leading cause of death worldwide and leading cause of death from a single infectious agent, ranking above HIV/AIDS. It affects the lungs and results in poor lung compliance secondary to diffuse fibrotic changes to lung tissue.[1]

Pathogenesis in TB cases include the formation of caseous granuloma, tissue liquefaction, and formation of pulmonary cavities.[2] From these changes there are residual lesions remain in many patients which results in pulmonary sequelae, such as bronchovascular distortions, bronchiectasis, emphysema, and fibrosis.[3] These changes affects the calibre of the airways, increases its strength, and decreases airflow.[4] Through the mechanism of cicatricial fibrosis, there is also a reduction of total lung capacity.[5] Post-TB patients may have limited exercise tolerance and significant disability which may affect daily activities.[6]

Pulmonary Function Tests objectively quantify lung function and impairment, and are used to evaluate persons with chronic lung disease.[7]

Studies performed to asses lung function in post PTB patient have been of highly selected populations, such as inpatient TB treatment, referred for preoperative evaluation and persons sufficiently well to be currently employed in mining.[3,6] These patients do not completely represent the populations affected by TB. In these studies lung function assessment was done by spirometry. None of the studies used DLCO for the assessment of lung function.

Rationale of this study is to estimate the lung function including DLCO, exercise capacity by 6MWT and symptoms severity by Mmrc dyspnea scale in cured PTB cases.

2. Materials and methods

After approval of institutional ethics committee, a hospital based observational descriptive study was conducted from January 2019 to 30th June 2020 in Out Patient Department of Respiratory Medicine, at a tertiary care hospital in Delhi NCR.

2.1. Sampling

Systematic sampling was done every k patient of cured PTB was selected (k = 3).

2.2. Sample size

According to Cruz Rde C et al,[8] 89.6% cured PTB patients presented with pulmonary sequels. Calculated using formula

$N = \frac{3.84pq}{d^2}$ p = prevalence, q = 1-p, d = permissible error (6%)

So p = 89.6 and q = 10.4 and hence sample size was 99.34. So we recruited 100 patients in our study.

2.3. Inclusion criteria

Patients with age ≥18 years but <70 years, established cases of PTB at the time of initiation of treatment, who had been treated and declared cured, irrespective of gender, educational status and socioeconomic status were enrolled in the study.

Table 1 – Demographic profile of the patients.

Sex distribution (n = 100)	
Males	53%
Females	47%
Age distribution	
18–30 years	27
31–40 years	14
41–50 years	18
51–60 years	28
61–70 years	13
Occupation distribution	
Skilled	4
Semi-skilled	95
Unskilled	1

2.4. Exclusion criteria

PTB cases with doubtful diagnosis at the time of initiation of anti tubercular treatment (ATT), treated inadequately or incompletely, extrapulmonary TB, cases associated with any other co morbid conditions such as heart disease, COPD, renal disease, immunocompromised patients and smoker were excluded.

After written consent, each patient was evaluated via detailed history and thorough clinical examination. Chest X ray, KFT, Blood sugar, ELISA for HIV 1 and 2, ECG and sputum smear for AFB were used to rule out any associated co morbidities or active TB. Finally PFT including spirometry and DLCO to evaluate the lung function, mMRC for symptoms severity and 6MWT to evaluate the exercise capacity were used.

The data from patients was collected using a pre-formed paper sheet, entered in the MS Excel sheet and cleared. Analysis was done by using statistical software Statistical Programme for Social Sciences (SPSS) Version 16. Categorical data was presented in the form of percentages and continuous data was presented in the form of means and standard deviation. Interpretive algorithms were used in determining restrictive or obstructive patterns and spirometry results were analysed.

3. Results

Of 100 patients selected for the study, 53 were males and 47 were females (Male: Female ratio = 1.13:1). Average age was

Table 2 – Pattern of lung function on Spirometry.

Type of disease	Number of Patients
Normal	17 (17%)
Restrictive	32 (32%)
Mixed	34 (34%)
Obstructive Total 17 Cases (17%)	Mild -1 case
	Moderate-10 Cases
	Severe-4 Cases
	Very Severe-2 Cases

Table 3 – Pattern of DLCO in cured PTB patients.

DLCO(%predicted)	Category	Number of Patients
80–120	Normal	31
60–80	Mild	22
40–60	Moderate	43
<40	Severe	4

Table 4 – DLCO in various groups of spirometry.

Spirometry Results	DLco Results	
Normal	Normal	8
	Reduced	9
Restrictive	Normal	4
	Reduced	28
Obstructive	Normal	12
	Reduced	5
Mixed	Normal	7
	Reduced	27

Table 5 – prevalence of various symptoms and severity of dyspnea (mMRC grading).

Symptoms (n = 100)	
Shortness of Breath	100%
Dry cough	29%
Cough with expectoration	18%
mMRC Grading	
Grade 1	15
Grade 2	15
Grade 3	41
Grade 4	29

44.18 years. Maximum number of patients were in the age group of 51–60 years (28%), followed by 18–30 years age group (27%). Average BMI was 22.6 Kg/m^2. No patient was obese and muscle wasting was found in 5% of patients. Most of the patients in this study were semi-skilled workers (95%), followed by being in skilled profession (4%) (Table 1).

On spirometry, most of the patient (34%) had mixed pattern followed by restrictive pattern (32%). Among the patients having obstructive pattern, mostly had moderate obstruction (Table 2).

Most of the patient (43%) had Moderate decrease in DLCO (Table 3).

More than half of the patients having normal spirometry values had reduced DLCO (Table 4).

90 (90%) patients had taken anti-tubercular drugs for 6 months, 3 (3%) for 7 months, 1 (1%) for 8 months, 5 (5%) for 9 months and 1 (1%) patient had taken for 12 months.

All participants had complaints of shortness of breath of either grade. Dry cough was more common than productive cough. Most of the patients had Grade 3 dyspnea according to mMRC (Table 5).

Borg's scale was used for assessment of dyspnea in 6MWT, in the patient having grade 4 dyspnea test was terminated as Spo 2 fell below 80%. In 6MWT tachycardia, tachypnea and fall in Spo2 increased as the severity of symptoms increased (Table 6).

4. Discussion

Despite the availability of effective drugs against *Mycobacterium tuberculosis*, it has not been possible to limit the permanent pulmonary tissue scarring and damage left by PTB. The destructive nature of the disease leads to a reduction in vital capacity, and bronchiectatic changes, along with bronchial stenosis causing airway obstruction.

On spirometry test, 34% patients had mixed pattern, 32% had restrictive pattern, 17% had obstructive pattern and 17% had normal spirometry. In a study by Mohamed Manji[9] et al, 42% patients had obstructive pattern which is in contrast to our study. While in a study by Kurt J. Daniels et al,[10] it was observed that 52% of patients had normal lung function followed by 21% had obstructive, 25% had restrictive and 2% had mixed patterns.

Literature was searched for DLCO in cured PTB cases but proper literature was not found.

70% patients had MMRC Grade 3 (41%) or Grade 4 (29%) and only 30% patients presented with Grade 1 or Grade 2 MMRC. In contrast, in study by Mohamed Manji et al[9] 81.2% patients had MMRC score of 1 or 2.

Major weakness in this study was that, the only patients who presented to our institute either for routine follow-up or with respiratory symptoms were included. This meant the exclusion of a large subset of patients who had not sought treatment for symptoms or who were symptom-free. The incidence of different post TB cure respiratory impairment could thus undergo upward or downward revision if applied to all cured patients. This can only be confirmed by a more comprehensive study that follows up a cohort of all cured patients over a period of time. The main strength of this study is that DLCO was performed to evaluate the lung function of cured PTB patients which have not been ever performed in previous studies.

Table 6 – Pre and post pulse rate, respiratory rate, SpO_2, distance covered and Borg's scale in 6MWT.

mMRC grade	Pulse Rate		Respiratory Rate		SpO_2		Distance walked (mean) (metres)	Borg's	
	Pre	Post	Pre	Post	Pre	Post		Pre	Post
Grade 1	76.53	79.73	13.9	16.93	96.9	95.7	361	0,0.5	1,2
Grade 2	76.2	97.4	16.07	26.4	96	88.53	286	0,0.5	1,2
Grade 3	85.48	130.43	19.5	36.6	92.24	82.39	179	0.5,1,2	3,4,5,6
Grade 4	114.93	Terminated	27.3	Terminated	83.3	Terminated	78	3,4,5,6	Terminated

5. Conclusion

Pulmonary tuberculosis results in poor lung compliance due to diffuse fibrotic changes in the lung tissues. Findings of our study suggest that even the patients who have successfully completed treatment for PTB suffer from impaired lung function, decreased exercise capacity and poor quality of life. Whether these impairments resolve over time is also not clear. What remains clear is that we clinicians have to realize that a holistic management of PTB patients is required, even after microbiological cure. At present, pulmonary rehabilitation guidelines for post-TB patients do not exist.

To conclude, there is a need for long-term follow-up, appropriate diagnosis and optimal treatment even after microbiological cure of TB. Such cases should be actively searched and treated. These findings should be considered in the future evaluation of interventions to minimise long-term disability and the global assessment of the burden of disease associated with TB.

Author's Contribution

Mohan Bandhu Gupta: Concept, Design, Literature search, Data analysis, Statistical analysis, Manuscript editing, Manuscript review, Guarantor. Sharad Bagri: Concept, Design, Data acquisition, Data analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor. Ankur Garg: Literature search, Data acquisition, Manuscript preparation, Manuscript review. Devendra Kumar Singh: Concept, Design, Data acquisition, Data analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor. Prashant choudhary: Concept, Manuscript preparation, Manuscript editing, Manuscript review. Subah Sahni: Data analysis, Manuscript preparation.

Conflicts of interest

The authors have none to declare.

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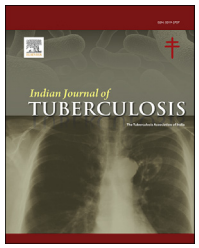

Original article

Role of Magnetic Resonance Imaging in evaluation of tuberculous tubo ovarian mass

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ABSTRACT

Objective: Role of Magnetic Resonance Imaging (MRI) in diagnosis of tuberculous tubo-ovarian (TO) mass.

Methods: MRI was performed on 33 patients of tuberculous TO mass of female genital tuberculosis (FGTB).

Results: Mean age, BMI, and parity was 27.5 ± 4.2 years, 22.7 ± 3.6 kg/m^2, and 0.27 ± 0.13. All patients (100%) had infertility; primary infertility (72.72%) and secondary infertility (27.23%) with mean 5.8 years. Abdominal/pelvic pain 33 (100%) cases, abdominal lump 4 (12.12%), adnexal mass 33 (100%). MRI findings showed pelvic masses 33 (100%), bilateral TO masses 11 (33.33%), cystic lesion 4 (12.12%), solid cystic lesion 3 (9.09%) with bilateral pyosalpinx 1 (3.3%), homogeneous content with ascites 1 (3.03%), rim enhancing lesion abutting pelvic wall in 1 (3.03%). Right adnexal mass 11 (33.33%), right adnexal cyst 2 (6.06%), right adnexal cystic mass in 1 (3.03%), right sided complex TO mass 1 (3.03%), right sided hydrosalpinx in 1 (3.03%) case, right sided TO mass in 4 (12.12%) cases and right sided para-ovarian cyst in 2 (6.06%). Left sided adnexal mass was seen in 11 (33.33%), cystic lesion in 1 (3.03%), ovarian cyst in 3 (9.09%) cases, left sided hydrosalpinx in 2 (6.06%), left ovarian cyst 2 (6.06%) cases, left sided ovarian cyst with encysted ascites 1 (3.03%) case and with left sided paraovarian cyst 2 (6.06%) case. Miscellaneous finding were generalised ascites (6.06%), encysted ascites (3.03%), pelvic (1; 3.03%) and mesenteric lymphadenopathy 1 (3.03%). Incidental finding were fibroid 3 (9.09%) and adenomyosis 1 (3.03%) case.

Conclusion: MRI appears to be useful diagnostic modality for tuberculous TO masses where differential diagnosis is malignancy but molecular diagnosis remains the gold standard.

