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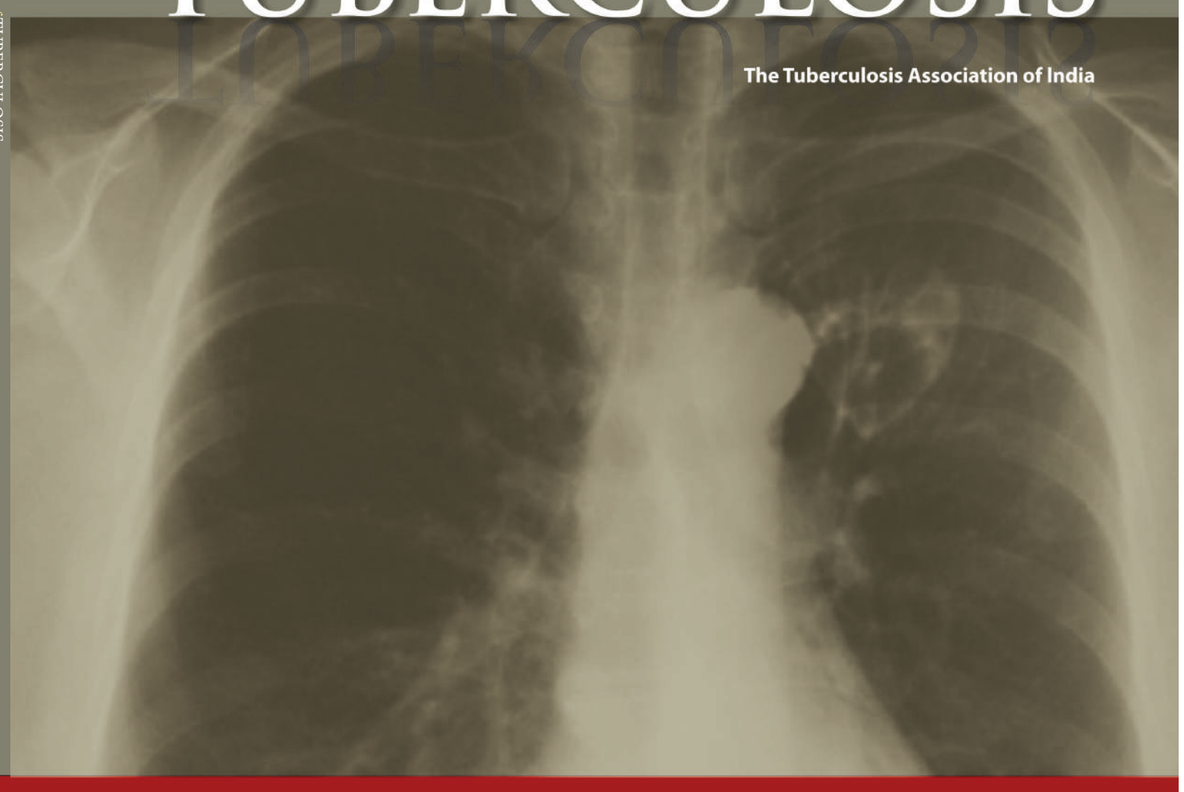

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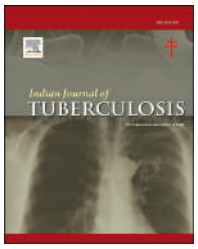

Editorial

BCG centenary: Lessons learnt

Keywords:
Bacille Calmette-Guérin (BCG) vaccine
TB prevention
TBI

Tuberculosis (TB) still remains a major public health problem worldwide with about a quarter of the world's population believed to be infected with *Mycobacterium tuberculosis* (MTB) and nearly two billion people having a persistent state of immune stimulation with no evidence of clinically manifest active TB, referred as TB infection (TBI). The risk of activation and developing into clinical disease is 5–15% in a lifetime in the general population while the risk of 5–15% is yearly in people living with HIV(PLHIV)/AIDS. An estimated 10.6 million people became newly sick with TB disease in 2021, out of which men, women and children (aged <15 years) accounted for 56%, 32% and 12% respectively. There were an estimated 1.6 million deaths among HIV-negative and positive combined.[1] Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, ranking above HIV/AIDS. Tuberculosis still continues to be one of the top 10 causes of death worldwide. Reductions in the reported number of people diagnosed with TB in 2020 and 2021 suggest that the number of people with undiagnosed and untreated TB has grown, resulting first in an increased number of TB deaths and more community transmission of infection and then, with some lag-time, increased numbers of people developing TB.[1] The WHO's End TB Strategy has time framed goals and targets which can only be achieved by adopting a multipronged approach with multi-sectorial involvement focusing on uniform easily accessible strategies, not only for proper management of TB cases but also a greater emphasis on the prevention strategies.[2] This is infact the need of the hour. Preventive strategies should include not only treatment of TB infection (TBI) but also prevention of infection and latency through vaccination. Bacille Calmette-Guérin (BCG) vaccine, the only WHO approved TB vaccine is one such prevention strategy[2] and is on the WHO's List of Essential Medicines.[3] It is the world's oldest approved most widely used vaccine, with billions already vaccinated and in use for a century now.[4]

Bacillus Calmette-Guérin (BCG) is the deliberate administration of a suspension of live attenuated *Mycobacterium bovis*—Calmette and Guérin strain to produce immunity against a possible subsequent tubercular infection.[5]

The original M.*bovis* strain was first isolated by Edmond Nocard in 1902 from a cow with tuberculous mastitis, known as 'lait Nocard' strain. Almost 230 successive subcultures on bile, glycerine and potato medium performed by Albert Calmette and Camille Guérin over 13 years, from 1908 to 1921, led to the attenuation of this strain to preduce the BCG strain. It was later lyophilized at the Pasteur Institute and distributed to different countries.[5] Different ways of subsequent sequential cultures using different culture medias, harvesting after varying number of passages performed in these countries led to the origin of different sub-strains with recognized genetic mutations over a 40-year period all having distinct morphological, biochemical and immunological differences.[5,6] Thus, the sub-strain brought by Julio Elvio Moreau to Uruguay in 1925, known as *Moreau strain*, nominated as a primary seed lot (1924–1925), had already undergone two mutations.[6] BCG vaccine brought to Brazil by Arlindo de Assis in 1927 was actually a daughter strain of the Moreau BCG vaccine, hence nominated as the BCG *Moreau - Rio de Janeiro strain*. This Brazilian strain is regarded as one of the most immunogenic.[6] Other sub-strains are namely, Pasteur or French strain; BCG *Tokyo* with an unusually high colony count and the only lyophilized heat resistant strain in the world[6]; BCG *Gothenburg* (Copenhagen), the only strain kept as per Calmette's instructions, being closest to the original but was replaced in 1975 by the Danish 1331 strain due to its high rate

of adverse effects, especially osteitis[6]; BCG *Danish1331 strain*, being used as primary seed lot in the Universal Immunization Programme (UIP)of India; BCG Russia *Moscow 368 strain*; BCG Bulgaria *Sofia SL222*; BCG Tokyo; BCG *Tice*, named after the doctor who received the sub-strain in the USA (1933-34), now being used for urinary bladder carcinoma,[6] strains being used in Canada are BCG *Montreal,* BCG *Connaught* and the BCG *Glaxo*.[6]

The first human BCG vaccine was administered as an oral vaccine on 18 July 1921 to a newborn whose mother had died of pulmonary TB. The infant did not develop the disease nor had any adverse events.[5] The oral vaccine was then largely used in Europe between 1920 and 1930. In 1930, in Lubek, Germany, the oral BCG vaccine lot caused 73 deaths among 250 vaccinated children resulting in a negative impact on the BCG vaccination drive. However, analysis showed it to be an isolated event due to unintentional contamination of the vaccine with a virulent TB bacillus as there were no similar occurrences elsewhere. Intra-dermal BCG vaccination and multiple puncture technique were introduced in 1927 and 1939 respectively. The first clinical trials evaluating the protective efficacy of the first dose of BCG began after 1930 and results of several studies ready by late 1940.[5] Due to the favorable results obtained in these clinical trials, the vaccination programmes were encouraged throughout the world by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF). WHO stated that the major internationally used BCG sub-strain stestedin 1989, Danish, Glaxo, Pasteur and Tokyo, all are equally suitable for immunization against tuberculosis.[5,7] Approximately 1.5 billion people were vaccinated between 1948 and 1974.[7] In 1974, the WHO launched the Expanded Program on Immunization. Presently, BCG vaccine is being produced from the attenuated *M. bovis* sub-strains by several laboratories globally, used mostly for tuberculosis prevention but some also for other non-tubercular indications, with more than 4 billion doses administered till 2013.[5,8] These formulations are regularly approved and monitored for safety, quality and efficacy, as per product characteristics of the lyophilized seed lot system, set by the WHO Expert Committee on Biological Standardization.[7] WHO has recognized more than 10 vaccine substrain preparation still now, depending on the sub-strain used as these have different viability, residual virulence, potency and overall efficacy. The number of particles cultivated per dose is also different, for example, varies from 37,500 to 500,000 in the Pasteur sub-strain and from 200,000 to 3,200,000 in the Copenhagen Danish1331 sub-strain.[7]

Currently, globally BCG vaccination covers almost 85% newborns and infants in high endemic countries. It is estimated that billions of people have been vaccinated till date, with almost 100 million children being vaccinated every year.[4,8] It has been estimated that widespread routine infant BCG vaccination programmes achieving a high global coverage (90%) could prevent over 115000 TB deaths per birth cohort in the first 15 years of life.[8] Despite being routinely administered globally to infants in many countries, the vaccination campaigns have had little impact on the occurrence of infectious adult pulmonary TB thereby in breaking the chain of transmission of this disease.[9]

1. Protective efficacy of BCG

The efficacy and effectiveness of BCG vaccination against TB has been found to differ considerably between studies and populations. Several case control studies and clinical trials have been carried out in various countries after 1930 to assess the protective efficacy of BCG vaccine against all forms of TB including pulmonary TB.[10–13] These studies showed large discrepancies in the protective efficacy against all forms of TB depending on the geographic areas and the study design, with the efficacy rate ranging between 0 and 80%,[10,11] whereas protection against tuberculous meningitis and miliary tuberculosis[12] was consistently high (greater than 50%) in all studies. BCG offered no protection against post-exposure prophylaxis. These studies differed in the study designs, logistics and study location which could have influenced the results obtained. The largest community-based 15 years follow–up double blind randomized controlled trial was carried out in Chingle put, Madras by the Indian Council of Medical Research (ICMR)[13] enrolling 366,625 participants. Out of these, 281,161 were vaccinated with BCG or placebo by random allocation. Two strains of BCG were used, the French and Danish, with a high dose (0.1 mg/0.1 ml) and low dose (0.01 mg/0.1 ml)of each strain. The entire population was followed up for 15 years by means of resurveys every 30 months. The interim incidence rate results at the end of $7^{1/2}$ years in the three "vaccination" groups were similar pointing towards the complete lack of protective efficacy. Final results also showed no protection against TB in adults and a low level of overall protection (27%; 95% C.l.-8 to 50%) in children. The findings of high infection rates and high nonspecific sensitivity at 15 years highlighted that BCG did not offer any protection on its own against the adult form of bacillary pulmonary TB. This lack of protection could not be explained fully by the methodological shortcomings, or the effect of prior sensitization by non-specific sensitivity, or because most of the cases arose as a result of an exogenous re-infection.[13] This fueled uncertainties about the protection accorded by the vaccine, making the subject highly controversial and opening a gateway which led to the complete re-evaluation of BCG and global vaccination strategies against TB.Three meta analyses namely by Rodrigues LC et al[14] (1993), Colditz GA et al[15,16](1994,1995) were carried out to analyze the different results obtained in all the studies available on BCG vaccine till that time. The results were homogeneous for the protective efficacy of BCG vaccine against tuberculous meningitis (TBM) and miliary TB, ranging between 72 and 100%, with a summary estimate of 86%, and above 80% for the different strains of the vaccine (Copenhagen, Moreau, Glaxo).[14] Colditz et al, quantified the summary estimates for randomized clinical trials (RCT) and case–control studies. The efficacy of BCG vaccine in prevention of TB was50% while it was 71% for meningitis, disseminated disease and TB deaths. Protection was uniform across all ages.[15] Nevertheless, the protective efficacy of BCG vaccine against pulmonary tuberculosis (PTB) were quite heterogeneous, as several RCTs revealed rates ranging between 0% and 80%, overall reduction in incidence at best only 50%.[15,16] Two recent systematic reviews and meta-analyses were conducted in 2014. Mangtani et al, analyzed

18 RCTs on incidence of PTB and found 3 distinct variables that influenced the vaccine efficacy, namely age at time of vaccination, prior TST positivity and distance from the equator. They found that neonatal BCG provided good protection against dissemination, severe disease and pulmonary TB while efficacy was variable for pulmonary TB in adults when BCG was given later in life, indicating prior infection with *M. tuberculosis* or exposure to environmental mycobacteria decreases the level of protection. The higher apparent protection against PTB in settings away from the equator was reduced in the multivariable analysis ($P < 0.054$), which could be due to the fact that TST screening does not exclude exposure to environmental mycobacteria. Further, efficacy was not related to the BCG strain used.[17]

Similar high protection from BCG vaccination of neonates and moderate protection of school-ageTST-negative children was found in another systematic review and meta-analysis of 12 cohort studies. Protection for PTB ranged from 44% to 99%, with 82% (RR0.18,95%CI:0.15–0.21) and 64% protection after neonatal vaccination and in school-ageTST-negative children respectively in 11 studies, with no protection in one study.[18]

Another separate meta-analysis, respectively of 6 RCTs and 14 case control studies, indicated a high degree of vaccine efficacy, reduction in severe disseminated disease, tuberculous meningitis and miliary TB in neonates and TST-negative school-age children, with 90% protection, highest for those immunized in the neonatal period.[19] There was little evidence of protection against severe disease if vaccination was done of school-age or older children not stringently TST screened. However, their estimates could be imprecise as numbers were small (0–3 cases).[17,19]

It was thought till recently an important limitation of BCG is that though it definitely prevents dissemination and development of the severe, dangerous forms of TB disease i.e. tubercular meningitis and miliary tuberculosis, it is not very effective at prevention of primary infection and persistent TBI. Roy et al, analyzed 14 studies on vaccinated and unvaccinated children aged less than 16 years with known recent exposure to patients with pulmonary tuberculosis. Children were screened using only interferon gamma release assay, a much better indicator of infection than the Mantoux test using PPD. Protective efficacy against TB infection ranged 19–27% while reduction in progression to active disease was 58–71%, indicating that BCG protects against both, M tuberculosis infection and its progression to active disease.[20] This has important implications for high burden countries where a huge population pool has latent TBI and especially, countries like Africa, where TB-HIV co-infections are high and there is a higher likelihood of progression from TBI to an active TB disease.

Further, although the efficacy of BCG varies, the vaccine has led to a significant drop in TB cases worldwide.[4] The protective effect of BCG among children against the primary infection is about 20% and it protects almost half from getting active disease.[20] BCG administration in children under 5 years has particularly resulted in reduction of infant mortality by providing strong protection against the severe forms of TB, particularly tuberculous meaning it is, disseminated disease and pulmonary TB.[17–20] However, the protection of adolescents and adults wanes over time. In Great Britain, the Medical Research Council conducted a study between 1950 and 1970; including 54,239 participants aged 14–15 years, to assess the length of protection. The analysis carried out every five years revealed that protection decreased from 84% in the first five years, to 59% between 10 and 15 years and zero after 20 years, highlighting a timed reduction in the protective efficacy.[21] A placebo-controlled clinical trial on American Indians and Alaska Natives revealed a decline in efficacy from 77% at the beginning of the follow-up period (1935–1947) to 52% at the end of six-decade follow-up period (1948–1998), an evidence of only a slight waning in the protection even 60 years after immunization.[22] A more recent study in Nordic countries with low risk of tuberculosis also confirmed a higher (61–64%) protection in 15–29-year-olds, in tuberculin-negative vaccinees.[23] This has been confirmed by other studies also. Studies using the same BCG strain in different countries report different levels of protection, higher in places far away from the equator, hypothesized to be due to infrequent exposure to environmental mycobacteria (EM) but this is just a hypothesis, without any conclusive proof.[17,20] Vaccine efficacy may also be reduced by pre-exposure to helminths.[24] Systemic reviews have concluded that the protective efficacy of neonatal BCG lasts for about 15 years hence BCG has a limited role in prevention of re-infection or reactivation of TB in adults in tropics, while it varies from 20-60 years in northern Europe and North America.[18,19,23]

The length of protection and efficacy of a vaccine, burden of infection and disease all play an important role in adopting and establishment of different national vaccination policies. Revaccination of children and adolescents was a practice followed by some TB high burden countries to boost immunity in an effort to protect against adult tuberculosis. A review in Hungary from 1959, when TB was a serious epidemic with high incidence rates, neonatal vaccination was followed by compulsory revaccination to tuberculin-negative children and adults aged 10 and 20 years. In lieu of a reduction in incidence rate, it was decided in 1983 to continue the compulsory revaccination policy to strengthen immunity of children and adults in Hungary,[25] while in a nationwide survey in Brazil focusing on school-age children, revaccination showed no protective effect which led the Brazilian government to suspend their revaccination programme and limit it only to health care professionals and contacts of TB or leprosy.[26] A study in Malawi found that revaccination with BCG in both children and adults conferred and additional 49% protection (95%CI:0–75%).[27] But majority of trials, cohort and case-control studies have not shown any beneficial effect of BCG revaccination in adolescents and adults after primary BCG vaccination in infancy, either on protection against *M. tuberculosis* infection, TB disease or post-exposure prophylaxis for prevention of leprosy.[11,26] Such differences between studies and populations may reflect different patterns of natural exposure to a variety of mycobacterial species.[27]

Though a systematic review is underway but most of the evidence regarding revaccination is based on observational

studies that after the second dose of BCG vaccine was discontinued in PPD-nonreactive children, the number of cases did not increase, compared to the cohort of children revaccinated with BCG vaccine.[28] Hence, WHO recommends only one dose of neonatal BCG vaccine against TB in high burden countries, given the lack of supporting systematic evidence of proven efficacy of repeat dosages.[2,4] Presently, however, there is a growing interest in the use of BCG vaccine for its 'off-target' effects with future studies on novel applications which might involve revaccination of a vaccine which is otherwise safe.[28] BCG is recommended in HIV immunologically stable seropositives though there is a1% higher risk of developing local, regional and disseminated BCG disease and mortality in severely immune-compromised HIV-infected infants and children.[29]

Different countries have different vaccination policies. As per WHO recommendations, neonatal vaccination with single dose of the BCG to neonates is recommended in TB and/or leprosy high burden countries as there is lack of evidence supporting any protection provided by the use of additional doses. Currently, this is being followed in all Asian, African, Central and South American countries as a universal national BCG vaccination programme. BCG vaccine is not recommended in most European countries except high burden eastern European countries, some even have a policy for revaccinating school-age or older children who test negative in the purified protein derivative (PPD) tuberculin test. Low incidence countries including USA, United Kingdom, Western Europe and Canada vaccinate only high risk groups such as health professionals working in endemic areas, homeless people and infants/children who have risk of coming into contact with TB or have high risk of developing TB.[8]

BCG vaccination induces an immune response and is an immune modulator. Thus, it has been used in atopy, ancylostomiasis and other helminthic infections and for other indications with conflicting results.[5,9,30] Chief among these is the prevention of leprosy[27] and Buruli ulcer disease (BUD),[31] caused by M.*ulcerans*.Studies though no significant evidence of a protection offered by routine BCG vaccination on the risk of developing either BUD or severe forms of BUD.[31] In the US, it is used in the treatment of bladder cancer, where BCG is instilled intravascularly as the primary treatment modality.[32] BCG has also been investigated for its possible protection against other respiratory pathogens, including COVID-19, as its use appears to be associated with a lower risk of viral respiratory illness in some populations.[33,34] Although there have been ecological associations between BCG vaccination and a lower risk of COVID-19,[34] clinical trials are still ongoing. BRACE Trial, a Phase III, two group, multicentre, randomized controlled trial is underway to assess the efficacy of BCG as an immune booster for health workers during the Covid19 epidemic.[35] The WHO does not currently recommend that BCG be used for protection against COVID-19as its diversion from TB for this purpose could exacerbate existing global BCG shortages.[36]

Prevention of any disease is much more important than cure in controlling it, the same applies to TB also especially with emerging strains resistant to multiple drugs. The Bacillus Calmette-Guerin (BCG), the only licensed vaccine against TB, is not effective against adult pulmonary TB, the highly contagious form of TB though there is a clear benefit to its use for the prevention of severe forms of TB in young children. Hence, there is a desperate need for a more effective vaccine against *M. tuberculosis*.A new more efficacious TB vaccine can be one of the best strategies to end this menace at the global level. Targeting and fast pacing the development of a vaccine better than BCG is thus an important goal.Though a lot of research is going on and since long time to find a promising vaccine but there has been a lack of an effective vaccine strategy. In the past two decades or so, many novel TB vaccines have been developed, and some of them evaluated in clinical trials.[37] However, the lack of validated immune correlates to assess the clinical relevance of novel TB vaccines before their entry into costly efficacy trials is a huge challenge to the field of TB vaccine development.[37]

Till now only four vaccine candidates seem promising, out of which three have recently completed or are currently undergoing phase III trials: *M.vaccae*trial, a heat-killed preparation of *Mycobacterium vaccae*, has been completed and results pending publication while a Phase III Prevention of Disease (POD), double-blind, three arm with placebo, randomized controlled trial is already underway to evaluate the efficacy and safety of two vaccine candidates, VPM1002, a recombinant BCG and Immunovac/MIP, a heat-killed *Mycobacterium indicuspranii*, a non-pathogenic non-tuberculous mycobacterium in healthy household contacts of newly diagnosed sputum positive pulmonary TB patients. This Phase III trial is underway in India supported by the Indian Council of Medical Research (ICMR)to study effect of two vaccines in preventing spread of tuberculosis in persons living with new TB patients.[37] Preliminary results from a trial of the M72/AS01$_E$ vaccine candidate—a recombinant protein/adjuvant subunit vaccine containing the immunogenic mycobacterial fusion protein M72—suggest that this is another promising candidate for preventing the development of TB disease among adults who are already infected with *M. tuberculosis*.[38] Phase III trials of this novel vaccine are being planned. With the boon in vaccine investment and development for COVID-19, it is hoped there will be additional vaccine candidates developed and tested for TB, along with adequate funding to test and deploy any successful candidates.[37,39]

2. Conclusion

The current BCG vaccination for the prevention of severe and disseminated forms of TB in young children represents a remarkable achievement in terms of vaccine development and deployment. Its ability, however, to prevent adult forms of TB remains extremely limited and there is a pressing need for better vaccine candidates to tackle the global TB crisis, which has only worsened in the era of COVID-19. Historically, the dogged work that was done to both develop and test the BCG vaccination is a reminder of the capacity and political will that can be generated for TB vaccine trials. In these modern times, it is hoped that the innovations developed for COVID-19 vaccines can be applied to TB with the same sense of urgency and level of funding, but with a far greater eye toward access and equity for those most at risk. Millions of lives depend on it, as modeling estimates show that without a novel vaccine strategy, global aspirations to end TB will not be reached.

Conflicts of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Sangeeta Sharma reports writing assistance was provided by National Institute of Tuberculosis and Respiratory Diseases. Dr. Sangeeta Sharma reports a relationship with National Institute of Tuberculosis and Respiratory Diseases that includes: employment. Dr. Sangeeta Sharma has patent pending to Not Applicable. Not Applicable.

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Sangeeta Sharma
National Institute of TB and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, 110030, India

Vijay Kumar Arora*
Tuberculosis Association of India, India

**Corresponding author.*
E-mail address: vijaykumar1945@gmail.com

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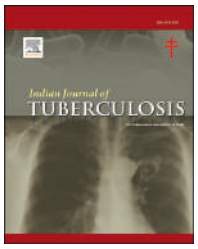

Viewpoint

Yes, we have the power to end TB!

Nibedita Rath [a,b], *Chaitali Nikam* [a,c], *Stephanie Seidel* [d], *Anindya Sinha* [e,f], *Vijay Kumar Arora* [a,g,*]

[a] *Union Lung Health, South East Asia Region, New Delhi, India*
[b] *Open Source Pharma Foundation, National Institute of Advanced Studies, Faculty Block, Indian Institute of Science Campus, Bangalore, 560012, India*
[c] *Thyrocare Technologies Limited, Navi Mumbai, 400703, India*
[d] *TB Alliance, New York, USA*
[e] *National Institute of Advanced Studies, Indian Institute of Science Campus, Bangalore, 560012, India*
[f] *Institute of Public Health Bengaluru, 3009, II-A Main, 17th Cross, Krishna Rajendra Road, Banashankari Stage II, Bangalore, 560070, India*
[g] *The Tuberculosis Association of India, New Delhi, 110001, India*

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ABSTRACT

Robust efforts are essential to sustain and increase the advancements made in battling TB, as well as to tackle persistent issues that have caused the fight against the disease to be uneven. The End TB Strategy proposes that new technologies are to be developed by 2025 to encourage a quick growth in TB occurrence diminishment. This calls for a cross-sectoral focus on creating and distributing suitable medical and programmatic modernizations in a fair manner. However, many difficulties and differences still exist in the realms of research and development regarding vaccines, drugs, technical advances, and services related to TB. Therefore, priority needs to be given to overcoming these difficulties and discrepancies for a better future.

On World TB Day 2023, SEAR Union, TB Alliance, the National Institute of Advanced Studies (NIAS) and Open Source Pharma Foundation (OSPF) gathered to discuss an important topic under the heading: "YES, WE HAVE THE POWER TO END TB!" With a commitment to putting the patient first and increasing their collective efforts, the organizations recognized that it is possible to make this goal a reality. The organizations involved in the discussion have declared their commitment to engaging in collaborative efforts to end TB globally. They advocate for strengthening access to TB services, controlling and preventing TB, improving surveillance and drug resistance management, and investing in research and development. Furthermore, they recognize the importance of reducing stigma and integrating patient voices in this endeavour. This Round Table serves as a framework to build on and ensure that the goal of ending TB is achievable.

* *Corresponding author.*
E-mail address: vijaykumar1945@gmail.com (V.K. Arora).
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1. Introduction

Ending the global TB epidemic is attainable if drastic steps are taken to reduce TB deaths and cases, as well as the economic and social burden of TB. If successful, this would result in immense health benefits for individuals and the global population. However, if ignored, the consequences would be dire. The World Health Organization (WHO) targeted to reduce worldwide TB incidence by 90% by 2035.[1] Early initiation of effective treatment based on susceptibility patterns of the *Mycobacterium tuberculosis* Complex (MTBC) is considered key to successful TB control in countries with high drug-resistant TB (DRTB) incidence.

The Round Table was designed to explore the strategies, actions, and partnerships necessary to reach the Sustainable Development Goal (SDG) of ending Tuberculosis (TB) epidemic by 2030.[2,3] The panel members, consisting of experts, decision-makers and those affected by TB, shared their expert views and innovative practices to protect people from TB. The event culminated with participants joining hands and reaffirming the commitment to working together to end TB and stand for justice and equity to protect those affected by the disease. Overall, the Round Table appeared to be a successful event, bringing together a diverse range of stakeholders from the public and private sectors, civil society and the community. The event was an opportunity to strengthen partnerships and galvanise collaboration amongst stakeholders and provide an interactive platform to discuss solutions, challenges and approaches in the fight against tuberculosis. The Round Table also reiterated the importance of putting the patient first and taking collective action if we are to achieve the goal of ending TB.

The Round Table was an initiative to foster collaboration and dialogue between experts, decision-makers, and communities, which seeks to promote greater inclusivity, equity, and use of evidence in TB care around the world. It also works to emphasize the importance of a collective effort between public and private institutions, civil society, and the community to effectively address the challenges of TB in India.

2. Discussion

The political will to combat tuberculosis (TB) needs to be strengthened on all levels of the political spectrum – local, national, and global.[4] Local politicians need to be informed of the issue and act on an individual level to raise awareness about TB. National level politicians also need to be influenced upon to provide policies, funding, and support for local governments to tackle the issue at a larger scale. Finally, global leaders must come together to coordinate international strategies in the fight against TB. To strengthen TB patient groups, digital technologies can be used to bring together individual TB patient voices from around the globe into a connected online platform, allowing them to share resources, experiences, and support. Additionally, local and national governments could provide guidance and assistance to local TB patient groups in the form of educational resources, advocacy, access to healthcare, and financial support. Various global organizations and initiatives should be utilized to leverage funds, expertise, and other resources to support TB patient groups and spread awareness of the issue.

The BRICS (Brazil, Russia, India, China, and South Africa) TB Fund is an innovative financing mechanism that would enable partners to tackle the global tuberculosis (TB) pandemic together.[5] The partnership can be committed to taking collective action to strengthen TB prevention, care, and control and to accelerate the global response to TB. By pooling the resources, expertise and capacity of BRICS countries, the initiative can create new, effective approaches to address TB while by working together, one can expand the possibilities and leverage the power of collective action to make a lasting impact on TB in the BRICS countries as well as in other affected regions of the world.

Balancing the risk of latent tuberculosis disease and the risks of treatment for individuals who have undergone testing but fall outside the above categories, the decision to treat latent TB infection should be individualized, as the risk may outweigh the potential benefits. Balancing the risk of disease with the risks of treatment becomes an important consideration when treating individuals who have undergone testing for TB infection but do not fall into the predefined categories mentioned above. Careful consideration of the potential benefits and risks of treatment should be made on a case-by-case basis, as an individual's risk of disease may not be adequately weighed against the potential risks of treatment. With adequate engagement of the TB patient, and a treatment decision-making process that involves the patient, a health professional, and the patient's support system such as family, can help to ensure that risks are fully understood and managed. The decision to treat should be based on the individual's age, TB status, underlying health conditions, lifestyle, and other factors. Disease management strategies should be tailored to each individual's unique needs in order to ensure the most effective outcome with the least amount of risk.

Case finding, an important parameter in the fight against TB has always remained a challenge despite advances in diagnostic modalities, access to health care and administrative commitment.[6] We are still far from reaching the goals set in the End TB Strategy and the Indian National Strategic Plan 2017–2025, and case finding is of paramount importance for achieving the said targets.

In this connection, it is important to focus on active case finding (ACF) for tuberculosis elimination.[7] We must find all (or nearly all) of the cases, not just the "low hanging fruit" or the high risk groups. We must not just rely on volunteers and must test everyone, not just those with symptoms. Sputum molecular tests, such as Xpert, or radiology should be used as the first screening test since many with infectious TB may not have symptoms. All found cases must be linked to appropriate and effective therapy and regular testing must be sustained for at least 5–10 years until the prevalence and incidence of TB is low. The measure of success should be the impact on incidence (or number of cases NOT found) as this will demonstrate the effectiveness of the ACF programme in eliminating TB.

It is also important to remember that ACF for TB elimination is not a one-time intervention. It must be sustained over a period of time to ensure that all cases are identified and linked to appropriate care. This means that active surveillance must be conducted regularly, with contact tracing and case finding

activities conducted as soon as a new case is identified. Additionally, high-risk groups must be identified and targeted for screening and testing, as well as engagement and education about TB. Finally, the programme must be supported by adequate funding and resources to ensure it is sustained over the long-term. By remembering to focus on active case finding, and regularly conducting case finding activities, we can make progress towards TB elimination. With adequate resources, support and partnership with TB-affected community stakeholders, we can make a significant impact on the burden of TB in our communities and ultimately achieve elimination.

A patient-centric approach has been seen to benefit those affected by tuberculosis, as it improves adherence, reduces lost to follow-up, decreases deaths, and increases treatment outcomes.[8] Examples of such schemes and local initiatives are plentiful. To ensure the systematic implementation of such an approach, the expansion of social protection schemes is necessary to combat poverty, vulnerability, and social exclusion. Engaging patients and TB-affected communities is critical to the planning and redesign of services to ensure a patient-centric focus. Furthermore, systematic documentation of outcomes and impacts of these approaches is urgently needed.

A patient-centered model of care prioritizes engaging the patient, taking into account local social, structural and cultural factors, and tailoring the patient's education and counselling accordingly. It should include mental health professionals, social workers, and behavioral counsellors to cover the full range of clinical, socio-economic and structural issues facing each patient. Evidence shows that this approach can lead to better engagement in care, improved patient outcomes, and greater satisfaction with overall treatment.

The emergence of data science is being propelled by the increasing digitalization of our world, which has resulted in a surge of 'big' data, along with the capability to process it. Such data encompasses numerical, textual, visual, auditory, haptic, and more, and when properly mined, it can lead to novel insights and solutions to current healthcare problems. Data Science is the science of translating data into information, meaning, interpretation, knowledge, action, feedback, and learning. Currently, the design of TB care systems is dominated by the biological, medical, economic, and social sciences, which each address a significant part of the problem. Despite large-scale, long-term efforts with micro-biological diagnoses, medical treatments, economic incentives, and social protection for TB care in India, the issue of TB epidemiology and endemicity still persists. By complementing these traditional sciences with data science, it can help to transform the TB care system in various ways, such as by delivering timely care.[9]

It should also be noted that early diagnosis of pulmonary tuberculosis is critical for disease management. Early diagnosis is a step towards reducing the chances of further disease transmission in the community and the overall wellbeing of patients and the public. Digital health or 'healthtech' is growing exponentially, and technology is transforming the way healthcare is delivered. Advances in AI algorithms has unveiled great promises in identifying the presence and absence of TB.[10] As of late, many attempts have been made to formulate the strategies to increase the classification accuracy of TB diagnosis using the AI and machine learning (ML) approaches. AI-driven pulmonary TB diagnostics has the potential to transform the healthcare landscape by providing accurate and timely diagnosis, improving disease control, and speeding up the treatment of TB.