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1. Introduction

Tuberculosis continues to be a major public health problem all over the world but is more common in developing countries like India.[1,2] Although pulmonary TB remains the most common and infectious form, extrapulmonary TB (EPTB) is becoming more common especially due to concomitant HIV infection and more liberal immigration.[3] Female genital tuberculosis (FGTB) is a type of EPTB with very low incidence (0.69%–1%) in USA and upto 19% in India.[4–6] In a recent study in USA on 323 infertility women, the authors observed 7.7% positive quantiferon gold test but less than 1% positive culture or histopathology for FGTB.[6]

FGTB causes infertility through involvement of fallopian tubes (blockage), endometrium (Asherman's syndrome) and ovaries (decreased ovarian reserve).[4,5,7,8] Abdominopelvic TB may present as abdominal or pelvic lump with or without ascites and may mimic ovarian cancer with raised CA 125 levels.[9]

Traditionally diagnosis of FGTB is made by demonstration of acid fast bacilli (AFB) on microscopy or culture of endometrial or peritoneal biopsy or demonstration of positive gene Xpert on endometrial biopsy or epithelioid granuloma on histopathology of endometrial or peritoneal biopsy.[4,5,7,8] However these tests are positive in small percentage of patients and thus diagnosis of FGTB may be missed in many cases.[4,5] Interferon gamma release assay (IGRA) and Quantiferon gold have been observed to have higher sensitivity and specificity than standard tuberculin skin testing in USA and China.[6,10]

Polymerase chain reaction (PCR) though very sensitive but has high false positivity and alone is not recommended to diagnose FGTB or to start antituberculous treatment.[4,5,7] Wang et al[11] from China have developed high sensitivity Taq Man based PCR for specific diagnosis of *Mycobacterium tuberculosis* in both pulmonary and extrapulmonary TB. Munne et al[12] have observed newer molecular tests like PCR, TB-LAMP(loop mediated isothermal amplification), Xpert and line probe assays to be useful in diagnosis of FGTB. Gupta et al[13] have observed role of nuclear receptors in diagnosis of FGTB in association with standard diagnostic methods.

Endoscopic procedures like laparoscopy and hysteroscopy have been used to directly visualise TB lesions and can pick up more cases but are invasive, need general anaesthesia and are associated with higher complications.[4,5,14] Radiological imaging is useful especially for tuberculous tubo ovarian masses but has lesser sensitivity for FGTB without adnexal masses.[4,5,15,16]

Ultrasound through economical and easily available can pick up cases of tubo ovarian masses but has lesser sensitivity.[15,16] Computed tomography (CT) has also been used for tuberculous adnexal masses, ascites and pelvic lymphadenopathy with varying results.[15,16] Magnetic resonance imaging (MRI) has highest sensitivity in detecting tuberculous lesions particularly in even slightly enlarged lymph nodes, thickening of endometrium or omentum, involvement of small and large intestine, associated ascites and can help to differentiate between tuberculous tubo ovarian masses from ovarian cancer.[15,16] Patil et al[17] in a comparative study on CT scan and MRI for EPTB including FBTB observed MRI to be more useful than CT scan for some type of EPTB but equal for other EPTB. The present study was conducted over 33 cases with adnexal masses out of total 175 cases of FGTB to evaluate the role of MRI in detection of FGTB with tubo ovarian masses.

2. Methods

It was a prospective study on 175 infertile women diagnosed to have FGTB on composite reference standard from a tertiary referral centre over 4 year period from July 2016 to August 2019. Out of 175 women, a total of 33 women (18.85%) who had tubo ovarian mass or clinical examination were invited in this study on which MRI was performed. The study was part of our large FGTB project for which ethical clearance was obtained from the institute. Written informed consent was taken from all patients. The diagnosis of FGTB was made on composite reference standard (CRS) which included positive acid fast bacilli on microscopy or culture of endometrial biopsy, positive gene xpert or positive epitheloid granuloma on histopathology of endometrial or peritoneal biopsy or definite (tubercles, caseous nodules or beaded tubes) or probable (hydrosalpinx, pyosalpinx, peritoneal, pelvic, abdominal or perihepatic adhesions, shaggy areas, encysted ascites, tubo ovarian mass) findings of FGTB on laparoscopy.

All women underwent detailed history taking and clinical examination including abdominal or gynaecological examination and baseline investigations including complete hemogram, erythrocyte sedimentation arte (ESR), blood sugar, X-ray chest. Endometrial biopsy was taken between day 21 to day 23 (premenstrual phase) in all the cases using number 4 Karman's cannula and sample was sent in normal saline for demonstration of AFB on microscopy, culture, PCR, gene Xpert also called cartridge based nucleic acid amplification test (CB-NAAT) and in formalin solution for demonstration of epithelioid granuloma on histopathology and for type of phase of cycle for ovulation.

MRI was done in all patients of FGTB with tubo ovarian masses only. It couldn't be done in all patients of FGTB due to financial and logical constraints as MRI was more expensive and has longer waiting periods. Axial T2 weighted MRI of pelvis and abdomen was performed for various findings of FGTB with adnexal mass. In selected patients, gadolinium enhanced, axial MRI with T1 weighted image with fat suppression was done for better resolution of multiple and smaller hypovascular nodules. The MRI findings like unilateral or bilateral tubo ovarian masses, ovarian cysts, hydrosalpinx, pyosalpinx, cystic lesion, solid lesion, any associated ascites or lymphadenopathy or associated anovulation, peritoneal adhesions was also noted.

2.1. *Statistical analysis*

Data analysis was carried out using STATA software v 12.0. Continuous variables were tested for normality assumption using KOLMOGOROV- SMIRNV test. Descriptive statistics such as Mean, Standard Deviation, Range values were carried for normally distribution dates. Comparison of two groups means were tested using Student's 't' independent test.

Categorical data were presented as frequency and percentage values. Comparison of categorical values were tested using Chi-Square/Fischer's exact test.

The MRI findings were tabulated using SPSS version 12.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. A p value of less than 0.05 was taken for significance.

3. Results

Out of total 175 cases of FGTB, diagnosed on composite reference standard (combination of various tests), a total of 33 (18.18%) patients were found to have tubo ovarian masses. The characteristics, symptoms, sign and baseline investigations of the patients are shown in Table 1. The age ranged from 24 to 36 years, with mean being 27.5 ± 4.2 years. The body mass index (BMI) ranged from 17.5 to 31.8 kg/m^2, with mean being was 22.7 ± 3.6 kg/m^2, while parity ranged from 0 to 3, with mean being 0.27 ± 0.13. History of TB contact was seen in 14 (42.42%) cases, while history of BCG vaccination was seen in 25 (45.45%) cases. The infertility was seen in all cases (100%) being primary infertility in 24 (72.72%) and secondary infertility in 9 (27.27%) cases with duration of infertility, ranging between 2 and 13 years, mean being 5.8 ± 2.65 years(Fig. 1).

Various menstrual symptoms, menstrual dysfunction and constitutional symptoms are shown in Table 1. Various general physical examination findings, gynecological examination findings and baseline investigations are also shown in Table 1.

Diagnosis of FGTB in tubo ovarian masses is shown in Table 2. AFB were demonstrated on microscopy or culture of endometrial biopsy in 5 (15.15%) cases, positive gene Xpert was seen in 6 (18.18%) cases, being 4 (12.12%) in endometrial biopsy and 2 (6.06%) in peritoneal biopsy cases, positive polymerase chain reaction (PCR) was seen in 32 (96.96%), epithelioid granuloma was seen on histopathology in 7 (21.21%) cases, being 4 (12.12%) in endometrial biopsy and 3 (9.09%) in peritoneal biopsy cases. Definite findings (tubercles, caseous nodules, beaded tubes) of FGTB were seen in 15 (45.45%) cases, while probable findings (hydrosalpinx, pyosalpinx, pelvic, abdominal or perihepatic adhesions, encysted ascites)were seen in 18 (54.54%) cases. During laparoscopy peritoneal biopsy or biopsy from TB lesions like caseous nodules or tubercles was taken in selected cases.

Various MRI findings are shown in Table 3. Thus, masses were seen in all (33.33%) patients with bilateral tubo ovarian masses in 11 (33.33%) cases, with cystic lesions in 4 (12.12%) cases, with solid cystic lesion in 3 (9.09%) cases with bilateral pyosalpinx 1 (3.3%) cases, with rim enhancing adnexal lesion abutting pelvic walls in 1 (3.03%) cases, bilateral complex adnexal mass with thick walls in 1 (3.03%) case while with bilateral ovarian cyst with homogeneous content with ascites in 1 (3.03%) case. Right sided adnexal mass on MRI was seen in 11 (33,3%) cases, being right adnexal cyst in 2 (6.06%), right adnexal cystic mass in 1 (3.03%) case, right sided complex tubo ovarian mass in 1 (3.03%), right sided hydrosalpinx in 1 (3.03%) case, right sided tubo ovarian mass in 4 (12.52%) cases and right sided paraovarian cyst in 2 (6.06%) cases. Left sided adnexal mass was seen in 11 (33.33%) cases being with cystic lesion in 1 (3.03%) cases with ovarian cyst in 3 (9.09%) cases, left ovarian cyst alone in 2 (6.06%) cases, left sided hydrosalpinx in2 (6.06%) cases with encysted ascites in 1 (3.03%) case and with left sided paraovarian cyst in 2 (6.06%) case. Various miscellaneous findings were generalised ascites (2; 6.06%), encysted ascites (1; 3.03%), pelvic (1; 3.03%) and mesenteric lymphadenopathy (1; 3.03%). Incidental finding were fibroid in 3 (9.09%) and adenomyosis in 1 (3.03%) case.

4. Discussion

FGTB is a type of extrapulmonary tuberculosis and contributes for about 9% cases of EPTB.[1,2] It is usually secondary to pulmonary or other EPTB cases with infection mainly spreading hematogenously.[3–5] Fallopian tubes are involved most commonly followed by endometrium and ovaries and thus causing infertility or tubo ovarian masses.[4–8] It can cause peritoneal and abdominal disease with peritoneal and omental deposits and ascites and resembles ovarian cancer and may necessitate unnecessary laparoscopy or laparotomy to confirm the diagnosis.[9] Traditionally diagnosis is made by gold standard demonstration of AFB on microscopy or culture of endometrial or peritoneal biopsy or positive gene xpert or positive epithelioid granuloma on histopathology of endometrial or peritoneal biopsy.[4,8,10–12] But unfortunately these tests are positive in small percentage of patients and thus diagnosis may be missed.[4,5] PCR though highly sensitive has high false positivity and alone is not enough to diagnose FGTB or to initiate treatment.[4–8] Newer Taq Man based PCR has been observed to be better than traditional PCR in a Chinese study[11] but is still experimental.

Diagnostic laparoscopy can be used to diagnose FGTB and abdominal TB even in early disease and may show definite findings of TB like tubercles, caseous nodules and beaded tubes and probable findings of FGTB like hydrosalpinx, pyosalpinx, peritubal, pelvic, abdominal or perihepatic adhesions, shaggy areas (white deposits), encysted ascites, tubo ovarian mass.[4,5,14] In the present study, CRS (composite reference standard) was used to diagnose FGTB which can pick up higher number of cases by combining many tests as has been done for all types of EPTB including FGTB.[18] For FGTB, it combines demonstrating AFB on microscopy or culture of endometrial biopsy, positive gene Xpert or epithelioid granuloma on histopathology of endometrial and peritoneal biopsy and definite or probable findings of FGTB on laparoscopy. The diagnosis of FGTB is always a dilemma due to its paucibacillary nature and lack of gold standard. But CRS can pick up higher number of cases.[19]

Radiological imaging has been used for detection of FGTB.[4,5,15–17] Hysterosalpingography though avoided in active TB, can detect tubal and endometrial disease and may diagnose hydrosalpinx, tubal blockage and endometrial adhesions.[20] Ultrasound especially 3 dimensional ultrasound can detect subtle changes of FGTB in expert hands but has lower resolution than CT scan and MRI.[15,16] Computed tomography has been used in diagnosis of tubercular tubo ovarian mass, ascites, pelvic and abdominal lymphadenopathy and to detect peritoneal and omental thickening and can help to differentiate between tuberculosis and malignancy, but has lower

Table 1 – Characteristics, clinical features and basic investigations of the patient.

Serial no.	Characteristics	Number N = 33	Percentage
1	Age (years)		
	Range	24–36	
	Mean ± SD	27.5 ± 4.2	
2	Body Mass Index (Kg/m^2)		
	Range	17.5–31.8	
	Mean ± SD	22.7 ± 3.6	
3	Parity (numerical)		
	Range	0–3	
	Mean ± SD	0.27 ± 0.13	
4	History of TB Control	14	44.42
5	History of BCG Vaccination	25	
6	Type of Infertility	33	100
	I. Primary Infertility	24	72.72
	II. Secondary Infertility	9	27.27
7	Duration Of Infertility (years)		
	Range	2–13	
	Mean ± SD	5.85 ± 2.65	
8	Menstrual Symptoms		
	I. Normal Menstruation	14	42.42
	II. Menstrual Dysfunction	19	57.57
	III. Abnormal Uterine Bleeding	2	6.06
	IV. Hypomenorrhoea	8	24.24
	V. Oligomenorrhoea	7	21.21
	VI. Amenorrhoea	2	6.06
	VII. Dysmenorrhoea	9	27.27
9	Anorexia	12	36.36
10	Weight Loss	14	42.42
11	Pyrexia	11	33.33
12	Dyspareunia	9	27.27
13	Vaginal Discharge	11	33.33
14	Abdominal or Pelvic Pain	33	100
15	Abdominal or Pelvic Lump	33	100
	Examination Findings		
1	Pallor	11	33.33
2	Lymphadenopathy	9	27.27
3	Abdominal Lump	4	12.12
4	Speculum Examination		
	1. Abnormal Discharge	23	69.69
	2. Adnexal Mass Or Tenderness	33	100
	a. Unilateral	18	54.54
	b. Bilateral	15	45.45
	Baseline Investigations		
1	Anemia	11	
2	Erythrocyte Sedimentation Rate(ESR)		
	mean ± SD (mm/hour)	33.4 ± 11.48	
3	Leucocyte count (per cubic mm) mean ± SD	6128 ± 2854	
4	Infectious montoux test (>10mm)	14	42.42
5	Abnormal X ray Chest	9	27.27
6	CA125 Level (IU/Ml)		
	Range	15–500	
	Mean ± SD	75.4 ± 38.5	

resolution than MRI and may not detect smaller lymph nodes.[15,16,21]

In the present study of MRI, on 33 women with tuberculous tubo ovarian masses, we observed bilateral tubo ovarian mass in 11 (33.33%) cases, right sided mass in 11 (33.33%) cases and left sided mass in 11 (33.33%) cases. Other findings included tubo ovarian mass with cystic lesions, solid cystic lesions, pyosalpinx, hydrosalpinx, rim enhancing adnexal lesion abutting pelvic walls, encysted or generalised ascites, pelvic and mesenteric lymphadenopathy. We also observed some incidental non tubercular findings also like fibroid in 3 (9.09%) cases, (intramural in 2 and subserosal in 1 case) and adenomyosis in 1 case. MRI can detect whether the mass is simple or complex. It can also detect calcification in the TB lesions.[21]

Shore et al reported a case of tubal granuloma due to FGTB confined to have tubal calcifications with complex debris on MRI in their case.[22] Da Rocha et al[21] observed significant role of

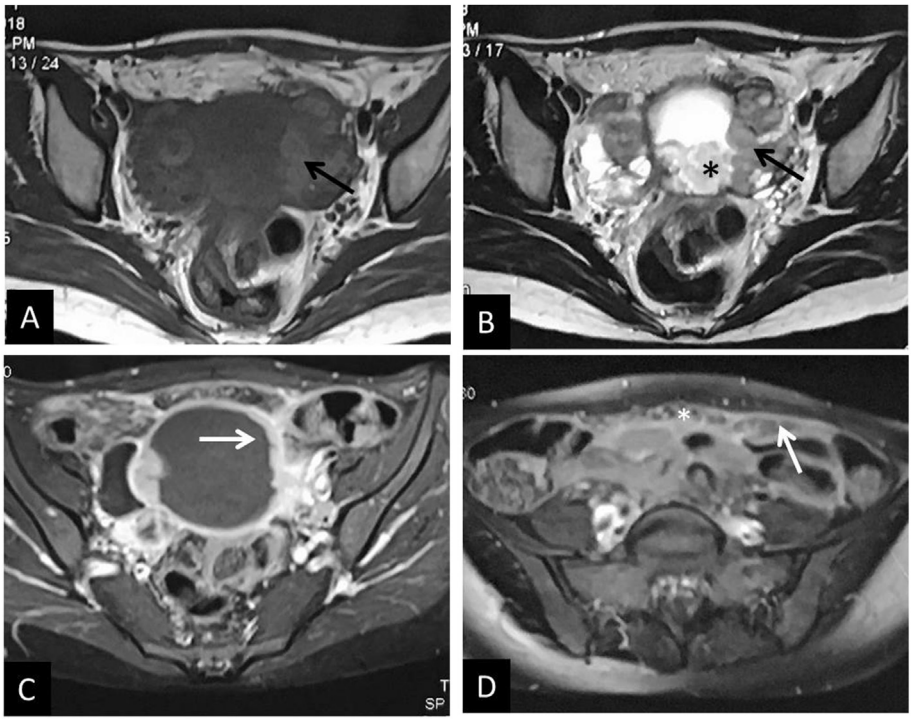

Fig. 1 – MRI Tuberculosis. A. Axial T1WI (A), T2WI (B) and post contrast axial T1WI (C) reveals bilateral adnexal tubo-ovarian lesions. The tubal component shows T1 hyperintense and T2 Intermediate signal intensity contents (black arrow in A and B). The lesions are cystic with internal debris (* in B) and thick enhancing walls (arrow in C). D. Axial post contrast MRI at a more cranial level reveals peritoneal enhancement (arrow in D) and omental nodularity (* in D).

Table 2 – Diagnosis of FGTB in tubo ovarian masses.

S.no.	Test	Number(n)	Percentage (%)
1	AFB on microscopy or culture of endometrial biopsy	5	15.15
2	Positive gene Xpert on endometrial or peritoneal biopsy	6	18.18
	a. Endometrial biopsy	4	12.12
	b. Peritoneal biopsy	2	6.06
3	Positive polymerase chain reaction	32	96.96
4	Epithelioid granuloma on histopathology of endometrial or peritoneal biopsy	7	21.21
	a. Endometrial biopsy	4	12.12
	b. Peritoneal biopsy	3	9.09
5	Definitive findings of tuberculosis on laparoscopy	15	45.45
6	Probable findings of tuberculosis on laparoscopy	18	54.54

MRI and CT scan in diagnosis of abdominal TB including differentiation between wet and dry ascites and fibrinous peritonitis. They also observed psoas abscess and ascites in their study of MRI in abdominal tuberculosis.[21] MRI has been observed to be better in diagnosis of TB of solid organs like liver, spleen, kidney etc and has superior soft tissue resolution and multiplanar acquisition.[21,22] Using new advances like respiratory compensation, breath hold acquisition, fat suppression and orally administered contrast agents high quality MR images can be obtained through the entire abdomen.[21,22] MRI has better resolution and can pick up abdominal TB with lymphadenopathy, bowel TB and omental TB.[21,22] It is also a useful modality for diagnosis of hepatic, splenic, adrenal TB in abdomen.[17,18,21,22] Recently MRI was found useful in diagnosing pelvic masses in covid 19 patients also.[23]

Bomanji et al[16] reported that MRI was better than other modalities in diagnosis of abdominal and genital TB in the form of conglomerate mass which is unilocular or multilocular with thickened wall that may show enhancement on contrast enhanced MRI. Other authors also favoured MRI to be useful in FGTB and pediatric EPTB.[24,25]

In the present study on 33 cases with tubo ovarian masses out of 175 cases of FGTB, we didn't find any unique specific or pathognomic finding that would substantiate the diagnosis of FGTB any more than transvaginal scan or CT scan. Infact MRI being a more sensitive imaging modality for delineating soft tissue structures, it would have been more appropriate to include FGTB cases without tubo ovarian masses as MRI might have detected some cases with adnexal lesions missed by ultrasound or CT scan. Unfortunately MRI could not be done in

Table 3 – MRI findings in tuberculous tubo ovarian mass.

S.No.	MRI findings	Number(n)	Percentage (%)
1	**Bilateral tubo ovarian masses**	11	33.33
	Bilateral tubo ovarian mass with cystic lesions	4	12.12
	Bilateral tubo ovarian mass with solid cystic mass	3	9.09
	Bilateral tubo ovarian mass with bilateral pyosalpinx	1	3.03
	Bilateral rim enhancing adnexal cystic lesion abutting pelvic walls	1	3.03
	Bilateral complex adnexal masses with thick wall	1	3.03
	Bilateral ovarian cyst with homogenous content with ascites	1	3.03
2	**Right sided adnexal mass**	11	33.33
	Right adnexal cyst	2	6.06
	Right cystic mass	1	3.03
	Right sided complex tubo-ovarian mass	1	3.03
	Right sided hydrosalpinx	1	3.03
	Right tubo ovarian mass	4	12.12
	Right sided par ovarian cyst	2	6.06
3	**Left sided adnexal mass**	11	33.33
	Left sided tubo ovarian mass with cystic lesions	1	3.03
	Left sided tubo ovarian mass with ovarian cyst	3	9.09
	Left ovarian cyst	2	6.06
	Left sided hydrosalpinx	2	6.06
	Left sided ovarian cyst with encysted ascites	1	3.03
	Left sided para-ovarian cyst	2	6.06
4	**Miscellaneous finding** [a]		
	Ascites (generalized)	2	6.06
	Encysted ascites	1	3.03
	Pelvic lymphadenopathy	1	3.03
	Mesenteric lymphadenopathy	1	3.03
	Incidental fibroid	3	9.09
	a. Intramural	2	6.06
	b. Subserous	1	3.03
	Incidental adenomyosis	1	3.03

[a] Note-some patients had more than one MRI findings.

FGTB cases without tubo ovarian masses due to financial and logistic constraints which is a major limitation of the study.

In the present study we only observed adnexal masses with cystic and internal debris and also peritoneal enhancement and omental nodularity which are not specific for TB and can be seen in other conditions also like chronic pelvic inflammatory disease necessitating tissue biopsy for confirmation of diagnosis. Hence MRI alone can't be used for diagnosis of FGTB but can only be used as an adjunct to molecular tests.

However molecular diagnosis remains gold standard for the diagnosis of FGTB, imaging being only an aid to molecular diagnosis. Higher imaging like CT scan and MRI have better resolution than ultrasound and can be useful in advanced abdominopelvic TB where differential diagnosis is ovarian or peritoneal cancer. Unfortunately there are no known randomised controlled trials in the world literature to the best of our knowledge comparing best modalities for diagnosis of abdominopelvic TB or FGTB.

MRI for FGTB is a useful adjunct only when there is strong need to explore a differential diagnosis of a benign or malignant mass. It has got no diagnostic or prognostic value in clinical management of pelvic TB. The low cost and easily available ultrasound should be the first modality while MRI should be restricted to cases where malignancy is to be ruled out due to its higher cost and non availability at many places. MRI also has a role for research purpose for abdominopelvic TB and FGTB. It can also be extrapolated in advanced FGTB before any surgical intervention is sought especially in drug resistant cases.

We took caseous nodules, beaded tubes and tubercles as definitive finding of TB but caseation is usually is a feature of histopathology, large whitish and yellowish nodules on laparoscopy can be called caseous nodules and are a definitive finding of TB. Similarly beaded tubes is usually a finding on hysterosalpingography but it can be seen during laparoscopy also with alternate constriction and dilatation of tubes. Similarly tubercles are not definitive for TB till confirmed by histopathology as similar macroscopic picture can be seen in other granulomatous lesions. All these are limitations of one study. We don't recommend routine use of MRI for all cases of FGTB or abdominopelvic TB but where malignancy is a differential diagnosis or where ultrasound findings are not helpful.

Hence, MRI appears to be a useful but limited modality in diagnosis of selected cases of FGTB with tubo ovarian masses where differential diagnosis is malignancy as it has better resolution.

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Conflicts of interest

The authors have none to declare.

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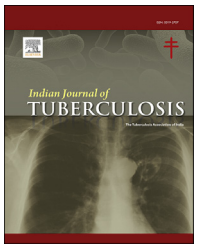

Original article

Enrolment under of Nikshay Poshan Yojana among tuberculosis patients in a tertiary care hospital of Delhi

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ABSTRACT

To mitigate malnutrition among tuberculosis burden, Government of India launched Nikshay Poshan Yojana in 2018, providing incentive of INR500 per month to each enrolled patient. Our study tried to find out the status of the scheme and its target benefits amongst the beneficiaries in a tertiary care hospital in Delhi, and also the facilitating factors and barriers towards enrolment, and their knowledge, attitude and practice towards nutrition in TB.

Methods: A cross-sectional study was undertaken at a tertiary care hospital involving a calculated sample of 188 patients. The subjects were interviewed on a pre-designed, semi-structured, validated questionnaire and data was analysed on SPSS v.21.

Results: Enrolment rate for the scheme was 81.4%, of which only 10% of the participants received any benefit. Of the 35 participants who were not enrolled or were not aware of their enrolment status, 22 were interested in enrolment. The reason for non-enrolment by the 21 participants who were not enrolled were lack of awareness, lack of time or lack of a bank account.

Conclusion: The study found that most of the patients attending DOTS treatment were enrolled under the scheme, but 90% were not receiving any incentive promised under it.