The impressive performance of ML methods in many areas of science, technology, and medicine has seen a dramatic increase in their use to design or discover drugs to treat different diseases. As TB is an infectious disease with high mortality rate and present problems of treatment efficacy and rapidly emerging resistance, finding new, effective, and safe anti-TB drugs has become a high priority. Structural biology-based approaches, when coupled with ML methods, make for a powerful combination that can help devise drugs to effectively treat TB in the near future.[11]

By integrating evidence from the biological, medical, social, and economic sciences pertaining to an entity, data science can be used to enable timely counselling. This will help to reduce the cycle time and personalize the counselling, as well as to adapt it to the different stages of TB care, manage the dosage, and focus on the individual, family, community, or public receiving the counselling. By using feedback and learning from those counselled, effective counselling and a continuum of care can be provided under various socio-economic circumstances.

By using data science, timely corrections can be made to proactively identify instances of non-compliance with treatment protocols and patient non-adherence to help prevent the progression of TB. Decision support tools can then be utilized to detect deviations from the expected trajectory, analyze the causes of the deviation, and suggest an appropriate future course of action based on the feedback. Furthermore, improvements can be reinforced, normal movements continued, and deteriorating conditions redirected in order to effectively manage the entity.

The traditional drug discovery model is not the best suited model for diseases of the of the Global South, such as tuberculosis. There is a need for an alternate paradigm for drug discovery to provide affordable and accessible health care.[12] The current model of collaboration and resource-sharing, while protecting confidentiality, hinders the opportunities to bring expertise from different fields and thus limits potential cost savings on expensive drug discovery processes. The open source model, on the other hand, can empower researchers by providing unrestricted access and greater opportunities for communication, consultation, integration and collaboration to discover new drugs that are cost-effective and targeted towards the diseases of the developing world. Creating a Global Open Digital TB Research Platform can provide access to open data sources relevant to digital TB research and global health. The platform can allow users to aggregate and store data from various locations, as well as search and visualize the data to gain further insights into the disease biology. This data can be an inclusion of TB incidence and prevalence, geographic concentration, and risk factors, as well as patient outcomes and treatments. Moreover, the Global Open Digital TB Research Platform can provide an online platform for researchers, clinicians, and policymakers to share ideas, collaborate, and engage in meaningful conversations about TB research and interventions.

The high financial burden historically placed on multi drug-resistant TB (MDR-TB) patients can be attributed to the lack of a

coordinated and multi-sourced financing model to secure access to expensive drugs, and the lack of a safety-net for those with low ability to pay.[13] Innovative models for reducing cost and increasing access of novel TB treatments, such as the volume guarantee announced by MedAccess and Viatris in late 2022 for new DR-TB treatments, greatly help to address these barriers.[14] Additional key intervention areas need to be identified and pursued, such as continuing to make novel drugs more affordable and available, providing incentives for pharmaceutical companies to expand indications of established medicines, implementing public initiatives to support the use of repurposed medicines, and engaging with civil society to best understand and advocate for the needs of TB-affected communities. A comprehensive approach is necessary and a safety-net should be established to ensure universal access to MDR-TB medication at a more affordable cost.

Shortening treatment regimens to 3–4 months with existing or repurposed drugs is an important component of TB control as it improves patient compliance and reduces the cost of therapy. BPaL is the combination of three antibiotics—bedaquiline, pretomanid, and linezolid—developed by TB Alliance.[15] The combination was explicitly created to treat drug-resistant forms of tuberculosis. The regimen received its first regulatory approval from the United States Food and Drug Administration in August of 2019.[16] Since the launch of updated World Health Organization Guidance on the treatment of Drug-resistant TB, recommending the use of this therapy for all forms of drug-resistant TB (BPaLM/BPaL) this combination therapy has been procured and used by 109 countries around the world.[17]

The Sustainable Development Goal of ending the tuberculosis epidemic by 2030 builds upon the progress made under the Millennium Development Goals, with more specific targets set out in the WHO Global Strategy and Targets for Tuberculosis Prevention, Care and Control after 2015. The main specific targets for 2030 include ensuring that no family is financially burdened by TB-related expenses, and achieving a 90% reduction in TB deaths and an 80% reduction in TB incidence as compared to the rates in 2015, with further reductions (95% and 90% respectively) expected by 2035.[18] Despite these goals, however, there exists a huge gap between current reality and the SDG vision.

India is striving to eradicate TB by 2025, five years ahead of the United Nations Sustainable Development Goals' (SDGs) global objectives.[19] To reach this target, the government of India has launched a National Strategic Plan that encompasses a multifaceted approach to ending the TB epidemic, four key strategies: detection, treatment, prevention, building and strengthening policies, empowered institutions, and human resources with enhanced capacities.

Conflicts of interest

The authors have none to declare.

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Viewpoint

Elimination of tuberculosis requires prior control of silicosis including sub-radiological silicosis

Dharmendra Singh [a], *Bidisa Sarkar* [b], *Kamalesh Sarkar* [c,*]

[a] *Department of Biochemistry, ICMR-National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra, Uttar Pradesh, India*

[b] *Department of Community Medicine, Gouri Devi Institute of Medical Sciences & Hospital, Durgapur, West Bengal, India*

[c] *ICMR-National Institute of Occupational Health, Ahmedabad, Gujarat, India*

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ABSTRACT

India is committed to the elimination of tuberculosis by 2025. But its achievement appears to be difficult as India has a huge burden of silicosis as well as sub-radiological silicosis, which was never given its required attention. Silicotic subjects are highly vulnerable to pulmonary tuberculosis due to the progressive decline of lung immunity. A study among vulnerable glass factory workers in Firozabad, Uttar Pradesh, revealed that silicotic workers were 7.5 times more at risk of pulmonary tuberculosis compared to non-silicotic subjects. Since India has a huge burden of silicosis and sub-radiological silicosis, the elimination of tuberculosis needs prior attention on silicosis. This article may be viewed as an eye-opener for understanding the necessity of dual control of both silicosis as well as tuberculosis by integrating both together.

ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra conducted a cross-sectional study to investigate the existence and magnitude of pulmonary tuberculosis among glass industry workers in Firozabad district, Uttar Pradesh (see Table 1). The industry comprises a large number of different-sized factories producing glass bangles and various other glass items. Institute Ethics Committee approved the study before initiating it. A total of 380 workers (205 workers working in glass industries and another 175 workers working in non-glass industries) were included in this study from their workplaces for assessing pulmonary tuberculosis as well as underlying silicosis as they are continually exposed to respirable glass clouds of dust. Silicosis was assessed by chest x-ray as per International Labour Organisation (ILO)'s guidelines.[1] Participants were asked to collect their sputum samples in sterile 50ml falcon tube containers. Their sputum was examined by Cartridge-based nucleic acid amplification test (CB-NAAT) for detecting tubercular infection using standard procedure.[2] The result revealed that the detection rate of pulmonary tuberculosis was 4% among non-glass factory workers and the same was unusually high among the glass factory workers, in the tune of 46% (Table 1). Among the studied glass factory workers (n = 205), 114 subjects were detected to have silicosis. Surprisingly they had a detection rate of pulmonary tuberculosis of 66.6%, whereas the same was 20.8% among non-silicotic subjects (Table 2). This may be viewed as an eye-opener as India has a very high burden of silicosis as well as sub-

* Corresponding author.
E-mail addresses: singhd72@yahoo.co.in (D. Singh), bidisa18@gmail.com (B. Sarkar), kamalesh.sarkar@gmail.com (K. Sarkar).
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Table 1 – Distribution of pulmonary tuberculosis among glass and non-glass factory workers in Firozabad, Uttar Pradesh, India (N = 380)

Study Participants	Infected with Pulmonary Tuberculosis	Not infected with Pulmonary Tuberculosis
Glass factory workers (n = 205)	95 (46%)	110 (54%)
Non-glass factory workers (n = 175)	7 (4%)	168 (96%)
Total participants (n = 380)	102	278

radiological silicosis. Subradiological silicosis is often overlooked and never got its required attention. A study carried out on sub-radiological silicosis in South Africa revealed two important findings – out of every 100 silicotic workers, 57 were subradiological silicosis (diagnosed by autopsy after death) and subradiological silicotic workers were 2–3 times more at risk of developing silicotuberculosis compared to non-silicotic subjects.[3] This indicates probably India too have a bigger burden of sub- radiological silicosis compared to the burden of radiologically detectable silicosis, which was never tried to be explored from the tuberculosis elimination point of view.

If detection of silicosis at sub-radiological stage is missed due to ignorance or other reason/s, a golden opportunity to prevent silicosis and tuberculosis will be missed. Usually, if workers are occupationally exposed to silica dust for less than 10 years, he usually suffers from sub-radiological silicosis and x-ray shows no abnormality. But he remains vulnerable to tuberculosis and silicosis (radiologically detectable).

Chemically glass dust contains silicon dioxide (SiO_2).[4] Workers working in these glass industries become vulnerable to silicosis by continuous or intermittent inhalation of respirable glass (silica) dusts. The increased risk of tuberculosis in silica exposed populations has been established for both those with and without radiological evidence of silicosis.[5–7] Silicotic workers are vulnerable to silicotuberculosis due to their declined lung immunity by continuous destruction of lung macrophages by the inhaled silica particles.

Respiratory public health is currently facing several challenges – increasing air pollution, growing chronic obstructive pulmonary diseases (COPDs), emergence of newer strains of influenza viruses, the problem of multidrug-resistant tuberculosis and a highly distressing pandemic causing SARS-CoV-2 virus with continuously evolving newer variants etc. Against this backdrop, it is unacceptable that a preventable occupational disease such as silicosis would threaten the health and human lives of so many persons throughout the world.[8] On the other hand, India is committed to eliminating tuberculosis by 2025. But considering the huge burden of silicosis as well as sub-radiological silicosis in India, it appears that elimination of tuberculosis is difficult to achieve unless prior control of silicosis is achieved. It is observed that the presence of silicosis (including sub-radiological silicosis) causes difficulty in the diagnosis of pulmonary tuberculosis, the uncertainty of treatment outcome and there is a higher chance of development of multidrug-resistant tuberculosis.[9]

Silicosis is a neglected disease, usually diagnosed at an advanced stage, when nothing much can be done to improve the patient's health or prolonging his/her life. Consequently, silicotuberculosis is also diagnosed at a late stage facilitating further transmission in the community and/or to colleagues at work places, including few are by the multidrug resistant organisms. Hence, prior control of silicosis is extremely important for elimination of tuberculosis. In 1995, the WHO and the International Labour Organization started a public campaign of awareness and prevention with the aim to eliminate silicosis from the world by 2030.[10] But due to various reasons, it is not progressed to the expected extent in most Asian countries including India.

Considering the above, early detection of silicosis and silicotuberculosis is the need of the day using some suitable predictor/marker. Indian Council of Medical Research – National Institute of Occupational Health (ICMR-NIOH) has recently identified a proxy biomarker known as club cell protein 16 or CC-16 (a lung protein) that could indicate silicosis including sub-radiological silicosis at an early stage if periodic screening is done using the said marker among silica dust exposed workers.[11] Following its discovery, scientists of ICMR-NIOH and ICMR-National Institute of Virology (ICMR-NIV) jointly have developed a point of-care, semi-quantitative, user's friendly and low-cost device, CC-16 detection kit for this purpose. Indian Council of Medical Research, Ministry of Health & Family Welfare, Govt. of India has approved the kit for mass use.[12]

India is said to have the greatest number of drug-resistant tuberculosis patients. Poor primary health care infrastructure, rural areas with inadequate resources to combat TB in many provinces, uncontrolled private health care, and a lack of strong political will are all major TB control difficulties in India. The diagnosis and treatment of tuberculosis have been advanced rapidly since the turn of the century. The situation has altered the tuberculosis control scenario

Table 2 – Distribution of pulmonary tuberculosis among silicotic and non-silicotic glass factory workers in Firozabad, Uttar Pradesh, India (n=205).

Study Participants	Infected with Pulmonary Tuberculosis	Not infected with Pulmonary Tuberculosis	Odds Ratio
Silicotic workers based on chest x-ray (n = 114)	76 (66.6%)	38 (33.3%)	7.57 (Confidence Interval at 95% level) 4.00–14.34
Non-silicotic workers based on chest x-ray (n = 91)	19 (20.8%)	72(79.1%)	
Total participants (n = 205)	95	110	

with newer approaches and more strong medications like Bedaquiline and Delamanid. This appears to be a start in the right direction, with the potential to have a bigger influence. But to date TB programme management authority has not yet focussed on the required attention to the huge burden of silicosis in India. Growing multidrug resistance in India as mentioned above, could be due to the huge burden of silicotuberculosis, which has never been attended to adequately. There is no statement about the control of silicotuberculosis for the sake of elimination of tuberculosis. Sub-radiological silicosis needs to be considered too emphasising their early detection and necessary intervention through an effective programmatic approach. The initiation of the National Silicosis Control Programme is an urgent need. Integration of both silicosis, as well as tuberculosis control activities, appears to be an essential step towards this to avoid various adverse outcomes of silicosis on pulmonary tuberculosis.[13] Elimination of TB activities appears to gain faster momentum if it is integrated with silicosis control activities. Suitable legislation along with political commitment is necessary. This study was funded by the Indian Council of Medical Research as an intramural project.

Authors' contribution

- Dharmendra Singh (DS) contributed by supervising the collection of biological samples from the study subjects, supervising the laboratory work and drafting the laboratory portion of the article.
- Bidisa Sarkar (BS) contributed by designing this study, analysing data and initially drafting of this article.
- Kamalesh Sarkar (KS) contributed by designing the study, supervising the entire fieldwork, and writing and approving the final article.

Conflicts of interest

The authors have none to declare.

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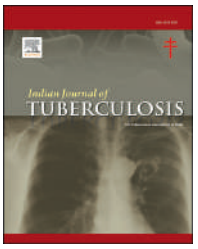

Review article

Chronic pulmonary aspergillosis in a tertiary tuberculosis institute: A common entity missed commonly

Ritu Singhal [a], *Amitesh Gupta* [b], *Neeta Singla* [c], *Rupak Singla* [d,*], *Ritika Jha* [d], *Shweta Raina* [a], *Madhumita Paul Choudhary* [d], *Nilotpal Bhattacherjee* [d]

[a] *Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases, New Delhi - 110030, India*

[b] *Department of Pulmonary Medicine, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi*

[c] *Department of Epidemiology, National Institute of Tuberculosis and Respiratory Diseases, New Delhi - 110030, India*

[d] *Department of Tuberculosis and Chest Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi - 110030, India*

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ABSTRACT

The disease chronic pulmonary aspergillosis (CPA), which has 3 million cases globally, has a substantial impact on global health. The morbidity and mortality it cause are also rather severe. Patients with modest immune suppression or those with underlying structural and chronic lung illnesses are more likely to develop this condition. CPA pose a diagnostic and management challenge to clinicians. The condition causes patients to have persistent respiratory difficulties, which lowers their quality of life, and the therapy is lengthy and offers few choices. Particularly in a nation like India, where tuberculosis (TB) is prevalent and patients exhibit identical signs and symptoms, a strong index of suspicion is required. Treated pulmonary TB patients, presenting with symptoms or chest x-ray abnormalities, especially those with presence of cavity are also more prone to develop CPA. The constellation of symptoms together with presence of microbiological criteria and suggestive radiology can help to reach at the diagnosis. The field of mycology has made major developments, but there is still much to understand about this illness and to establish timely diagnoses and make the best use of the existing treatment choices. The burden of CPA in patients with treated TB is highlighted in this article along with the most recent research and clinical guidelines.

* *Corresponding author*. Department of Tuberculosis and Chest Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi - 110030, India. Tel.: 91-9891168908.
E-mail address: drrupaksingla@yahoo.com (R. Singla).

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1. Introduction

Three million persons worldwide have chronic pulmonary aspergillosis (CPA), a disease complex that has been identified as a serious health burden in the last ten years. It is a serious condition since untreated patients have a 50–85% five-year survival rate.[1,2] Typically, people with underlying lung illness who have weakened immune system get this condition.[3]

Pulmonary tuberculosis (PTB) constitutes over 85% of global TB burden with post-TB CPA estimated at 1.2 million cases annually.[4,5] Almost 43% of diagnosed PTB cases are microbiologically negative, which could include clinically diagnosed TB cases and missed CPA cases, wrongly diagnosed as TB.[5] Patients with and without cavities following PTB were likely to develop CPA in 22% and 2% respectively as per another study.[4]

India is one of the countries with the largest TB burden, accounting for 26% of all TB cases worldwide.[5] CPA-complicating TB is estimated to occur in 27,000 to 170,000 cases annually in India, with a high TB incidence of 2.1 million cases and a prevalence of 24 cases per 100,000.[6] A prospective observational study carried out at the author's center revealed that, of all the post-TB sequelae patients with persistent symptoms included in the study, 57% of patients had been identified as CPA. On chest x-rays, 67% of these patients with CPA had cavities.[7]

Two more illnesses, sarcoidosis and allergic bronchopulmonary aspergillosis (ABPA), cause 71,907 and 411,000 instances of CPA worldwide, respectively.[8,9] With an occurrence rate of 3.9–16.7%, non-tuberculous mycobacteria (NTM) lung illness following CPA is also becoming more widely known.[10] CPA can also occur in people who have a pneumothorax or underlying chronic obstructive pulmonary disease (COPD).[9]

Aspergillus, a common opportunistic fungus that thrives in many environments and is known for inflicting lung fungal infections in immunocompromised people, is the source of the spectrum of CPA. In patients with diseased lungs, inhaled Aspergillus spores can become a focus for infections causing symptoms, possibility of which increases with time.[4,11,12]

In initial stage, clinico-radiological symptoms and presentation of aspergillosis is subtle and becomes apparent as disease progresses but still not distinct. There is a risk of death from CPA when the diagnosis is incorrect and treatment is not initiated promptly on time.[13] If CPA patients receive prompt diagnosis and treatment, they may benefit from surgery or long-term antifungal therapy.[14]

2. Classification of CPA

A wide spectrum of appearances, including aspergilloma, aspergillus nodule, chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), and subacute invasive pulmonary aspergillosis (SAIA), fall under the umbrella term of CPA. However, these illness patterns frequently overlap one another. When the disease lasts more than three months, acute and subacute types of CPA can be distinguished from one another.[14,15]

2.1. *Aspergilloma*

It is a fungal ball made up of fungal hyphae, cellular debris, mucous and fibrin that develop within an already existing pulmonary cavity. Although tuberculosis is the most prevalent cause of pre-existing pulmonary cavities, aspergillomas can develop in cavities brought on by sarcoidosis, bullous emphysema, or lung cancer. Simple aspergilloma is defined when an aspergilloma in a solitary cavity remains stable over months. Previously CCPA was mistakenly referred to as "complex aspergilloma".[14]

2.2. *Aspergillus nodule*

In immunocompetent hosts, Aspergillus nodules may be single or multiple, with or without cavitation, and variable F-fluorodeoxyglucose uptake. Histopathologically they have central necrosis which may contain fungal hyphae surrounded by granulomatous inflammation.[16]

2.3. *Chronic cavitary pulmonary aspergillosis (CCPA)*

CCPA involves formation and expansion of single or multiple cavities. It usually begins as focal or diffuse consolidation which later forms cavities which may be associated with fungal balls. In contrast to simple aspergilloma, the cavities coalesce and increase in size. Nodules that may eventually cavitate may surround these cavities.[14]

2.4. *Chronic fibrosing pulmonary aspergillosis (CFPA)*

CFPA is seen subsequent to chronic cavitary pulmonary aspergillosis with development of extensive fibrosis distorting the lung structure.

2.5. *Subacute invasive pulmonary aspergillosis (SAIA)*

SAIA (erstwhile also referred to as chronic necrotizing pulmonary aspergillosis) progresses over 1–3 months in patients having immune suppression, e.g., diabetes mellitus, advanced age, prolonged steroid therapy, other immunosuppressive therapy, human immunodeficiency virus (HIV) infection etc. Areas of consolidation may be present in the surrounding which subsequently may cavitate.

3. Diseases and conditions mimicking chronic pulmonary aspergillosis

The signs and symptoms in patients of CPA are usually similar to other respiratory diseases which may also be present along with CPA or may be pre-existing to CPA. However, alternative diagnosis should be ruled out before labeling the patient as CPA. Various differential diagnosis of CPA and their differentiating features with CPA are summarized in Table 1 below:

4. Clinical features

Most of the patients of CPA have signs and symptoms of preexisting or concurrent pulmonary disease. However, few patients may be asymptomatic and may have radiological

Table 1 – Summary of various differential diagnosis of CPA and their differentiating features with CPA.

Radiological feature	Differential diagnosis	Differentiating feature of CPA
Cavity	CPA PTB NTM Lung cancer Necrotizing pneumonia Other endemic fungi	In CPA usually more than one cavities are present usually in the upper lobe which coalesce and expand
Cavity with intracavitary material	CPA (Aspergilloma) Hydatid cyst Bronchogenic carcinoma Lung infarct Lung abscess Infected bullae	In CPA usually a single cavitay lesion with fungal ball forming an air crescent sign remaining stable over months is observed
Pleural thickening	CPA Lung cancer PTB Endemic mycosis	In CPA there is focal pleural thickening overlying a cavity
Pericavitary infiltration	CPA TB NTM	In CPA there is marked pericavitary inflammation which often merges with surrounding fibrosis

Abbreviations: CPA: Chronic Pulmonary Aspergillosis, PTB: Pulmonary Tuberculosis, NTM: Non-Tubercular Mycobacteria.

worsening only.[17] The most frequent risk factor for the development of CPA is lung cavities.[3,7,18] These underlying disorders may contribute to the development of these cavities.

a) pulmonary tuberculosis
b) bullae or cystic lung disease
c) pulmonary fibrosis
d) sarcoidosis
e) lung abscess
f) pulmonary infarction
g) non tubercular mycobacterial infection
h) previous Pneumocystis pneumonia
i) bronchogenic carcinoma with cavity

5. Case definition of CPA

Most of the previous studies used to define a period of 3–6 months for duration of disease for defining CPA.[14,18–22] However, the latest expert panel consensus by Denning et al defined presence of symptoms and signs for a minimum of 3 months as a diagnostic criteria for CPA.[17] Any increase in severity of signs and symptoms, in patients already having some chronic pulmonary disease and risk factors as mentioned above, is considered as the beginning point of this three month duration.[17] In patients having lack of any clinical signs and symptoms, but radiological progression in form of any one of the following in form of new consolidation or cavitation, increase in the size of pre-existing cavity, peri cavitary infiltrates or fibrosis, new development of a fungal ball and or fungal ball may also be taken as the initiation of disease process.[17]

6. Signs and symptoms

More than 90% of patients of CPA have clinical signs and symptoms although few have only radiological progression.[23] Following symptoms may be present in patients of CPA.

a) Hemoptysis- It is the most alarming symptom which may be present in 12%–43% of the patients.[12,16,20,22] The amount may vary from blood streaking to life threatening hemoptysis.
b) Chest pain- Mild but persistent dull aching pain is experienced by more than 30% of patients.[17]
c) Weight loss and fatigue- These are common but not seen in all and are non-specific.
d) Cough- It is usually productive but non-specific and may be present in other respiratory diseases as well.
e) Dyspnea- It is common in patients with advanced disease on chest x-ray but is non-specific.
f) Fever- It is uncommon. If present, alternative diagnosis or invasive disease is to be suspected.
g) Night sweats- These are rare and do not help to distinguish CPA from other pathology.

7. Radiologic features

The expert panel consensus by Denning et al recommended radiological criteria based on chest x-ray due to limited availability of computed tomography (CT).[17] However, pulmonary nodules, numerous cavities, disease in the retrocardiac space and lung apices, and fungal balls can all be seen on a CT scan more clearly than on a chest x-ray.[24] CT is to be done where chest x-ray is not helpful in making the diagnosis in presence of clinical and microbiological features suggestive of CPA.[17] The radiological findings of CPA are due to pre-existing chronic lung condition superimposed with changes subsequent to infection by Aspergillus spp. and immune response by the body.[25]

7.1. *Cavitation*

The presence of single or multiple cavities is one of the cardinal features of CPA. These may be small or large with

commonly having thick and rarely thin walls; and usually lie close to the pleura.[24,26] As the disease progresses, the cavities may grow larger and perhaps congregate. Pleural thickening and parenchymal fibrosis may be present in conjunction with formation of intracavitary fungal wall. The "air-crescent" sign, or aspergilloma, is typically seen in the upper lobe as an opaque shadow in a cavity that may be circular or oval and is partially encircled by a strip of air. It is mobile when lying prone.[27]

7.2. Pleural thickening

Pleural thickness is the primary CPA diagnostic sign. Patients with TB or other infectious disorders rarely experience pleural thickening.[28] It consist of two features-pleural fibrosis adjacent to cavity or consolidation and inward shifting of extra-pleural fat, however, these are difficult to identify on chest x-ray.[17]

7.3. Peri cavitary infiltration

Areas of active inflammation that are present near the cavity are called peri cavitary infiltration. These regions could also have fibrosis and pleural thickening nearby. If seen on plain chest x-ray these are conclusive indication for therapy.[26,29] In reactivation of TB or NTM infection these infiltrates are usually extensive and diffuse.

7.4. Nodules

CT chest is required for distinct diagnosis of Aspergillus lung nodules.[30] These nodules may be solitary or multiple with a diameter ranging from 5 to 50 mm. with solid or cystic component in form of a cavity. Sometimes nodules larger than three centimeters also may be present. However, to rule out other various causes of lung nodules, radiology guided biopsy is required however, its presence is limited in resource constrained settings. Hence expert panel report by Denning et al has removed it from their operational definition of CPA.[17]

8. Microbiological diagnosis

Properly collected good quality specimen is crucial for accurate microbiological diagnosis of CPA. Specimens of choice for diagnosis include aspirates such as bronchial wash, bronchoalveolar lavage (BAL), pleural fluid, lung tissue and three sputum samples collected on different days. Aspergillus is usually found in large airways, rather than alveoli, hence bronchial wash specimen is superior to BAL.[11] Swab should be avoided it has a very little material.[31]

8.1. Microscopy

Aspergillus spp. are usually seen as thin dichotomous branching septate hyphae on direct microscopy on wet mount and if present is highly indicative of CPA.[11,31] Hyphae from sterile samples like BAL, pleural fluid and lung biopsy confirms infection and should be communicated to treating physician immediately. Sensitivity of microscopy is largely dependent on quality and type of specimen and personal expertise. Wet mount using 10% of potassium hydroxide (KOH), is the commonest used microscopy technique, as it is simple to perform, inexpensive and requires least regents. Its sensitivity can be further increased using fluorescent dyes and fungal-specific stains.[31,32]

8.2. Culture

Culture is essential for identifying clinically relevant fungal species by macroscopic and microscopic examination from primary cultures and also for antifungal susceptibility. Amount of growth has no association with disease severity, however isolation of *Aspergillus fumigatus* is associated more with infection.[11] Blood cultures are almost always negative for *Aspergillus* spp.

8.3. Serology test

Most antigens are secreted by *Aspergillus* spp. while it is growing and are within or adherent to the cell wall and stimulate production of Aspergillus IgG antibodies.[33] Antibody detection thus is decisive of Aspergillus infection rather colonization and is key diagnostic feature of CPA.[11,14] Studies in CPA show sensitivity of Aspergillus IgG from ~70% to 90%, depending on the assay and cut-off used.[14]

Antibodies against Aspergillus spp. have been found in the serum of CPA patients using immunodiffusion (ID) and its quick modification, counter immuno-electrophoresis (CIEP). It is important to keep in mind that ID and CIEP are less sensitive than enzyme linked immunosorbent assays (ELISA).[34,35]

ELISA is based on colorimetric reaction obtained on formation of antigen–antibody complex, which is measured spectro-photometrically and provides quantitative result of IgG antibody. ELISAs are currently most sensitive and reliable technique for detection of IgG antibodies, however require expensive regents and special equipments such as ELISA reader or Immucocap machine as shown in Fig. 1. Bio-Rad and Bordier Affinity Products SA are manual ELISA assays which detect Aspergillus antibody IgG, with sensitivity varying from 86 to 97.4% and specificity from 90.3% to 98.2%. Automated versions and high-throughput ELISAs include PhadiaImmuno CAP and Immulite with sensitivity varying from 83.8 to 99.3% and specificity of 96%–99.3%.[32,34] ELISAs need to be done as batch testing and in manual format are labor intensive.

LDBio Aspergillus ICT is only commercially available lateral flow assay for Aspergillus IgG and IgM antibodies detection. It fulfills World Health Organization (WHO) standards as inexpensive, user-friendly, rapid, equipment free, test with possibility of being point-of-care test.[32] Lateral flow has comparable sensitivity of 88.9–91.6% and specificity 96.3–98% to ELISA however doesn't give quantitative results.[35] (Fig. 2).

8.4. Galactomannan antigen detection

Galactomannan (GM) is a polysaccharide antigen, which is increased and released during invasive Aspergillus infections and is detectable in serum and body fluids.[31] Sensitivity and specificity of GM ELISA is 80–90% for all forms of invasive aspergillosis (IA) and ~75% for CPA, which is considerable

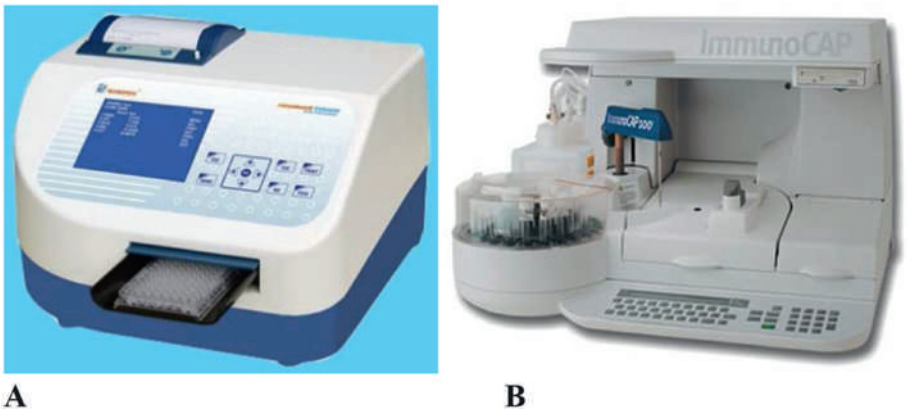

Fig. 1 – (A) ELISA reader. (B) PHADIAImmunoCAP (Thermofischer).

more than culture or microscopy on BAL. Test is rapid and can be reported within 24–48 hours, though serum samples require some pre-processing. BioRad, Dynamiker, Era Biology, Vircell are various galacomannan antigen detection ELISAs.

A lateral-flow GM assay from Immy has also been launched recently, which provides result within one hour, can be read visually or with a reader and can be done as single sample testing. Performance is similar to ELISA assay.[11]

8.5. *Polymerase chain reaction (PCR)*

These methods are faster than culture identification due to direct detection in clinical samples and from cultures, have better sensitivity of around 80% and high specificity of 80%.[32]

CPA is most reliably diagnosed by positive Aspergillus antibodies, which indicate immune response against *Aspergillus* spp. If use of microscopy and culture or antigen or nucleic acid amplification (PCR) detects Aspergillus in the airways, it indicate the presence of only *Aspergillus* spp. but not necessarily infection.[11] (Table 2).

9. Diagnostic criteria

CPA diagnosis requires presence of certain clinical, radiological and microbiological spectrum findings along with ruling out alternative diagnosis especially active mycobacterial disease by appropriate laboratory methods. As per published literature these criteria have evolved over the time since 2003 and are summarized in Table 3 below:[14,17,18,20–22]

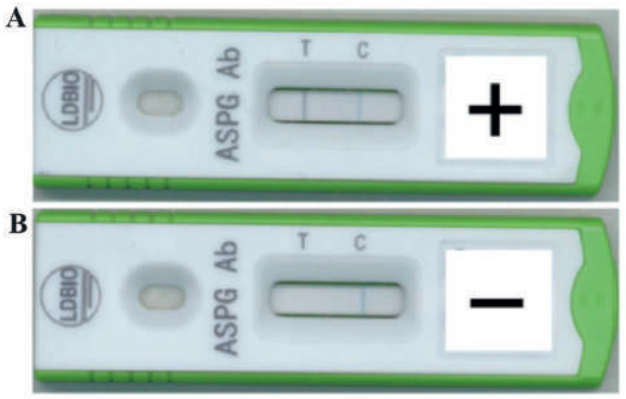

Fig. 2 – Example of positive (top) and negative (bottom) immunochromatographic (LDBIO test results.

9.1. *Medical treatment*

The main objectives are symptom relief, decreasing hemoptysis episodes, preventing pulmonary fibrosis and preserving lung function. To achieve this, three approaches are to be followed: a) control of Aspergillus spp.; b) treating the complications; c) control of underlying lung disease. Currently, there is no literature that guides CPA treatment.

9.2. *Triazole therapy*

Triazoles are the mainstay of medical treatment for CPA. As they inhibit ergosterol formation from lanosterol and also disorganize fungal cell membranes, they have both fungicidal and fungistatic effects on Aspergillus. Two randomized control trials have supported the use of triazoles.[21,36] A study by Kohno et al[21] compared intravenous (IV) voriconazole (VRCZ) at 6 mg/kg twice daily on day 1 and at 4 mg/kg twice daily on day 2 with once a day IV micafungin (MCFG) with a dose of 150–300 mg. A combination of clinical, mycological, radiological, and serological responses was used to assess the treatment effectiveness two weeks after initiation and also at the end of the treatment. Two weeks after MCFG and VRCZ treatment initiation, there were no significant differences in efficacy rates (68.0% vs 58.7%). Another study compared oral azole therapy with no antifungal treatment and found that patients with CCPA significantly responded better to itraconazole (76.5%) than supportive care (35.7%) ($p = 0.02$).[36] A parenteral Voriconazole dose of 6 mg/kg IV every 12 h for one day, followed by 4 mg/kg IV every 12 h is also recommended by the Infectious Disease Society of America (IDSA) (2016) in patients with invasive aspergillosis.[37] Patients who have encountered adverse medication responses or have developed resistance to both itraconazole and voriconazole should be treated with posaconazole.[38] No study has been conducted till now comparing these two oral triazole drugs head-to-head. An extended spectrum triazole with good oral bioavailability

Table 2 – Diagnostic sensitivity of rapid tests for aspergillosis (all figures are rounded for ease of comprehension).