1. Introduction

It is well established that nutritional deficiency is associated with impaired immune functions, increasing susceptibility to infections.[1] Moreover, infections can lead to nutritional stress and weight loss, further aggravating immune system and nutritional status of an individual.[2] This is clearly represented by the high double-burden of malnutrition and tuberculosis infection in India, which already harbours 27% of Global TB cases.[3]

Multiple studies have shown that malnutrition is one of the major risk factor of TB.[4,5] It disproportionally affects the poor, undernourished, vulnerable and marginalized populations.[6] Furthermore, TB is known to causes significant economic burden on patients and their households.[7–9] World Health Organization (WHO) in its END TB strategy also emphased that

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no TB-affected families should face catastrophic expenditure by 2020.[10]

In line with this strategy, the National Strategic Plan (NSP) for TB Elimination in India 2017–25 envisages instituting various patient support and social protection measures as well as provide free diagnosis and treatment services.[11] To strengthen this approach, the Government of India launched a scheme called 'Nikshay Poshan Yojana' in March 2018 to provide nutritional support to TB patients. Under this scheme, all TB patients notified and treated as on or after 1 April 2018 are eligible to receive a benefit, which is either in kind (for example, a food basket or dry ration) or in cash (500 INR per month). The cash benefit is transferred electronically to the bank accounts of the beneficiaries through Direct Benefit Transfer (DBT).[12]

To avail this incentive the patients are required to submit details of their bank account, Aadhar card, phone numbers and address. As few studies have shown, some patients may not fulfil these pre-requisites; Further, cases of unwillingness of patients to reveal their bank account details and their qualms about insufficient incentive may pose a challenge to the aim of the scheme.[13,14]

By better understanding these challenges and assessing the coverage of this scheme, it will benefit the patients, and also the policy makers in their decisions to achieve the envisaged vision of the National Tuberculosis Elimination Programme.

In this cross-sectional study, we aimed to assess among the TB patients receiving treatment at a Directly Observed Treatment Short (DOTS) course centre of a tertiary care hospital in Delhi, the enrolment status of patient in the Nikshay Poshan Yojana scheme, the facilitating factors and barriers towards it. We also seek to study the knowledge, attitude and practise of the TB patients regarding nutrition in tuberculosis.

2. Methods

The was a cross-sectional, descriptive study conducted at a DOTS center of a tertiary care hospital of Delhi- Safdarjung Hospital. The study participants included the adult TB patients attending treatment at the centre. The sample size for the study was calculated based on a study conducted by Nirgude et al. in Dakshina Kannada who found the rate of enrollment to be 49.9%.[15]

Relative error of 15% was taken, and a non-response rate of 10% was added to the calculated sample to achieve a sample of 188 patients.

All the enrolled adult TB patients in their Continuous Phase of DOTS treatment during July to December, 2019 were approached and interviewed for the purpose of the study, after taking informed consent. For this purpose, a predesigned, semi structured questionnaire was used containing questions on socio-demographic profile, knowledge about Nikshay Poshan Yojana and DBT and their enrolment under it, obstacles faced by the patients in availing the program benefits, and Knowledge, Attitude and Practise regarding nutrition in TB. The nutritional knowledge was assessed as per the Guidance document on nutritional care and support for patients with tuberculosis in India.[16] Ethical approval was taken from the Institute Ethics Committee prior to the study.

All data was collected in Excel sheets and analysed on licensed SPSS V.21.

3. Results

A total of 188 TB patients were approached and interviewed for the purpose for the study and their data was collected and analysed.

The mean age of participants was 29 years (S.D. ± 12.3) having 57.5% male participants and 54.8% married. Majority of them lived in Pillanji (37, 19.9%), RK Puram (35, 18.6%) and Mohammad Puri (29, 15.4%). Almost all (183, 97.3%) were Hindu.

Majority of the patients were educated till intermediate or less. Thirty seven percent of the study participants were employed in private jobs, 27% were students, 17.6% were housewives, 11.2% were unemployed, 7.4% had government jobs and one was a retired pensioner.

On average one participant lived with 4.8 other people, with minimum being none and maximum being 11. Eighteen percent of the participants possessed a BPL card (Table 1).

Ninety percent of the study participants were on DOTS treatment for Category 1 TB. The mean weight of the study patients at the time of enrolment was 48.7 ± 11.4 kg with minimum and maximum weight of 30 kg and 79 kg respectively (Table 2).

Of the 188 participants interviewed, 88.8% were enrolled in Nikshay Poshan Yojana, and most (141, 75%) were aware of the scheme. Of the three fourth who were aware, 128 (90.7%) of them got to know about it from health staff or the doctor, the rest came to know about it from their family, friends, neighbours or advertisement. Of those who were aware, almost all (98%) got to know of it during the treatment onset.

The study found that only 9% of the enrolled participants received any incentive under NPY scheme. Of those who got the incentive, they got it for a period of 2 months, i.e., an amount of Rupees 1000; and they spend it on fruits, regular food, vegetables, transport etc.

Most (86%) of the enrolled patients faced no difficulties during enrolment. Fifty five percent (92) of the enrolled participants knew why the incentive is being provided. When asked if they knew what they should buy for nutrition, 106 (63.5%) said yes, and a total of 89 (53.3%) of the enrolled participants said that they were informed what to buy for the nutrition.

The reason for non-enrolment by the 21 participants who were not enrolled were lack of awareness (12), lack of time (5) and not having a bank account (4). Of these 18 (85.7%) were interested in enrolment. When they were asked why, buying food was cited as a major reason along with needing money for other causes. Of those who were not interested, less amount of money along with no requirement and lack of a bank account were cited as reasons (Table 3).

Table 1 – Distribution of study participants according to socio-demographic characteristics (N = 188).

	Number (%)
1. Age group[a] (In Years)	
15–24	86 (45.7)
25–34	50 (26.6)
35–44	27 (14.3)
45–59	17 (9.0)
≥60	8 (4.2)
2. Sex	
Male	108 (57.5)
Female	80 (42.5)
3. Marital status	
Unmarried	85 (45.2)
Married	103 (54.8)
4. Address	
Pillanji	37 (19.9)
R K Puram	35 (18.6)
Mohammad Puri	29 (15.4)
Safdarjung Enclave	20 (10.6)
Ambedkar Nagar	18 (9.6)
Sarojini Nagar	10 (5.3)
Others	39 (20.7)
5. Religion	
Hindu	183 (97.3)
Muslim	3 (1.6)
Christian	2 (1.1)
6. Education	
Illiterate	32 (17)
Up to Middle School	46 (24.5)
Matriculation	39 (20.7)
Intermediate	38 (20.2)
Graduate or Postgraduate	24 (12.7)
Other Professional Degree	8 (4.2)
PhD	1 (0.5)
7. Occupation	
Private Sector Job	69 (36.7)
Student	50 (26.6)
Housewife	33 (17.6)
Unemployed	21 (11.2)
Government Service	14 (7.4)
Retired	1 (0.5)
8. Number of cohabitants[b]	
0	2 (1.1)
1	4 (2.1)
2	18 (9.6)
3	19 (10.1)
4	51 (27.1)
5	38 (20.2)
6	20 (10.6)
7	16 (8.5)
≥8	20 (10.6)
9. Below poverty line card	
No	152 (80.9)
Yes	34 (18.1)
Don't know	2 (1.1)

[a] Mean age = 28.99 years; S.D. = ±12.22; Max = 81; Min = 15; Range = 66.
[b] Mean cohabitants = 4.8; S.D. = ±2.12; Max = 11; Min = 0; Range = 11.

Table 2 – Distribution of study participants as per category of DOTS and weight (N = 188).

	Number (%)
1. Dots category	
Category 1	170 (90.4)
Other categories	18 (9.6)
2. Weight category at enrolment (kg)[a]	
25–34	7 (3.7)
35–49	96 (51.1)
50–64	62 (33)
65–75	19 (10.1)
>75	4 (2.1)

[a] Mean weight = 48.7 kg; S.D. = ±11.4; Max = 79; Min = 30; Range = 49.

When enquired on the knowledge of need for any special diet during TB 157 (83.5%) patients said yes, 11 (5.9%) said no (incorrect response) and rest were unaware. Most of those who said yes had correct knowledge of what to eat, when compared against the guidance document on nutrition in TB.

Similarly, When patients were questioned on the need to avoid anything during treatment course, 91 (48.4%) said yes. Of these the correct responses included smoking (25.3%), oily and fried food (24.2%), cold foods like ice-creams, cold drinks (22%) and others. The incorrect responses were sour food, curd, rice, milk and urad dal. A total of 32 (17%) patient also erroneously stated that TB patients do not need to avoid anything.

Most (153, 88.4%) participants who had not received the incentive were willing to change their diet when they get it. While others said they will not change their diet, or said that there is no need of the incentive as it is minuscule. Similarly, almost all (184, 97.9%) were willing to change their data on doctor's advice.

Only 9 (4.8%) of the participants were practising nutritional care for TB patients by buying any kind of food supplement, which were mostly protein supplements (Table 4).

4. Discussion

Our study reports status of Nikshay Poshan Yojana amongst 188 TB patients attending DOTS treatment in a tertiary care hospital of Delhi.

We recorded that only 88% of all the TB patients contacted were enrolled in the NPY scheme. Awareness of the incentive scheme was reported by three fourth of the participants, lower in comparison to what Begum et al found (91.5%) in their telephonic interview based study conducted in Andhra Pradesh.[17] The difference though not substantial could be due to self-selection of informed respondents in telephonic interviews. The various sources of information reported in studies were majorly healthcare staff and then family, friends and neighbors. Further in our study, we found that 98% of the participants who were aware got to know it during the enrollment process, indicating that healthcare workers were actively informing the patients about the scheme.

Of the participants who were enrolled only 9% reported getting any DBT incentive. In a study conducted in Dakshina Kannada, Nirgude et al found 28.7% of the study participants got the incentive out of 50% who were approved for the payment.[15] Similarly Begum et al reported DBT transfer in only 17

Table 3 – Distribution of study participants as per enrolment in Nikshay Poshan Yojana.

	Number (%)
1. NPY enrollment (N = 188)	
Yes	167 (88.8)
No	21 (11.2)
2. Awareness of NPY (N = 188)	
Aware	141 (75.0)
Source of Information (n = 141):	
• Doctor/health staff	128 (90.7)
• Family/friends/advertisements	13 (9.3)
Time of getting information (n = 141):	
• During enrolment	138 (98.0)
• After enrolment	3 (2.0)
Not Aware	47 (25.0)
3. Receiving NPY incentive (n = 167)	
Yes	15 (8.9)
For how many months (n = 15)	
• 2 months	15 (100)
How much amount (n = 15)	
• INR 1000 (INR500/month)	15 (100)
Spending on[a] (n = 15):	
• Fruits	6 (40.0)
• Regular food	5 (33.3)
• Vegetables	4 (26.6)
• Transport	4 (26.6)
• Chicken and Egg	3 (20.0)
• Milk and paneer	2 (13.3)
• Does not spend	2 (13.3)
No	124 (74.3)
Don't know	28 (16.8)
4. Experience of Enrolment (n = 167)	
No difficulty	144 (86.2)
Not aware as relative did the enrollment	20 (12.0)
Difficulty due to lack of documents	3 (1.8)
5. Awareness of reasons for incentive (n = 167)	
Aware	92 (55.0)
Not aware	75 (45.0)
Total	167 (100)
6. What to buy for nutrition (n = 167)	
Aware	106 (63.5)
Not aware	61 (36.5)
7. Whether informed what to buy for nutrition (n = 167)	
Yes	89 (53.3)
No	78 (46.7)
8. Reasons for non-enrollment (n = 21)	
Not aware	12 (57.1)
No time	5 (23.8)
No bank account	4 (19.1)
9. Wish to enrol if not enrolled (n = 21)	
Yes	18 (85.7)
Reasons[a](n = 22):	
• For food	10 (47.6)
• Need money	8 (38.1)
• Transport	2 (9.5)
No	
Reasons (n = 4):	2 (9.5)
• Don't need it	1 (25)
• No bank account	1 (25)
Don't know	1 (4.8)

[a] Not mutually exclusive

Table 4 – Distribution of study participants as per their Knowledge, Attitude and Practice related to nutrition in TB.