Test	Turnaround time (Hours)	Acute invasive (%)	Subacute invasive (%)	Chronic cavitary (%)	Aspergillus nodule (%)	Allergic (%)	Aspergillus bronchitis (%)
Culture on respiratory samples	48–96	<30[a]	<30[a]	<30	<10	<30	80[b]
Microscopy on respiratory samples	<6	<10	<10	<10	<5	<25[b,c]	10–25
Antigen on BAL	2-48[d]	70-95[b]	70-90[b]	75[b]	ND	ND	ND
Antigen on serum	2-48[d]	20-80[b,e]	20–30	10–65	ND	ND	ND
IgG antibody	48–96	<25	30–60	80-90[b]	65	80-90[b]	60-80[b]
IgE antibody	48–96	ND	ND	60–70	ND	90-100[b]	ND
Beta d glucan on serum	2–48[f]	50–75	ND	20–75	ND	<5	75[b]
Aspergillus PCR on respiratory samples	24–72	75-90[b]	75-90[b]	60	ND	60	ND
Aspergillus PCR on serum/blood	24–72	60-80[b,e]	ND	<20	ND	ND	ND

Reference: Denning DW. Diagnosing IJTLD 21.
BAL- Bronchoalveloar lavage, ND-no data, Ig-immunoglobulin, PCR-polymerase chain reaction, ELISA-enzyme-linked immunosorbent assay, ABPA-allergic bronchopulmonary aspergillosis, COPD chronic obstructive pulmonary disease.
[a] COPD patients show higher yield with invasive aspergillosis-not carefully studied;
[b] Most recommended test to request in these clinical circumstances;
[c] ABPA causes sputum plugs that usually show fungal hyphae, eosinophils and charcotlayden crystals;
[d] Lateral flow antigen tests are less time taking. Single sample Aspergillus ELSIA is <2 hours, other ELISA assays are batched;
[e] Profoundly neutropenic patients without mould active prophylaxis show highest sensitivity;
[f] Single sample assays are quick otherwise batch tested.

and minimal side effects, Isavuconazole is a newly discovered triazole with a broad spectrum.[15]

Oral triazole therapy when given on outpatient basis provides therapeutic benefit in patients of CPA with symptoms, particularly in decreasing the chances of life-threatening hemoptysis.[39] Long-term treatment with itraconazole may be helpful in improving the overall general condition but with minimal relief in breathlessness.[18]

9.3. *Duration and dosage of oral antifungal therapy for CPA*

CCPA usually responds slowly, and it is recommended to continue the therapy for a minimum of six months and response to be assessed at the end of 4th month of therapy. The outcome is considered as a failure when there's no response. In few cases if the response is slow, then the treatment can be extended to nine months.[38] In studies, indefinite suppression therapy is shown to improve outcomes in those who respond. It is important to take into account respiratory disability, the effects of medications, ongoing other medications with potential drug interactions, and cost as necessary, depending on the case-to-case circumstances.[36] The recommended dosage is 200 mg of itraconazole or 150–200 mg of voriconazole twice daily.

10. Other routes of therapy

10.1. *Parenteral therapy*

Amphotericin B or echinocandins are other antifungal drugs which are given by intravenous route. In patients with progressive disease with triazole resistance or intolerance, these drugs are indicated and is also used in invasive pulmonary aspergillosis. Liposomal Amphotericin B is reasonably safe, as it alter the permeability of fungal cell membranes that leads to the death of the fungal cells. According to a study, liposomal amphotericin B after prior azoles was clinically effective in 65% of cases with a mean dose of 3 mg/kg for 17 days.[40] However, there are a few limitations to the use of amphotericin B in elderly and diabetic patients because of its high cost and the likelihood of developing acute kidney injury in 33% of these patients.[17,40]

Echinocandins, micafungin and caspofungin are the other alternative drugs that can be used. They kill Aspergillus cells by disrupting their cell wall. As with liposomal amphotericin B, the indications are similar. As per few studies they are non-inferior to triazoles when given for duration of 3–4 weeks. Patients with invasive aspergillosis or sarcoidosis complicated by CPA benefit most from these treatments.[21,41,42]

10.2. *Intracavitory instillation*

There are relatively few studies showing use of intracavitary amphotericin B alone as a paste or solution, miconazoles, itraconazoles, sodium iodide, or nystatin with amphotericin B paste in patients who cannot undergo surgery.[15,43] As per literature success rate varies from 70% to 100%.[43] As far as treatment options go, amphotericin B (50 mg in 20 mL of 5% dextrose solution) is still the preferred drug. There are many complications reported, including cough, chest pain, pneumothorax, and endobronchial reflux. Using a needle or catheter placed into the cavity with aspergillomas, antifungal agents can be instilled through bronchoscopic guidance via endobronchial catheters, or through a transthoracic route.[43]

Table 3 – Summary of various diagnostic criteria for CPA as per past published literature.

	Denning et al[18] 2003	Kohno et al[21] 2010	Cadranel et al[22] 2012	Jhun et al[20] 2013	Denning et al[14] 2015	Denning et al[17] 2019
Clinical signs & symptoms	More than one of the following symptoms for three months: Weight loss, productive cough, hemoptysis in absence of immunosuppression	More than one of the following symptoms with no definitive symptom duration: fever, weight loss, sputum production, cough, hemoptysis, fatigue, shortness of breath	All of the following symptoms should be present for one to six months: fever, cough, sputum production, weight loss	More than one of the following symptoms for three months: weight loss, productive cough, hemoptysis in absence of immunosuppression	Significant pulmonary and/or systemic symptoms for three months or more with no specific symptoms listed	Hemoptysis and/or persistent cough, and/or weight loss for more than three months; other symptoms likefatigue, chest pain, dyspnea and sputum production may be present but not required,
Chest imaging	More than one of the following: cavitary lesion with paracavitary fibrosis, new or expanding cavity on serial chest radiology	More than one of the following: new infiltrates, cavity formation, expansion of preexisting cavities; with or without the following: peri cavitary infiltrates, adjacent pleural thickening	Cavitary pulmonary lesion with evidence of peri cavitary infiltrates and adjacent pleural thickening with/without fungal ball	More than one of the following: cavitary lesion with paracavitary fibrosis, new or expanding cavity on chest radiology	Both required: more than one pulmonary cavity with either thick or thin wall, possibly containing aspergilloma; evidence of radiologic progression over more than three months required in the form of new cavities, increasing peri cavitary infiltrates, or increasing fibrosis	Progressive cavitation on chest imaging and/or intracavitary fungal ball and/or pleural thickening or peri cavitary fibrosis or infiltrates all adjacent to cavities
Microbiological criteria	Any of the following: positive precipitins, or isolation of Aspergillus in culture from pulmonary or pleural cavity	More than one of the following: platelia serum galactomannan index >1.0, positive precipitins, positive (1,3)-β-D-glucan, evidence of Aspergillus spp. by molecular diagnosis, culture or pathological findings	Essential criteria: Culture from sputum or BAL positive for Aspergillus,	Any of the following: elevated Aspergillus-specific IgG, isolation of Aspergillus in culture from pulmonary or pleural cavity Either raised *Aspergillus*-specific IgG or culture from pulmonary or pleural cavity	Aspergillus-IgG/precipitins or other evidence of Aspergillus if presence of fungal ball. Fungal ball absent but multiple cavities, then any of the following: Aspergillus-specific IgG, Aspergillus precipitins, strongly positive Aspergillus antigen or DNA in respiratory fluids, percutaneous or excision biopsy showing fungal hyphae on microscopy,	Positive Aspergillus-specific IgG and/or sputum microscopy results showing hyphae consistent with Aspergillus and/or Aspergillus growth on >2 sputum or other respiratory samples
Laboratory markers	Raised levels of either: CRP, ESR, plasma viscosity	More than one of the following: raised: leukocyte count, CRP, ESR	No need	Raised levels of either: CRP, ESR	Not need	Not need
Exclusion of other causative agents	Pathogens like mycobacteria, endemic mycoses	Lack of improvement with more than three-day course of broad-spectrum antimicrobial drugs required; patients with infectious diseases other than aspergillosis excluded	Pathologies like TB, other mycoses, granulomatosis with polyangiitis, ABPA, invasive aspergillosis, simple aspergilloma to be excluded	Not required	Pathologies like TB, atypical mycobacteria, necrotizing lung cancer, pulmonary infarction, vasculitides, rheumatoid nodule, histoplasmosis/ coccidioidomycosis/ paracoccidioido-mycosis in those with relevant travel history to be excluded	Mycobacterial infection ruled out with smear, GeneXpert, and/or mycobacterial culture

Abbreviations: BAL: Bronchoalveolar Lavage, CRP: C Reactive Protein, ESR: Erythrocyte Sedimentation Rate, TB: Tuberculosis, ABPA: Allergic Broncho Pulmonary Aspergillosis.

11. Surgical treatment of CPA

In light of the relatively small number of overall respiratory patients affected by CPA, there are only a few case reviews and case series describing surgery as a treatment option.[16,44,45] Surgery is used as a treatment modality for alleviating life-threatening symptoms like recurrent hemoptysis and, as a cure in a limited number of patients. The most common surgical procedure performed in CPA patients is lobectomy. Pneumonectomy, decortication, sub-lobar resection, segmentectomy, thoracoplasty, cavernostomy, bullectomy, and pleurectomy are among the other known surgical treatments that are used.[16,44,45] Complications following major thoracic surgery include hemorrhage, secondary infection, respiratory failure, empyema, and chronic air leaks with or without formation of a bronchopleural fistula.[16,44,45] Video assisted thoracic surgery (VATS) can reduce morbidity and recovery time in patients with simple aspergilloma undergoing lobectomy. In contrast, VATS-assisted pneumonectomy increases hospital stay and post-operative pleural drainage.[45,46] At the end of one month following surgery, the mortality risk differs between hospitals depending on their experience, ranging from zero to 4.3%.[16,44,45] Increased morbidity and mortality are associated with pneumonectomy, severe hemoptysis before surgery, advanced age, and loss of weight, while a better outcome is seen in females and those with forced expiratory volumes above 75%.[44,45] A study found that patients with CPA experienced a 26% recurrence rate after surgery.[16]

12. Other alternative therapies

Table 4 summarizes the other alternative medical and surgical therapies that have been described and used in literature shown below:[47–49,46,50]

13. Follow up

Assessing the treatment response involves chest imaging, including chest x-rays and CT chest scans, along with improvement in clinical well-being. The use of chest imaging is recommended after initiation of antifungal treatment every 3–6 months.[22,38] The signs of improvement include reduction in pleural thickening, reduction in fluid inside the cavity, a smoother interior wall of the cavity, as well as a decrease in the size of the nodule or peri cavitary consolidation. Progressive disease is characterized by increasing cavity size, formation of new cavities, coalescing cavities, aspergillomas, and new consolidation around cavities.

14. TB-related chronic pulmonary aspergillosis

After the completion of antitubercular therapy, 5%–35% of patients suffer from CPA as a result of PTB.[51] Globally, 1.17 million patients with pulmonary tuberculosis (PTB) sequelae developed CPA in 2019.[4,52] There are limited data on CPA prevalence in post-TB sequelae in India. Approximately 170,000 incident cases of post-TB CPA were estimated by one modeling study from India.[6] This type of data is especially important in resource constrained and high burden settings where CT scans are not readily available. As a result of overlapping symptoms and radiological patterns, CPA is difficult to diagnose in patients with active or treated PTB. In post-TB individuals with a combination of positive Aspergillus serology, cavitation or pleural thickening on chest radiography demonstrated a sensitivity and specificity of 92.3% and 98.5% respectively for CPA.[52] In our previous study, high percentage of symptomatic post TB patients attending the outpatient clinic were diagnosed to have CPA.[7] Presence of

Table 4 – Summary of alternative treatment options and surgery in CPA.

	Indication	Dose	Intention of treatment
Corticosteroids	Patients with comorbid diseases such as sarcoidosis, rheumatoid arthritis, COPD, ABPA or asthma	5–30 mg/day	Symptom control
Interferon-γ immunotherapy	CPA patients with immune deficit with low levels of IFN-γ	50–60 μg subcutaneously, three times weekly	Clinical improvement in certain group of patients
Tranexamic acid	CPA patients with hemoptysis	500 mg three times a day	Symptom control
Bronchial artery embolisation	CPA patients with recurrent life-threateninghemoptysis	–	Symptom control
Lobectomy or any other segmental resection/VATS	Single/simple aspergilloma	–	Cure and prevention of life-threatening haemoptysis
Lobectomy followed by Pneumectomy or Thoracoplasty with simultaneous cavernostomy and muscle transposition flap	Extensive CCPA disease refractory to medical Management	–	Improved control of disease, possibly cure

Abbreviations: CPA- Chronic pulmonary aspergillosis, CCPA: Chronic cavitary pulmonary aspergillosis, ABPA: Allergic bronchopulmonary aspergillosis, IFN-γ: interferon gamma, COPD: Chronic Obstructive Pulmonary Disease.

cavity on chest x-ray and multiple number of ATT courses taken were found to be independent significant risk factors for CPA.[7] In view of this finding of high occurrence of CPA at the authors' institute, it becomes very important to investigate treated TB patients with new onset symptoms or radiological worsening for presence of CPA. Clinical prediction scores need to be developed that can be used to differentiate TB disease, post-TB sequalae, and co-infection of TB and CPA especially in settings with limited resources.

15. Conclusion

Globally prevalence of CPA is estimated at almost three million cases. There is an increased risk of CPA in patients with PTB sequalae, NTM, sarcoidosis, and ABPA due to their underlying lung injury. The disease CPA is frequently mis-diagnosed as relapse of PTB or smear negative TB due to similar signs and symptoms and geographical distribution. Consequently, symptoms persist, and death may result from inappropriate treatment. Timely and accurate diagnosis of CPA using latest modalities is therefore imperative to initiate treatment with anti-fungal and or surgery, which helps in reducing economic burden of disease.

Conflicts of interest

The authors have none to declare.

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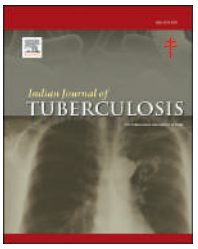

Review article

Issues with the current drugs for *Mycobacterium tuberculosis* cure and potential of cell envelope proteins for new drug discovery

Pratik S. Mahajan [a], *Payal Girigosavi* [a], *Vijay Chauware* [a], *Nitin D. Mokashi* [b], *Vijay Nema* [a,*]

[a] *Division of Molecular Biology, ICMR-National AIDS Research Institute, Pune, 411026, India*
[b] *Postgraduate Institute, Yashwantrao Chavan Memorial Hospital, Pune, 411018, India*

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ABSTRACT

Mycobacterium tuberculosis has been the smartest pathogen ever and a challenge to drug development. Its replicative machinery is unique, so targeting the same for killing the pathogen remains a challenge. Our body typically throws out the drugs before they see the bacterium multiply. The pathogen has also learned how to remove drugs from its internal chambers and not allow them to reach their targets. Another strategy for Mtb is the mutation of the targets to reject drug binding and bypass the drug's inhibitory actions. In this review, we tried to explore possible targets on the outer side of the bacterial cell. We have also explored if those targets are promising enough and if there are drugs or inhibitors available. We also discuss the essential proteins and why they remain to be a good target. We concluded that the cell envelope has got a few proteins that can be targeted in isolation or maybe along with other machinery while making the outer environment more conducive for penetration of current drugs or newly proposed drugs.

1. Introduction

Tuberculosis (TB) is caused by a highly contagious bacterium called *Mycobacterium tuberculosis* (Mtb), which resides inside the phagosomes of alveolar macrophages and inhibits the phagosomes and lysosome fusion. It is believed that Mtb has been present in nature for the last 15,000 years. Tuberculosis remains one of the world's deadliest infections and in co-infection with HIV, TB cure is a very challenging task. Tuberculosis's two-thirds burden is on only eight countries, namely India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.[1] One of the most challenging tasks in TB treatment is to cure multidrug-resistant (MDR) Mtb. In 2019–20, 465,000 new MDR-TB cases were reported worldwide with resistance to Rifampicin and Isoniazid. The number of people suffering from MDR-TB in the last few years increased, and current therapy lacks promising drugs to cure MDR-TB.[2] MDR-TB is one of the most critical threats to human life, and it can bypass the current treatment

* *Corresponding author*. Division of Molecular Biology, National AIDS Research Institute, 73 G MIDC Bhosari, Pune, 411026, India. Tel.: +91 20-27331200
E-mail address: vnema@nariindia.org (V. Nema).
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by adding new systems to resist and survive into the presence of an anti-tuberculosis drug. As revealed by the molecular epidemiology approach the rate of dissemination in Mtb is quicker than in other pathogens; therefore, it creates a challenge for control and treatment strategies.[2] TB treatment requires six months to cure and prevent the re-development of infections and disease symptoms. This treatment strategy is effective but time-consuming, needs adherence and the use of a high concentration of combination drugs. The therapy induces various toxicities and improper use or discontinuation of these drugs due to toxicity leads to drug resistance in the Mtb.[3]

Development of resistance, long-term treatment and toxicity issues emphasises the need to explore more and better molecules for faster and more effective treatment to reduce the MDR and XDR load on tuberculosis management. Globally only the Bacillus Calmette Guerin (BCG) vaccine is in use since 1992 for the prevention of TB, but known data suggest that the BCG vaccine does not effectively prevent the development of severe and fatal forms of TB in young children and is not effectively able to resist the development of infectious pulmonary cases in adults. Hence, effective and potent TB vaccine development is necessary.[3] However, the vaccine development process is time-consuming (At least 20 or more years of work to bring a reliable vaccine to market). There are a few vaccines in the developmental pipeline but have not shown promise as of now. If we consider this time interval of the vaccine development, each year, 1.4 million people will die from the disease; in this interval, 28 million lives will be lost by TB.[1] This scenario also goes in favor of new drug development and various efforts are ongoing.

As the existing targets of the drugs in use have seen mutations in various strains of Mtb, there is always a chance for the development of drug resistance during therapy. The alternate target site and potential drug for that target can aid in the cure of the Mtb infection. The abundance of lipids, glycolipids and polysaccharides make the cell envelope waxy and thick. This barrier becomes a difficult hurdle for drugs to cross and reach their targets inside the cellular matrix of Mtb to stop its functions in order to kill it. Once Mtb enters the host macrophages, it can stay there for an indefinite time in a dormant or persistent state with a potential to reactivate as active tuberculosis (TB) when conditions become favorable. The favorable conditions for pathogen to get reactivated may be described in many ways like immunocompromising conditions due to the elderly, HIV infection, suppressive medical treatments in case of an organ transplant, chemotherapy and long-term use of steroids. Malnutrition and illness or injury in the digestive system, systemic diseases such as diabetes and many other reasons are also responsible for the reactivation of latent TB into a disease condition.[4] For the development of a new drug, the Mtb cell envelope is an ideal candidate because it provides the potential target sites that are essential for the growth and development of Mtb.[3] The unique cell envelope of Mtb provides it extra stability to survive under drug pressure and resist the adverse conditions in the host.[5] On the cell envelope number of potential targets are present like Protein kinases B (PknB), Mycobacterial Membrane Protein Large 3 (MmpL3), Fibronectin Binding Proteins C (FbpC), CpsA1 (Rv3267), CpsA2 (Rv3484), EmbC and others. In this review, we discuss challenges and issues with current drugs, the nature of cell envelope, potential target present on a cell envelope and the currently available cell wall targeting drugs for the treatment of TB.

2. Challenges and issues with current drug therapy for M.tb treatment

To treat *Mycobacterial* infection, several drugs are reported, but only a limited number of drugs effectively cure the Mtb infection. Various factors affect drug efficiency and stability. In this section, we discuss the issues and challenges of current drugs like resistance, toxicity, and longtime treatment of Mtb drug.

2.1. *Resistances*

In 2019–20, almost 10 million new cases were reported worldwide, and almost 465,000 new cases of resistance against at least the first line of the drug were reported.[1] Various factors are responsible for the emergence of drug resistance in Mtb under drug pressure. After the drug exposure, Mtb increases the number of the transporter on the cell envelope, which is responsible for the efflux of the drug and lowering the intracellular concentration of a drug. At the transcriptional level, WhiB transcription factors and sigma factors work as stress regulators and upregulate the drug efflux pump synthesis.[6] It is also observed that at the post-transcriptional level toxin-antitoxin systems neutralize the toxin and small regulatory RNAs actively participate in drug tolerance mechanisms by regulating various genes which are directly involved in efflux pumps, transport, metabolism, cell envelope synthesis.[7] Mtb resides inside the macrophage. Human cells also contain the ABC and SLC superfamily transporter which are also responsible for the efflux and influx of drugs. Hence human cell also reduces the intracellular concentration of drugs. Mtb adapts to some physiological and metabolic changes that help sustain in the hostile environment. After exposure to drugs, the metabolic slowdown in energy-producing pathways like the TCA cycle, ATP synthesis, and aerobic respiration is observed.[8] Under drug pressure, metabolic shifting is an important strategy for M.tb to achieve tolerance. In metabolic shifting upregulation of energy storage pathways like triacylglycerol synthesis takes place. An enzyme like isocitrate lyases is upregulated under RIF pressure. Mtb contains a very high lipid concentration and creates an almost impermeable cell envelope, which is responsible for providing a barrier to the drugs. A unique feature of the Mtb cell envelope is also responsible for resistance. After exposure, Mycobacterial cell envelope thickening is observed. The thickening of the cell envelope reduced the lipophilic drug uptake and almost blocks the cell envelope for hydrophilic drugs. In normal conditions, hydrophobic molecules enter through the porins available on the envelope but under drug pressure thickening of the envelope takes place restricting the porins to allow hydrophobic molecule transportation.[9] Experimental data suggest that upregulation of mycobacterial porin gene *mspA* increases drug susceptibility of Mtb against the INH, EMB, and streptomycin. It is also observed that Mtb dysregulates the host immune system and

sometimes mutation in the drug target site, which changes the binding affinity of the active drug site or resists the drug to bind. Ethambutol is the first-line drug against Mtb but resistance cases are also in very high numbers; it is generally seen that mutation at 306 positions in the *embB* gene is responsible for ethambutol resistance, this mutation replaces Methionine with isoleucine or valine.[10] Drug resistance is acquired in Mtb by various mechanisms like adaptability to a drug or phenotypic drug tolerance, in some cases, slow growth of Mtb is also responsible for drug resistance. Various gene mutations are responsible for resistance. Mutations in *katG* and *inhA* gene are responsible for Isoniazid resistance, a mutation in *pncA, rpsA,* and *panD* gene are responsible for the resistance of Pyrazinamide, a mutation in *rpoB* gene is responsible for the Rifampicin, and mutation in *gyrA, gyrB* genes are responsible for the resistance to Fluoroquinolones class drugs like Levofloxacin, Moxifloxacin and Gatifloxacin resistance.[9,10] Apart from that many other factors play role in inducing resistance against one or multiple drugs. Non-adherence is one of the major factors responsible.

2.2. *Toxicity*

Hepatotoxicity is a significant toxic effect of first-line drugs except for ethambutol. For treatment of TB, at least six months of treatment is required with the first-line drugs. The mechanism of drug toxicity and its impact on liver function is unknown and unpredictable, but the combined effect of the drugs is the main reason for liver toxicity. Liver toxicity happens due to its participation in digestion and active role in drug metabolism. It is observed that orally administrated drug generally causes liver toxicity; the drugs are covalently attached to liver proteins and induce the production of excess oxidative metabolites, which are directly responsible for the cause of permanent liver damage. General liver toxicity symptoms are abdominal pain, vomiting, nausea, and induced jaundice probability.[11] In TB treatment, chances of liver toxicity are increased in case of malnutrition, advanced age, female sex, alcoholism, and any other per-existing liver disorder. Most Anti-TB drugs show effects like rash, neurological syndrome, and visual disturbances; mostly seen with ethambutol treatment. After administration, isoniazid goes through various metabolic processes like acetylation, hydrolysis, conjugation, and oxidation and converts it into hydrazine. Hydrazine is mainly responsible for causing neurological disorders.[12] Pyrazinamide is converted into 5-hydroxypyrazinoic acid by xanthine oxidase, actively participating in toxicity and responsible for rash, hyperuricemia, and arthralgia. Another drug is Rifampicin which is deacetylated by microsomal enzymes. Rifampicin induced coloration of body fluids, shortness of breath, rash, arthralgia nephrotoxicity; many other side effects are observed due to the anti-Mtb drug.

2.3. *Slow growth challenge*

Mtb is slow-growing pathogenic bacteria and most of the time, it is in a dormant condition and its doubling time is 16–22 hours. For TB treatment, some drugs target the enzyme directly involved in the replication, but due to their slow growth rate and non-replication state, the drug does not effectively inhibit the replication enzyme, and the Mtb survives under the bactericidal environment.[13] The half-life of the drug and replication cycle time is directly correlated with the treatment of Mtb. For the treatment of Mtb for a long time, the drug must be available in blood with their LC_{50} or should work on other essential elements of the pathogen system which are not dependent on replicative machinery.

3. The cell envelope of Mtb

Mycobacterium tuberculosis has a unique cell envelope that contains a variety of biomolecules like lipids, proteins, lipoproteins, glycolipids, and polysaccharides. They provide the architectural complexity to the Mtb and this complexity distinguishes the mycobacterial genus from all other microbes. These cell envelope components play an essential role in the interaction and virulence of Mtb. In Mtb cell envelope mainly three components viz. Mycolic acid (MA), Peptidoglycan (PG), and arabinogalactan (AG) are present (Fig. 1). This complex architecture allows very low permeability and enhanced rigidity to the cell envelope. In the cell envelope, lipid represents almost 40% dry cell mass; this lipid richness in Mtb provides additional character to grow in clumps and acid-fastness. Mycobacterial cell envelope mainly consists of the plasma membrane, cell envelope core, and outer membrane (Capsule). Alongside, the cell envelope also contains periplasmic space, which helps in the metabolic process, and it is located between the plasma membrane and cell envelope core. Cell envelope thickness range between 30 and 40 nm.[14] The cell envelope is directly involved in the influx of essential nutrients for growth and development. It's also responsible for the efflux of toxic metabolic molecules. In the attachment of Mtb with lung cells, the cell envelope plays a very critical role. Fibronectin Binding Proteins are available on the Mtb surface which directly participates in the attachment process. In the cell envelope, various metabolic processes take place providing a number of the potential targets which are directly or indirectly involved in the metabolic regulation of the cell envelope.[15]

3.1. *The plasma membrane*

The plasma membrane (PM) is composed of the lipid bilayer, the innermost membrane of the Mtb cell that separates the inner cytosol from the rigid arabinogalactan and peptidoglycan layer (Fig. 1). It provides a barrier to all anabolic and catabolic processes necessary for cell activity. PM is composed of the lipid bilayer. Generally, all other microbial cell membranes consist of glycerol-based phospholipids, but in the Mtb specifically, phosphatidylethanolamines and phosphatidylinositol mannosides type of lipids are present, also tuberculostearic acid and palmitic acid are present in the considerable quantity.[16] Here also, some glycol-conjugates like lipomannan and lipoarabinomannan are attached to the cell membrane and extended up-to-the-cell wall core, which plays the leading role in the virulence of TB. In PM, various poly terpene-based products like menaquinones, leprotene, and carotenoids are present and protect from photolytic damage. The outer leaflet of the plasma membrane is attached with arabinogalactan and

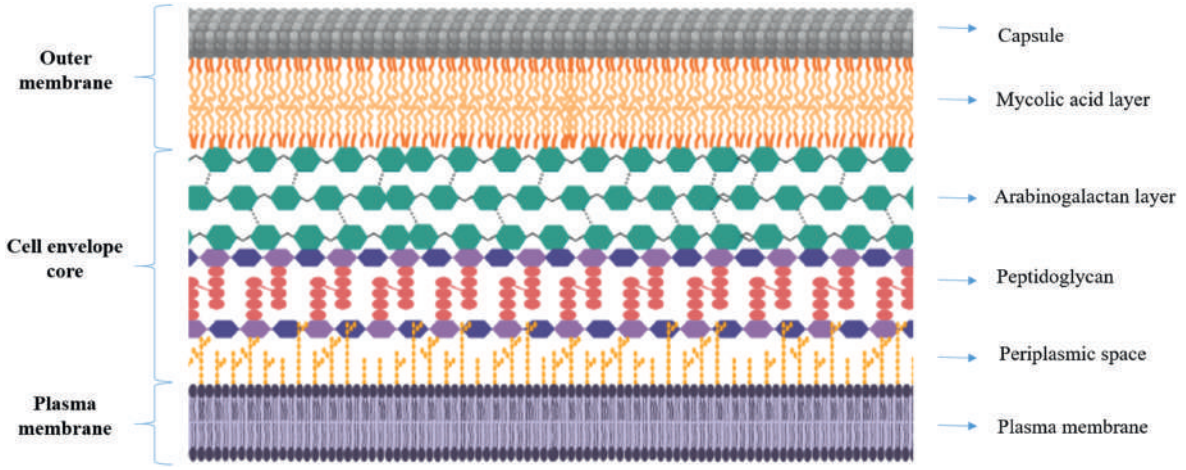

Fig. 1 – Schematic diagram of Cell envelope of *Mycobacterium tuberculosis* with three compartments and layers.

peptidoglycan complex. In the membrane, polar lipid, phospholipid, and protein are assembled.[17]

3.2. *Cell envelope core*

The cell envelope core plays a significant and essential role in the shape and osmotic stability of Mtb. In the cell wall core, two primary types of heteropolysaccharides are present, namely, arabinogalactan (AG) and peptidoglycan (PG), which are non-covalently bound with periplasm and covalently bonded with each other via phosphoryl-N-acetylglucosaminosyl-rhamnosyl (Fig. 1). In core member, PG is highly cross-linked with peptide and provides extra rigidity to the cell wall of Mtb other than any organism.[18] PG synthesis plays a very critical role in the Mtb growth and stability however, the PG synthesis pathway of Mtb remains unclear.[18] The main component in PG is N-acetylglucosamine β (1 → 4) N-glycolylmuramic acid disaccharide and peptide. The presence of AG in the cell wall core is a unique feature of mycobacteria.[19] The cell envelope core creates an almost impermeable covering on the cell cytosolic chamber and provides additional support in reducing the drug diffusion. Hence targeting the cell envelope core will be a good strategy for the new drug discovery.

3.3. *Outer membrane*

In the outer membrane of the Mycobacterium, Mycolic acid (MA) is a significant constituent, and it forms a thick lipophilic membrane. The presence of MA in the outer membrane is a particular type of character observed in mycobacteria (Fig. 1). MA is essential for the survival of the Mycobacterium genus and has a significant role in fluidity regulation. Mycolic acid is present in free form and esterified with the AG layer. The outer membrane of Mtb is covered by an outermost and a loosely bound compartment called the capsule. It comprises polysaccharides and peptides and provides additional resistance in the presence of bactericidal substances or antibiotics. In the capsule, α-glucan is the main component and in small quantities, mannan, arabinomannan, lipid and proteins are present. In capsule, the lipid concentration varies about 2–3%, and only phenolic and phthiocerol dimycocerosates type of glycolipids are present.[20] In the outer membrane of Mtb, generally α-Mycolic acid, Keto-Mycolic acids, and Methoxy-Mycolic acid are present. In the inner leaflet, mycolic acid is covalently attached to AG while in the outer leaflet, it is connected with the capsule. Due to the thickness and lipid richness in the cell envelope, it is impermeable to small polar molecules. This is compensated by the presence of specialized proteins called porins which are responsible for the transport of nutrition.[21]

4. Potential target present on cell envelope of mycobacterium

The proteins present on the cell envelope of Mtb play various roles including transportation of metabolites and drug moieties. They also provide linkages between multiple layers of the biomolecules and provide the cell with structural stability. Some of these proteins are essential for their cellular functions related to energy metabolism and the exchange of nutrients necessary for cell survival. These essential targets are discussed below to describe how they become important for drug discovery and if there are toxicity issues due to similarities with host proteins.

4.1. *Kinases*

The Mycobacterium genome sequence suggested that there are 11 types of Serine/threonine-protein kinases (STPK) present. Protein kinases (Pkn) A to L are all transmembrane proteins except PknG and PknK. However, in all kinases, only PknA and PknB are known to participate actively in cellular processes like cell wall synthesis, cell metabolism, cell division, and control of cell shape. PknB is a receptor-like STPK encoded by the *pknB* gene. It is not only conserved but also essential for the growth, signaling, and survival of Mtb. It is reported that PknB senses the environmental changes and passes the signal to the inner compartments of the cell; it also plays a vital role as a replication switch for hypoxic conditions and also in reactivation from the hypoxia condition.[22] It is also observed that the knockout of the PknB gene inhibits the growth of Mtb, and

overexpression of PknB downregulates growth and notable changes in the cell morphology. PknB contains five domains; four are on the extracellular side, and a single transmembrane helix connects one catalytic domain on the intracellular side. On the extracellular side, it contains four penicillin-binding proteins and serine/threonine kinase-associated domains (PASTA) with the ligand-binding site on the fourth domain (Fig. 2a).[23] In the Intracellular domain site, N-terminal contains ATP binding site and the C-terminal lobe helps in the substrate stabilization. For the auto-phosphorylation and kinase activity, front-to-front or back-to-back dimerization of PknB is essential. PASTA domains are attached with PG and provide additional strength to cell envelope stability; it is reported that all PASTA domains are essential for the growth of Mtb. In extracellular PASTA domains, dimerization of the third and fourth domains is necessary to activate the catalytic domain.[24] PknB contains a total of 627 amino acids; in the catalytic domain, 279 residues and extracellular site 273 residues are present. N-terminal Leu17, Gly18, Val25, Ala38, Met92, Glu93, Try94, Val95, and C -terminal Met145 and Met155 of PknB catalytic domain create an active groove, which is essential for the kinase activity. In PASTA 4 domain Trp571, Lys589, Asn601, and Phe624 are essential residues for the ligand binding and dimerization of PASTA domains. Many STPK genes are also present in the human genome. However, the exciting thing is that Mtb STPK genes show only 30% identity with the human STPK genes family members. For PknB inhibition, several drugs are reported. The IMB-YH-8 is a synthetic drug, Demethylcala-Baxanthone, Cryptolepine hydrochloride, dictamine, scoparone, Ermanin and NuBBE964 are the phytomolecules extracted from various plants.[25] VI 16212, VI 16641, VI-15662 are chemically modified compounds. But all these drug shows inhibition at a high concentration and not a single drug could pass through the drug development pipeline. Hence, the PknB qualifies all criteria for being a significant target site for inhibition of Mtb growth and demands more work for its inhibition.

4.2. *Transporters*

In Mtb Mycobacterial Membrane Protein Large (MmpL) family proteins function as transporters and they actively participate in the export of lipid to the outside of the PM (Fig. 2b). In Mtb various MmpL proteins are present. Mtb genome encodes 13 MmpL proteins but only MmpL3 is reported to be essential for survival. At the same time, mycolic acid plays a crucial role in the growth and survival of Mtb, being a necessary component for building the outer membrane of Mtb.[26] Mycolic acid biosynthesis starts in the cytoplasm via FAS II and I pathway, and finally, it is translocated into the periplasmic membrane of Mtb with the help of MmpL3 in the form of trehalose monomycolates. MmpL3 is a plasma membrane-based mycolic acid transporter and belongs to Resistance, Nodulation, and Division (RND) protein superfamily. Only a few members of Actinobacteria viz., Mycobacterium and Actinomycetes use the MmpL family transporter to translocate product to the periplasmic or outer membrane. MmpL3 is a monomeric protein, and it consists of 12 transmembrane helices and two periplasmic subdomains PD1 and PD2 which form the periplasmic pore. In MmpL3, a ligand-binding site is present between the center of transmembrane helix numbers IV, V, VI, X, XI, and XII.[27] Helix numbers IV and X form hydrogen bonds between Asp256-Tyr646 and Asp645-Tyr257; the Asp–Tyr hydrogen bond is a conserved feature of MmpL family transporters (Fig. 2b). It is reported that knockdown of MmpL3 led to an accumulation of trehalose dimycolate precursor in the cytoplasm, inhibition in cell division and death of Mtb.[28] Hence, proving that MmpL3 is essential for Mtb growth, cell replication, and regulation. MmpL3 deletion affects the osmoprotection and homeostasis of Mtb and induces the up-regulation of 47 genes and downregulation of 23 genes which are directly related to the energy, mycolic acid and essential to biomolecules production. Due to the importance of MmpL3 in Mtb growth and stability, it is a promising target for anti-TB drugs.

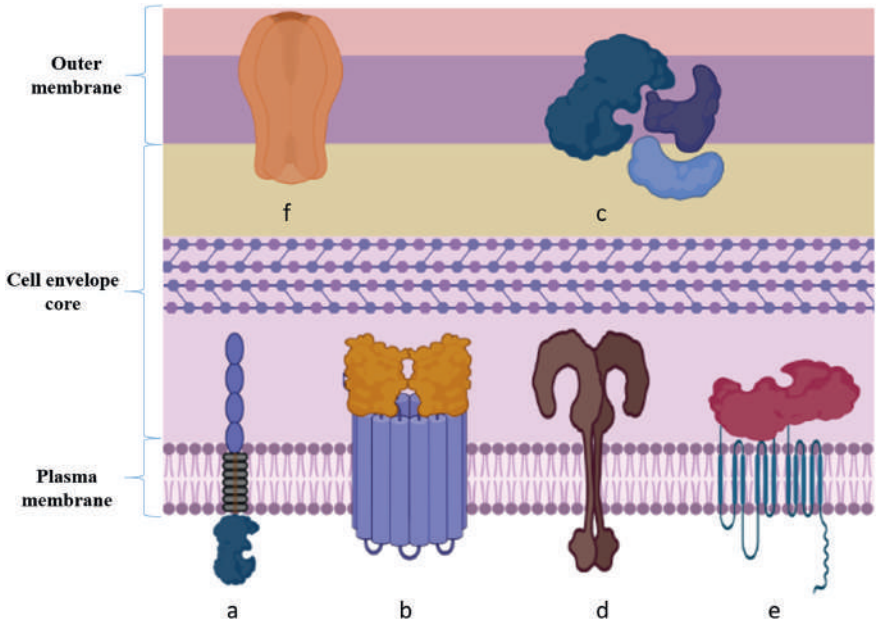

Fig. 2 – Cell envelope of *Mycobacterium tuberculosis* with potential drug targets a) PknB, b) MmpL3, c) FbpA, B and C d) CpsA1 and CpsA2 e) EmbC f) Porin.

4.3. *Fibronectin Binding Proteins (Fbp)*

In Mtb A, B, and C types of Fbp are found. Fbps are highly antigenic and help in the establishment of disease through their binding capacity. Fbp A, B, and C are present in a complex known as antigen 85 (Ag85). FbpA, B, and C each protein act as mycolyltransferase and are involved in the final transfer of α,α'-trehalose monomycolate to mycolic acid. Fbp complex maintains Mtb cell envelope integrity.[29] Fbp is located in the MA and AG layer of the Mtb cell envelope and fibronectin-binding specificity proves their involvement in receptor-mediated phagocytosis of Mtb (Fig. 2c). Fbp A, B, and C encode by *fbpA, fbpB*, and *fbpC* genes, respectively.[29] It is reported that downregulation of FbpC decreases the mycolic acid content by up to 40% and alters the Mtb cell envelope permeability. Downregulation of FbpA and FbpB has no significant effect on the cell envelope of Mtb, but FbpC is indicated to be directly involved in the cell permeability; hence it becomes a potential and essential targets candidate for the discovery of new anti-mycobacterial drugs.

4.4. *Ligase*

For the survival of Mtb, the cell envelope plays a unique role. Two major heteropolysaccharides AG and PG are present in the cell envelope core. AG and PG are covalently attached, and for their attachment, CpsA1 (Rv3267) and CpsA2 (Rv3484) play an essential role as a ligase. CpsA1 and CpsA2 belong to the LytR-CpsA-Psr (LCP) family. These enzymes play an important role in glycolpolymer transfer reaction in various microbes.[30] The *cpsA1* and *cpsA2* genes encode CpsA1 and CpsA2. Knockout experimental data for *cpsA1* and *cpsA2* indicated their essentiality. Individual knockout downregulates the growth and combined knockout is responsible for the death of Mtb. In most microbes, *cpsA1* downregulation is insufficient for the dramatic changes in the morphology and composition of cell envelopes. These proteins are transmembrane enzymes and contain one transmembrane helix, cytoplasmic N terminal tail, and C terminal extracellular domain for ligation (Fig. 2d). Mtb resides in macrophages that have an antimicrobial capacity, but lysosomal degradation success depends on the phagosome lysosome fusion. M.tb inhibits phagosomal trafficking to avoid lysosomal degradation. For decades, the mechanism that impairs phagosomal trafficking has been unclear, but some research groups have identified that CpsA2 active participation impairs phagosomal trafficking. The macrophage LC3-associated phagocytosis (LAP) pathway is actively involved in the phagolysosomal fusion for lysosomal degradation of microbes. In the LAP pathway, LC3 interacts with the microbe-containing phagosome. In trafficking, Macrophage pathogen recognition receptors activate the LAP-associated degradation in the presence of microbes containing phagosome, for phagolysosomal fusion NADPH oxidase and RUBCN/RUBICON is essential. CpsA2 interferes with the recruitment of CYBB/NOX2 on microbe containing phagosome. LAP is the canonical pathway for macro-autophagy; it requires autophagy-related same proteins. The autophagy of microbes containing cells is called xenophagy. In LAP, LC3 is attached to a single-membrane phagosome; in LAP activation toll-like receptor-2 and C-type lectin receptor signaling play a critical role, which recruits NADPH oxidase on the phagosome. NADPH oxidase promotes the production of reactive oxygen species, which are directly involved in LAP activity. NADPH oxidase is stabilized in the presence of RUBCN, and this xenophagy is dependent on the presence of RUBCN and the stabilization of NADPH oxidase.[31] M.tb effectively controls this lysosomal trafficking pathway. To check the clinical importance of the CpsA2 enzyme in M.tb more studies about the active site and critical residues are necessary. More information about gene essentiality, conservation pattern, identification of potential inhibitors, and creating new drug discovery opportunities is required.

4.5. *Transferases*

In Mtb cell envelope Mycolic acid-arabinogalactan-peptidoglycan complex is essential for survival. In this complex formation, arabinofuranosyltransferases A, B, and C (EmbA, B, and C) play an important role and this enzyme is encoded by *embA, embB, embC* genes receptively. This protein belongs to the glycosyltransferase superfamily, this type of protein is involved in the Glycosyltransferases or Oligosaccharyltransferases and polymerization of sugar.[32] This is a transmembrane protein that contains 13 transmembrane helixes and one c terminal extracellular domain (Fig. 2e). Data suggest that knockout of *embB, embC* is lethal for the Mtb, but these all have different functions EmbA and EmbB downregulation inhibits the AG synthesis but does not affect lipoarabinomannan (LAM) concentration. At the same time, the downregulation of EmbC affects the LAM concentration.[33] The catalytic site of EmbC contains a disulfide bond, Cys749-Cys993 and Trp985 are critical for enzymatic activity. The Anti-Mtb drug ethambutol inhibits the activity of the Emb enzymes. Nevertheless, as the number of drug resistance mutations increases against ethambutol, the new drug discovery is essential. Hence, EmbC remains a potential target for new and effective drugs for tackling drug resistance.[33]

5. Reported inhibitor for Mtb cell envelope components

Drug discovery efforts have identified the above-discussed targets as important and various workers are reporting the efficacy of different molecules as new drugs. Several drugs are reported, some of them are natural compounds like Ermanin, Cryptolepine hydrochloride, Demethylcala-baxanthone, dictamine, Glycyrrhizin, scoparone are extracted from *Tanacetum microphyllum, Cryptolepis sanguinolenta, Garcinia mangostana, Pilocarpus grandifloras, Glycyrrhiza glabra, Khaya senegalensis,* respectively. Some other potential compounds are synthesized by using a computational approach (Structure-based drug design) like IMB-YH-8, SQ109, BM212, I3-AG85, THPP and various others which are reported as potential inhibitors for targets present on the cell envelope.

Table 1 enlists a few of these targets with the reported inhibitors and some of their properties as given in the literature.

Table 1 – List of cell envelope targets in Mtb and their proposed inhibitors.

Sr. No	Target Name	Drug/Inhibitor	Properties	Ref
1	PknB	IMB-YH-8	It is a 220.07 Molecular weight compound produced by synthesis; it shows activity against PknA and B IC_{50} is 44.3 μM and 20.2 μM, respectively. Block the auto-phosphorylation of PknB and phosphorylation of GarA. Causes damage in the cell wall of Mtb and minimum inhibitory concentration (MIC) is 0.25 μg/ml.	34
		Demethylcala-baxanthone	It is a 378 Dalton compound and shows binding energy with PknB as −8.06 kcal/mol. It is a xanthone derivative extracted from *Garcinia mangostana*, with a MIC value is 6.25 μg/ml against Mtb.	25
		Cryptolepine hydrochloride	It is a 232 Dalton compound and shows binding energy with PknB as −7.58 kcal/mol. It is an alkaloid in nature and extracted from West African medicinal plant *Cryptolepis sanguinolenta*, and MIC is 16 μg/mL agents Mycobacterium.	25
		Ermanin	It is a 314 Dalton compound and shows binding energy with PknB as −6.90 kcal/mol. It is extracted from *Tanacetum microphyllum*, which is commonly used as Spanish traditional medicine.	25
		3-hydrazinyl-3-oxo-propanamide derivatives	All compounds are synthetic in nature and show −7.44 to −6.24 kcal/mol binding energy agents PknB. The molecular weight ranges 197 to 283 Dalton and it shows MIC range between 12.5 and 100 μg/mL against Mtb H37Rv strain.	35
		Mitoxantrone	It is bound in the adenine-binding pocket of PknB, it is a DNA reactive agent and used in cancer treatment and MIC is 400 μg/mL against Mtb	36
		MRT68606	It is a synthetic compound with IC50 against PknB as 0.519 μM and MIC against Mtb as 16.0 μM	37
		NuBBE936 (dictamine)	It is quinolone alkaloids extracted from *Pilocarpus grandifloras*. MIC is 30 μg/ml against Mtb	38
		NuBBE1180 (scoparone)	A coumarin, extracted from *Khaya senegalensis*. MIC is 388 μg/ml against Mtb	
		NuBBE1045	Flavone, extracted from *Conchocarpus heterophyllus*.	
		NuBBE105	Indole alkaloid, extracted from *Chimarrhis turbinata*.	
		NuBBE964	Quinoline alkaloids, extracted from *Balfourodendron riedelianum*	
		NuBBE598	Quinoline alkaloids, extracted from *Balfourodendron riedelianum* and *Esenbeckia Grandiflora*.	
		VI 16212	It is 364.45 Dalton, synthetic compound with IC50 is 0.82 μM and MIC against Mtb is ≥20.00 μg/mL.	39
		VI 16641	It is 404.52 Dalton, synthetic compound with IC50 of 0.258 μM and MIC against Mtb is 20.00 μg/mL.	
		VI-15662	It 396.45 Dalton compound with IC50 against PknB is 0.870 μM and shows 82.4% of inhibition at 10.00 μM	
		17494	It 497.44 Dalton compound with IC50 against PknB is 0.129 μM and shows 97.6% of inhibition at 10 μM	

2	MmpL3	SQ109	This compound is analog of ethambutol and synthesized using combinatorial chemistry; it shows IC50 at 5–15 μM and MIC in between 0.76 and 1.56 μg/ml and SQ109 is undergoing phase II clinical trials.	40
		HC2091	It is (N-[2-(4-chlorophenyl) ethyl]-4-thiophene-2-yloxane-4-carboxamide) shows EC50 at 6.4 μM and MIC at 19.3 μM. It is also capable of killing Mtb inside the Macrophage and shows minimal cytotoxicity.	41
		CCI7967	CCI7967 is 1-(4-Chlorophenyl)-2-[4- methyl-6-(2-it the action of MmpL3 and shows MIC at 1.0 μM	42
		THPP	THPP is tetrahydropyrazolo [1,5-a] pyrimidine-3-carboxamides, shows MIC of 0.3 μM against Mtb and has the potential to kill Mtb inside the macrophage with MIC80 = 0.16 μM	
		Indole-2-carboxamides	Has a potential anti-mycobacterial activity with 0.93 μM MIC value and also shows a low toxicity	43
		Benzothiazole amides derivative - 50 and 51	It shows high lipophilicity and nonspecific binding, MIC against Mtb is 4 μg/ml and 0.12, respectively.	44
		AU1235	It is a derivative of Adamantyl ureas and shows MIC 0.01 μg/ml against Mtb, and its binding mode is similar to SQ109.	45
		BM212	It is a derivative of Pyrroles and capable of killing the replicating and non-replicating Mtb with MIC 5 μM and 18.5 μM, respectively—also, potentials to kill MDR and XDR Mtb.	
		PIPD1	PIPD1 is obtained from Piperidinol and show MIC of 0.32 μM against Mtb, and it is non-toxic in nature	
3	FbpC	Ebselen	It is a small molecular Wight molecule with a thiol-reactive selenium atom, and it shows a MIC of 20.00 μg/ml against drug sensitive and resistant Mtb. It binds at conserved Cys-209 residue of FbpC.	46
		NIH415032	This compound has the potential to bind FbpA, B, and C and show the IC_{90} of 20 μg/ml (53.023 μM) against Mtb	
		2-amino-6-propyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile (I3-AG85)	I3-AG85 is a synthetic drug capable of killing the Mtb inside the macrophage and show significant inhibition against the drug-resistant/multidrug-resistant Mtb. MIC ranges between 100 and 200 μM against MDR Mtb	
		6-azido-6-deoxytrehalose (ADT)	This drug can inhibit all three Fbp proteins and show MIC of 200 μg/ml against Mtb and IC_{60} is 100 μg/ml against FbpC.	
		cyclipostins and cyclophostin derivatives (CyC)	It represents monocyclic enolphosph(on)ate compounds class type and can inhibit the Mtb inside the macrophage. CyC7β, CyC8β, and CyC17 are bind to the active side of FbpC and inhibit its activity. CyC7β show extracellular and intracellular MIC_{50} of 16.6 and 3.1 μM respectively, CyC8β show intracellular MIC50 ~ 11.7 μM and CyC17 show MIC_{50} ~ 0.5 μM against Mtb	47
		Diethyl Phosphate (DEP)	It binds in the active site if FbpC with binding energy −4.15 kcal/mol and shows stable MD simulation with less than 0.1 nm RMSF value at 100 nm.	48

(*continued on next page*)

Table 1 – (*continued*)

Sr. No	Target Name	Drug/Inhibitor	Properties	Ref
4	EmbC	Ethambutol	It is the first line drug used for Mtb treatment and it inhibits Emb A, B, and C, but the change in EmbB expression due to mutations is directly correlated with Ethambutol resistance. MIC ranges between 1.9 and 7.5 μg/ml	49
		Glycyrrhizin	It is reported bioactive compound against Throat Infection, Chickenpox, Gastric Ulcer, And Antiviral. It is extracted from the roots of *Glycyrrhiza glabra*.The in-silico study shows a promising result, C score is five and H- bonds number is 14, MD simulation was stable interaction with around 0.55 nm RMSD value.	50
		Amikacin	It shows that the Glide docking score is −10.71 (kcal/mol) and −58.93 energy is (kcal/mol). In MD simulation study show stable interaction with RMSD around ~0.25 nm and RMSF 0.139 nm.	
		Terlipressin	It shows that the Glide docking score is −11.39 (kcal/mol) and −91.34 energy is (kcal/mol). In MD simulation study show stable interaction with RMSD around ~0.2 nm and RMSF at 0.107 nm.	
5	CpsA1 and CpsA2	No drug reported as per the accessible literature	–	-

6. Discussion and conclusion

M. tuberculosis is one of the most successful pathogens in the history of medicine. The uniqueness of the cell envelope of Mtb plays an important role in the success of this pathogen. It provides a protective defense mechanism against the drugs/inhibitors. In the current regimen for treating TB, most of the drug targets are present in the intracellular compartment except ethambutol. During the treatment, the Mtb cell envelope provides additional resistance against drug penetration and allows a limited number of drugs to penetrate. In the cell envelope, the presence of AG, PG, and MA layers are the special features of the Mycobacterium genus. MA is a lipid-rich compartment of the cell envelope and only allows the passage to the hydrophobic drugs. Due to these impermeability issues, the demand for a concentration of drugs and treatment period increases. This high demand for the drugs challenges the human metabolic machinery and gives rise to the side effects like vomiting, gastrointestinal microbial flora disturbances, joint pain, liver toxicity, and neurotoxicity. Hence, for the treatment of TB, a new drug target the very contact point of the bacterium, the cell envelope would be an ideal target. This would avoid complexities for drug penetration and may help other drugs to move in smoothly if there is a compromised cell envelope presented with the help of these membrane manipulating drugs. That was a driving force for us to discuss the targets present outside the plasma membrane with essentiality for Mtb growth and development. However, other drug discovery efforts with newer targets remain significant and their importance in supporting overall drug discovery is indispensable.

Author contributions section

PM: searched the literature and contributed to writing the draft and reviewing the same. VN: conceptualized the review and contributed to writing the draft, reviewing, and final approval. PG: supported PM and VN in arranging literature, corrections in the manuscript and overall review. VC: supported PM and VN in arranging literature, corrections in the manuscript and overall review. NDM: Reviewed the manuscript and gave the final approval. All authors approved the final version of the manuscript.

Ethics approval

This is a review article and has not used personal information, human samples, or animals. Ethics approval and consent to participate are not applicable here.

Availability of data and materials

The literature and raw data used in this article are available with the corresponding author.

Conflicts of interest

The authors have none to declare.

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Original article

Prevalence of depression and anxiety in pulmonary tuberculosis patients and its association with unsuccessful treatment outcome: A prospective cohort study

Siwadon Kumpuangdee [a], *Chutima Roomruangwong* [b], *Jiratchaya Sophonphan* [c], *Pirapon J. Ohata* [c], *Gompol Suwanpimolkul* [a,d,e,f,*]

[a] *Department of Medicine, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand*
[b] *Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand*
[c] *HIV-NAT, Thai Red Cross AIDS Research Centre, Bangkok, Thailand*
[d] *Centre of Excellence in Tuberculosis, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[e] *Division of Infectious Disease, Department of Medicine, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand*
[f] *Emerging Infectious Diseases Clinical Center, Thai Red Cross Society, Bangkok, Thailand*

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ABSTRACT

Background: Pulmonary tuberculosis (TB) remains a major public health problem in Thailand. TB causes chronic disease which may cause physical disability, mental and socioeconomic problems in TB patients. Mental disorders may occur after TB infection or co-exist with the disease. This study assessed the prevalence of depression and anxiety among pulmonary TB patients and its association with treatment outcome.

Methods: This is a single-center prospective study. Pulmonary TB patients who were treated at a tertiary hospital, in both outpatient and in-patient settings, were enrolled into the study. Demographic data and Thai Hospital Anxiety and Depression Scale (HADS) score at baseline and at least 2 months after diagnosis were collected to evaluate the probability of depression and anxiety. Logistic regression model was used to analyze the data. Association between suspicious mental disorder and treatment outcome were evaluated at the end of each participant's treatment.

Results: One hundred and three participants were enrolled into the study on March 2018 to October 2019. The prevalence of probable depression and anxiety (Thai HADS score ≥11 from both test) were 7.8% and 6.8%, respectively. Unsuccessful treatment outcome rate was

* *Corresponding author*. Division of Infectious Diseases, Department of medicine, Faculty of medicine, Chulalongkorn university, 1873 Rama 4 Road, Pathumwan, Bangkok, 10330, Thailand. Tel.: +662 256 4578 9; fax: +6682 2564000 3419.
E-mail address: sgompol@gmail.com (G. Suwanpimolkul).
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10.7% (11/103). From the multivariate analysis, people previously treated/relapsed (aOR (95%CI): 7.04 (1.19–41.85), p = 0.03) and probable depression/anxiety with Thai HADS score ≥11 (10.12 (1.54–66.45), p = 0.02) were associated with unsuccessful treatment outcome.
Conclusions: In this study, Thai HADS score could identify probable depression and anxiety among pulmonary TB patients, and its association with unfavorable treatment outcome. Clinicians should keep in mind that pulmonary TB can affect the mental status of the patients and therefore, should evaluate them and provide appropriate treatment.

1. Introduction

Pulmonary tuberculosis (TB) is an important national and global public health problem. According to the World Health Organization (WHO) report in 2019, TB is still the number 1 out of 10 leading causes of death worldwide and the Southeast Asia region has the highest reported cases.[1] In Thailand, there are 153 cases of TB per 10,000 population with a total of 86,949 cases reported in 2018. Although treatment success rate was 84% but Thailand is still ranked in the top 30 countries in the world to have high prevalence of TB.[1]

In order to have a successful treatment of TB, the duration and continuity of treatment are important. The duration of standard treatment regimens ranges from 6 to 9 months. It is crucial for the patients to receive complete course of medication to have a good treatment outcome and avoid treatment failure or emergence of drug-resistant strain.[2]

Pulmonary TB is a chronic infectious disease which may cause disability and affect the mental status, and socioeconomic status of infected people. It has been reported that patients with chronic physical illness are more likely to have emotional disorders,[3,4] especially depression and anxiety.[5,6] Depression itself can affect the patients by increasing the feeling of pain and fatigue which isolated the patients from their family and society, resulting in poor adherence to treatment and unsuccessful treatment outcome.[5,6] A previous multicenter study of 1502 TB patients found that psychological distress or K-10 score (Kessler Psychological Distress scale −10) of more than 30 increased the risk of unsuccessful treatment outcome by 2.29 times.[7] Another study found that TB patients with depression were 3.54 times at risk for unsuccessful treatment.[8]

The main objective of this study was to find the prevalence of probable depression and anxiety in pulmonary TB patients and identify risk factors associated with this mental disorder. In addition, the secondary objective evaluated the association between depression and anxiety with unsuccessful treatment outcome in the Thai population.

2. Material and methods

2.1. Design and study population

This was a single center prospective study that was conducted at a tertiary hospital, in both outpatient and in-patient settings. Patients diagnosed with pulmonary TB from any methods, including microbiological evidence or clinical diagnosis, were eligible to join the study. This study was approved by the Ethics Committee of the institution. All participants provided signed, written informed consent. This study was conducted according to the most current version of the Declaration of Helsinki and Good Clinical Practice.

This study enrolled patients who were older than 18 years old and able to complete the questionnaire. Patients who were pregnant, younger than 18 years old and could not complete the questionnaire were excluded from the study.

2.2. Study protocol

After the participants were enrolled into the study, basic information such as age, sex, height, weight, educational status, smoking, and alcohol drinking were collected. The Thai HADS (Thai Hospital Anxiety and Depression Scale) has been validated in Thailand and used to screen for probable depression and anxiety.[9] This questionnaire has 14 questions. After the participants have completed the questionnaire, the scores are ranked as follows: normal (scores 0–7), borderline abnormal or borderline case (scores 8–10) and abnormal case (scores 11–21). We evaluated the study population with this tool at 2 time points, at first visit (within 2 weeks after diagnosis) and at least 2 months after the first time.

All of the participants were followed until the end of treatment. The final treatment outcome was categorized as successful or unsuccessful which included lost to follow-up (incomplete course of treatment), death, and participants who met "treatment failure" criteria as defined in Thailand TB Practical Guideline 2013 (patient with persistent positive microscopic identification of TB or positive culture of TB at 5 months of treatment).

2.3. Definitions

Persistent depression/anxiety score ≥8 indicated that the participant had a score more than 7 at both time points.

Persistent depression/anxiety score ≥11 indicated that the participant had a score more than 10 at both time points.

2.4. Outcomes

The primary outcome was the prevalence of probable depression and anxiety among the participants. Secondary outcome was the risk factors associated with mental disorders

in pulmonary TB participants as well as the association between probable depression and anxiety with unsuccessful treatment outcome.

2.5. *Statistical analysis*

Demographic and clinical data were described. Continuous variables were expressed as median (interquartile range: IQR) and percentage for categorical variables. Differences in the depression and anxiety scores between unsuccessful and successful groups were assessed using a Wilcoxon rank sum test. Chi-square test or fisher exact test was used to compare the prevalence of depression and anxiety. The logistic regression was used to determine the factors associated with unsuccessful treatment. Covariates with $p < 0.1$ in the univariate models were adjusted in the multivariate models. All P-values reported were two-sided. Statistical significance was defined as $P < 0.05$. Stata version 15.1 (Stata Corp., College Station, Texas) was used for analysis.

3. Results

One hundred and three participants who were diagnosed with pulmonary TB and treated at the King Chulalongkorn Memorial Hospital between March 2018–Oct 2019 were enrolled into the study. Baseline characteristics of the study population are shown in Table 1. The median age was 50 years and more than 90% of the participants were newly infected with pulmonary TB. Most of the participants had an educational level below a bachelor's degree (71/103: 68.9%). Half of the participants had smear positive results. Participants who had smear negative results were diagnosed by either polymerase chain reaction (PCR) or culture for mycobacterium tuberculosis. Eleven percent of the participants were co-infected with HIV (Human Immunodeficiency Virus) and 60% of the participants had comorbidities (Table 1).

Table 1 – Baseline characteristics of the study population.

Variables	N = 103
Median (IQR) age (years)	50 (35–64)
Sex; N (%)	
Male	57 (55.3%)
Female	46 (44.7%)
Marital status; N (%)	
Single	33 (32.0%)
Married	59 (57.3%)
Divorced	9 (8.7%)
Education; N (%)	
Below bachelor degree	71 (68.9%)
Bachelor degree	27 (26.2%)
Master degree	2 (1.9%)
PhD	1 (1.0%)
Income/month; N (%)	
less than 10,000 bath/month	44 (42.7%)
10,000 bath/month and more	59 (57.3%)
Active alcohol drinking; N (%)	17 (16.5%)
Active tobacco smoking; N (%)	14 (13.6%)
BMI; N (%)	
underweight (BMI≤18.4)	29 (28.2%)
normal BMI (BMI 18.5–22.9)	50 (48.5%)
overweight (BMI 23.0–24.9)	11 (10.7%)
obese (BMI≥25.0)	13 (12.6%)
Hypoalbuminemia (serum albumin <2.5 gm/dL); N (%)	11 (10.7%)
Sputum AFB positive patient; N (%)	55 (53.4%)
HIV co-infection; N (%)	11 (10.7%)
Category case; N (%)	
newly infected participant	93 (90.3%)
previously treated	2 (1.9%)
relapsed case	8 (7.8%)
Have comorbidity; N (%)	62 (60.2%)

3.1. *Prevalence of probable depression/anxiety in the study population and its significance was associated with unsuccessful treatment outcome*

All participants were evaluated for depression and anxiety by using the Thai HADS score at 2 time points, approximately 2 months apart as shown in Table 2. The prevalence of probable depression at the first visit was 22.3% (depression score ≥8) and at 2 time points was 13.6% (persistent depression score ≥8). When the cut-off score of ≥11 was used, this increased the probability of having probable depression. The prevalence of probable depression at the first visit was 12.6% (depression score ≥11) and at 2 time points was 7.8% (persistent depression score ≥11).

The prevalence of probable anxiety at the first visit was 24.3% (anxiety score ≥8) and at 2 time points was 15.5% (persistent anxiety ≥8). When the cut-off score of ≥11 was used, this increased the probability to detect probable anxiety. The prevalence of probable anxiety at the first visit was 8.7% (anxiety score ≥11) and at 2 time points was 6.8% (persistent depression anxiety ≥11) (Table 2). Eleven out of 103 participants (10.7%) had unsuccessful treatment outcome. Four out of 11 (36.4%) participants had persistent depression/anxiety score ≥11. On the other hand, among successful treatment outcome group, the prevalence of probable depression/anxiety was less than 5% (persistent depression/anxiety score ≥11).

3.2. *Risk factors associated with unsuccessful treatment outcome*

The following factors were associated with unsuccessful treatment outcome: older than 60 years old, the female sex, single or divorced status, people with lower education level, citizens with low income (income less than 10,000 baht/month), people who regularly consumed alcoholic beverages, smokers, population with abnormal BMI (BMI less than 18.5 or greater than 22.9), people with hypoalbuminemia (serum albumin less than 2.5 gm/dL), stigma, people with positive sputum microscopic examination (Acid-Fast Bacilli or AFB positive), people co-infected with HIV, people previously treated or had relapsed pulmonary TB, comorbidities, people treated in an inpatient setting, and had persistent depression/anxiety score ≥11 (Table 3).

From 103 participants, 92 participants had successful treatment outcome (89.3%) and 11 had unsuccessful treatment outcome (10.7%). Those that had unsuccessful treatment outcome had not completed the course of TB treatment and were lost to follow-up (5 participants), discharged as treatment failure (4 participants) and died before completing the treatment course (2 participants).

Table 2 – Prevalence of depression and anxiety in the study population.

	Total (N = 103)	Treatment successful (N = 92)	Treatment unsuccessful (N = 11)	P-value
Median (IRQ) Depression score				
1st	4 (1–7)	3 (1–6)	11 (5–13)	0.001
2nd	3 (1–6)	2 (1–6)	11 (3–13)	0.004
Depression score ≥ 8; N (%)				
1st	23 (22.3)	17 (18.5)	6 (54.6)	0.007
2nd	16 (16.3)	12 (13.2)	4 (57.1)	0.01
Persistent Depression score ≥8	14 (13.6)	10 (10.9)	4 (36.4)	0.04
Depression score ≥ 11; N (%)				
1st	13 (12.6)	7 (7.6)	6 (54.6)	<0.001
2nd	9 (9.2)	5 (5.5)	4 (57.1)	0.001
Persistent Depression score ≥11	8 (7.8)	4 (4.4)	4 (36.4)	0.004
Median (IRQ) Anxiety score				
1st	4 (2–7)	4 (2–7)	8 (6–14)	0.001
2nd	4 (1–6)	3 (1–6)	14 (6–16)	<0.001
Anxiety score ≥ 8; N (%)				
1st	25 (24.3)	19 (20.7)	6 (54.6)	0.002
2nd	17 (17.4)	12 (13.2)	5 (71.4)	0.01
Persistent Anxiety score ≥8	16 (15.5)	12 (13.2)	4 (36.4)	0.07
Anxiety score ≥ 11; N (%)				
1st	9 (8.7)	5 (5.4)	4 (36.4)	0.007
2nd	8 (8.2)	3 (3.3)	5 (71.4)	<0.001
Persistent Anxiety score ≥11	7 (6.8)	3 (3.3)	4 (36.4)	0.002

Persistent is defined to have abnormal scores (score ≥8 or ≥ 11) at 2 time points.

Table 3 – Risk factors associated with unsuccessful treatment outcome.

	Univariate OR (95%CI)	p-value	Multivariate aOR (95%CI)	p-value
Age: >60 vs < 60 years	0.67 (0.17–2.70)	0.57		
Sex: Female vs male	1.04 (0.30–3.63)	0.96		
Marital status				
Single or divorced	0.74 (0.20–2.71)	0.65		
Education				
Below Bachelor Degree	0.77 (0.20–2.83)	0.65		
Bachelor degree or higher	Ref			
Income				
< 10,000 bath/month	1.71 (0.49–6.0)	0.41		
≥10,000 bath/month	Ref			
TB stigmata	0.82 (0.09–7.40)	0.86		
BMI				
Normal	Ref			
Underweight	1.17 (0.30–4.56)	0.82		
Overweight	0.32 (0.04–2.81)	0.30		
Hypoalbuminemia	3.94 (0.87–17.86)	0.08	3.07 (0.45–20.77)	0.25
Sputum AFB positive	2.18 (0.59–7.95)	0.24		
HIV co-infection	0.82 (0.09–7.10)	0.86		
Previously treated or had a relapse	8.19 (1.86–36.03)	0.005	**7.04 (1.19–41.85)**	**0.03**
Had a comorbidity	7.69 (0.95–62.60)	0.06	5.18 (0.58–46.20)	0.14
Past admission for TB treatment	1.35 (0.26–6.97)	0.72	**10.12 (1.54–66.45)**	**0.02**
Persistent Depression score ≥11	12.60 (3.14–50.63)	<0.001		
Persistent anxiety score ≥11	4.61 (1.28–16.74)	0.02	Collinearity with depression	

OR =Odds ratio, aOR = adjusted Odds ratio.