	Number (%)
1. Do TB patients need special diet (N = 188)	
Yes	157 (83.5)
What special diet[a] (n = 157)	
Correct responses:	
• Meat	82 (52.2)
• Eggs	75 (47.8)
• Fruits	55 (35.0)
• Green vegetables	55 (35.0)
• Milk	37 (23.6)
• Pulses	31 (19.7)
• Paneer	7 (4.5)
No (incorrect)	11 (5.9)
Don't know	20 (10.6)
2. Do TB patients need to avoid any food item (N = 188)	
Yes	91 (48.4)
What all items[a] (n = 91):	
Correct responses[b]:	
• Smoking	23 (25.3)
• Oily and fried food	22 (24.2)
• Cold food (ice creams, cold drinks, etc)	20 (22)
• Spicy food	12 (13.2)
• Alcohol	11 (12.1)
• Fast food	5 (5.5)
Incorrect responses[b]:	
• Sour food	8 (8.8)
• Curd	7 (7.7)
• Rice	7 (7.7)
• Milk	2 (2.2)
• Urad Dal	2 (2.2)
No (incorrect)	32 (17.0)
Don't know	65 (34.6)
3. Will you change your diet if you get DBT (N = 173)	
Yes	153 (88.4)
No	11 (6.4)
Money not enough	5 (2.9)
Don't know	4 (2.3)
4. Will you change your diet if doctor tells you (N = 188)	
Yes	184 (97.9)
No	3 (1.6)
Don't know	1 (0.5)
Total	188 (100)
5. Are you buying any supplement (N = 188)	
No	179 (95.2)
Yes	9 (4.8)
Type of supplement (n = 9)	
• Protein powder	7
• Calorie/Mineral supplement	1
• Calcium supplement	1
Total	188 (100)

[a] Not mutually exclusive.
[b] As per Guidance document on nutritional care and support for patients with tuberculosis in India.[16]

of their 83 cases (22.4%), and in a study conducted by Patel et al in Vadodara the proportion of DBT beneficiaries was 42.2%.[17,18] In another study conducted in Delhi by Kumar et al which included 57 patients found 30 (52.6%) patients got at least one incentive during their study.[19] Thus there is a significant variation between various regions and treatment centers in dispersion of timely DBT incentive, which could be due to administrative problems specific to each location in terms of regular submission, review, edit of beneficiary list

and dispersion through PFMS. Further the minuscule reporting could also be due to patient related factors as DOTS beneficiaries at the study center mostly comprise of migrant workers who might not being able to access their bank account regularly.

Furthermore, those who received the benefit got a thousand rupees, that is two months of incentive. Studies of Begum et al and Kumar et al also found most beneficiaries got DBT after two months of treatment, against the scheduled payment wherein first installment has to be made on case notification.[17,18] Also, those who received the benefit mostly spent it on food products, as reported elsewhere too.[17]

Eighty six percent of the enrolled participants reported facing no difficulty during enrolment, while others were either unaware as someone else in the family was involved in the enrolment process or they faced difficulty due to lack of required documents. Begum et al reported 58.8% of the participants facing no difficulty in getting DBT, highlighting the large variation in experiences of the beneficiaries between different locations and healthcare staff involved therein.[17]

When we enquired about awareness of the patients on reason(s) for the incentive and what they should be buying for nutrition, only 55% and 63.5% respectively reported in affirmative. It points that TB healthcare staff might not be informing each patient in detail regarding the nutrition, presumably due to increased workload. Further, the study found only 53.3% of patients were informed what to buy for nutrition, which further strengthens the point.

The reasons for non-enrolment cited in the study were lack of awareness (12, 57.1%), lack of time (5, 23.8%) and lack of bank account (4, 19.1%). The issue of lack of bank account which stalls the objective of NPY, has been reported in varying number in other studies depending on their study population.[15,18]

We were further able to report that majority (18, 85.7%) of the non-enrolled patients wished to enroll in the scheme citing the need for buying food, or requiring money for other reasons, showing a gap in the demand and implementation of the scheme.

During the assessment of Knowledge, Attitude and Practice of the participants we found the majority (157, 83.5%) of them knew that TB patients need special diet and were able to enlist what special food they should consume. On the other hand, when they were asked if TB patients need to avoid any food, 17% denied the need of it, 34% said they do not know, and from the rest 48% majority were able to enlist correct items to be avoided as per the Guidance document on nutritional care and support for patients with tuberculosis. This strengthens the case of the need for nutritional counselling for TB patients along with nutritional incentive.

The attitude as presented by 88.4% of patients who had not received the DBT was positive towards changing their diet when they do get it. Rest said they will not as they were already consuming what they should be, and few said that the money is not enough to change the diet. Similarly almost all (98%) of the participants reported willingness to alter their diet if their doctor advises them, showing that the patients can improve their diet if adequate incentive is provided to them on the right time and with right dietary advice.

Even though majority of the patients were aware of what they should be consuming, only 4.8% of them were actually buying any kind of supplement, which necessitates having a mechanism to check if patients are actually spending the incentive on nutrition.

5. Conclusion

Enrolment rate under NPY is not universal but 88.8% only and the actual DBT is at 8.9%. Only about half (55%) of the enrolled participants are aware why incentive is being given under NPY.

5.1. *Limitations*

1. The study did not include the service providers in the study.
2. Results are applicable to the study population served by the institution, and may not be applicable to countrywide centres for which a multicentric study is warranted.

5.2. *Strengths*

1. The study conducted under programmatic settings highlights the gaps and hurdles in the implementation of the nutritional incentive scheme.
2. We tried to assess the knowledge, attitude and practice of the patients regarding nutrition in TB which has not been done in other studies.
3. We had calculated the sample size for the study using a scientific formula and were able to interview all the calculated number of participants.

5.3. *Recommendations*

1. Enrolment rate needs to be improved amongst the patients by addressing the issues and generating more awareness about it.
2. Alternative mechanisms need to be explored for those lacking necessary documents.
3. Dispersal of incentive needs to be regularized ensuring it is transferred to all the patients receiving treatment. Furthermore, the transfer must take place on a monthly basis as erratic dispersal may not benefit patients' nutrition.
4. A feedback system needs to be established assuring that patients are actually withdrawing the incentive and spending it on their nutrition.
5. Information, Education and Communication (IEC)
   i. IEC is required to make people aware about the reasons behind NPY and the reasons behind it.
   ii. Patients need to be informed by the service providers what to buy for nutrition, and also what all food items they should be consuming and avoiding in TB.
6. Behaviour Change Communication
   a. There is a need to improve patient willingness towards improving their nutrition on receiving DBT.
   b. There is need to fill the gap between patient knowledge on nutrition in TB and their actual practise.

7. There is a need to conduct a mixed method studies including the service providers interviews in details regarding problems faced in the implementation of NPY and DBT at various DOTS centres across the country.

Conflicts of interest

The authors have none to declare.

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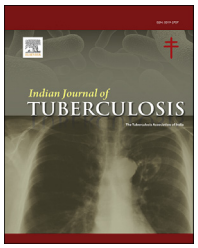

Original article

Role of Gene Xpert in smear negative pulmonary tuberculosis

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ABSTRACT

Background: Tuberculosis is a major health problem contributing to significant morbidity and mortality. Early diagnosis and treatment is the key for TB control. Sputum microscopy is a rapid and inexpensive test but due to low and variable sensitivity, many cases can be missed. Culture is considered to be the gold standard but is time consuming. Gene Xpert is a novel and rapid cartridge based nucleic acid amplification test (CBNAAT) that can be used for prompt diagnosis.

Aim: To compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Gene Xpert with culture in diagnosing tuberculosis in sputum smear negative patients.

Methods: The study is a prospective observational study conducted from December 2017 to January 2019 on 189 patients, who were sputum smear negative but had signs and symptoms suggestive of tuberculosis. Their respiratory samples were taken (either sputum or bronchoalveolar lavage) and sent for Gene Xpert. The results were compared with culture, which was taken as the gold standard, and diagnostic accuracy was assessed.

Result: A total of 189 patients were included in the study. In 25 patients sputum was taken and in 164 patients BAL was taken (which included 22 patients in whom sputum Gene Xpert was negative but there was high clinical suspicion of tuberculosis). The sensitivity, specificity, PPV and NPV of Gene Xpert in diagnosing smear negative pulmonary tuberculosis was found to be 96.3%, 81.3%, 87.5% and 94.2% respectively.

Conclusion: Gene Xpert can be used as a rapid diagnostic tool in patients who are sputum smear negative but have clinical features highly suggestive of tuberculosis. It additionally helps in detecting rifampicin resistance. But every Gene Xpert positive case does not necessarily mean an active disease, therefore, past history of tuberculosis along with radiological signs of disease activity are to be considered. In case of negative Gene Xpert but high clinico-radiological suspicion of TB, patients should be followed up on regular intervals, while awaiting their culture.

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1. Introduction

Tuberculosis is a communicable disease that most commonly affects the lungs (85% of the cases), though other sites may also be affected.[1] It is a bacterial disease caused by *Mycobacterium tuberculosis*, which is an acid fast, aerobic, non spore forming rod shaped bacillus. The incidence was estimated to be 10.0 million in 2018 with an estimated 1.2 million deaths in non HIV patients with an additional 251,000 deaths in HIV TB. South East Asia contributes 44% of the total TB cases with India accounting for 27% followed by China with 14%.[2] It is one of the top ten causes of death worldwide and leading cause of death from single infectious agent (ranking above HIV/AIDS).[2] Prompt diagnosis of TB is the key for TB control, for the patients and also as a public health intervention.

Chest X-ray is useful but not specific for diagnosis. Also, TB can present with varied and atypical findings.[3,4] Sputum microscopy is quick and inexpensive test but has low and variable sensitivity, ranging from 22 to 80%, depending on various factors like the bacillary load, the type of stain used, and also the expertise of the laboratory technicians.[5,6] Conventional fluorescence microscopy is more sensitive than the Ziehl Neelson (ZN) staining but is expensive and requires regular maintenance.[7] LED (light emitting diode) microscopy has also been found to be more sensitive than conventional ZN microscopy. Mycobacterial culture is considered to be the gold standard for diagnosis but takes 2–4 weeks, which could result in the delay in the initiation of treatment. In case of smear negative patients, results may take 4–8 weeks. Liquid culture techniques also take about 21 days which is still long for a diagnostic test to be effective in curbing transmission.[8] Gas chromatography detects tuberculostearic acid in samples but requires high performance liquid chromatography and technical expertise. Latex particle agglutination test detects antibodies against lipoarabinomannan, but it is not specific.[6]

Various Nucleic Acid Amplification (NAA) tests have been developed for rapid detection of *Mycobacterium tuberculosis* (MTB) in various clinical specimens.[9] In December 2010, WHO endorsed Gene Xpert for diagnosis of tuberculosis.[9] Gene Xpert is a novel and rapid automated cartridge based nucleic acid amplification test (CBNAAT) that apart from detecting TB bacilli also detects resistance to rifampicin within two hours of collection.[10] This test requires minimal hands on technical times and also as the reagent used for processing is bactericidal and tubercle bacilli are inactivated in vitro, the test is safe. Though the sensitivity of the test is more in smear positive cases, the International standard for TB care recommended that Xpert MTB/RIF should be performed in smear negative patients.[11]

2. Aims and objectives

To compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Gene Xpert with culture in diagnosing tuberculosis in sputum smear negative patients.

3. Methodology

The study was done on patients attending the postgraduate department of respiratory medicine at Govt. Chest Disease Hospital, GMC Srinagar. It is a prospective observational study conducted from December 2017 to January 2020. A total number of 189 patients who fulfilled the inclusion criteria were included in the study.

Approval for the study was taken from institute's ethical committee.

Patients who were smear negative with symptoms suggestive of tuberculosis like persistent cough >2 weeks, fever >2 weeks, night sweats, weight loss, loss of appetite or hemoptysis and supportive chest X-ray findings like upper lobe consolidation, bilateral diffuse infiltrates, cavitatory lesions or on resolving consolidation were included in the study. We excluded the patients who were smear positive and those having a history of lung malignancies or fungal infections.

A proper and detailed history was taken from the patient attending the OPDs at Govt. Chest Disease hospital, Srinagar. All the baseline investigations were done. A Chest X-ray was also done. In case the clinical history and X-Ray findings were suggestive of TB, sputum for AFB was advised. The sputum smear was examined by Ziehl Neelson staining. All those who tested negative but had the clinical signs and symptoms of TB were advised a CT scan of the lung. They were tested for tuberculosis by Gene Xpert MTB/RIF. The sample for the test included either sputum or bronchoalveolar lavage (BAL) obtained from bronchoscopy, depending on the whether the patient was able to produce good quality sputum or not. Also in certain cases where there was high suspicion of tuberculosis and sputum was negative for Gene Xpert, bronchoscopy was done and BAL was taken. The sample was also sent for MTB culture, which was used as a Gold standard to compare the result of Gene Xpert MTB/RIF with.

For Gene Xpert MTB/RIF, about 2 ml of sample is taken and is treated with a buffering solution comprising 4% NAOH +20% Isopropyl alcohol. After thorough mixing, 2 ml of the sample is taken and put in the cartridge. The cartridge is put in the Gene Xpert module. Results are obtained within two hours.