From the univariate analysis, the risk factors associated with unsuccessful treatment outcome were previously treated or had relapsed pulmonary TB (odds ratio 8.19 (1.86–36.03); p = 0.005), persistent depression score ≥11 (odds ratio 12.60 (3.14–50.63); <0.001), and persistent anxiety score ≥11 (odds ratio 4.61 (1.28–16.74); p = 0.02). From the multivariate analysis, the risk factors that were significantly associated with unsuccessful treatment outcome were previously treated or had relapsed pulmonary TB (odds ratio 7.04 (1.19–41.85); p = 0.03) and persistent depression/anxiety score ≥11 (odds ratio 10.12 (1.54–66.45); p = 0.02).

4. Discussion

Patients with pulmonary TB may have depression and anxiety. In this study, the Thai HADS score was used to screen people who were at risk of probable depression and anxiety. When a cut-off value of 11 was used, the sensitivity and specificity to diagnose depression were 85.71% and 91.3%, respectively, and for anxiety, the sensitivity and specificity were 100% and 86%, respectively.[10] However, if the score was between 8 and 10, then the participant was considered to be a doubtful case. These participants must be re-evaluated or sent to a medical professional. In this study, the prevalence of probable depression at 2 time points was 7.8% and the prevalence of probable anxiety at 2 time points was 6.8% when the cut-off value ≥ 11 was used (Table 2). However, when a cut-off of ≥8 was used, the prevalence of probable depression and probable anxiety at 2 time points increased to 13.6% and 15.5%, respectively. According to our result, the Thai HADS score may be used as a screening tool to provide appropriate and timely mental health treatment to the patients.

Previous studies have reported the prevalence of depression in patients with pulmonary TB to be 16.8%–54.0%,[11–16] and the prevalence of anxiety to be 38.3%–47.2%.[11,13–15,17] The prevalence of depression and anxiety varied between the studies. Even though the results from this manuscript does not reveal any new finding, yet it is the first study to be done in Southeast Asia using the Thai HADS score to evaluate the prevalence of depression among TB patients. The prevalence of depression vary among the regions because different screening questionnaires were used. We would like to encourage the physician to use this questionnaire because it is easy, simple and takes less than 5 minutes to complete. This questionnaire can help early detect and treat TB patients with depression. Any patient with a positive result or at risk should be referred to the psychiatrist for definite diagnosis.

In a recent systematic review and meta-analysis [17], an association between depressive symptoms and unfavorable TB treatment outcome (OR = 4.26; CI 95%:2.33–7.79) was detected. Depressive symptoms were also associated with lost to follow-up (OR = 8.7; CI 95%: 6.5–11.64). However, most of the data were from cross-sectional studies and may have some selection bias. Not only that, but Southeast Asians were not included in these studies. For this study, the treatment failure and loss to follow-up may be associated with depression. TB patients already face many problems such as financial problems and health problems. Depression can exacerbate these problems and may impact the patients' compliance and adherence during treatment.

Our study is the first to assess the prevalence of probable depression and anxiety in pulmonary TB patients in Southeast Asia and its correlation with unsuccessful treatment outcome. The participants were followed until the course for TB treatment was completed. There were 11 cases with unsuccessful treatment outcome for pulmonary TB (10.7%) of which 5 participants were lost to follow-up (so they did not complete their treatment for TB), 4 participants were discharged as treatment failure, and 2 participants died before completing their treatment (dead by pulmonary TB). The percentage of unsuccessful treatment supports the results reported by WHO. When analyzed by the Chi-square test, both persistent depression and anxiety were factors associated with unsuccessful treatment. According to the univariate analysis, hypoalbuminemia, patients who had been previously treated or had recurrent pulmonary TB, and patients with comorbidity were associated with unsuccessful treatment but these factors were not significant in the multivariate analysis.

The strength of this study was that it was a prospective study and lacked selection bias. In addition, the data collection was accurate. Immediately after the questionnaire was completed, a staff checked to make sure there were no missing data or contradicting data while the participant was still in the clinic. This guaranteed that the data was accurately recorded. The data was collected at 2 time points, at the first visit and 2 months after TB treatment. By collecting the data at 2 time points, this increased the specificity as well as increased the true positive diagnosis of anxiety and depression among the active TB patients.

However, there were some limitations in the study. First, the sample size was small. Additional study with a larger sample size is needed. Second, we did not enroll MDR-TB patients so the findings from this study is only applicable to drug susceptible TB patients. Last, the diagnosis of anxiety/depression for all patients were not confirmed by a psychiatrist. Nevertheless, the HADS score higher than 11 had an 85% sensitivity and specificity to diagnose anxiety/depression, and thus does not need a confirmation from a psychiatrist.

5. Conclusion

Pulmonary TB is a chronic infectious disease which may cause physical disability and affect emotional and socioeconomic status of those infected with the disease. This study has found that probable depression and anxiety are risk factors for unsuccessful treatment outcome. Therefore, the authors suggest that the doctors, medical personnel and staff involved in pulmonary TB care should consider mental disorder among TB patients.

Non psychiatrists can use the Thai HADS score to screen TB patients for mental disorder and if the screening result is positive, provide treatment or refer the patient to an expert.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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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.2022.05.007.

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Original article

A study to evaluate the hepatoprotective effect of N- acetylcysteine on anti tuberculosis drug induced hepatotoxicity and quality of life

Deepasree Sukumaran [a,*], *P. Usharani* [a], *G.K. Paramjyothi* [b], *M.V.S. Subbalaxmi* [c], *K. Sireesha* [a], *Mohammed Abid Ali* [a]

[a] *Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad*
[b] *Department of Pulmonary Medicine, Nizam's Institute of Medical Sciences, Hyderabad*
[c] *Department of General Medicine, Nizam's Institute of Medical Sciences, Hyderabad*

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ABSTRACT

Background: Drug induced liver injury (DILI) is a serious adverse effect caused by first-line anti-TB (ATT) drugs, limiting the TB-treatment. The tissue inflammation induced by free radical burst and poor dietary intake in TB induces oxidative stress, which was proposed as one of the mechanisms responsible for ATT induced DILI. *N*-acetylcysteine (NAC) exerts a hepato-protective effect by enhancing the cellular antioxidant defense mechanism. There are few studies evaluating the effect of NAC on ATT induced DILI in Indian-population.

Methods: This is a prospective, randomized, double-blind, placebo-controlled, parallel-group study. Thirty-eight newly diagnosed TB patients on first-line ATT with normal liver function test (LFT) were recruited and randomized to receive either NAC 600 mg tablet or placebo twice daily for 4 weeks and followed-up for next 4 weeks. LFT [AST, ALT, ALP and Total bilirubin] was assessed at baseline, 2, 4 and 8 weeks. Oxidative-stress biomarkers [Malondialdehyde (MDA), Nitric Oxide (NO), Glutathione (GSH)] and quality of life (QOL) by SF-36 questionnaire were assessed at baseline, 4 and 8 weeks. Adverse Drug Reactions (ADRs) were monitored at every visit. Compliance was assessed by pill-count method.

Results: Baseline characteristics were homogenous among both the groups. In the NAC group, there was significant reduction in ALT ($p < 0.01$), ALP ($p < 0.01$), total bilirubin ($p < 0.001$) at 4 weeks compared to baseline. AST, MDA and NO showed a reduction of 19%, 21.6% and 5.5% respectively from baseline and GSH at showed an increase of 2.6% from baseline at 4 weeks in the NAC group, however these were not statistically significant. These effects in LFT and oxidative biomarkers persisted even at the end of 8 weeks.

Significant improvement from baseline in QOL was observed in both the groups ($p < 0.05$). Between group analysis showed, significant reduction in ALT ($p < 0.05$) and AST ($p < 0.05$) in NAC group at 4 weeks, whereas bilirubin, MDA, NO and GSH showed improvement at 4 weeks compared to placebo in NAC group, however it was not statistically significant. This improvement in the LFT and oxidative biomarkers continued even at

* *Corresponding author*. Tel.: +91 8078136365.
E-mail address: deepasree.sukumaran@gmail.com (D. Sukumaran).
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the end of 8 weeks. Itching and rashes were the most common ADRs, with similar incidence in both the groups. Compliance to treatment was good in both the groups.
Conclusion: Significant improvement in liver function parameters is suggestive of hepatoprotective effect of NAC. This observed effect at 4 weeks was found to be persistent at 8 weeks, which signifies prolonged hepato-protective effect of NAC. Long duration studies with large sample size are required for further confirmation of hepato-protective action of NAC.

1. Introduction

Tuberculosis (TB) is a major health problem in developing countries like India.[1] With the advent of highly effective combination therapy for TB which includes Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PYZ) and Ethambutol, it has been possible to attain cure for TB to a greater extent. This benefit is limited by the development of adverse effects of which, hepatotoxicity is one of the common adverse effects. Drug induced liver injury (DILI) caused by anti-TB (ATT) drugs is a serious adverse effect of first line ATT drugs, limiting the treatment of TB.[2,3] Oxidative stress was proposed as one of the mechanisms responsible for ATT induced hepatic injury.[4] Oxidative stress is closely associated with decrease of glutathione levels.[5] *N*-acetylcysteine (NAC) exerts a hepatoprotective effect by replenishing glutathione stores and enhancing the cellular antioxidant defense mechanism.[6] It is well established that by augmenting cellular antioxidative defense system, especially by increasing glutathione (GSH), cells can be protected against oxidative stress induced injuries produced by various drugs and chemicals. NAC has been extensively studied and used for many years in the treatment of paracetamol-induced hepatotoxicity, with good evidence of efficacy and safety. By improving systemic hemodynamics and tissue oxygen delivery, NAC was found to be beneficial in non-paracetamol induced acute liver failure.[7,8] It was demonstrated that, in the treatment of non-paracetamol induced acute liver failure, the mortality was reduced to 28% in the NAC group compared to 53% in the placebo group.[9] Animal studies have shown that ATT induced oxidative injury can be prevented by supporting the cellular antioxidant defense mechanism by NAC.[7] Few clinical trials showed that patients receiving NAC along with their standard ATT had significantly lower rates of drug-induced hepatotoxicity.[6,10] There is paucity of similar studies from India, where TB is a major public health concern.

There is no proven therapy till date for treatment or prevention of ATT induced DILI. In the present clinical scenario, if hepatotoxicity is suspected, the three hepatotoxic drugs (INH, RIF, PYZ) are discontinued and they are restarted when the LFT falls to near normal.[11] Use of steroids in immune mediated DILI and UDCA (Ursodeoxycholic acid) in cholestatic liver injury has shown controversial results. In a randomised trial with 177 patients with non – paracetamol DILI, a 72 hour infusion of NAC has shown significant increase in survival in patients with low grade encephalopathy in a subgroup analysis.[12] Drugs such as NAC, Glutathione, steroids, UDCA are often prescribed as offlabel for treatment for non paracetamol DILI. However, efficacy of any of these drugs to reduce the severity of liver injury is not proven yet.[11] Our study throws light into this area of unmet medical need.

In this study, N- acetylcysteine was evaluated for hepatoprotective effect against first line ATT induced liver injury. The primary objective was to study the effect of NAC on liver function tests and secondary objectives were to study the effect of NAC on oxidative stress biomarkers and quality of life (QOL) and to assess the safety and tolerability of NAC.

2. Materials and methods

This was a prospective, randomized, double blind, placebo controlled, parallel group study. This study was conducted in Department of Clinical Pharmacology and Therapeutics in collaboration with Department of Pulmonary medicine and General medicine at Nizam's Institute of medical Sciences after approval by NIMS ethics committee (EC/NIMS/2298/2019 dated 18.06.2019) and also registered in CTRI (No: CTRI/2020/05/024,995). Study was conducted in accordance with Declaration of Helsinki and Good Clinical Practice Guidelines issued by the Government of India.

3. Methodology

Voluntary written informed consent was taken from all the study participants prior to enrollment. The study population included newly diagnosed TB patients (pulmonary and extra pulmonary) of either gender, taking first line ATT, aged between 18 and 65 years, with normal liver function tests (LFT). Patients with history of hypersensitivity to NAC, chronic liver disease, kidney disease, asthma, COPD, chronic alcoholism and smoking, pregnant and lactating women and those who participated in any clinical trial within the last 3 months were excluded from study. Screening was done by medical history, clinical examination and necessary laboratory investigations and eligible subjects were randomized.

At the baseline visit (0 weeks), history and physical examination including vitals were done. Ten ml of blood was collected for LFT [AST, ALT, ALP and total bilirubin] and estimation of oxidative stress biomarkers [Malondialdehyde (MDA), Nitric oxide (NO), Glutathione (GSH)]. QOL was assessed by SF -36 questionnaire which includes 8 domains of

health namely, physical functioning, role limitation due to physical health and emotional problems, energy/fatigue, emotional well being, social functioning, bodily pain and general health. The subjects were randomized by simple randomization into 2 groups by computer generated random numbers. Group I subjects received first line ATT along with NAC tablet 600mg twice daily and Group II subjects received first line ATT along with identically looking placebo twice daily. The study medication was given for a period of 4 weeks and a post treatment follow up was done for the next 4 weeks. Follow up visits were done at 2, 4 and 8 weeks. LFT was repeated at all the visits. Oxidative stress biomarkers and QOL was assessed at 4 and 8 weeks. Compliance to the study medication was assessed at 2 and 4 weeks by pill count method. All the patients were monitored for safety and tolerability till 8 weeks.

3.1. *Statistical analysis*

Statistical analysis was performed using Graph Pad Prism version 9.0.2 (161) and Microsoft Excel. Data were presented as mean ± standard deviation. Safety data was represented as numbers (percentage).Comparison within the group was performed by paired t test and comparison between the groups was performed by un-paired t test. Categorical data was analyzed by Chi-square test. P value < 0.05 is considered as statistically significant.

For efficacy analysis, modified intention to treat population (those who have at least one post randomization assessment) was included. Primary efficacy parameter was change in LFT and secondary efficacy parameters were change in MDA, NO, GSH and QOL. Missing data was handled by multiple imputation method. For safety analysis, intention to treat population (all the randomized subjects) was included.

Sample size was calculated based on a study conducted by Farazi et al.[10] Taking the mean difference of ALT value at 4 weeks between NAC treated and placebo group as 9.4 with an anticipated standard deviation of 10, nineteen subjects were needed in each group to reject the null hypothesis. Considering 10% dropout rate and 10% screen failure rate, 46 subjects were needed to be screened. Power of the study was 80%. Type I error probability was 0.05.

4. Results

Out of 42 subjects screened, 38 eligible participants (19 subjects in each group) were randomized. Fig. 1 given below represents the participant flow diagram.

The demographic and efficacy data are presented as mean ± standard deviation. The safety data is represented as numbers (percentage).

The demographic details of the study population is shown in Table 1 below.

The mean age of the study population was 33.9 ± 11.6 years in the NAC group and 35.7 ± 11.8 years in the placebo group. The demographic characteristics were homogenous among the groups with respect to age and BMI.

The baseline liver function parameters and oxidative stress biomarkers are represented in Table 2 given below.

At baseline, the liver function tests (AST, ALT, ALP and bilirubin) and oxidative stress biomarkers (MDA, NO and GSH) were homogenous among both the groups.

The SF − 36 domain scores at baseline are represented in Table 3 given below.

The baseline SF-36 scores of the 8 domains were similar in both the groups.

The primary outcome measure was comparison of mean values of LFT parameters at baseline, 2, 4 and 8 weeks within the group and between the groups. The mean values of LFT parameters at baseline, 2, 4 and 8 weeks are represented in Fig. 2.

Subfigures 2A, 2B, 2C and 2D shows the mean values of ALT, AST, ALP and bilirubin respectively at baseline, 2, 4 and 8 weeks in both the groups.

ALT showed significant reduction from baseline of 16.4% at 2 weeks ($p < 0.01$), 37.4% at 4 weeks ($p < 0.01$) and 35.7% at 8 weeks ($p < 0.01$) in the NAC group. AST showed a reduction of 18.8% and 18.4% from baseline at 4 and 8 weeks respectively, however it is not statistically significant. ALP showed significant reduction from baseline of 20.9% at 4 weeks ($p < 0.01$) and 28.6% at 8 weeks ($p < 0.05$) in the NAC group. Bilirubin showed significant reduction from baseline of 41.6% at 2 weeks ($p < 0.05$), 42.7% at 4 weeks ($p < 0.001$) and 48% at 8 weeks ($p < 0.001$) in the NAC group.

At 4 weeks, ALT ($p < 0.05$) and AST ($p < 0.05$) showed significant reduction in the NAC group compared to placebo group. There was reduction in the mean ALT and AST values at 8 weeks and mean bilirubin values at 4 weeks in the NAC group compared to placebo, however it was not statistically significant. At 8 weeks, there was significant reduction in the mean bilirubin in NAC group compared to placebo ($p < 0.05$).

The secondary outcome measures were comparison of mean values of oxidative stress biomarkers (MDA, NO and GSH) and mean SF-36 scores at baseline, 4 and 8 weeks within the group and between the groups. The assessment of safety and tolerability of NAC at every visit was also a secondary parameter.

Fig. 3 shows the mean values of oxidative biomarkers at baseline, 4 and 8 weeks in the NAC and placebo groups.

Subfigures 3A, 3B and 3C shows the mean MDA, NO and GSH values respectively at baseline, 4 and 8 weeks in both the groups.

In the NAC group, MDA showed a reduction of 21.6% from baseline at 4 weeks and a significant reduction of 29.9% from baseline at 8 weeks ($p < 0.05$), whereas in the placebo group, there was significant increase in MDA at 8 weeks from baseline ($p < 0.05$). NO showed a reduction of 5.5% and 2.1% from baseline at 4 and 8 weeks respectively in the NAC group. GSH showed an increase of 2.6% and 3.2% from baseline at 4 weeks and 8 weeks respectively in the NAC group, whereas there was a reduction of 7.3% and 17% from baseline in the placebo group at 4 weeks and 8 weeks respectively.

At 4 weeks, there was reduction in mean MDA levels in NAC group compared to placebo group at 4 weeks, though not statistically significant. There was significant reduction in MDA ($p < 0.01$) in the NAC group compared to placebo at 8 weeks. NO showed reduction and GSH showed increase at both 4 weeks and 8 weeks in the NAC group compared to placebo, however it is not statistically significant.

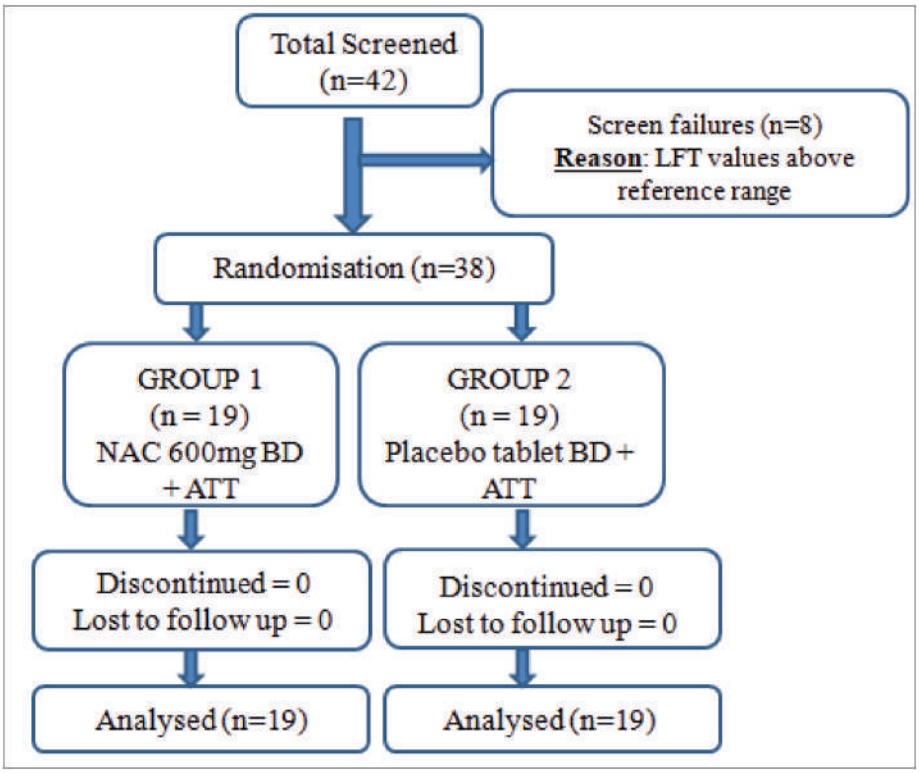

Fig. 1 – Showing the participant flow diagram.

Table 1 – Showing the demographic characteristics of the study population.

SL:NO	Parameter	NAC group (n = 19)	Placebo group (n = 19)	P value
1.	Age (years)	33.9 ± 11.6	35.7 ± 11.8	0.64
2.	Male: Female ratio	15:4	6:13	0.003
3.	BMI (kg/m^2)	19.8 ± 3.4	21.8 ± 6.9	0.26

Table 2 – Showing the baseline liver function parameters and oxidative stress biomarkers of the study population.

SL:NO	Parameter	NAC group (n = 19)	Placebo group (n = 19)	P value
1.	Mean AST (U/L)	27.2 ± 12.2	25.4 ± 14.3	0.68
2.	Mean ALT (U/L)	23.8 ± 11.1	18.05 ± 6.2	0.05
3.	Mean ALP(U/L)	127.2 ± 66.63	112.6 ± 51.02	0.46
4.	Mean total bilirubin (mg/dL)	0.89 ± 0.5	0.64 ± 0.3	0.08
5.	Mean MDA (nmol/mL)	11 ± 4.9	10.5 ± 6.6	0.82
6.	Mean NO (μmol/mL)	12.6 ± 4.6	13.3 ± 4.2	0.62
7.	Mean Glutathione (μmol/mL)	497.2 ± 171.8	545.4 ± 226.6	0.46

Table 3 – Showing the baseline Quality of life scores of the study population as assessed by SF-36 questionnaire.

SL:NO	SF -36 domain	Scores in NAC group (n = 19)	Scores in placebo group (n = 19)	P value
1.	Physical Functioning	76.1 ± 24.7	76.1 ± 24.7	>0.99
2.	Role limitation due to physical problems	43.4 ± 48.5	38.2 ± 48.9	0.74
3.	Role limitation due to emotional problems	64.9 ± 47.8	54.4 ± 50	0.51
4.	Vitality (Energy/fatigue)	63.2 ± 27.6	59 ± 23.9	0.62
5.	Mental Health (Emotional well being)	73.8 ± 22.8	76.4 ± 15.6	0.68
6.	Social functioning	77 ± 26.1	75 ± 22.8	0.81
7.	Bodily pain	77.2 ± 21.2	73.9 ± 25.3	0.66
8.	General health	62.6 ± 21.0	57.9 ± 23	0.51

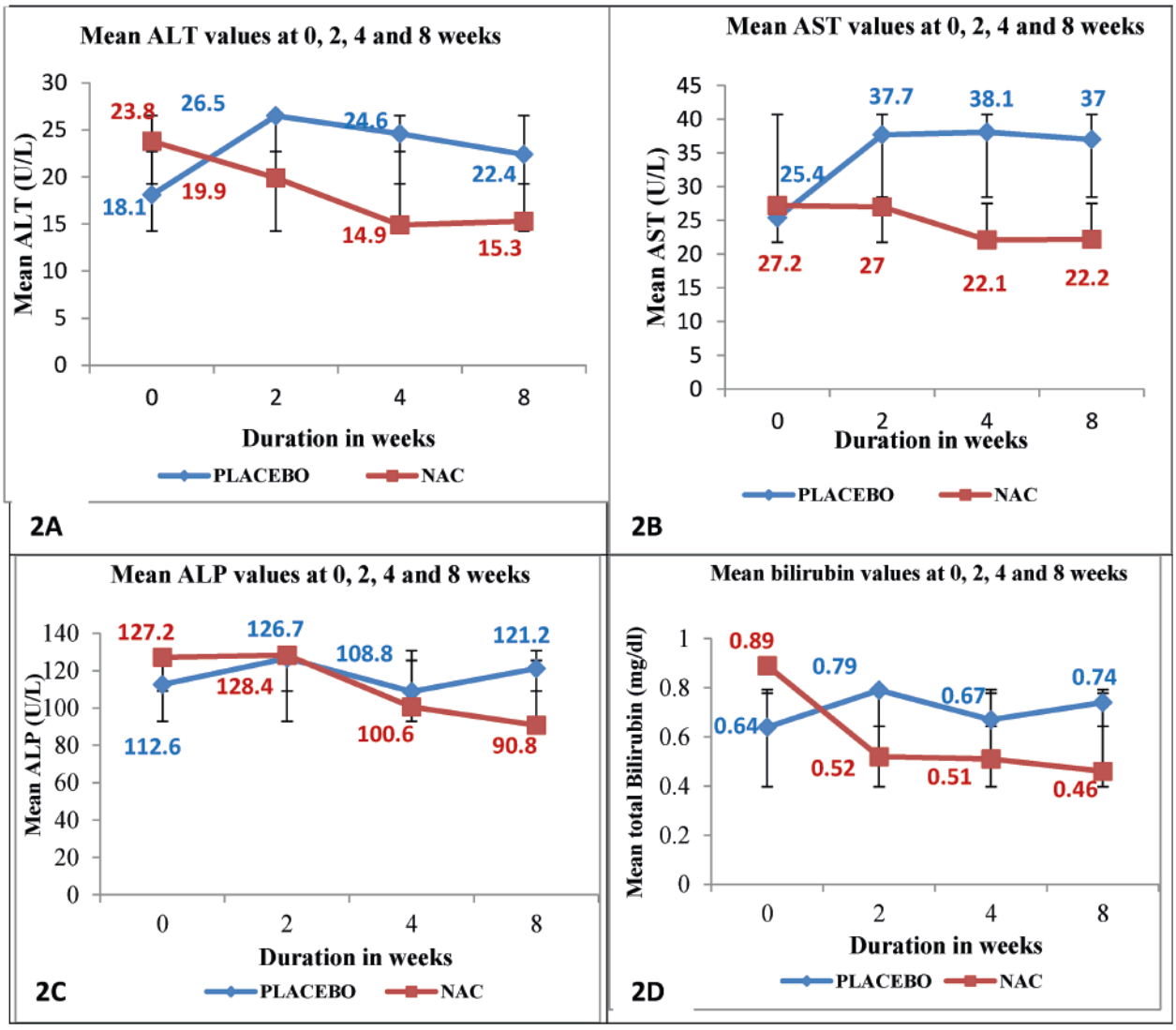

Fig. 2 – Showing the mean values of LFT parameters at baseline, 2, 4 and 8 weeks respectively in the NAC and placebo groups. The mean values of ALT, AST, ALP and bilirubin have been represented in subfigures 2A, 2B, 2C, 2D respectively.

Table 4 shows the mean SF-36 scores indicating the QOL at baseline, 4 and 8 weeks in the NAC and placebo groups.

At 4 weeks, there was significant improvement in the QOL scores in the domains of physical function, role limitation due to physical health and emotional problems, vitality, social function, mental health and bodily pain in the NAC group, whereas with placebo group, significant improvement was observed in only social domain.

At 8 weeks, there was significant improvement in the QOL scores in all the eight domains of health in the NAC group, whereas in placebo group, significant improvement was observed in domains of physical function, role limitation due to physical health and emotional problems, vitality, social function and mental health.

The adverse drug reactions were monitored and recorded in both the groups throughout the study. The incidence of adverse drug reactions (ADR) has been presented in Table 5.

A total of 24 ADR were noted. Itching and rashes were the commonest ADR, followed by gastritis. There was similar incidence of adverse reactions among both the groups ($p > 0.5$).

Compliance to treatment assessed by pill count method was found to be good in both the groups.

5. Discussion

In our study, we evaluated the effect of NAC in the prevention of hepatotoxicity in patients on ATT. The efficacy parameters evaluated were LFT parameters (AST, ALT, ALP and bilirubin), oxidative stress biomarkers (MDA, NO and GSH) and QOL scores assessed by SF- 36 questionnaire.

We observed that, there was a significant reduction from baseline in mean ALT, ALP and bilirubin values and nearly 20% reduction in mean AST value after 4 weeks of NAC treatment. These results corroborate with the results of previous similar studies.[6,8] In a study by Baniasadi et al,[6] which evaluated the protective effect of NAC on ATT-induced hepatotoxicity, it

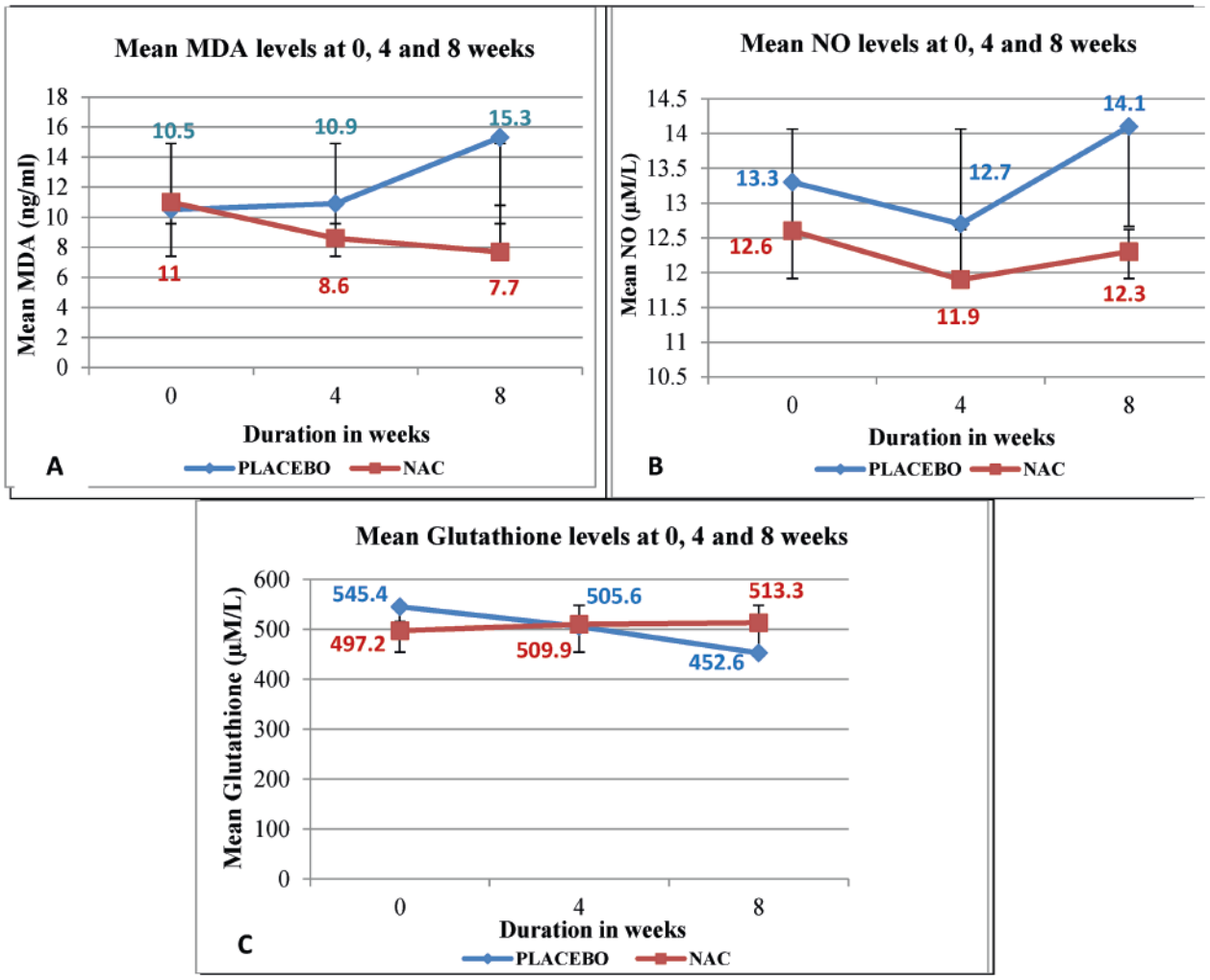

Fig. 3 – Showing the mean oxidative biomarker levels at baseline, 4 and 8 weeks respectively in the NAC and placebo groups. The mean values of MDA, NO and GSH have been represented in subfigures 3A, 3B, 3C respectively.

Table 4 – Showing mean SF 36 scores at baseline, 4 and 8 weeks in NAC and placebo groups.

SF 36 Domains	Study group	Baseline (0 weeks)	At 4 weeks	At 8 weeks	P value (0 Vs 4 weeks)	P value (0 Vs 8 weeks)
Physical Functioning	NAC	76.1 ± 24.7	86.6 ± 18.0	93.7 ± 11.8	0.001	0.0004
	Placebo	66.2 ± 24.6	77.9 ± 24.1	82.1 ± 22.3	0.063	0.013
Role limitation – Physical health	NAC	43.4 ± 48.5	68.4 ± 47.8	88.2 ± 31.6	0.035	0.0007
	Placebo	38.2 ± 48.9	54 ± 50.2	76.3 ± 42.1	0.062	0.0025
Bodily pain	NAC	77.2 ± 21.2	85.8 ± 14.5	89.9 ± 14.1	0.015	0.04
	Placebo	73.9 ± 25.3	81.3 ± 17.2	79 ± 17.8	0.143	0.47
General Health	NAC	62.6 ± 21.0	68.7 ± 19.1	73.7 ± 10.5	0.134	0.032
	Placebo	57.9 ± 23	67.2 ± 19	66.8 ± 18.7	0.055	0.087
Vitality	NAC	63.2 ± 27.6	74 ± 19.1	83.42 ± 11.1	0.006	0.003
	Placebo	59 ± 24	67.5 ± 21	67.4 ± 21.4	0.056	0.03
Social Functioning	NAC	77 ± 26.1	89.5 ± 17.3	94.7 ± 10.5	0.011	0.001
	Placebo	75 ± 22.8	88.2 ± 16.4	87.5 ± 16.7	0.005	0.004
Role limitation – Emotional problems	NAC	64.9 ± 47.8	84.2 ± 32.2	94.7 ± 22.9	0.023	0.011
	Placebo	54.4 ± 50	64.9 ± 47.8	80.7 ± 39	0.34	0.048
Mental Health	NAC	73.8 ± 22.8	82.7 ± 12.8	90.3 ± 8.6	0.04	0.0005
	Placebo	76.4 ± 15.6	82.1 ± 16.1	85.2 ± 11.8	0.07	0.013

Table 5 – Showing the incidence of ADR in both study groups.

S.N	Adverse reactions	NAC group n (%)	Control group n (%)
1.	Gastritis	3 (23.1)	2 (18.2)
2.	Itching and Rashes	4 (30.8)	4 (36.4)
3.	Nausea	2 (15.4)	2 (18.2)
4.	Vomiting	1 (7.7)	1 (9.1)
5.	Headache	1 (7.7)	2 (18.2)
6.	Fatigue	2 (15.4)	0
Total		**13**	**11**

was shown that the mean AST and ALT values were significantly lower in the NAC treated group compared to the control group at 1 and 2 weeks of treatment. In a study by Farazi et al,[10] where NAC was administered for a period of 4 weeks in the test group, significant reduction in AST and ALT were observed at 2nd week, 4th week and 6th week and additionally ALT showed significant reduction at 8th week also.

It is well known that patients on first line ATT are at risk of developing hepatotoxicity. Though elevation of liver enzymes (ALT, AST) indicates hepatocyte inflammation, ALT is considered as more specific for hepatocellular injury than AST.[2] In this study, we have observed significant reduction in ALT and bilirubin from baseline at 2, 4 and 8 weeks with NAC treatment, signifying its hepatoprotective effect. It was demonstrated that, when NAC was administered prophylactically, along with anti-TB drugs in patients with normal liver function, it prevented the elevation of LFT parameters, limiting the risk of development of hepatotoxicity.

We observed that, MDA showed more than 20% reduction from baseline after 4 weeks of NAC treatment. Improvement was also observed with NO and GSH at 4 weeks of treatment with NAC. These effects persisted at 8 weeks even after stopping NAC at 4 weeks. This could be attributed to improvement of the total antioxidant status of the body by early supplementation of NAC, which is a potent anti-oxidant, along with TB treatment that improves the disease status leading to improvement of nutrition and overall QOL.[13]

Another secondary outcome measure was QOL assessment by SF-36 scores. After 4 weeks of NAC treatment, there was significant improvement in all the domains of health except general health domain, whereas with placebo, only social domain showed significant improvement at 4 weeks. Further, at 8 weeks, there was significant improvement observed in all the 8 domains in NAC group compared to 6 domains in the placebo group. This showed that, with NAC treatment, there was a better improvement in QOL at 4 weeks and 8 weeks. The QOL improvement with placebo could be attributed to TB treatment, which gradually cures TB and improves the overall health status of the patients. This result correlates with other similar studies. In a study by Farazi et al[10], it was shown that TB patients on ATT with concomitant NAC for 4 weeks, had an improved QOL at the end of 4 weeks compared to placebo group. In a study by Li et al,[14] where QOL among TB patients within 2 years of completion of ATT was compared with healthy population without TB, it was found that, both group of patients demonstrated similar QOL. This was attributed to TB treatment which resulted in complete cure of TB and improved the overall health status and thereby improved the QOL.

There were no serious adverse events observed in this study. Itching and rashes, followed by gastritis were the commonest adverse events reported which resolved on symptomatic treatment. The incidence of side effects was similar in both the groups. Therefore, NAC demonstrates good safety and tolerability among TB population.

To the best of our knowledge, there are no published studies in literature, which evaluated the effect of NAC treatment on oxidative stress biomarkers in TB patients. Our study is the first of its kind in India in evaluating the effect on NAC on prevention of hepatotoxicity among the TB patients. From this study, co-administration of NAC along with ATT has demonstrated hepatoprotective effect by preventing the elevation of liver function parameters and maintaining the oxidative stress balance in the body, thereby reducing the incidence of ATT induced hepatotoxicity.

The limitations of the study are smaller sample size and shorter duration. Further studies are required with longer duration and larger sample sizes to confirm the therapeutic potential of NAC in prevention of hepatotoxicity among TB patients. The hepatoprotective effect of NAC can further be explored in patients those who are at high risk of developing heptotoxicity.

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Conflicts of interest

The authors have none to declare.

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Original article

Effect of age and gender on high - Sensitivity C - Reactive protein levels serum on health worker with latent tuberculosis and healthy control

Refi Yulistian [a,*], *Nur Ahmad Tabri* [a], *Harun Iskandar* [a], *Syakib Bakri* [a], *Idar mappangara* [a], *Agus Sudarso* [a], *Arifin seweng* [b]

[a] *Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia*
[b] *Department of Biostatistics, Public Health Faculty, Hasanuddin University, Makassar 90245, Indonesia*

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ABSTRACT

Background: Latent tuberculosis is defined as a state of persistent immune response stimulated by Mycobacterium tuberculosis antigens with no evidence and signs of active TB . Health workers have a high risk of developing latent TB disease due to occupational exposure from patients. High sensitivity CRP (hs-CRP) assays have been developed for special values that may indicate low-grade inflammatory lesions as is true in measurement of latent tuberculosis infection. Factors that affect CRP levels are gender and age. Our study is conducted to asses effect of age and gender on Hs- CReactive protein leves serum on health worker with latent tuberculosis and healthy control.

Method: This research is a cross sectional study using primary data. The research was conducted at Wahidin Sudirohusodo Makassa Hospital and Community Center For Lung Health In South Sulawesi. Studied subject were recruited by consecutivesampling, in which the patient who met the inclusion criteria and then the serum HsCRP test was measured. Data analysis was performed using SPSS version 25.

Result: During the study period , 80 subjects met the inclusion criteria. At age $\leq$ 32 years, the mean HsCRP was found to be lower in latent TB than in healthy controls, but not statistically significant (p>0.370). At age >32 years, the mean HsCRP was found to be higher in latent TB than in healthy controls, but not statistically significant (p>2.49). In males, the mean HsCRP was found to be higher in latent TB than in healthy controls, but not statistically significant (P =0.584). In women, the mean HsCRP was found to be lower in latent TB than in healthy controls, but not statistically significant (P =0.712).

Conclusion: Serum HsCRP levels were found to be higher in latent TB subjects with increasing age and male gender but not statistically significant.

* *Corresponding author*. Internal Medicine Department , Faculty Of Medicine, Hasanudin University , Perintis Kemerdekaan St, Makassar 90245, south Sulawesi, Indonesia
E-mail address: Refiyulistian@gmail.com (R. Yulistian).

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Latent tuberculosis is defined as a state of persistent immune response stimulated by *Mycobacterium tuberculosis* antigens with no evidence and signs of active TB.[1] According to the World Health Organization (WHO) it is estimated that 2–3 billion people are infected latent with *M. tuberculosis*, and 5%–15% progress to active tuberculosis.[2] (Tables 1e4, Fig. 1)

Health workers have a high risk of developing latent TB and active TB disease due to occupational exposure from patients.[3] The prevalence of about 2028 health workers in 14 different hospitals in Germany is 9.9%.[4] In Malaysia the prevalence of latent TB in health workers is 10.7% in administrative personnel, 13.7% in medical personnel.[5] An Indonesian study reported a significant association between the interferon-gamma release assay (IGRA) and workplace, as evidenced by positive IGRA results in 37 (37.4%) hospital health workers involved in this study.[3]

In recent years, high sensitivity CRP (hs-CRP) assays have been developed for the sensitive quantification of CRP, which can detect minimal variation of serum CRP levels even within the normal range and have special values that may indicate low-grade inflammatory lesions as is true in measurement of latent tuberculosis infection. Results from several studies indicate that hs-CRP is a sensitive and reliable marker of low-grade systemic inflammation.[6] Several factors that affect CRP levels are gender, age, ethnicity, genetic polymorphism and others. Mild inflammatory stimuli such as cigarette smoke, air pollution and estrogen consumption also affect Hs-CRP levels.[7]

Age is strongly associated with hs-CRP levels, with increasing age.[8] Oxidative stress is a common pathophysiological mechanism in many inflammatory and hypoxic conditions, and is also part of the natural process of aging.[9] In several studies in China and in Pakistan, serum HsCRP levels were found to be higher in men than women. This could be due to the presence of the hormone estradiol in women where the anti-inflammatory effect of estradiol on CRP is consistent with the effect of estradiol on inflammation in many types of tissues.[10]

To the best of the researchers' knowledge, this study has never been reported in Indonesia and based on the above background, the researchers are interested in conducting research on the effect of age and sex on serum hs-CRP levels in health workers with latent TB and healthy controls.

1. Subjects and methods

1.1. Study population

This research is a cross sectional study using primary data. The research was conducted at Dr. Hospital. Wahidin Sudirohusodo Makassar and Makassar Pulmonary Center from August 2021 until the number of samples is reached. Sample testing was carried out in the research laboratory of the Unhas Hospital. Inclusion criteria: Age >18 years, health workers diagnosed with latent TB and healthy controls, willing to participate in the study (see Table 1)

Table 1 – Baseline characteristics (n = 80).

Variabel	n	%
Gender		
Male	38	47.5
Female	42	52.5
Age		
≤32 years old*	50	62.5
>32 years old	30	37.5

Note: age categories based on median, n: total.

Table 2 – Serum HsCRP levels in Latent TB and healthy controls by Gender.

Gender	group	N	Mean	SD	p-value
Male	Latent Tb	4	1.67	2.58	0.584
	healthy controls	34	1.40	1.58	
Female	Latent Tb	10	1.13	1.42	0.712
	healthy controls	32	1.24	1.59	

Table 3 – HsCRP levels in Latent TB and healthy controls by Age Group.

Age	Group	n	Mean	SD	p-value
≤32 years old	Latent tb	9	0.64	0.72	0.370
	healthy controls	41	1.46	1.68	
>32 years old	Latent tb	5	2.46	2.46	0.113
	healthy controls	25	1.10	1.39	

Analysis Bivariat

Variable			Group		Sig.
			Tb Laten	control	
Gender	Male	Mean	1.670	1.404	0.767
		Std. Deviation	2.582	1.580	
		N	4	34	
	Female	Mean	1.133	1.243	0.846
		Std. Deviation	1.422	1.590	
		N	10	32	
Age	≤32 years	Mean	0.649	1.538	0.191
		Std. Deviation	0.833	1.722	
		N	7	38	
	>32 years	Mean	1.924	1.039	0.178
		Std. Deviation	2.204	1.325	
		N	7	28	

1.2. Study designs

Sampling was done by consecutive sampling, where subjects who met the inclusion criteria were included in the study until the number of samples was reached. Health workers were given an explanation about the research. The explanation given includes the procedures and research objectives. Each research subject who is willing to participate in the study signed a letter of consent in the form of an informed consent. Anthropometric examinations, blood pressure and laboratory data tracing were carried out in the form of routine blood, blood chemistry, blood glucose. Examination of IGRA test data on research subjects and chest X-rays through history taking. Health workers are medical and paramedical personnel on duty in the TB treatment room (outpatient and inpatient TB) and the emergency room. The diagnosis of latent tuberculosis was a subject with a positive IGRA and from the anamnesis, a normal chest radiograph was obtained. Healthy controls were subjects with a negative IGRA and from the anamnesis, a normal chest X-ray was obtained and then the serum Hs CRP test was measured.

Table 4 – HsCRP levels in latent TB and controls.

Group	Variables	n	Min	Maks	Mean	SD	p
TB Laten	HsCRP	14	0,22	5,54	1,29	1,73	0.667
Controls	HsCRP	66	0,07	5,20	1,33	1,57	

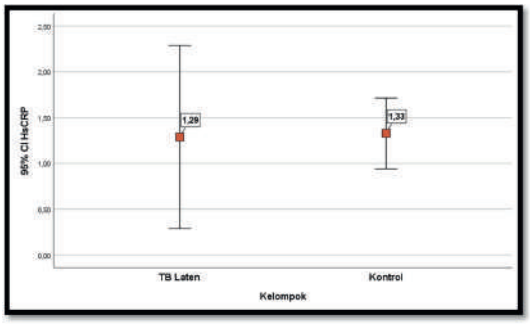

Fig. 1 – HsCRP levels in latent TB and controls.

2. Clinical laboratory methods

2.1. *Measurement of serum high-sensitivity C-reactive protein*

Hs-CRP was measured by Chemiluminescent Immunometric technique, Immulite product no. E1798Hu catalog, with Immulite 2000 tool. Briefly, 50μl/well each of the reconstituted hs-CRP Kit standard and test serum samples were dispensed into appropriately labeled high-affinity 96-well plastic microplates pre-coated with the corresponding specific monoclonal anti-hs-CRP antibody and the mixture incubated for 2hrs at 370C. The solution was discarded and the microplates washed x4times with 400μl/well Kit wash buffer with the aid of auto-washer. Next, 100μl/well of hs-CRP conjugate was added to all wells (standard and sample wells) and the micro-ELISA strip plate covered with a sealing tape and incubated for 1hr at 370C. The mixture was discarded and microplates washed x4times with Kit wash buffer and the liquid mixture aspirated. Then, 50μl/well each of Kit chromogen solution A and Kit chromogen solution B substrate were added and incubated for 15mins at 370C(in the dark) with gentle shaking with automatic shaker and then washed x4times with the Kit wash buffer and liquid aspirated.

2.2. *Statistical analysis*

Data analysis was performed using SPSS version 25. In this study, the statistical tests used were the Kolmogorov–Smirnov test and the Mann–Whitney test to assess the normality of the data. The results of the statistical test were significant if the p value < 0.05. The results obtained will be displayed in the form of a narrative equipped with tables and pictures.

2.3. *Ethical approval*

The study was approved and acknowledged by the Ethics Medical Committee of Hasanuddin University, with reference number: 528/UN4.6.4.5.31/PP36/2021.

3. Results

During the study period, 80 subjects met the inclusion criteria. Majority were females (52.5%) and aged 21–53 years. In males, the mean HsCRP was found to be higher in latent TB than in healthy controls, but not statistically significant (P = 0.584). In women, the mean HsCRP was found to be lower in latent TB than in healthy controls, but not statistically significant (P = 0.712). At age ≤32 years, the mean HsCRP was found to be lower in latent TB than in healthy controls, but not statistically significant (p = 0.370). At age >32 years, the mean HsCRP was found to be higher in latent TB than in healthy controls, but not statistically significant (p = 2.49) (see Tables 2-4, Fig. 1).

4. Discussion

It is well known that inflammation plays a central role in the initiation and development of latent TB.[6] In the setting of latent TB infection, there is a persistent subclinical inflammatory state in which a small number of Mycobacterium TB bacilli are not sufficient to manifest clinically.[11] In this condition, low-level inflammation is found that is systemic, subclinical and chronic. Low-grade inflammation defined as a mild increase in CRP levels. Low CRP levels can be measured accurately, and thus make it possible to identify individuals with low-grade inflammation, defined as a CRP measurement above 3 mg/L but below 10 mg/L.[8]

In this study, it was found that the mean HsCRP was higher in men with latent TB than in healthy controls, but not statistically significant (p > 0.05). This difference can be caused by factors that affect the increase in HsCRP levels, namely age and gender. The same study was conducted in China by Ying Tang (2018) on a total of 6060 healthy adults, of which 3672 subjects were male and 2388 female, and ages from 18 years to 89 years where the serum hs-CRP levels increased with increasing age and gender of male.[12] Tomasic et al in a study in Poland in 2015 in assessing the dynamics of changes in HsCRP values in healthy individuals ranging from 20 to 90 years of age where higher hcCRP was found with increasing age. Aging is thought to be related to the inflammatory process. Various studies have shown that several cytokines, especially IL-6, TNF alpha and CRP, increase with age in the absence of acute infection.[9] Ferruci et al in 1998 in Italy in their study of 1270 people found a significant increase in IL-6, sIL-6, IL-1ra, IL-18, CRP, and fibrinogen with increasing age. Oxidative stress is also positively associated with aging. Older people are often affected by a low-grade proinflammatory state characterized by elevated levels of cytokines and acute phase proteins. Cytokines are intercellular signaling proteins that exert pro- and anti-inflammatory activity through ligation of specific receptors or stimulate the production of acute

fake proteins in the liver, such as C-reactive protein (CRP) and fibrinogen.[13]

In this study, it was found that the mean HsCRP was higher in men with latent TB than in healthy controls, but not statistically significant. In women, the mean HsCRP was found to be lower in latent TB than in healthy controls, but not statistically significant ($p > 0.05$). Research conducted in China by Ying Tang (2018) on a total of 6060 healthy adults, of which 3672 subjects were men and 2388 women where the serum HsCRP values were significantly higher in men than women.[12] It is possible that the role of female hormones is that the anti-inflammatory effect of estradiol on CRP is consistent with the effect of estradiol on inflammation in many tissue types, because estrogen has a negative effect on inflammatory cell migration and production of inflammatory markers in various non-reproductive and non-immune tissues. Estrogen has also been shown to reduce levels of tumor necrosis factor-alpha, a major proinflammatory cytokine., estrogen can act as an antiapoptotic agent in various cell types including endothelial cells by preventing the release of cytochrome c from mitochondria, thereby reducing subsequent vascular inflammation. These results support the hypothesis that endogenous estradiol may have an anti-inflammatory effect.[10]

The limitations of the study are the small number of samples and this study only carried out one measurement of HsCRP in patients with latent TB, whereas in studies with long-term inflammatory conditions it may not be optimal if only one measurement is carried out.

Conflict of interest

The authors have none to declare.

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Original article

Direct out-of-pocket expenditure of tuberculosis treatment in intensive phase in Kalutara District, Sri Lanka

Nithin Ranawaka [a,*], *Sumal Nandasena* [b], *Sunil De Alwis* [c]

[a] *Postgraduate Institute of Medicine, University of Colombo, Sri Lanka*
[b] *Regional Director of Health Services, Kalutara District, Sri Lanka*
[c] *Ministry of Health, Nutrition and Indigenous Medicine, Sri Lanka*

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ABSTRACT

Background: Tuberculosis (TB) is a communicable disease. Financial risk protection is a key target to achieve in end TB strategy. Out-Of-Pocket Expenditure (OOPE) consisted of expenses bore by patients for their illnesses after subtracting third-party payments such as insurance. Despite the free health care in Sri Lanka, TB patients have to pay for various expenses (e.g., expenses for travel, food, drugs, medical investigations, and cost of accompanied person/bystander).

Objectives: The main objective of this study was to estimate direct OOPE and find the association between direct OOPE and noncompliance to TB treatment in intensive phase.

Methods: A cross-sectional study was conducted with TB patients who were registered in Kalutara-district chest clinic for period of six months (n = 267). Interviewer-administered questionnaire (consisted of sections on socio-demographic characteristics, treatment compliance, sources and amount of OOPE, etc.) was used to collect data. Mean median, minimum, maximum and interquartile range were calculated in each component of OOPE.

Results: Questionnaire were administered for 252 patients (male = 160, 63.5%). Mean total direct non-medical cost for one DOTS visit (without accompanied person) was 435.40 (IQR = 420.00) Sri Lankan Rupees (SLR) (i.e., 2.45 United State Dollars (USD)). A patient without an accompanying person spent 26124.00 SLR (435.40 per day into 60 days) (i.e., 146.76 USD) for transport and food during the intensive phase. During the intensive phase, the mean medical cost for one patient was 6444.66 LKR (IQR = 6400) (i.e., 36.21 USD). OOPE was not associated with noncompliance to TB treatment in intensive phase (p = 0.29).

Conclusions: There was no association between OOPE and noncompliance. The direct OOPE for TB treatment in the intensive phase was high. Therefore, it is necessary to develop strategies to reduce OOPE during TB treatment especially in intensive phase.

* *Corresponding author*. No 123 Andadola Wathugedara Sri Lanka.
E-mail address: ranawakanithin@yahoo.com (N. Ranawaka).

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1. Background

Tuberculosis is "an infectious disease caused by the bacillus - *Mycobacterium tuberculosis* and occasionally by *Mycobacterium bovis* and *Mycobacterium africanum*. Tuberculosis (TB) commonly affects the lungs but can affect any other organ in the body".[1]

TB is a one of main public health concern all over the world. It is estimated that 10 million people contracted with TB in 2019 worldwide. One point two million TB deaths were reported among HIV-negative people.[2] The number of TB patients reported in Sri Lanka was 8846 in 2018 and TB related mortality, excluding TB-HIV, was 3.2/100,000. Out of the total number of TB patients in Sri Lanka, district of Kalutara reported 6.97% of TB patients (n = 617).[3] In fact, Sri Lanka is a low TB burden country. TB incident in Sri Lanka is 64 patients per 100,000 population.[4]

"National Program for Tuberculosis Control and Chest Diseases" (NPTCCD) is a national institution liable for TB and other chest disease control activities in Sri Lanka. For administrative purposes, Sri Lanka is divided into districts (n = 25). There is a District Chest Clinic (DCC) in each district responsible for all TB control activities of the district. NPTCCD and the regional directorate of health services provide administrative and technical support to DCC. Under each DCC, there are several branch chest clinics, microscopic centers, and Directly Observed Treatment – Short course (DOTS) centers. If patients are diagnosed with TB, they will be directed to the DCC for registration. After registration, those patients will allocate to the DOTS center according to the patients' preferences. DOTS provider will observe the drug intake by patients.

OOPE generally refers to direct payments by patients or their families for diseases after subtracting third-party payments such as insurance. OOPE consisted of direct expenditure such as expenditures for travel to clinic or DOTS center, expenditure for foods during clinic or DOT visit, spending for a person go along with patient (direct non-medical cost), expenditure for drugs or investigation (direct medical cost), and income loss due to clinic/DOTS visit (Indirect cost).[5] Even though the government provides TB drugs and investigations free of charge, patients may need to spend money for vitamins/drugs used to control side effects of anti TB drugs, or the patient may do investigation by private laboratories. TB patients may need to spend a comparatively high amount of money, especially on direct non-medical costs, because they need to attend daily to the DOTS center for drug intake during the intensive phase.

As TB patients need to take treatment for at least period of 6 months, patient or family had to spent considerable amount of money during and after the treatment.[6] Studies from other countries show that the high cost for TB may implicate on loss of compliance, blockade and delay the access to care, increase vulnerability and risk of contracting TB, and aggravation of poverty.[7,9,10] Despite free health care delivery, many patients lose substantial working days and money due to TB, especially in the intensive phase.[8] The poor compliance among TB patients leads to poor health outcomes, increasing healthcare service utilization, resulting in high cost to government and individuals. The main objective of this study was to estimate the direct OOPE and to find its association with noncompliance to TB treatment in intensive phase in Kalutara district, Sri Lanka.

2. Methodology

Cross-sectional study was conducted at DCC Kalutara; the only DCC for the entire district of Kalutara. All the patients diagnosed with TB within Kalutara district were referred to DCC Kalutara for treatment. Referred patients will be registered in District Tuberculosis Register (DTR) and allocated for the DOT center. Some patients may take DOTS from DCC Kalutara. This study aimed to calculate OOPE for intensive phase. Therefore, patients who have finished the intensive phase but have not completed the continuation phase are considered for study. Study was started from January 2018. Thus, patients registered from 1st of October 2017 to 31st March 2018 were included to study (e.g. Patient who was registered to DCC 1st of October will finish intensive phase December 1st and will finish continuation phase in April 1st, patient who were registered to DCC on March 31st, will end intensive phase May 31st and will end continuation phase September 31st). As describe above, this research was carried out from January to October in 2018, though study population comprised the TB patient registered in DTR from 1st of October 2017 to 31st March 2018.

All TB patients (aged 18 years and above) registered in TB Register of Kalutara District (n = 304) (1st of October, 2017 to 31st March, 2018) were included for the study. Some patients were excluded from the study, such as previously treated patients, hospitalized patients, and patients not in sound mind to give consent. Entire eligible study population (267 TB patients) was selected for study. Therefore, no sampling methods were used.

The interviewer-administered questionnaire was used to collect necessary information. Some data was extracted from registers and clinic records (i.e., TB register, TB treatment card and TB follow up card). Registered patients are required to attend chest clinic (Kalutara) for a consultation by chest physician. Interviews were conducted during those visits to DCC. The questionnaire consisted of information on sociodemographic characteristics, treatment compliance, and OOPE. The TB treatment noncompliance was defined in the present study as "any patient missing or interrupting of taking anti TB drugs consecutively or intermittently for four days or more per week within intensive phase". Data were entered to the "Epidata" software. Then data exported to Statistical Package for the Social Sciences (SPSS) and analyzed by SPSS version 22. The OOPE profile for patients was calculated and presented as mean and median. Values were originally calculated in Sri Lankan rupees (SLR) and converted to US dollars (USD) based on the exchange rates at the time of data collection. Then association between noncompliance and OOPE was assessed by Chi-square test. To assess the association between noncompliance and OOPE, patients were categorized into two groups by the median value of indirect cost component for the patients. Ethical approval for this study was gained by the Postgraduate Institute of Medicine (PGIM), University of Colombo.

3. Result

About 90% (n = 227) of patients expended for transport and 36.9% (n = 93) of patients expended for food for themselves (Table 1). Sizable proportion of patients spent expenses of travelling (33.4%, n = 84) and food (29.8%, n = 75) of an accompanied person. Without an accompanied person, patient spent SLR 435.40 (IQR = 420.00) (i.e., 2.45 USD) for attending a DOT center. On the other hand, with an accompanied person, patient spent SLR 682.74 (IQR = 610.00) for attending a DOT center (i.e., 3.84 USD, IQR = 3.42 USD) (Table 1). Therefore, during the intensive phase, the total amount of money spent by a patient without an accompany person was SLR 26124.00 (435.40 per day for 60 days) (i.e., 146.76 USD). Without an accompany person, a patient spent 7.5 folds higher amount of money, if the patient expected to visit for daily DOTS instead of weekly DOTS (SLR 26124.00 vs. 3483.20).

About 10% (n = 27) of patients have expended money for drugs. In addition 27% (n = 68) of patients expended for investigation. The average drug cost was SLR 2576.00 (IQR = 2200) (i.e., 14.47 USD) and average investigation cost was SLR 3868.66 (IQR = 4000) (i.e., 21.73 USD). According to the result, on average, one TB patient has spent a total of SLR 6444.66 (IQR = 6400) (i.e., 36.21 USD) (Table 1) for drugs and investigations (mean direct medical cost) during the intensive phase. . Further, 41.8% (n = 61) of patients loss their income due to DOTS (Table 3). According to the result of the current study, there was no association between OOPE and noncompliance with TB treatment in intensive phase (χ^2 = 1.0788, p = 0.299) (Table 2).

4. Discussion

The current study has aimed to estimate the OOPE and its' association with noncompliance to TB treatment during the intensive phase. Sri Lanka has free health system, though TB patients spend a considerable amount of money during intensive phase of TB treatment. According to the result, about 90% of participants have borne direct non-medical cost and only 27.0% of participants (Table 1) have borne the direct medical cost compared to study done in India 40.3% of study subjects bore direct non-medical cost and 7% of study subjects bore direct medical cost.[11] It revealed that a larger proportion of TB patients have borne OOPE in Sri Lanka than India in the intensive phase, especially the non-medical cost component. Unequal/inadequate distribution of DOT centers or reluctance of the patients to visit DOT centers near their accommodations due to stigma may be the reason for the high percentage of non-medical cost in Kalutara district. This need to confirmed or further elaborate by another research.

TB patient has to spent 435.40 LKR (i.e., 2.45 USD) (Table 1) for transport and food (total mean non-medical cost) per one DOTS visit compared to India, 60 Indian rupees (0.78 USD). Total average medical cost for a TB patient in Sri Lanka was 6444.66 LKR (i.e., 36.21 USD) (Table 1) compared to India, 300 Indian rupees (3.93 USD).[11] This revealed that direct cost (non-medical and medical costs separately and in total) bore by TB patients in Sri Lanka were considerably higher than in India. However, TB patients in some countries have spent substantially higher OOPE in intensive phase compared to Sri Lanka.[12],[13] There are significant differences in health systems between the countries and other contributing factors to cost components such as transport cost. Therefore, it is difficult to compare OOPE between different countries.

It was hard to find any previous studies on OOPE for TB treatment in Sri Lanka to compare with the current study. According to the current study, there was no any significant association between OOPE and noncompliance to TB treatment (χ^2 = 1.0788, p = 0.299) (Table 2). However, some studies revealed the association between OOPE and treatment adherence showing that higher OOPE was inversely associated with treatment adherence.[12]

According to the manual published by NPTCCD in 2016, all the TB patients should observe daily for drug intake by DOTS provider or health care worker at least in intensive phase. Though, in practice, some patients attended for daily DOTS. In contrast, some patients attended for weekly DOTS. If a TB patient attended for daily DOTS, the mean average direct OOPE (without accompanying person) needed to bear was

Table 1 – Direct out of pocket expenditure of TB patient in intensive phase.

	number n (%)	average (SLR)	median (SLR)	range min. - max	IQR
Direct non-medical cost component					
For patient					
Travelling	227 (90.1)	338.44	200.00	35.00–2000.00	380.00
Food	93 (36.9)	96.96	100.00	30.00–200.00	40.00
Sub total		435.40	300.00		420.00
For accompanied person					
Travelling	84 (33.4)	155.85	160.00	40.00–300.00	140.00
Food	75 (29.8)	91.49	100.00	30.00–200.00	50.00
Sub total		247.34	260.00		190.00
Total direct non-medical cost (one day)		682.74	560.00		610.00
Direct medical cost components					
Drug cost	27 (10.7)	2576.00	2000.00	1800.00–4000.00	2200.00
Investigation cost	68 (27.0)	3868.66	1000.00	600.00–20000.00	4000.00
Total direct medical cost		6444.66	3000.00		6400.00

Table 2 – Association between TB treatment noncompliance and out of pocket expenditure.

	Compliant n (%)	Non-compliant n (%)	Total n (%)	Significance df,χ^2, p
OOPE				
More than 200SLR	108 (79.4%)	28 (20.6%)	136 (100)	df = 1 χ^2 = 1.0788, p = 0.299
200 or less SLR	98 (84.5%)	18 (15.5%)	116 (100)	
Total	206 (81.7%)	46 (18.3%)	252 (100)	

Table 3 – Distribution of participants according to the loss of income due to DOTS attendance.

	Number	Percentage
Loosed income due to DOTS attendance	61	41.8
Not loosed	85	58.2
Total	**146**	**100.0**

26124.00 SLR (435.40 per day into 60 days) (i.e., 146.76 USD) compared to 3483.20 SLR (i.e., 19.57 USD) in weekly DOTS. Hence, patients who attended for daily DOTS bore substantially higher OOPE compared to patients who attended for weekly DOTS and expenses for daily DOTS would be catastrophic for some patients.

Patients may not be able to attend their jobs due to clinic or DOTS attendance. It increases the financial burden to the patient.[8] According to the current study, 41.8% of TB patients lose their earnings/wages due to DOTS compared to 46% in India.[8]

5. Conclusions

TB patients had to bear high OOPE for TB treatment in the intensive phase despite available free health care services in Sri Lanka. However, there is no statistically significant relationship between OOPE and noncompliance in intensive phase.

As OOPE is a financial burden for TB patients, it is necessary to provide monetary assistance or materials assistance by Government to the patients with low income.

6. Limitations

The present study limited to District of Kalutara and findings were summarized by descriptive statistics. So, the generalizability of the results to the entire country would be low. The study was conducted only for six months. Therefore, seasonal changes could be missed.

Declarations ethical approval and consent to participate

Ethical approval for the proposal was obtained from PGIM, University of Colombo, Sri Lanka. Written informed consent was taken from all the participants.

Consent to publish

Relevant administrative authorities and all co-authors have given consent to publish.

Availability of data and materials

Datasets and materials support to the finding of this study are available with the corresponding author.

Conflicts of interest

The authors have none to declare.

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Original article

Utilization of artificial intelligence for tuberculosis screening in Nepal

Data Ram Adhikari [a,*], *Young Ae Kang* [b], *Sujan Gautam* [c,**], *Padam Kanta Dahal* [d]

[a] *Ministry of Health and Population, Nepal*
[b] *Yonsei University College of Medicine, Severance Hospital, Seoul, South Korea*
[c] *Manmohan Memorial Institute of Health Sciences, Nepal*
[d] *School of Health, Medical and Applied Sciences College of Science and Sustainability, Central Queensland University, Sydney Campus, Australia*

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ABSTRACT

Backgrounds: : Tuberculosis (TB) is an infectious disease that needs to be diagnosed and enrolled for treatment. Artificial intelligence for TB (AI4TB) software screens TB suspected cases at the point of care and helps in quick diagnosis. This study aims to explore the significance and usefulness of AI4TB by comparing its performance with different diagnostic test results.

Methods: A cross-sectional study was conducted among 197 participants who had symptoms suggestive to TB. The chest X-ray images were analyzed by AI4TB software and human expert readers. The bacteriological test results were obtained, and Kappa test was applied to calculate the inter-reader reliability. The sensitivity, specificity, positive predictive value and negative predictive value was calculated and ROC curve was generated.

Results: Among 85 sputum smear microscopy, about 21% of the had sputum positivity rate. At 0.4 threshold: 62.4%, at 0.5 threshold: 58.4% and at 0.6 threshold: 50.3% symptoms suggestive cases were identified having abnormal X-ray images. Reader-I identified 28.4% and Reader-II identified 37.1% of the symptoms suggestive cases of TB as positive cases. There was a significant substantial agreement between two human expert readers (k-0.783, p-value: <0.001). The ROC curve explored the fair sensitivity accuracy of the AI4TB test results at 0.5 threshold level (AUC = 0.72) and at 0.6 threshold level (AUC = 0.77).

Conclusion: The sensitivity of the AI4TB was higher compared to different human readers. AI4TB can be the relevant screening tool for the TB symptoms suggestive cases prior to the laboratory test in the countries like Nepal with deficient health manpower.