3.1. *Statistical methods*

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Statistical software SPSS (version 20.0) and Microsoft Excel were used to carry out the statistical analysis of data. Continuous variables were expressed as Mean ± SD and categorical variables were summarized as percentages. Chi-square test or Fisher's exact test, whichever appropriate, was employed for comparison of categorical variables. The diagnostic accuracy of Gene Xpert MTB/RIF was assessed in terms of Sensitivity, Specificity, PPV and NPV, by taking 'Sputum/Bal Culture' as gold standard. A p-value of less than 0.05 was considered statistically significant. All p-values were two tailed.

4. Results

Out of the total 189 patients included in the study, maximum were in the age group of >40 years with the mean age of 40.6 ± 8.39. The youngest patient was 11 years old and the oldest patient was 68 years. 100 were males and 89 were females (see Tables 1–5).

Bronchial anthracofibrosis was seen in 24 patients, 18 patients were diabetic, 17 patients were hypertensive and 13 patients had chronic obstructive pulmonary disease (COPD). 3 patients in the study population showed CT findings suggestive of silicosis and had a significant occupational history.

All the 18 diabetics included in the study turned out to be positive for tuberculosis. 10 patients out of 18 had lower lobe involvement (55.5%), followed by diffuse involvement in 6 patients (33.3%) and upper lobe involvement in 2 (11.1%).

In our study, total of 109 patients tested positive for tuberculosis (105 by Gene Xpert and 4 additionally by culture).

Table 1 – Diagnostic accuracy of Gene Xpert.

Gene Xpert	Sputum/BAL Culture	
	Positive	Negative
Positive	**TP** = 105	**FP** = 15
Negative	**FN** = 4	**TN** = 65

Table 2 – Diagnostic accuracy of Gene Xpert.

Variable	Value	95% CI
Sensitivity	96.3	90.86–98.99
Specificity	81.3	70.99–89.13
PPV	87.5	80.19–92.83
NPV	94.2	85.81–98.40
Accuracy	89.9	84.83–93.47

Table 3 – Diagnosis in false positive patients.

Number (n)	Diagnosis
14	Post tubercular obstructive airway disease with infective exacerbation
1	Community acquired pneumonia

Table 4 – Diagnosis in true negative patients.

Number (n)	Diagnosis
30	Community acquired pneumonia
14	Bronchial anthracofibrosis without concomitant tuberculosis
11	Malignancy
4	Hypersensitivity pneumonitis
4	Bronchiectasis
1	Silicosis
1	Wegner's granulomatosis

Table 5 – Comparison of the diagnostic accuracy of Gene Xpert in smear negative pulmonary TB.

Study	Sensitivity	Specificity	PPV	NPV
Aggarwal M et al.[12]	79.1%	93.1%	67.8%	96%
Kumar A et al.[14]	55.77%	98.26%	78.4%	95.1%
Lombardi G et al.[15]	73.0%	99.0%	–	–
Kandi S et al.[16]	66.3%	–	–	–
Chopra V et al.[17]	98.3%	–	–	–
Sharma SK et al.[18]	77.7%	–	–	–
Sreekanth B et al.[19]	38.31%	–	–	–
Our study	96.3%	81.3%	94.2%	89.9%

The most common symptom in tuberculosis patients was cough followed by fever. Decreased appetite was present more frequently in the TB group (n = 47, 43.1%) as compared to the non TB group (n = 25, 31.3%). Hemoptysis was also more frequently present in the TB group (n = 32, 29.4%) with a p-value of <0.001 making it statistically significant.

The most common X-Ray abnormality both in the TB and non TB group was consolidation {(TB: n = 84, 77.1%) (non TB: n = 66, 82.5%)}. Cavity was present in 20 of the TB patients as compared to 2 in the non TB group with a p-value of 0.002 making it statistically significant.

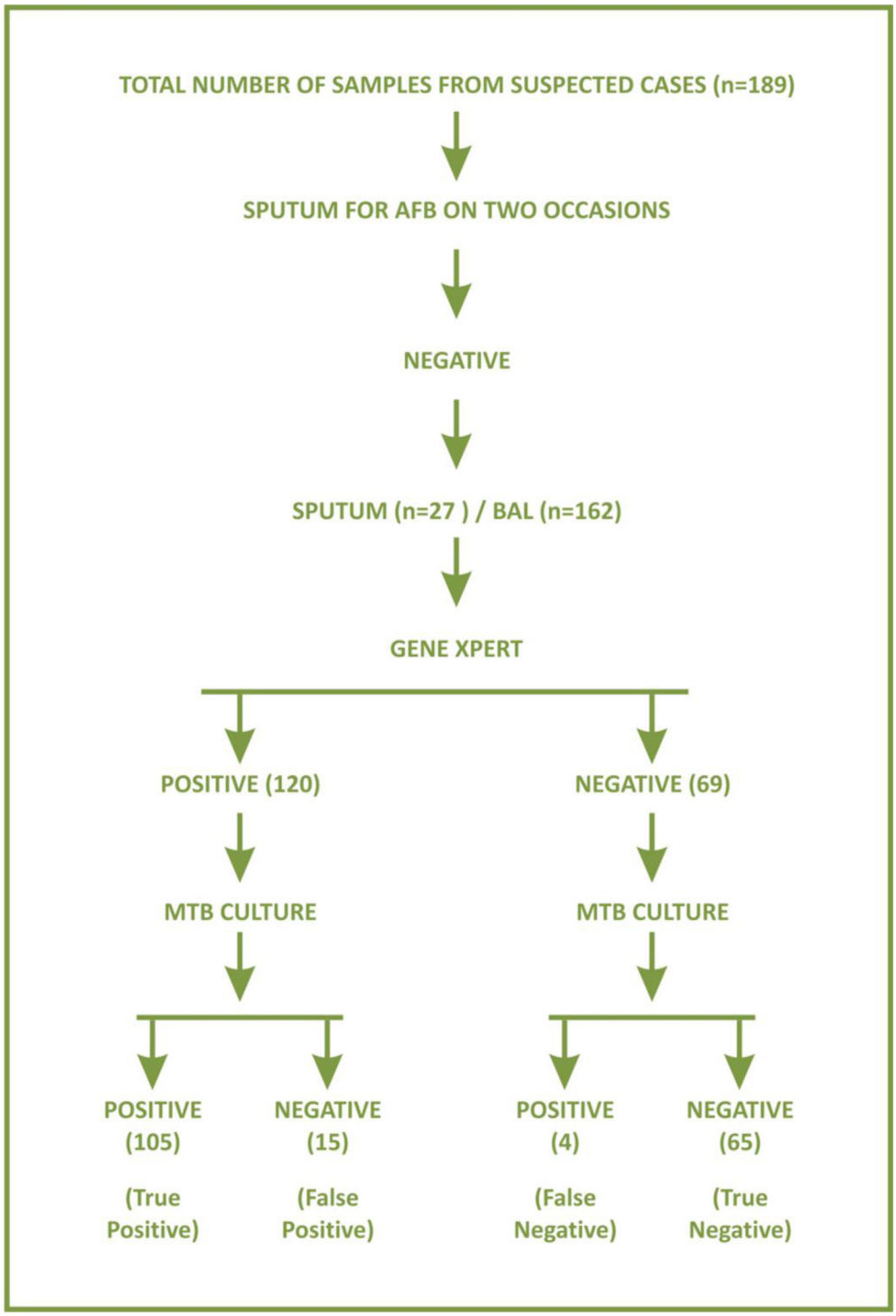
The most common CT findings in both TB and Non TB groups was consolidation {(TB group: n = 84, 77.1%) (Non TB group: n = 68, 85.0%)} followed by nodules {(TB group: n = 38, 34.9%) (Non TB group: n = 28, 35.0%)}. Cavity was seen in 32 (34.9%) of the TB patients as compared to 4 in the non TB group with a p-value of <0.001 making it statistically significant. 3 out of 4 patients with cavitatory lesions in the non TB group were later diagnosed squamous cell carcinoma and one was diagnosed as granulomatosis with polyangitis with c-ANCA positive.

Tree in bud opacities were also more common in the TB group (n = 19, 17.4%) as compared to the non TB group (n = 4, 5%) with a p value of 0.018 making it statistically significant.

Upper lobe involvement was seen more commonly in TB (n = 61, 56%) as compared to Non TB group (n = 21, 26.3%), with a p-value of <0.001, making it statistically significant for TB.

On comparing the bronchoscopic findings in the TB versus non TB group, no significant difference was found. Normal bronchoscopy was the most common finding in both the groups [(TB: n = 39, 45.9%) (Non TB: n = 37, 46.8%)] followed by inflammed mucosa in the TB group (n = 20, 23.5%) and increased secretions in the non TB group (n = 19, 24.1%).

Out of the total 189 patients, sputum was taken in 25 patients and BAL was taken in 164 patients (which included 22 patients in whom sputum for CBNAAT was negative but there was high clinical suspicion of tuberculosis). 120 samples tested positive by Gene Xpert (95 BAL and 25 sputum samples), out of which 15 had negative cultures. These were taken as false positives. 14 out of these 15 had a past history of tuberculosis. These 14 patients were followed up at regular intervals with chest x-rays and no deterioration was seen. Their symptoms improved with a course of bronchodilators and antibiotics. So the false positive results could be explained by the fact that CBNAAT cannot differentiate between the live and dead bacilli. It amplifies the DNA whether live or



dead.[12] 1 remaining patient was managed as Community acquired pneumonia and was followed up for one year with serial sputum samples and x rays. No clinical or radiological deterioration was seen. The Gene Xpert could have been positive probably because of contamination.

105 were taken as true positives (which included 15 patients whose sputum sample was initially negative for CBNAAT but BAL turned out to be positive).

4 patients, who tested negative by Gene Xpert, later were found to be positive on culture. They were taken as false negatives. This false negative result could be due to the fact that culture can pick up lesser no of bacilli as compared to Gene Xpert. (10–100 cfu/ml compared to 130–150 cfu/ml for Gene Xpert).[13]

65 patients were negative by both Gene Xpert and culture, taken as true negatives. In this group the most common

diagnosis was community acquired pneumonia (n = 30), followed by bronchial anthracofibrosis without concomitant TB (n = 14), malignancy (n = 11), hypersensitivity pneumonitis (n = 4), bronchiectasis (n = 4), silicosis (n = 1) and Wegner's granulomatosis (n = 1).

The sensitivity, specificity, PPV and NPV of Gene Xpert in diagnosing smear negative pulmonary tuberculosis is 96.3%, 81.3%, 87.5% and 94.2% respectively.

5. Discussion

In our study, Gene Xpert has been found to be a helpful tool in rapid diagnosis of smear negative pulmonary tuberculosis and initiation of appropriate treatment with a sensitivity of 96.3% and a specificity of 81.3%. It also helped to formulate a diagnostic plan in patients who turned out to be negative for tuberculosis.

In a study conducted by Agarwal M et al,[12] total of 170 patient samples were evaluated (149 BAL and 21 Sputum samples). The sensitivity and specificity in smear negative cases of pulmonary tuberculosis was 79.1% and 93.1% respectively with a PPV of 67.8% and a NPV of 96%.

In another study by Kumar A et al,[14] 598 patients were included. Sputum samples were sent for ZN staining, Gene Xpert and BACTEC culture (taken as gold standard). In smear negative cases, sensitivity, specificity, PPV and NPV was 55.77%, 98.26%, 78.4% and 95.1% respectively. Since only sputum samples were included in the study, low sensitivity in smear negative cases can be explained by the decreased bacillary load in sputum samples.

In another study by Lombardi G et al,[15] which was a five year retrospective study on 5170 samples, both respiratory and non respiratory samples were included. 386 culture positive samples were taken out of which 234 were sputum smear negative. Out of these 234, only 137 were respiratory samples. The sensitivity and specificity of Gene Xpert was found to be 73.0% and 99.0% respectively for these smear negative pulmonary cases with an overall sensitivity of 86.5% and specificity of 99.0% for respiratory samples.

In a study by Kandi S et al,[16] 200 samples were taken out of which 110 were respiratory samples (95 sputum samples and 15 BAL) and remaining 90 were extra-pulmonary samples. The overall sensitivity and specificity of Gene Xpert in pulmonary samples was found to be 79.2% and 89.5% respectively with a PPV of 79.2% and NPV of 89.5%. For smear negative cases the sensitivity was found to be 66.6%.

Chopra V et al[17] included 100 smear negative suspected TB patients in the study and found that Gene Xpert was positive in 58. Sputum of rest 42 patients was put on liquid culture, out of which just one showed growth of mycobacterium tuberculosis, making the test 98.3% sensitive.