* *Corresponding author.*
** *Corresponding author.*
E-mail addresses: datarama64@gmail.com (D.R. Adhikari), gautamsujan@gmail.com (S. Gautam).
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1. Introduction

Tuberculosis (TB) is a global public health problem and is one of the 13th leading cause of death and second leading infectious killer after COVID-19.[1] It is an infectious disease with high mortality and morbidity. Estimated around 10 million people were infected with TB, and 1.5 million people had died in 2020.[1] More than 95% of deaths occurs in low income countries.[2] According to World Health Organisation (WHO), TB deaths have increased during the COVID-19 pandemic in 2021 with fewer people diagnosed, treated and provided preventive services compared with the previous years.[3] Further, United State Dollar (USD) 13 billion were required annually for the TB prevention by 2022.[1] However, the causative organism was invented over hundred years ago and highly effective medicines and vaccine were also available.[4]

Currently, Nepal is facing the burden of TB where the prevalence is 416.3 per 100,000 population in 2019[5] and 9.9% among people living with HIV[6] and is sixth leading causes of death.[7] According to the national tuberculosis prevalence survey 2018/2019, over 117,000 people are living with TB and over 69,000 were newly diagnosed.[5] It's prevalence is 1.6 times higher in comparison to the previous estimate.[5] The survey indicated the higher TB prevalence among the senior citizen and higher burden than the previous estimate. However, report indicated that around 60% of infected cases need to be diagnosed and enrolled for treatment each year which indicated that there is the requirement of rapid screening and diagnostic system in health facilities, and medical expertise such as radiologist.[8] Further, more TB cases could be identified by using these latest screening techniques and tools.

WHO has introduced the artificial intelligence for TB (AI4TB) for diagnosis of TB in the high burden countries in 2010 and updated their TB screening guidelines in 2021.[9] The guideline recommended computer aided detection-based tuberculosis (CAD4TB) digital chest x-ray for tuberculosis screening and triage in individuals more than fifteen years of age.[9] In the first stage, it included digital radiography (Digital X-ray, DR) for taking chest x-ray which is necessary to collect digital communication in medicine (DCM) image files to operate AI4TB solution.[10] The DCM format CXR images files proceed to AI-based TB screening Algorithm (AI4TB) software to check abnormality by system picture archiving and communication system (PACS) to manage DCM data. Then, the AI4TB software suggests that it can automatically analyse Chest X-ray to detect anything abnormal and indicate the likelihood of active TB based on the abnormality score like 0%–100%. The "0" indicate the normal chest x-ray images and 100% indicates the highly likelihood of TB.[10] Thus, the score can be read without any skilled personnel and has better capacity to screening the symptoms suggestive TB cases rather than the clinically screening. It reduced time and cost, eliminates the subjectivity of the reader enables more objective image reading which might be useful for human resource scare countries with high prevalence of TB.[11,12] However, the artificial intelligence-based digital radiography chest x-ray reading system for screening of TB suspected cases are not available in Nepal due to poor socio-economic condition.[13,14] There are limited studies to understand the usefulness of AI4TB screening in the developing countries including Nepal. This will be one of the pioneer study to explore the usefulness of AI4TB in Nepal. Therefore, the objectives of this study are to a) assess significance and usefulness of artificial intelligence for tuberculosis screening, b) compare the performance of the AI4TB readings with the result of human experts reader of different experience level, and c) assess the sensitivity of AI4TB software.

2. Materials and methods

2.1. Study design and setting

This is a descriptive cross-sectional study. The data for this study was acquired from the Industry and Trade Cooperation Development Support Project, KOHEA, collected during 2016–2017 at two sites: Nepal Tuberculosis Center (NTC), Bhaktpaur, Bagmati Province; and District Hospital, Lamjung, Gandaki Province. NTC is the tuberculosis disease referral central hospital located in Bhaktapur district, proximal to capital city of Nepal, and Lamjung District Hospital is located in remote areas of Nepal, distant from the capital city. Lamjung district hospital is also the referral hospital however, TB cases are referred only from the peripheral health facilities such as sub-health posts, health posts and primary health care centers.

2.2. Study participants and data collection

A total of 197 participants aged above 15 years who had symptoms suggestive to tuberculosis attending both the study sites were the participants for this study. All the participants were undergone the chest X-ray. The chest X-ray images of all the participants were analyzed by the AI4TB software (considered as Reader-III in this study) and also the same set of chest X-ray images were read by the two human experts; Reader-I and Reader-II (both are pulmonologist). The human Reader-I had about 10 years of experience in tertiary level hospitals in South Korea, and Reader-II had more than 20 years of experience in TB field in South Korea. Similarly, 85 bacteriological laboratories test results (Microscopic AFB-73 and Xpert-12) of the total cases were obtained from NTC, Nepal. A total of 197 patients were selected purposively from both the study sites.

2.3. Data analysis

The data was analysed using SPSS version 24. The AI4TB threshold or cut-off points were set at level 0.4 (40%), 0.5 (50%) and 0.6 (60%) for classification of abnormality of chest X-ray images score. The AI4TB score was compared with the two human experts reader result. Frequency and proportions were reported. Further, the Cohen's Kappa statistics was calculated to compare the inter-reader agreement (inter reader reliability)[15] of the AI4TB (Reader-II) with two human readers (Reader-I and Reader-II) and AFB sputum smear microscopy or X-pert (Reader-IV). The AFB sputum smear microscopy or Gene X-pert test was considered as the Gold Standard test for the confirmation of TB. The sensitivity, specificity, positive

Table 1 – Demographic and clinical characteristics of screened cases with radiography (CXR) and laboratory test.

Variables			Number	Percent (%)	Mean age
Age	<20		37	19.8	35.49
	20–29		43	23.0	
	30–39		36	19.3	
	40–49		25	13.4	
	50–59		13	7	
	>60		33	17.6	
	Total		187[a]	100	
Sex	Male		107	57.2	–
	Female		80	42.8	–
	Total		187[a]	100	–
Hospital	NTC		191	97	–
	Lamjung		6	3	–
	Total		197	100	–
Reader-I	TB		56	28.4	–
	No TB		141	71.6	–
	Total		197	100	–
Reader-II	TB		73	37.1	–
	No TB		124	62.9	–
	Total		197	100	–
Reader-III (AI4TB)	0.4 (40%) (Threshold)	Abnormal	123	62.4	–
		Normal	74	37.6	–
	0.5 (50%) (Threshold)	Abnormal	115	58.4	–
		Normal	82	41.6	–
	0.6 (60%) (Threshold)	Abnormal	99	50.3	–
		Normal	98	49.7	–
	Total		197	100	–
Reader-IV (Bacteriological Lab Report)	TB		18	21.2	–
	No TB		67	78.8	–
	Total		85	100	–

[a] Ten cases had missing data on age and sex.

predictive value (PPV) and negative predictive value (NPV) of AI4TB and human readers were calculated explored with reference to the bacteriological test result. Similarly, Receiver operating characteristic (ROC) curve was generated to compare the detection of TB results among various readers.

2.4. *Ethical approval*

As the study was based on the secondary data collected from the KOHEA, no separate ethical approval was sought. Nonetheless, the ethical approval for this study was obtained from Institutional Review Board of the Graduate School of Public Health, Yonsei University, South Korea Similarly, permission to use the data were granted from the director of both study sites; NTC (Ref: 619/26, April 2018) and Lamjung district hospital.

3. Results

Of the total 197 symptoms suggestive cases of TB identified; 10 cases missed to report age and sex. Of the 187 cases, more than half (57.2%) were male. Most of the cases (23%) were at the age group 20–29 years (mean age: 35.79 years). Most of the cases (97%) had their test done at NTC. A total of 85 cases had bacteriological laboratory result (sputum AFB microscopy or Gene Xpert) with 21.2% positivity rate and 78.8% negative results. At 0.4 threshold: 62.4%, at 0.5 threshold: 58.4% and at 0.6 threshold: 50.3% symptoms suggestive cases were identified having abnormal X-ray images. Reader-I identified 28.4% and Reader-II identified 37.1% of the symptoms suggestive cases of TB as positive cases (Table 1).

We analysed the readings agreement of various reader (inter-reader agreement) and kappa-statistics was calculated. There was a significant substantial agreement between two human expert readers: Reader-I and Reader-II (k-0.783, p-value: <0.001). Similarly, there was also a significant moderate inter-reader agreement of human experts (Reader I and Reader II) with AI4TB at different threshold levels (Table 2).

Likewise, we also evaluated the different tests results (Human experts: Reader I and Reader II; and AI4TB) with the gold standard test (bacteriological test). The sensitivity of the AI4TB at different levels (94.4% at all the threshold levels) were

Table 2 – Inter-reader agreement, Reader-I vs Reader II, Reader-I vs AI4TB and Reader-II vs AI4TB.

		n = 197	
Readers		Kappa-value (k)	P-value
Reader-I vs Reader-II		0.783	<0.001
Reader-I vs AI4TB	0.4	0.386	<0.001
	0.5	0.422	<0.001
	0.6	0.504	<0.001
Reader-II vs AI4TB	0.4	0.485	<0.001
	0.5	0.513	<0.001
	0.6	0.594	<0.001

Table 3 – Performance comparison of Artificial Intelligence (AI4TB) with Human Expert Reader-I and Reader-II.

Diagnostic Test		Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
Laboratory test (Gold standard)		Reference	Reference	Reference	Reference	Reference
Reader-I		88.9%	86.6%	64.0%	96.7%	0.87 (0.78–0.97)
Reader-II		88.9%	76.1%	50.0%	96.2%	0.82 (0.72–0.93)
Reader-III (AI4TB)	0.4 (40)	94.4%	44.8%	31.5%	96.8%	0.69 (0.58–0.81)
	0.5 (50)	94.4%	50.7%	34.0%	97.1%	0.72 (0.61–0.84)
	0.6 (60)	94.4%	61.2%	39.5%	97.6%	0.77 (0.67–0.88)

higher compared to the different human readers (Reader I and Reader II: 88.9%) (Table 3).

Similarly, ROC curve was created and Area under the curve (AUC) was reported. The ROC curve explored the fair sensitivity accuracy of the AI4TB test results at 0.5 threshold level (AUC = 0.72) and at 0.6 threshold level (AUC = 0.77) (Fig. 1).

4. Discussion

This cross-sectional study is one of the pioneer studies for TB screening in Nepal aiming to explore the significance and usefulness of artificial intelligence for TB screening and comparing its results with different other test results. The selected sites were the public health institutions; one being the referral central TB centre; and other being the rural referral hospital in an area with high prevalence of TB. We analysed the abnormal chest X-ray images of the participants using different human expert reader and artificial intelligence for TB screening (AI4TB). We also evaluated the inter-reader agreement of different reader, and also compared the sensitivity and specificity of the TB screening test different test results with gold standard test (bacteriological test results).

In our study, the different human expert reader (Reader I: 28.4%; and Reader II: 37.1%) reported abnormal chest X-ray images as TB positive cases. Our findings were lower compared to the study done in South Africa which reported that 49% had abnormal CXRs by the radiological grading.[16]

Our study identified high proportion of chest X-ray images at the 0.4 threshold level cut off point of AI4TB (62.4%), revealing that the cut-off point and the abnormality proportion were directly proportional. Prior studies also indicated the similar findings, i.e., the higher threshold level had high chance of giving high scored.[9,11,16,17] Additionally, the findings of the bacteriological laboratory test (Gene Xpert/AFB microscopic test) of our study were close to the study done in Bangladesh.[18]

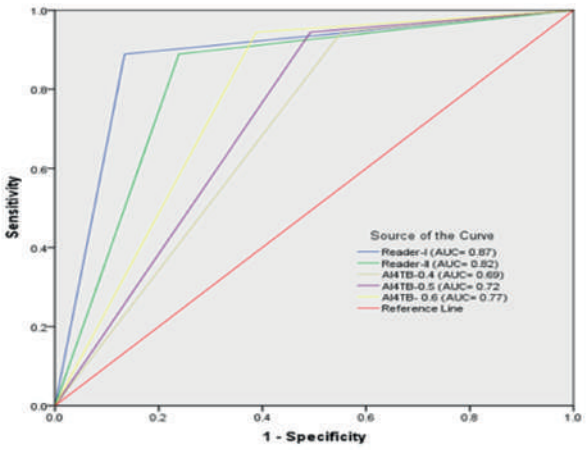

Fig. 1 – The ROC curves analysis for the detection of tuberculosis using the bacteriological laboratory test result reference.

Our study demonstrated the statistically significant relationship between human Reader-I vs Reader-II (k = 0.78). Further, the inter-reader agreement between AI4TB at different thresholds with two human expert readers had significant moderate agreement. This shows that the AI4TB system is very useful for TB screening prior to the bacteriological test. These findings were similar to the studies conducted in Lusaka, Zambia, where authors used same method (kappa test) to compare clinical officers and computer aided detection of tuberculosis (CAD4TB), which showed moderate (k = 0.49–0.67) to substantial (k = 0.61) inter-reader agreement with the radiological findings.[12]

The sensitivity and NPV of the AI4TB illustrated that the performance of the AI4TB was comparable with the human readers. The previous study showed slightly lower values of sensitivity and NPV, however values for specificity and PPV were slightly higher for CAD compared to X-pert test results.[11] Furthermore, a study by Qin et al., in Bangladesh showed that sensitivity and specificity are lower than CAD4TB (sensitivity:90.0%, 95% CI:89.0–91.0; and specificity: 72.9%, 95% CI: 72.3–73.5).[18] Qin Zhi Zhen et al., and his team explored sensitivity of CAD4TB is 0.95 (95% CI) at threshold level 57 (0.57) and specificity: 0.8 (95% CI) which is closely similar to our study report at 0.6 (60) threshold level. Thus, the use of the AI4TB screening system might be a good triage tool in passive and active case finding of tuberculosis.[19]

The area under the curve (AUC) in ROC curve for AI4TB sensitivity accuracy is almost more than 0.5, suggesting the strong ability of the software for TB screening and high chance of TB detection among the suspected case. For AI4TB, the ROC curve showed that at 0.5 threshold level, AUC was 0.72 (95% CI: 0.61–0.84) which was nearly close to performance of CAD4TB in previous study.[12] In the previous study by Philipsen et al., demonstrated that at 0.63 threshold, AUC was 0.74 (95% CI: 0.73–0.75). The study done by ZZ Quin et al., shows that three Deep Learning (DL) systems curves of the ROC were above the diagonal line in which Lunit (0.94, 95%CI:0.93–0.96), qXR (0.94, 95% CI: 0.92–0.97) and AUC point of CAD4TB (0.92, 95% CI: 0.90–0.95).[10] However, our study indicated the better AUC value (AUC: 0.77, 95% CI: 0.67–0.88) at even 0.6 threshold level.[20]

The sensitivity of the AI4TB was higher compared to different human readers. Similar was the findings in the previous studies conducted at similar settings showing that AI4TB has higher true positive and lower fall negative rates[10,19]. The features of ROC curve showed that the AUC of

AI4TB is comparable with the human reader AUC, which shows that AI4TB can be the relevant and complete screening tool for the TB symptoms suggestive cases in the country like Nepal with deficient health manpower.

The strength of this study is the direct comparison of the artificial intelligence ability with pulmonologists result. However, a smaller number of study site and samples are the limitations of the study.

5. Conclusion

AI4TB is an important digital technology to score chest radio images by using the artificial intelligence-based software program. It can determine the situation of CXRs based on the score, performance was higher and comparable with other human readers. Further to this, AI4TB help reduces the time and human resource for the diagnosis of TB. Therefore, it might be a useful tool prior to the laboratory test without human readers in high TB prevalence countries like Nepal.

Authorship contributions

Concept were proposed by Dataram Adhikari and Young Ae Kang. Data collection and analysis were conducted by Sujan Gautam, Padam Kanta Dahal and Dataram Adhikari. All authors equally contributed on manuscript preparation.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors have none to declare.

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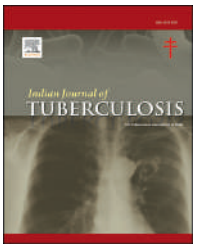

Original article

Effect of pandemic on DOTS treatment during COVID-19 lockdown- A cross-sectional study

Satish Kabbur [a,*], *Bhagyashree Patil* [b], *Mubashir Angolkar* [a], *Ashwini Narasannavar* [a]

[a] *Department of Public Health, J N Medical College, KAHER, Nehru Nagar, Belagavi, 590010, India*
[b] *Department of Respiratory Medicine, J N Medical College, KAHER, Nehru Nagar, Belagavi, 590010, India*

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ABSTRACT

Background: Tuberculosis (TB) is still the most common infectious disease globally, affecting 1.5 million people per year. Prior to COVID-19 outbreak, India was struggling with a rampant attack of Tuberculosis. With the surge of COVID-19 implementation of all national health programs including NTEP was disrupted. Prioritization of services, the challenges to reaching all types of communities and the role of stigmatization, and the possibility of increased disease transmission were few problems in the implementation of DOTS during the lockdown.
Aim: To assess effect of pandemic on DOTS treatment during COVID-19 lockdown.
Methods: A cross-sectional study was conducted among 254 tuberculosis patients who were under DOTS during Covid-19 lockdown in Belagavi district. Participants who were on DOTS during 2019–2021 period.
Result: Of 254 participants, only 5 (2.0%) were supervised while taking drugs, 67 (26.4%) of subject's empty blister packs were taken back by health personnel and 106 (41.7%) participants were regularly followed up for treatment by health department. The variables like gender, literacy status, socioeconomic status, and occupation were all significantly associated with hampered access to DOTS during the lockdown period at $p < 0.05$.
Conclusion: This study concluded that the participants had hampered accessibilities to DOTS during lockdown.

1. Introduction

Tuberculosis (TB) is still the most common infectious illness in the world, affecting 1.5 million people per year.[1] India is the world's largest TB burden country.[2] Before COVID-19 becoming a global pandemic, India was struggling with an outbreak of TB, which had infected 2.64 million Indians in 2019 and killed almost 4,50,000 individuals. That's approximately 1000 TB mortality every day.[3]

China first disclosed a group of instances of uncommon pneumonia associated with SARS-CoV-21 on Dec 31, 2019. The incidence of COVID-19 cases across India had begun to rise

* *Corresponding author*. Department of Public Health, J N Medical College, KAHER, Nehru Nagar, Belagavi, India.
E-mail addresses: satishkabbur637@gamil.com (S. Kabbur), bhagyashrisantosh@gmail.com (B. Patil), drmubi@gmail.com (M. Angolkar), drashwinimph2004@gmail.com (A. Narasannavar).
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since Mar 2020. India declared a lockdown to control it from Mar 25, 2020.[4]

India had achieved tremendous effort toward its goal of eliminating tuberculosis until March 24, 2020. When the country-wide lockdown was imposed due to COVID-19, it disrupted all important strategic interventions, resulting in a nearly 60% reduction in TB case detection during lockdown time.[5] Similarly, about 5.7 lakh instances (62% decline in notifications) were unable to be notified against the expected objective of NTEP from January to June 2020.[5]

During lock-down times, health facilities observed a reduction in the proportion of patients undergoing treatment on time due to the fear of getting SARS-CoV-2 virus infection. This might have had serious difficulties for tuberculosis control, particularly drug-resistant TB. The re-allocation of testing facilities and health care services to combat COVID-19 may have affected TB detection and treatment unintentionally. Other difficulties may have arisen under the TB program as a result of the pandemic, including a shortage of finance for Tuberculosis intervention, disruptions in utilization, and accessibility to drugs and supply.[6] Reduced availability of conventional modes of transportation and increased transportation expenses constituted hurdles to accessing healthcare centers for both healthcare practitioners and patients.[7] Lockdown has caused delays in diagnosis, treatment, and disease transfer to household contacts. It also has created disruption in treatment as many employees migrated back to their native places.[3] Even routine activities like TB case detection, active case finding, contact tracing, etc were held up not only in India but also in other countries. Different virtual approaches were considered to provide services.[5] The World Health Organization (WHO) recently released statistics from more than 80 nations that revealed a 21% drop in treatment during the 1st year of the pandemic compared to 2019. More than 469 million people had been infected with COVID-19 as of March 22, 2022, with over 6 million deaths. (WHO Report 2022) The COVID-19 pandemic has now surpassed all other health concerns around the planet. Hence this aims to assess the Effect of pandemic on DOTS treatment during COVID-19 lockdown.

2. Methodology

The present study was cross-sectional study conducted between November 2021 to April 2022. This study was conducted in 3 talukas of Belagavi (Hukkeri, Gokak & Belagavi). The sample size was calculated using the formula n = Z2 pq/d2 where p = drop of TB case notification during covid-19,[5] with precision of 10% and 95% of CI. Assuming 5% attrition the calculated sample size was 254.

Proportionate sampling technique was used to select participants from 3 talukas and from each taluka participants were recruited using simple random sampling after line listing. Data collection carried out by using a semi-structured questionnaire. Data obtained were tabulated, chi-square test was used to assess the effect of pandemic on DOTS treatment during lockdown.

2.1. *Inclusion criteria*

- Patients enrolled for treatment under NTEP (RNTCP) programme.
- Tuberculosis patients who were above 18 years above and willing to give informed consent.

2.2. *Exclusion criteria*

- Patients who were seriously ill.
- Patients who were not under DOTS during the lockdown period.

2.3. *Ethical committee*

- Ethical clearance was obtained from the Institutional Ethical Committee (I.E.C) of J.N. Medical College, KAHER, Belagavi. Ref no: MDC/DOME/263
- Permission was taken from State Task Force Operational Research Committee, NTEP to conduct a study. Ref no: LWSTC/NTEP/PPM/07/2021-22

3. Results

This present study was conducted between November 2021 to April 2022. Study included total 254 participants. Table 1 shows that the participant's ages ranged from >18 to 83 years. Most of the participants 147 (57.9%) were males and 107 (42.1%) were females. The majority of them, that is 83 (32.7%) had completed their primary education, 30 (11.8%) participants had completed their graduation, 94 (37%) of them had completed their higher secondary education, and 47 (18.5%) were illiterate. 105 (41.3%) were from joint families, and 149 (58.7%) were from nuclear families, majority were from rural region 175 (68.9%) and 79 (31.1%) inhabited urban regions.

Out of 254 participants 241 received DOTS treatment, only 5 (2.0%) of the all participants were supervised by a DOTS provider while taking drug, only 5 (2.0%) were supervised during the lockdown, 67 (26.4%) of participants empty blister packs were collected by health personnel, 97 (38.2%) of participants skipped their course one or more days, 119 (46.9%) of participants said that they were disappointed due to inaccessibility, 136 (53.5%) had been communicated by health personnel, Regular follow-up of treatment from the health department was 106 (41.7%) (Table 2).

- There were 7 questions related to hampered access to DOTS, mean of these 7 questions was considered to categorize in to three categories based on the mean and standard deviation (SD)
- Among 254 participants, the mean and SD of hampered access to DOTS was 4.6 and 1.27 during lockdown.
- Among all participants 15 had low hampered access to DOTS during lockdown. Similarly, 225 participants during

Table 1 – Demographic details of the study participants.

Variable	Category	Frequency	%
Age	≤ 30	93	36.6
	31–42	59	23.2
	43–54	42	16.5
	55–66	45	17.7
	67–78	14	5.5
Gender	Male	147	57.9
	Female	107	42.1
Literacy status	Illiterate	47	18.5
	Primary	83	32.7
	Secondary	94	37
	Graduate and above	30	11.8
Occupation	Labour	21	8.3
	Agriculture	74	29.1
	Private employee	42	16.5
	Government employee	8	3.1
	Others	109	42.9
Socio economic status	Upper class	15	5.9
	Upper middle class	82	32.3
	Middle class	91	35.8
	Lower middle class	61	24.0
	Lower class	5	2.0
Type of Family	Joint Family	105	41.3
	Nuclear Family	149	58.7
Residency	Rural	175	68.9
	Urban	79	31.1
Type of house	Pucca	71	28.0
	Kaccha	183	72.0

lockdown were in the category of average hampered. Similarly, 14 were high hampered.

During lockdown gender, occupation, literacy status, and socioeconomic status are the variables that are statistically significantly associated with hampered access to DOTS at $p < 0.05$ (Table 3). The remaining variables like age, type of family, type of house, and residency status were not statically significant.

4. Discussion

This chapter provides an insight into the discussions of the study's major findings while comparing it to the findings proposed in the previous studies.

4.1. *Socio-demographic variables of participants*

In the present study, the age of participants was in the range of 18–83 years. Surveys conducted in Brazil, Italy reported that study participants' age was between 18 and 60 years.[8,9]

In our study majority are males, similarly a study conducted in Zambia had 53% male participants and 47% females,[10] but a study in Brazil had 57% female participants and the remaining were male.[8]

The present study revealed that majority had completed primary and secondary education (32.7%, 37%), few studies had similar findings.[8,11]

In this study 8.3% were laborers, 29.1% were agriculturists, 16.5% were private employees and 3.1% were government employees. Another study conducted in Ethiopia shows that 19.8% were laborers by occupation, 17.0% were government employees and 0.9% were agriculturists.[12]

4.2. *During lockdown variable*

In our study, 69.5% of patients or their family members feared to reach the hospital during lockdown, according to the other study carried out by Alexandra J. Zimmer et al 55% of participants expressed that they had fear for to visit the hospital.[10] Study conducted in Ethiopia shows that 81.6% of participants expressed they were treated differently during Covid time.[12]

Among all 5.1% of participants experienced challenges in accessing DOTS TB treatment in our study, but 49% of participants had difficulty to access of TB treatment in a study conducted by Alexandra J. Zimmer et al.[10]

In the present study, most of the patients underwent Chest X-rays (33%) to confirm their TB, but a study conducted by the WHO European region expressed that, six countries used GeneXpert machines for COVID-19 testing instead of diagnostic testing for TB.[13]

In the current study, 86.2% of participants expressed they were panicked about being infected with coronavirus. In other studies, most of the participants feared contracting Coronavirus.[9,10]

In the current study, 9.85% of participants missed their follow up test at the end of their treatment course, which is less as compared to study conducted in Ethiopia where 41% of their participants missed their follow up test.[12]

Table 2 – Distribution of participants experiences during lockdown.

Sl No	Questions	During lockdown			
		Yes		No	
		Fq	%	Fq	%
1)	Did you receive DOTS treatment?	241	94.9	13	5.1
2)	Did the DOTS provider come to supervise while taking drugs?	5	2.0	249	98.0
3)	Have empty blister packs been taken back by health personnel while giving medicine?	67	26.4	187	73.6
4)	Did you ever discontinue taking TB drugs?	97	38.2	157	61.8
5)	Due to the inaccessibility of DOTS drugs were you depressed/ disappointed?	119	46.9	135	53.1
6)	Is there any Regular communication with health personnel?	136	53.5	118	46.5
7)	Is there any Regular follow up treatment from the health department	106	41.7	148	58.3

Table 3 – Association between Socio-demographic data with During Lockdown variables of Hampered access.

Sl no	Variables		During Lockdown			Chi-square	p-Value
			Low	Average	High		
1	Age in years	≤ 30	3	81	9	10.010	0.440
		31–42	4	52	3		
		43–54	2	40	0		
		55–66	5	39	1		
		67–78	1	12	1		
		79+	0	1	0		
2	Gender	Male	8	133	6	14.563	**0.021***
		Female	7	92	8		
3	Occupation	Labor	1	19	1	13.481	**0.006****
		Agriculture	3	71	0		
		Private employee	3	35	4		
		Govt employee	2	5	1		
		Others	6	95	8		
4	type of family	Joint	6	92	7	2.463	0.793
		Nuclear	9	133	7		
5	type of house	Pucca	4	62	5	3.449	0.799
		Kaccha	11	163	9		
6	Residence	Rural	9	155	11	1.166	0.558
		Urban	6	70	3		
7	literacy status	Illiterate	1	45	1	16.973	**0.001****
		Primary Education	9	72	2		
		Secondary education	1	85	8		
		Degree and above	4	23	3		
8	socio economic status	Upper class	4	10	1	19.844	**0.011***
		Upper middle class	6	70	6		
		Middle class	4	84	3		
		Lower middle class	0	57	4		
		Lower class	1	4	0		

Bold values are statistically significant values (<0.005).

5. Recommendation

It can be recommended that there should be a doorstep supply of medicines or special transportation facility for the vulnerable population like pregnant women, bedridden, and elderly people during a special situation like pandemic lockdowns.

Contribution details

Satish Kabbur: Concepts, Design, Definition of intellectual content, Literature search, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

Bhagyashree Patil: Concepts, Design, Definition of intellectual content, Literature search, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

Mubashir Angolkar: Concepts, Design, Definition of intellectual content, Literature search, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

Ashwini Narasannavar: Concepts, Design, Definition of intellectual content, Literature search, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

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Conflict of interest

The authors have none to declare.

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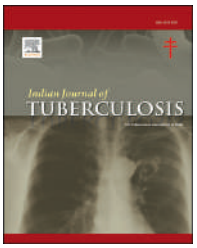

Original article

Determination of anti-tuberculosis activity of biosynthesized gold nanocompounds against *M. tuberculosis* H37RV

M.R.Kamala Priya [a], *Magizhaveni Balasubramanian* [b], *Christy Rosaline Nirmal* [b], *Azger Dusthakeer* [b], *Priya R. Iyer* [a,*]

[a] *Post Graduate and Research Department of Biotechnology, Women's Christian College, College Road, Chennai, 600006, Tamil Nadu, India*

[b] *Department of Bacteriology, National Institute for Research in Tuberculosis, Chennai, 600031, Tamil Nadu, India*

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ABSTRACT

Background: The biosynthesis of gold nanoparticles using medicinal plants as reducing and stabilizing agent for synthesis is an emerging area of research due to their cost effectiveness and further diversified applications in various fields. People with HIV are prone to these opportunistic infections like TB due to the immunocompromised condition. In the present study, the nanoparticles and nanoconjugates were screened for effective anti-mycobacterial efficiency against opportunistic infections.

Methods: Incidentally, the nanoparticles were biosynthesized using single plant extract. The biosynthesized nanoparticles were initially screened for effective anti-tuberculosis activity against *Mycobacterium tuberculosis*. Based on the effective antimicrobial activity, a nanoconjugate was biosynthesized combining three plant extracts for a cumulative activity.

Results: The biosynthesized gold nanoparticles and nanoconjugates showed MIC demonstrating for 99% inhibition and MIC_{99} was found to be 6.42 μg/ml. Among all the 15 nanoparticles tested, seven NPs showed exceptional anti-TB activities NP1, NP2, NP6, NP7, NP10, NP12 and NP15 and the other nanoparticles exhibited varying degrees of inhibition - anti-TB activities. In the 12 nanoconjugate tested, seven nanoconjugate demonstrated exceptional anti-TB activities such as NCC1, NCC2, NCC5, NCC6, NCV1, NCV6 and NCV4.

Conclusion: The objective of the study was to identify the nanoparticles and nanoconjugates which demonstrated potential activity against *M. tuberculosis* so that a single nanoparticle or nanoconjugate can be targeted to treat patients with TB. Minimum Inhibitory Concentration (MIC) of the biosynthesized gold nanoparticles and nanoconjugates were determined against *M. tuberculosis* H37Rv.

* *Corresponding author.*
E-mail address: brajuraj@yahoo.com (P.R. Iyer).

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1. Introduction

Tuberculosis is one of the chronic illnesses affecting the human community. People suffering with HIV/AIDS are more vulnerable to *Mycobacterium tuberculosis* infection since the condition proceeds at a rapid rate in immunocompromised host system. Around 1.4 million people died from TB in 2019 among which nearly 208 000 people were infected with HIV. Globally, Tuberculosis stands out to be one of the top 10 causes of death as reported by WHO (2020).[1] Multidrug resistance TB (MDR-TB) and extensively drug resistance TB (XDR-TB) are the two greatest challenges in the way of achieving effective treatment for the disease. Anti-tuberculosis therapy has two levels of treatment. The first-line therapy lasts for 6–9 months with four antibiotics such as isoniazid, rifampicin, pyrazinamide, and ethambutol in sequential combination. The second-line therapy lasts for 18–24 months with combination of much toxic and high cost second-line drugs such as aminosalicylic acid, fluoroquinolones, aminoglycosides, cycloserine, linezolid, and clofazimine, but is futile. Sloan *et al.*,[2] have reported on some of the new drugs which are currently under various phases of clinical trials such as Bedaquiline, Delamanid, Gatifloxacin, Moxifloxacin, and Rifapentine.

Tabaran *et al.*,[3] have stated on various studies which are actively involved in the development of new anti-mycobacterial drugs with high specificity and sensitivity. Metallic nanoparticles possess remarkable properties such as antiviral, antibacterial, anticancer, antiparasitic activities against vast number of diseases and disorders. Gold, silver, copper, zinc, magnesium and manganese are few of the metallic nanoparticles exploited as novel therapeutic molecules. Nasiruddin *et al.*,[4] have proposed nanoparticles to be a novel advantageous method of therapy. Nanomaterials act as site specific carrier molecules and effectively reach the targeted sites than larger molecules. Drug delivery results in a targeted and sustained release of drug for longer durations. The drug is encapsulated in liposomes or vesicles and administrated through oral, pulmonary or subcutaneous routes for effective delivery.

Mubarak and Ahmad,[5] have reported in detail on the applications of nanotechnology in biomedicine. Nanotechnology combines the two phenomenal aspects of biological research and physical sciences through different nanostructures and nanophases. The nanomaterials have made an impressive impact in biomedicine with proven notes as efficient drug delivery molecules. The size of the nanomaterials plays a crucial role in drug delivery wherein the dimensions between 1 and 100 nm are considered to be highly fruitful. Varghese *et al.*,[6] have reported on the major benefits over the nano-biotechnological innovations in drug delivery such as site specific targeting drug delivery, reduced toxicity, retention of therapeutic effects, biosafety, biocompatibility leading to the development and formulation of novel medications.