In a study done by Sharma SK et al,[18] 1492 patients were taken. Samples included sputum in 1141, endotracheal aspirate in 146, BAL in 128, induced sputum in 73 and bronchial washings in 4. The samples were sent for ZN staining, Gene Xpert and culture. Gene Xpert had a sensitivity of 95.7% and specificity of 99.3% for detecting MTB in pulmonary samples of patients with TB. The sensitivity in smear negative culture positive samples was 77.7%.

Contrary to our study, the sensitivity of Gene Xpert in detecting smear negative pulmonary cases was very low (15.38%) with an overall sensitivity of 38.31% in the study conducted by Sreekanth B et al.[19] Sputum samples were taken from 337 patients. The low sensitivity of Gene Xpert could be because only sputum samples were included in the study. In our study, we have included both sputum and BAL samples (majority being BAL), which has increased the diagnostic accuracy.

6. Conclusion

Though culture is considered to be the Gold standard, but since it is time consuming, it may lead to a delay in diagnosis and loss of follow up. Gene Xpert can be used as a rapid test for diagnosis of smear negative pulmonary tuberculosis. It has an additional advantage of detecting rifampicin resistance. In case of a past history of tuberculosis and a positive Gene Xpert result, radiological signs of disease activity are to be considered before starting the treatment. In case of negative Gene Xpert but high clinico-radiological suspicion of TB, patients should be followed up on regular intervals. Culture reports should always be checked for any discordance.

7. Limitations

- Ours was a prospective study conducted at only one center.
- Since majority of the samples were BAL, the diagnostic accuracy of Gene Xpert in sputum samples could not be assessed.
- Rifampicin resistance was not evaluated in our study.

Conflicts of interest

The authors have none to declare.

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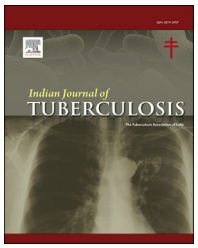

Original article

Non-adherence to anti-tubercular treatment during COVID-19 pandemic in Raipur district Central India

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ABSTRACT

Background: Non-adherence is major factor in failure of any drug regimen. The significance of non-adherence is so much that WHO states that increasing the effectiveness of Adherence Interventions may have far greater impact on health of population than any improvement in specific medical treatments. Incidence of non-adherence to Anti Tubercular Treatment (ATT) usually ranges from 8.4% to 55.8%. This study aims to find out the reasons of Non-adherence to ATT in patients receiving anti-tubercular treatment at DIRECTLY OBSERVED TREATMENT SHORTCOURSE (DOTS) Centre at District Tuberculosis Centre (DTC), Kalibadi, Raipur during COVID-19 pandemic.

Methods: A cross sectional study was conducted at Department of Pharmacology, Pt. JNM Medical College and DTC Kalibadi Raipur. 55 Patients taking ATT fulfilling inclusion and exclusion criteria were interviewed using structured questionnaire. The data obtained was analysed to know causes of non-adherence.

Results: Study was carried out between March & April 2020. In our study, 80% subjects were male and 20% were female. The main reasons for Non-adherence were Side-effects of drug in 36% cases, missing medication intentionally in 34% cases, lack of encouragement by family members in 32% cases, patient's unawareness of consequences of skipping medication in 25% cases, unaware of treatment duration in 22%, not feeling any change, forgetting to take medication, and burden of concomitant medication besides ATT, each in 20% cases, 13% cases had difficulty in procuring medication due to lockdown, 5% cases did not go to collect their medicine due to fear of contracting COVID-19 infection.

Conclusions: Our study shows reasons for Non-adherence are multi-factorial with drug side –effects & intentionally skipping medication being major factors.

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1. Introduction

TB is a major health concern.[1] Globally, an estimated 10 million people fell ill with TB in 2018, accounting for approximately 1.5 million deaths in 2018. These include 251,000 HIV positive patients,[2] thus making TB the leading cause of death in such patients. TB affects people of all age groups and both the sexes; however, incidence is maximum in men over 15 years of age.[2]

INDIA is a hot bed of TB with approx. 2.69 million new cases in 2018,[2] 92,000 out of those being HIV positive.[2] Poor adherence is very common despite multiple interventions aimed at improving treatment completion. Non-adherence not only leads to treatment failure and poor treatment outcome[3] but also is a major factor in drug resistance leading to emergence of MDR and XDR TB strains.[4]

1.1. *Classification of TB*[5]

TB can be classified in multiple ways depending on the anatomical site, history of treatment or drug resistance.

1.1.1. *Based on anatomical location*

Pulmonary TB—A case of TB involving the lung parenchyma or tracheo-bronchial tree is classified as Pulmonary TB,

Extra-Pulmonary TB—While case of TB involving pleura, lymph nodes or other body parts like intestines, bones, brain etc. is classified as Extra-Pulmonary TB.

1.1.2. *Based on history of treatment*[5]

New case—A patient who has never taken Anti-tubercular treatment in his life or one who has taken anti-TB treatment for less than 1 month is classified as a NEW CASE.

Previously treated cases—They have previously taken Anti-TB medication for more than 1 month and are of following types:

1. Recurrent case—A case successfully treated in the past but is subsequently found to be a confirmed case.
2. Treatment after failure—Previously treated case whose failed at recent most treatment.
3. Treatment after loss to follow up—Earlier treated patient lost in follow up who is now a confirmed case.
4. Others—They have been treated previously, but the outcome of their recent most treatment is unknown.
5. Transferred in case—A patient coming to a TB unit after having received treatment from other TB unit.

1.1.3. *Based on drug resistance*[5]

1. Mono-Resistance (MR)—Patient with resistance to any 1 1st line TB drug.
2. Poly Drug resistance (PDR)—Patient with resistance to more than 1st line TB drug other than both INH & RIFAMPICIN.
3. Multi Drug Resistance (MDR)—Resistance to both INH & RIFAMPICIN with or without resistance to other 1st line drugs.
4. Extensive Drug Resistance (XDR)—An MDR case additionally resistant to fluoroquinolones & 2nd line of injectable TB drugs like kanamycin or amikacin.

1.2. *Drug regimen*[5]

The Indian TB National Strategic Plan (NSP) 2017–2025[6] is the plan produced by the government of India (GoI) which states that even though India is engaged in TB control activity for >50 years yet it continues to be India's health crisis. It kills approx. 4,80,000 Indians every year, approx. 1400 per day.

Type of TB Case	Initiation phase	Continuation phase
New	HRZE (2 Months)	HRE (4 Months)
Previously treated	HRZES (2 Months) + HRZE (1 Months)	HRE (5 Months)

While diagnosing, the patient first has to be categorised as having Pulmonary or Extra Pulmonary Tuberculosis. All new TB patients in India should receive an internationally accepted first line regimen (A regimen is the prescribed course of treatment in the case of TB drugs).

For new patients the intensive phase should consist of eight weeks of the drugs isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) the continuation phase should consist of 3 drugs Isoniazid (H), Rifampicin (R), Ethambutol (E) given for another 16 weeks, this is alternatively written as 2HREZ/4HRE, there will be no need for extension of the continuation phase.[6]

The drug should be given according to the body weight of the patient. There are 4 weight band categories, under the new daily regimen TB patient will be given fixed dose combinations (FDC). FDC's are thought to prevent acquisition of drug resistance due to monotherapy which may occur with separate drugs. The number of tablets to ingest is smaller and thus encourage patient's adherence. FDC have equivalent efficacy to single pill and is more acceptable to patients. Standard regimen for new TB patients is 2 months HRZE and 4 months of HR daily dosing.

The National Strategic Plan 2017–2025 aims at rapidly ending the TB epidemic in India. NSP envisions a TB free India with zero death and disease. The goal of NSP is elimination of TB in India by the year 2025. The approach of NSP is based on Detect-Treat-Prevent-Build model.[6]

Detect—First and foremost there is need for early detection of all TB cases.

Treat—This is followed by not only treatment with appropriate drugs and regimen but also providing the patient with financial and nutritional support.

Prevent—In addition to all this there is focus on active case finding and contact tracing to prevent new cases.

Build—Lastly it stresses on management & upgradation of financial systems of TB control program.[6]

For the treatment to work all the medicines must be taken as per standard drug regimen. Drug side effects are a major

determinant to maintain patient compliance & adherence. Side-effects of ATT range from minor symptoms like nausea, vomiting, weakness, discolouration of urine to serious side effects like hepatotoxicity, peripheral neuropathy, changes in colour vision etc. In case treatment is interrupted or stopped early, failure can happen. This might also lead to development of drug resistant TB. Therefore, it is imperative to find the factors leading to non-adherence in tuberculosis patients in order to make strategic moves in countering TB in India.

2. Method

A cross sectional study was carried out after ethical clearance from institutional ethical committee at DTC, Kalibadi, Raipur. 55 Patients who were fulfilling the inclusion criteria were enrolled for the study. A written informed consent was taken and they were subjected to structured questionnaire. The data obtained was tabulated and subjected to statistical analysis.

2.1. Study design

A Cross sectional observational study of 8 weeks from 1st March 2020 to 30th April 2020 was conducted in Department of Pharmacology, Pt. JNMMC, Raipur and DTC, Kalibadi, Raipur. The institutional ethical committee approval was taken before study with reference no. 2020/198.

2.2. Enrolment of patient

Patient were selected with due consent from institutional ethics committee and also from the patient explaining to them the purpose of study and utility of data obtained from them. In case of a minor, consent is obtained from their parents.

2.3. Inclusion criteria

A. Patients Taking Anti Tubercular Treatment at District TB Centre, Kalibadi, Raipur for minimum one month.
B. Patients in age group of 15–75 years.
C. Those who missed at least 3 doses in a week.

2.4. Exclusion criteria

A. Psychosis
B. Previously Treated Patient
C. Active COVID infection.

2.5. Questionnaire

Q.1 Do you know how long will your treatment last?
Q.2 Are you aware of the dangers of skipping/stopping your treatment without doctor's advice?
Q.3 Do you feel any change (either positive or negative), ever since you started the treatment?
Q.4 Is there adequate supply of medicines at your DOTS Centre?
Q.5 Do your family members encourage you to continue taking your medicines?
Q.6 Do you sometimes, intentionally skip your medication? if yes, then what's the reason?
Q.7 Besides forgetting, is there any reason for you to miss your treatment?
Q.8 How often you have difficulty remembering to take your treatment?
Q.9 Besides att, are you taking any other medications?
Q.10. Did you have any trouble in procuring medication during COVID-19 pandemic?

2.6. Stat analysis

Sample size Can be estimated using the following formula:-

$$n = \frac{z^2_{1-\frac{\alpha}{2}}(1-P)}{d^2} \quad (1)$$

where P = anticipated proportion, d = absolute precision required on either side of proportion, p and d are expressed in fractions, z is a constant, its value for a 2 sided test is 1.96 for 95%.

3. Result

Our study was carried out in month of March & April 2020 with the sample size of 55 subjects. Out of 55 subjects 44 (80%) were males and 11 (20%) females.

In our study we found that the single largest factor responsible for non-adherence is drug side effects with 36% patient affirming it. Side effects in the form of nausea, vomiting, abdominal pain, generalised weakness, headache, itching skin rash, etc. make it difficult for the patient to adhere to the regimen, often resulting in skipping or stopping of treatment. Detailed information can be found in Table 1, Graph 1.

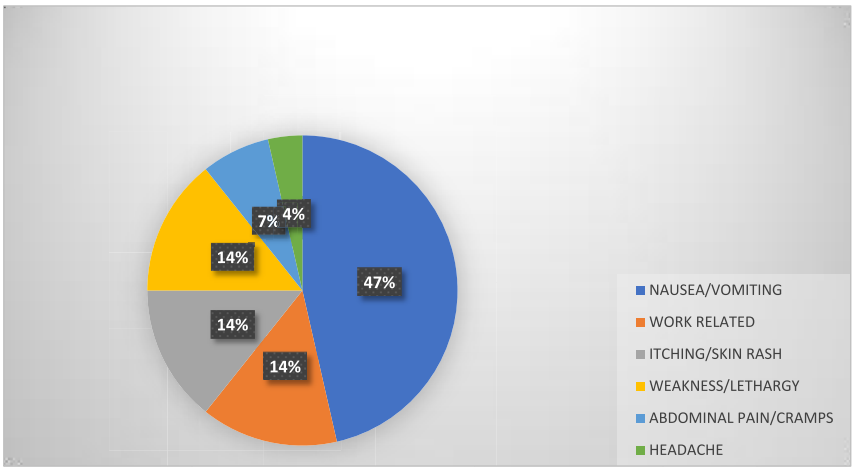
35% of patients in our study intentionally skipped medication.
32% of all patients cited lack of family support as the reason for non-adherence to medication.
25% of the patients were unaware of the consequences of stopping the treatment midway without doctor's advice.

Table 1 – Drug side effects.

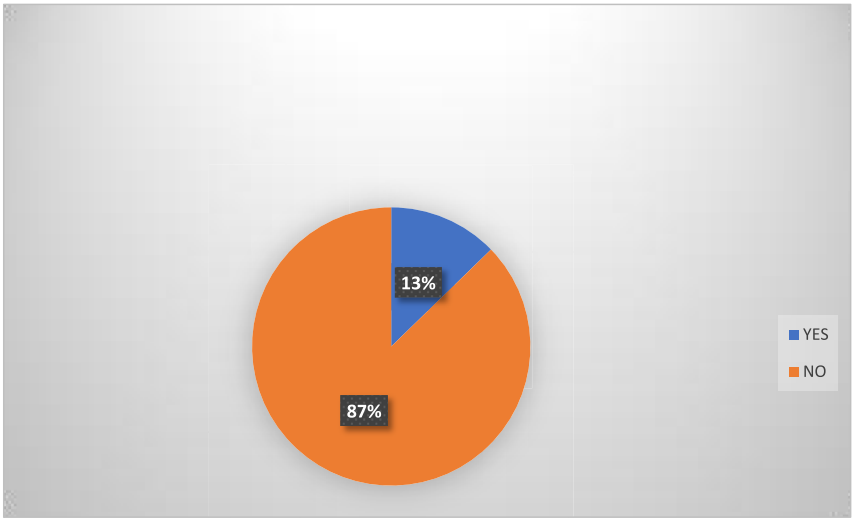
Drug side-effects	Number of patients (percentage)
Nausea/Vomiting	13 (47%)
Itching/Skin rash	4 (14%)
Weakness/Lethargy	4 (14%)
Abdominal pain/Cramps	2 (7%)
Headache	1 (4%)

Table 2 – Difficulty in procuring medication due to lockdown.

Difficulty in procuring medication due to lockdown	Number of patients (percentage)
Yes	7 (13%)
No	48 (87%)



Graph 1 – Shows percentage wise distribution of drug side-effects



Graph 2 – Shows difficulty faced in procuring medication due to lockdown.

22% patients were unaware of duration of their treatment plan which can range from 6 to 9 months.

20% of total patients were unresponsive towards the treatment and said that they didn't felt any change either positive or negative ever since the initiation of treatment.

20% of the patients forget to take Anti-tubercular medicines due to co-medications for Diabetes, Hypertension, etc.

13% of patients had difficulty in procuring medication due to commutation problem in lockdown period Table 2, Graph 2.

5% of patients did not contact healthcare facilities due to fear of catching COVID-19 infection Table 3, Graph 3.

4. Discussion

As per previous studies done earlier nationally as well as internationally the rate of Non-adherence to Anti Tuberculosis treatment in India is very high approximately 50%[7] The main reasons cited for the same are drug side effects, forgetting to take medication. Being away from home, lack of social family support, low socio-economic status of the patient, poor communication between patient and health care provider. The findings of our study (Table 4) are consistent with previous studies done on the same topic elsewhere in Ethiopia, Asia and Global annual TB reports published by WHO.[8,9]

While previous studies concentrated more on percentage of non-adherence, our focus was on finding the quantitative as well as qualitative distribution of the factors responsible.

Table 3 – Problem in collecting medication due to COVID-19 fear.

Did not collect medication from DOTS centre for fear of contacting COVID-19 infection	Number of patients (percentage)
Yes	3 (5%)
No	52 (95%)

Table 4 – Reasons for non-adherence.

Reasons of non-adherence	Percentage of patients
Drug side-effects	36
Intentionally skipping	35
Lack of family support	32
Unaware of consequences of treatment interruption	25
Not feeling any change	20
Forgetfulness	20
Taking other medicines	20
Difficulty in procuring medication due to lockdown	13
Did not collect medication from DOTS centre for fear of contacting COVID-19 infection	5

The success outcome of any drug regime depends upon the patient compliance and earnest adhesion to the prescribed drug regimen. As compared to the developed economies the medical adherence is found to be low in developing countries.[3]

Non-adherence to TB regimen study carried out in various countries over one month was found to be 20.8% in Southern Ethiopia, 25% in Uganda, in Kolkata India 40.55% and Jiangsu Province of China 12.2%.[10–13] In analysis of various studies - forgetfulness being key factor for non-adherence in continuation phase of therapy. TB patients who were asymptomatic were more likely to discontinue the medication. As per a study conducted in Kolkata India, the urge to leave treatment once patient started feeling better, was a significant determi1nant of non-adherence to Anti TB medication[12]

78% persons who took part in the study were aware of their TREATMENT duration, while 22% were not. 25% of them or roughly 1 out of 4 patients were not aware of the dangers of skipping/stopping their treatment without the doctor's advice. Approximately 20% patients said they did not felt any change since the initiation of the treatment and almost 33% of them received no support/persuasion by their family/friends to continue with DOTS medications. Major reasons for this behaviour as per patient's own admission were vomiting, general weakness, headache, lethargy, itching and skin rashes. For details please refer Table 1.

Finally non adherence to any chronic disease regimen as in our study is dependent upon interplay of various factors socio economic, literacy, empathy support of family members and employer towards patients, availability of health care facility to the patient, attitude and behaviour of health care provider. Lockdown restrictions during COVID-19 pandemic & fear of catching infection.

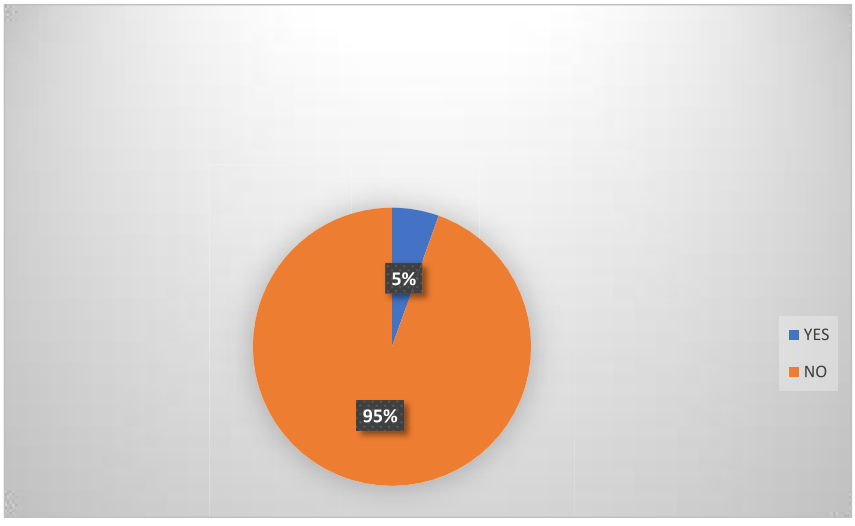
Promoting and hindering factors of non-adherence differ across the world and time to time. In given scenario. The study being cross sectional and hence had limitation of temporal relationship with some variables and won't be able to provide stronger causality.

4.1. *Strength of study*

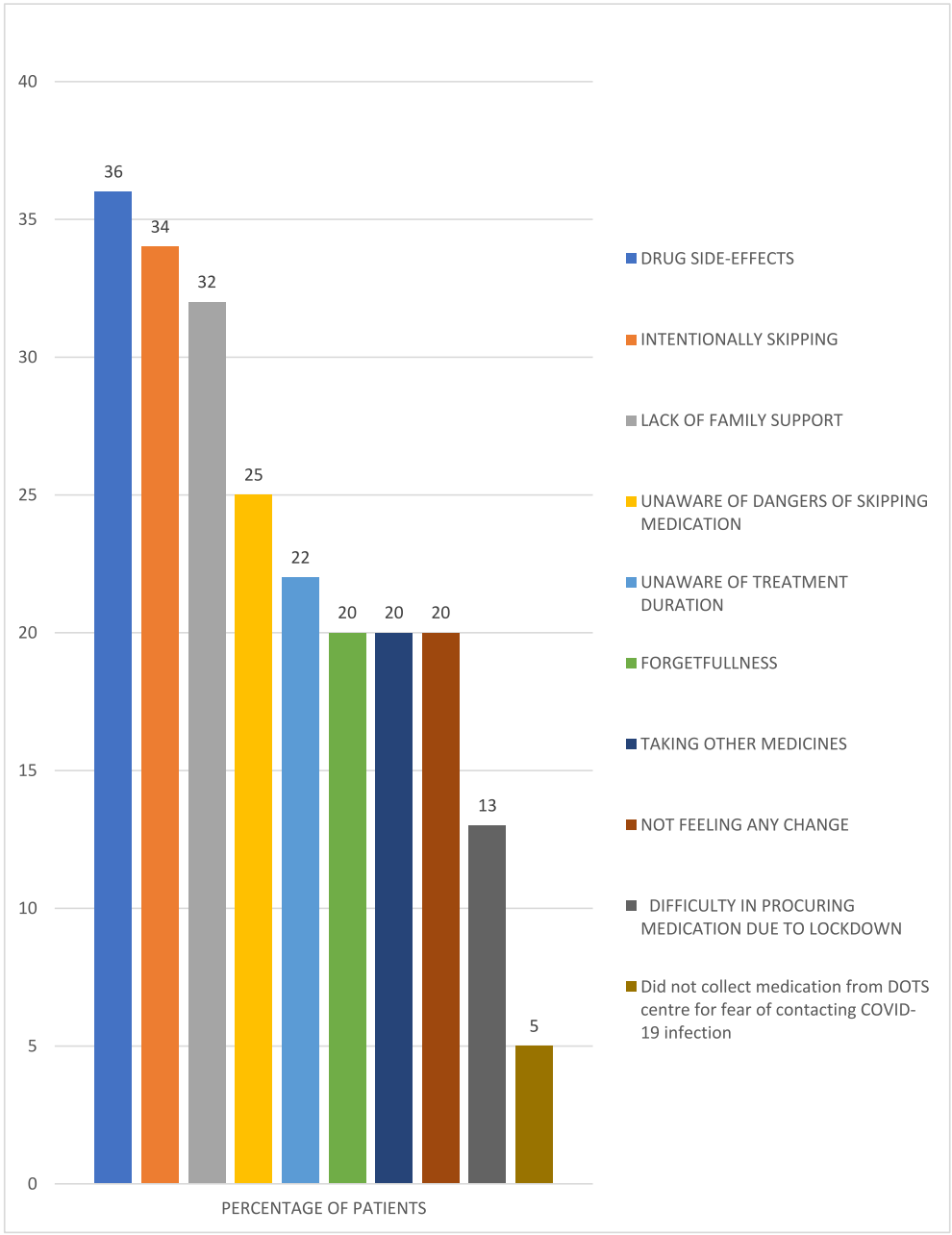
This is a pioneering work done to analyse the factors & reasons for non-adherence in COVID-19 pandemic in State of Chhattisgarh, Central India. This study will further strengthen the outcome & implementation of National Tuberculosis Elimination Programme.

4.2. *Limitation of study*

Short study duration.



Graph 3 – Shows whether patient didn't collect medication from TB centre for fear of contracting COVID-19 infection



Graph 4 – Shows various reasons for non-adherence in patients receiving anti-tubercular medication during COVID-19.

5. Conclusion

Non adherence is a complex phenomenon affected by the interplay of various modifiable and non-modifiable risk factors comprising of Human awareness, Socio-economic factors, family environment, etc. While previous studies on Tb non-adherence focussed more on the finding the percentage of patients who were non-adherent, we in our novel study aimed to find the reasons for the tubercular non-adherence and finding the contribution of all factors individually to non-adherence. The findings of our study being pioneering attempt in the state of Chhattisgarh, will enable the meticulous development of facility to provide congenial atmosphere for the implementation of Anti-tubercular regimen & minimising non-adherence.

Major factors responsible for non-adherence are common to ours as well as various other studies before us and can namely be identified as drug side-effects, forgetfulness, being away from home, personal reasons, lack of family support and lacking knowledge about consequences of non-adherence, lockdown restrictions & fear of COVID (Table 4, Graph 4). Also, there is an overlap among various causal factors with more than one factor being responsible in a single patient.

The individual single biggest factors were Drug side -effects & intentionally skipping of medication. So, by

counselling/guiding the patients by making them aware of all the possible side-effects along with dangers associated with skipping the medication without doctor's advice can go a long way in improving patient compliance & reducing non-adherence. Also including patient's family members & friends in this process who ensure better compliance. Also, patient must be persuaded to continue with the treatment even if he/she isn't feeling any change and regular reminders be given to the patient to take the medicines on time. Last but not the least, if possible, all concomitant medication, unless deemed necessary, be stopped.

Conflicts of interest

The authors have none to declare.

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