Baranyai *et al.*,[7] have stated on the various benefits of adapting nanoformulations as inhaler devices such as self-administration, easy to use and affordability that significantly brings down the cost of treatment and the use of complex medical equipment to a larger extent in anti-TB treatment. Further drug encapsulation of the antimicrobial drugs helps to increase drug solubility, prevents rapid clearance, enhancing bioavailability and to a certain extent in the reduction of undesirable side effects.

2. Methods

2.1. Bacterial culture used

Middle brook 7H9 medium supplemented with oleic acid, albumin, dextrose, and catalase (OADC) (7H9) was used to grow *M. tuberculosis*. In this study, *M. tuberculosis* H37Rv from the culture repository of the ICMR-National Institute for Research in Tuberculosis, Chennai, India was used.

2.2. Biosynthesized gold nanoparticles & nanoconjugates

The gold nanoparticles (AuNPs) and nanoconjugates (AuNC) were biosynthesized using green extracts of medicinal plants like *Andrographis paniculata, Catharanthus roseus, Cassia auriculata, Calophyllum inophyllum*, *Annono muricata*, and *Camellia sinensis* as discussed previously in our other reports.[8] The characterization was carried out to get an insight of the morphology and dimensions of the nanomaterials.[9] The effect of the underlying parameters such as temperature & pH were optimized, which played crucial role in the synthesis.[10] The applications of gold nanoparticles, *in vitro* cytotoxicity, and anti-cancer assay and anti HIV assay were carried out in different cell lines. The results demonstrated non-toxicity of the nanomaterials to normal cell line.[11–14]

2.3. Determination of anti-tuberculosis activity through Minimum Inhibitory Concentration (MIC) by broth dilution method

Minimum Inhibitory Concentration (MIC) of the biosynthesized gold nanoparticles and nanoconjugates were determined at the various concentrations ranging from 6.42, 3.21, 1.605, 0.8025, 0.40125, 0.200625, 0.1003125, 0.05015625 μg/ml. The dilution was made using 7H9 media without altering media composition. The widely studied *M. tuberculosis* H37Rv was used in the study. The culture suspension was adjusted to 1 McFarland standard as mentioned previously and diluted at 1:10 ratio so that each well contains 1×10^5 cells. Rifampicin at critical Concentration of 1 μg/ml was retained as drug control. Culture control and solvent control (DMSO) were also included in the assay. The plates were incubated at 37 °C for 21 days. Post incubation period, the plates were observed under microscope for determining gradation based on the serpentine cord formation of *M. tuberculosis* culture growth. All the tests were performed in triplicates in a microtiter plate. The concentration of nanocompounds which completely inhibit the growth of the *M. tuberculosis* cultures was considered as MIC.[15] The MIC_{80} value was determined as the concentration of the nanoparticle that inhibited 80% of the growth. The MIC_{80} value was calculated for each nanoparticles and nanoconjugate by comparison of the gradation between the treated and control wells. The amount of cord formed under inverted microscope

of the treated and control wells were observed and scored. The MIC_{99} value was determined as the concentration of the nanoparticle that inhibited 99% of the growth. The MIC_{99} value was determined for each nanoparticles and nanoconjugate by comparison of the gradation between the treated and control wells.

3. Results and discussion

3.1. *Biosynthesized gold nanoparticles & nanoconjugates (AuNPs & AuNCs)*

The biosynthesis of AuNPs was carried out using various medicinal plant extracts as reducing and stabilising agents. The biosynthesized AuNPs and AuNCs were observed to be pale pink to deep wine red in color indicating the characteristic color of gold nanoparticles. Fig. 1 represented the biosynthesized gold nanoparticles and nanoconjugates.

Fig. 2 represented the synthesized nanoparticles analysis under UV Vis spectrophotometer for characteristic Surface Plasmon Resonance peak for gold nanoparticles between 500 and 600 nm.

The electron micrograph analysis was carried out to elucidate the size and morphology of the biosynthesized nanoparticles. In Fig. 3, the size of the nanoparticles was observed to be 19 nm onwards. In Figs. 4 and 5, through SEM and HRSEM, the nanoparticles indicated predominantly spherical morphology. In Figs. 6 and 7, the TEM images represented a similar pattern of surface morphology in concordance to the SEM and HRSEM images.

The SAED pattern was recorded to determine the structural formation of the synthesized nanoparticles. In Fig. 8, the SAED pattern observed demonstrated crystalline structure of the nanoparticles.

In Fig. 9, the XRD pattern of nanoparticles represented the crystalline structure of the synthesized nanoparticles with characteristic peaks for gold nanoparticles.

In Fig. 10, the Zeta potential analysis showed that the nanoparticles were highly stable without aggregations with the zeta values at −30 mV indicating highly stable gold nanoformulations.

3.2. *Determination of anti tuberculosis activity for nanoparticles against* M. tuberculosis *H37Rv*

MIC was determined at various concentrations of the biosynthesized nanoparticles and nanoconjugates against *M. tuberculosis* H37Rv. After 21 days of incubation the plates were observed under microscope. The MIC_{99} was determined as the concentration of the nanoparticle that inhibited ≥99% of the growth and MIC_{80} was determined as the concentration of the nanoparticle that inhibited 80% of the growth. Based on the microscopic observation, the turbidity of the treated and control wells were compared and scored as N, SC, 1+, 2+, and 3+. The experimental set was demonstrated in Fig. 11 (see Table 1).

The microscopic observation of the titre plate was shown in Fig. 12. Fig. 12(a) showed the control growth of *M. tuberculosis* representing serpentine cord formation, Fig. 12(b) had *M. tuberculosis* treated with highest concentration of nanocompounds which showed a complete no growth compared to the control growth. This was observed to be the highest percentage of inhibition as 99% of inhibition and followed by further varying degrees of inhibition at consecutive concentrations of the nanocompounds in Fig. 12(c), (d), (e) and (f). In the present study, 15 biosynthesized gold nanoparticles were screened for effective anti-tuberculosis activity against the widely adapted *M. tuberculosis* H37Rv. Among all of the 15 nanoparticles tested, seven NPs showed exceptional anti-TB activities NP1, NP2, NP6, NP7, NP10, NP12 and NP15 and the

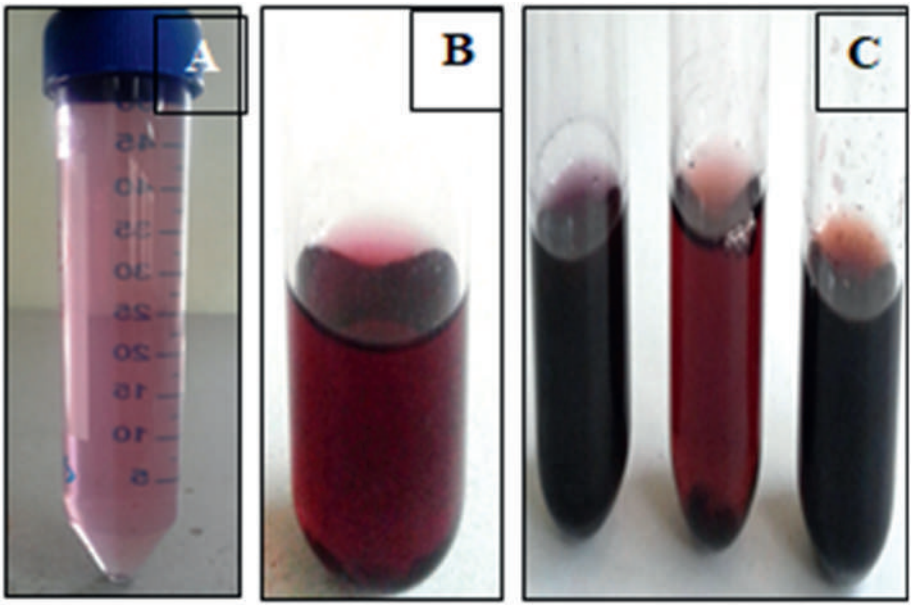

Fig. 1 – A, B – biosynthesized AuNPs, C – AuNCs.

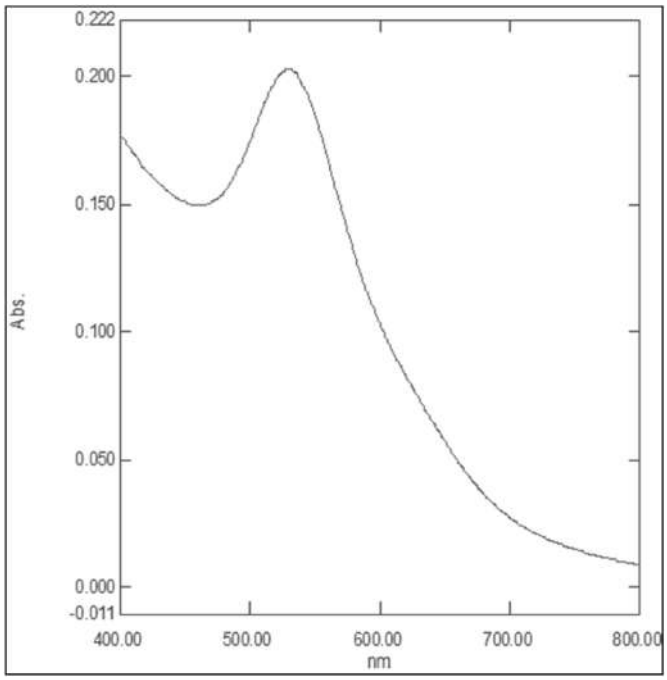

Fig. 2 – UV Vis absorbance of biosynthesized nanomaterials with SPR band at 536 nm.

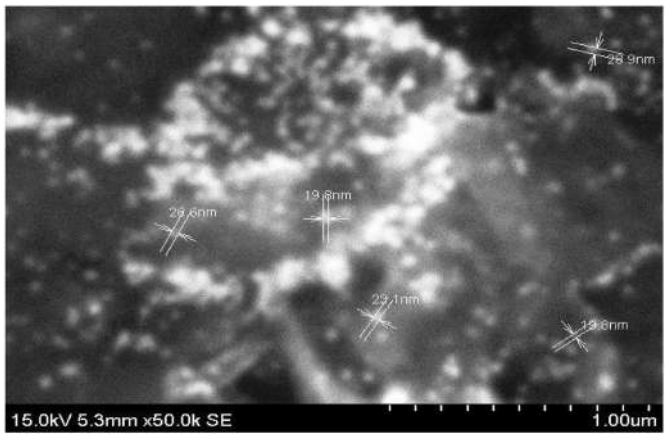

Fig. 3 – SEM image showing the size of nanoparticles synthesized.

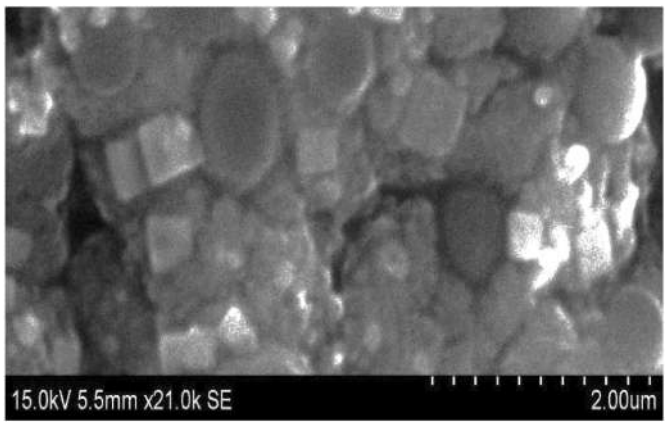

Fig. 4 – SEM image showing spherical morphology of the nanoparticles.

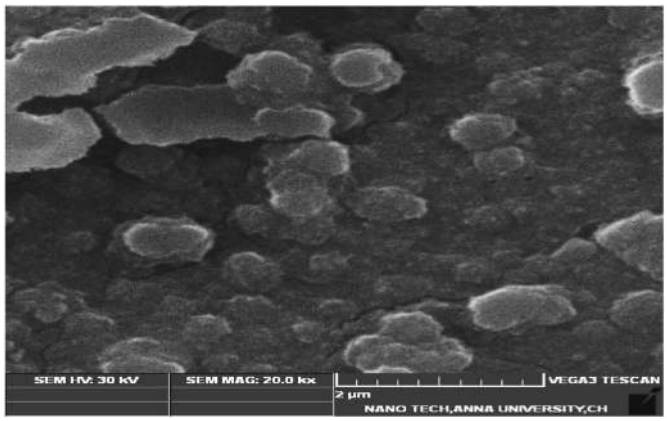

Fig. 5 – HRSEM micrograph of the biosynthesized nanoparticles.

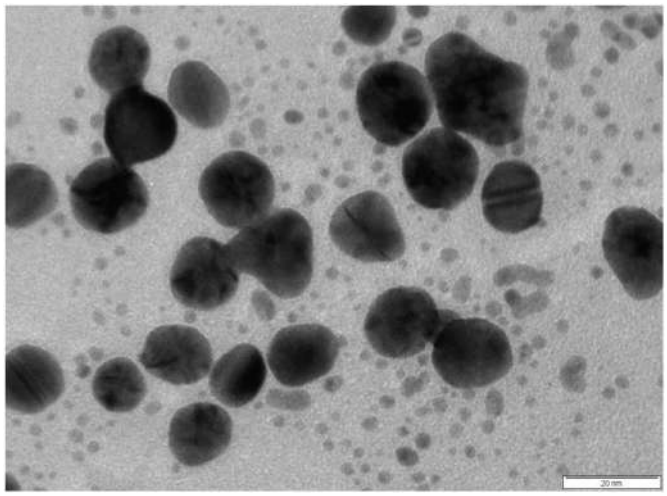

Fig. 6 – TEM image of the biosynthesized nanoparticles at 20 nm.

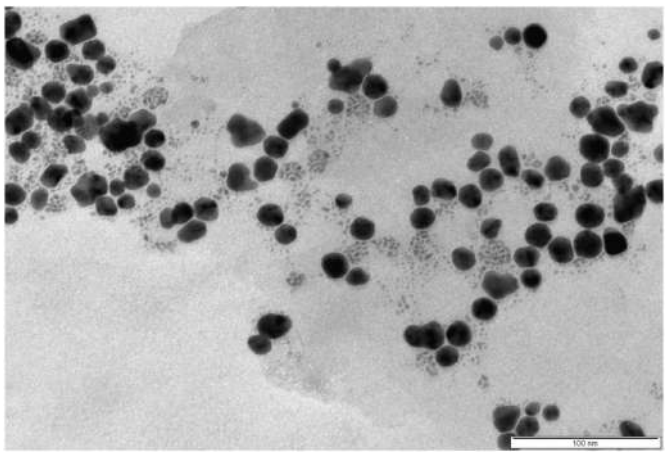

Fig. 7 – TEM image of the biosynthesized nanoparticles at 100 nm.

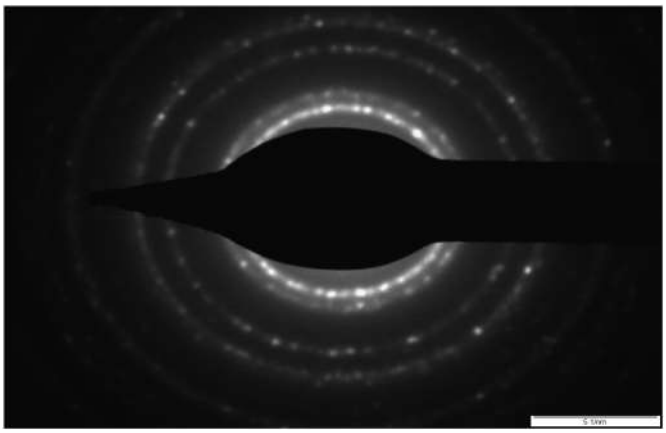

Fig. 8 – SAED pattern of the biosynthesized nanoparticles.

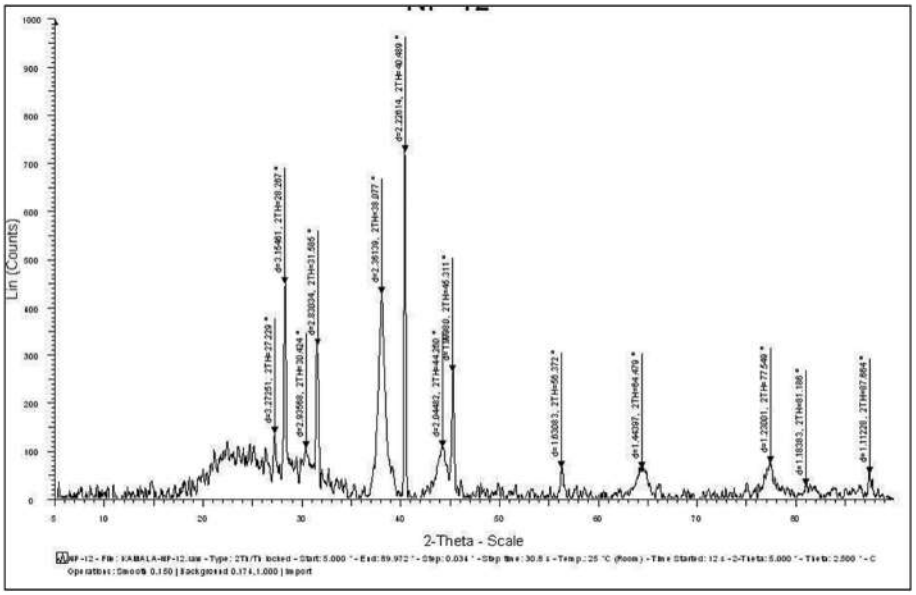

Fig. 9 – XRD pattern of the biosynthesized nanoparticles.

other nanoparticles exhibited varying degrees of inhibition - anti-TB activities (see Fig. 13).

3.3. *Anti-tuberculosis activity of nanoconjugates against* M. tuberculosis *H37Rv*

To achieve a synergistic effect of the nanoparticles, the gold nanoconjugate was synthesized. The biosynthesized gold nanoconjugates were screened for effective anti-tuberculosis activity against the widely adapted *M. tuberculosis* H37Rv. Among all of the 12 nanoconjugate tested, seven nanoconjugate demonstrated exceptional anti-TB activities such as NCC1, NCC2, NCC5, NCC6, NCV1, NCV6 and NCV4. All other nanoconjugate exhibited varying degrees of anti-tuberculosis activities (see Fig. 14; Tables 2–4).

Parakh,[16] has reported on the anti-tuberculosis activity of silver nanoparticles synthesised using *Coriandrum sativum*. The minimum inhibitory concentration was reported to be 1.56 μg/ml for AgNPs. Singh *et al.*,[17] have reported on the anti-tuberculosis activity of silver and gold nanoparticles synthesised using medicinal plants. It was found that silver-gold nanoparticles and silver nanoparticles exhibited anti-TB activities and gold nanoparticles did not exhibit appreciable anti-TB effects. The inhibitory concentration IC_{50} was reported to be <2.56 μg/ml. Banu and Rathod,[18] have reported on the anti-TB activity of the biosynthesized silver nanoparticles against *M. tuberculosis*. The inhibitory concentration IC_{50} was reported to be 12.5 μg/ml.

In the present study, biosynthesized gold nanoparticles and nanoconjugates exhibited commendable anti-TB activity against *M. tuberculosis*. The minimum inhibitory concentration demonstrating ≥99% inhibition, MIC_{99} was found to be 6.42 μg/ml. MIC99 was exhibited by two nanoparticles and three nanoconjugate such as NP2, NP7 and NCC2, NCC5,

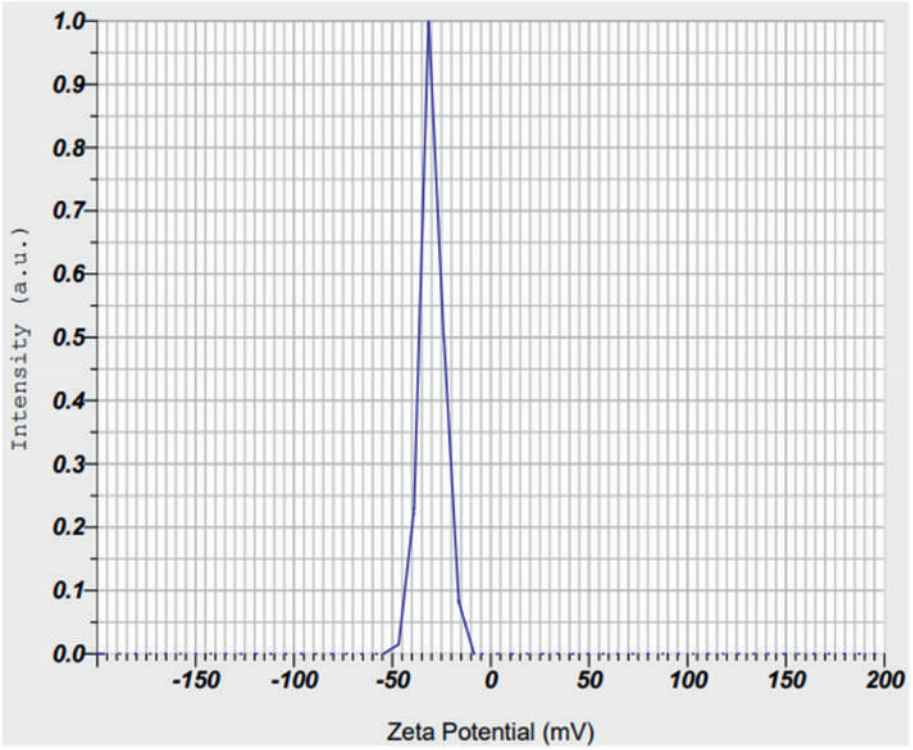

Fig. 10 – Zeta potential analysis of the biosynthesized nanoparticles.

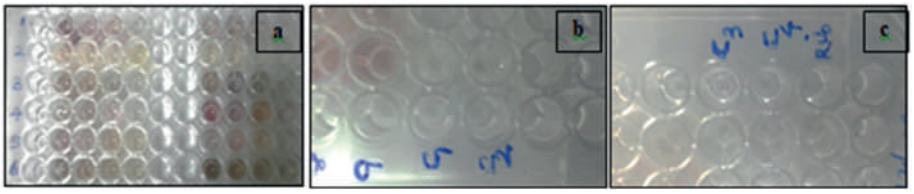

Fig. 11 – a) MIC study of different AuNPs against *M. tuberculosis* b) Culture control c) Drug control. Microscopic observation of Drug-Free & Drug Treated - *M. Tuberculosis*.

Table 1 – Anti-tuberculosis activity of nanoparticles – MIC assay.

AuNPs	Conc.1 6.42 µg/ml	Conc. 2 3.21 µg/ml	Conc. 3 1.605 µg/ml	Conc. 4 0.8025 µg/ml	Conc. 5 0.4012 µg/ml	Conc. 6 0.2006 µg/ml	Con. 7 0.1003 µg/ml	Conc. 8 0.0501 µg/ml
NP1.	1+	1+	1+	2+	2+	3+	3+	3+
NP2.	N	SC	1+	1+	1+	2+	2+	3+
NP3.	2+	2+	2+	2+	2+	2+	2+	3+
NP4.	2+	2+	3+	3+	3+	3+	3+	3+
NP5.	2+	2+	2+	2+	2+	2+	2+	2+
NP6.	1+	2+	2+	3+	3+	3+	3+	3+
NP7.	N	SC	1+	1+	1+	1+	2+	3+
NP8.	2+	2+	2+	2+	3+	3+	3+	3+
NP9.	2+	2+	2+	2+	2+	3+	3+	3+
NP10.	1+	1+	2+	2+	2+	2+	3+	3+
NP11.	2+	2+	2+	2+	2+	2+	2+	2+
NP12.	SC	SC	SC	SC	1+	1+	2+	2+
NP13.	2+	2+	2+	3+	3+	3+	3+	3+
NP14.	2+	2+	2+	2+	2+	2+	3+	3+
NP15.	1+	1+	3+	3+	3+	3+	3+	3+

*N – No Growth, 2+ - very less growth, SC – Scanty growth, 3+ - fewer growth, 1+ - Slight growth.

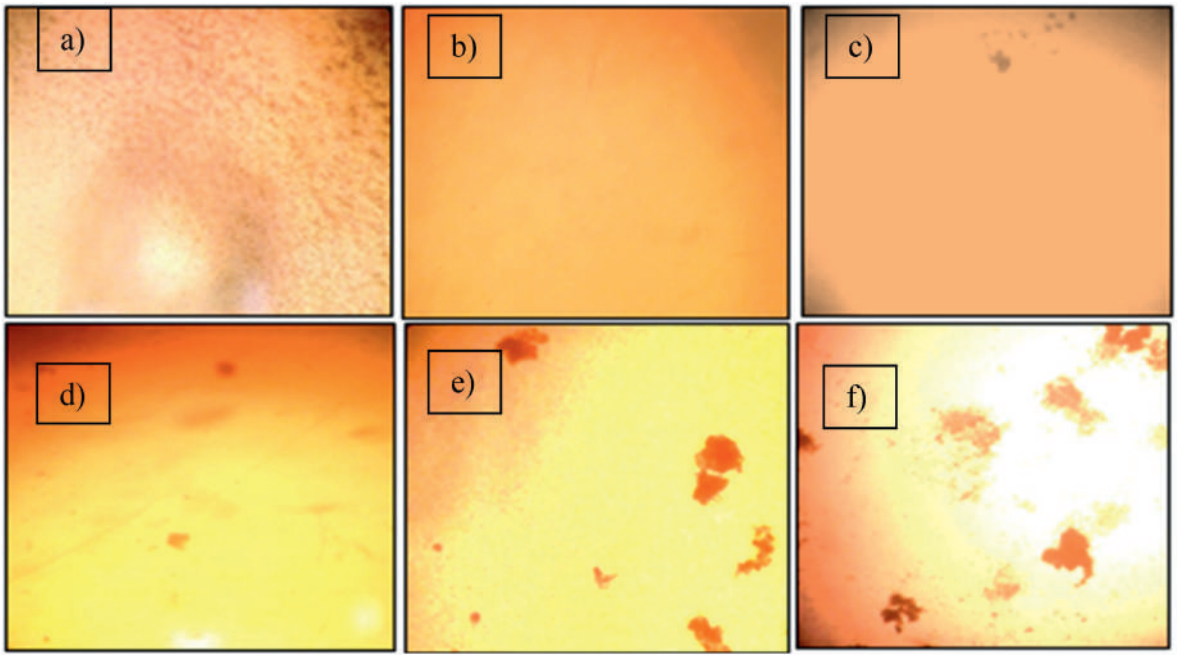

Fig. 12 – a) Control growth of *M. tuberculosis* b) AuNPs treated cells – No growth c) scanty growth SC d) 1+ growth e) 2+ growth f) 3+ growth.

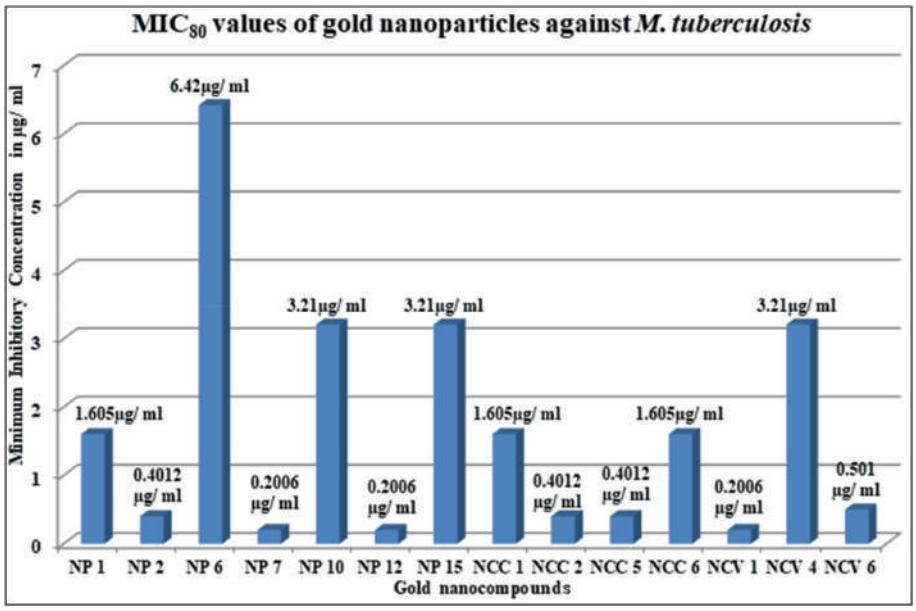

Fig. 13 – MIC_{80} values of gold nanoparticles against *M. tuberculosis* (µg/ml).

NCV1. The minimum inhibitory concentration demonstrating 80% inhibition, MIC_{80} was found to be 0.0501 µg/ml for NCV6, 0.2006 µg/ml for NP7, NP12 and NCV1, 0.4012 µg/ml for NP2, NCC2, NCC5, 1.605 µg/ml for NP1, NCC1, NCC6, 3.21 µg/ml for NP10, NP15 and NCV4, 6.42 µg/ml for NP6. NCV6 exhibited effectiveMIC_{80} at 0.0501 µg/ml.

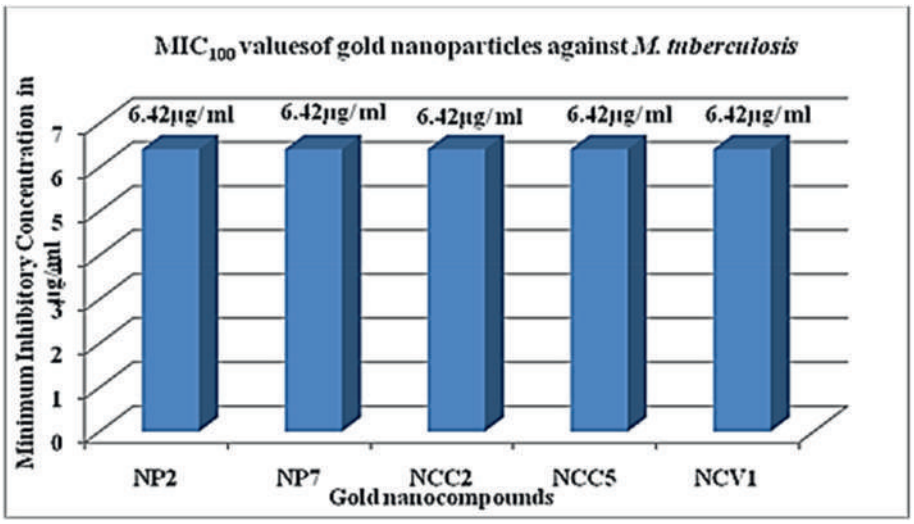

Fig. 14 – MIC_{99}values of gold nanoparticles against *M. tuberculosis* (µg/ml).

Table 2 – Anti-tuberculosis activities of nanoconjugates.

AuNC	Conc.1 6.42 µg/ml	Conc. 2 3.21 µg/ml	Conc. 3 1.605 µg/ml	Conc. 4 0.8025 µg/ml	Conc. 5 0.4012 µg/ml	Conc. 6 0.2006 µg/ml	Con. 7 0.1003 µg/ml	Conc. 8 0.0501 µg/ml
NCC1.	1+	1+	1+	2+	2+	3+	3+	3+
NCC2.	N	SC	SC	SC	1+	2+	2+	3+
NCC3.	2+	2+	2+	2+	2+	2+	2+	3+
NCC4.	2+	2+	3+	3+	3+	3+	3+	3+
NCC5.	N	SC	SC	1+	1+	2+	2+	2+
NCC6.	1+	2+	2+	3+	3+	3+	3+	3+
NCV1.	N	SC	SC	SC	1+	1+	2+	2+
NCV2.	2+	2+	2+	2+	3+	3+	3+	3+
NCV3.	2+	2+	2+	2+	2+	3+	3+	3+
NCV4.	1+	1+	2+	2+	2+	2+	3+	3+
NCV5.	2+	2+	2+	2+	2+	2+	2+	2+
NCV6.	SC	SC	SC	1+	1+	1+	1+	1+

*N – No Growth, 2+ - very less growth, SC – Scanty Growth, 3+ - fewer growth, 1+ - Slight growth, *NCC – Nanoconjugate formulated out of anticancer study, *NCV – Nanoconjugate formulated out of anti HIV study.

Table 3 – Minimum inhibitory concentrations MIC_{80} – biosynthesized gold nanocompounds.

S. No.	AuNPs/AuNC tested	Vs Microorganism	MIC_{80} values (µg/ml)
1.	NP 1	*Mycobacterium tuberculosis* - H37Rv strain	1.605 µg/ml
2.	NP 2	*Mycobacterium tuberculosis* - H37Rv strain	0.4012 µg/ml
3.	NP 6	*Mycobacterium tuberculosis* - H37Rv strain	6.42 µg/ml
4.	NP 7	*Mycobacterium tuberculosis* - H37Rv strain	0.2006 µg/ml
5.	NP 10	*Mycobacterium tuberculosis* - H37Rv strain	3.21 µg/ml
6.	NP 12	*Mycobacterium tuberculosis* - H37Rv strain	0.2006 µg/ml
7.	NP 15	*Mycobacterium tuberculosis* - H37Rv strain	3.21 µg/ml
8.	NCC 1	*Mycobacterium tuberculosis* - H37Rv strain	1.605 µg/ml
9.	NCC 2	*Mycobacterium tuberculosis* - H37Rv strain	0.4012 µg/ml
10.	NCC 5	*Mycobacterium tuberculosis* - H37Rv strain	0.4012 µg/ml
11.	NCC 6	*Mycobacterium tuberculosis* - H37Rv strain	1.605 µg/ml
12.	NCV 1	*Mycobacterium tuberculosis* - H37Rv strain	0.2006 µg/ml
13.	NCV 4	*Mycobacterium tuberculosis* - H37Rv strain	3.21 µg/ml
14.	NCV 6	*Mycobacterium tuberculosis* - H37Rv strain	0.0501 µg/ml

MIC_{80}values of AuNPs Vs *Mycobacterium tuberculosis* - H37Rv strain.

Table 4 – Minimum inhibitory concentration MIC_{99}–Biosynthesized gold nanocompounds.

S. No.	AuNPs/AuNC tested	MIC_{99}values (μg/ml)
1.	NP2	6.42 μg/ml
2.	NP7	6.42 μg/ml
3.	NCC2	6.42 μg/ml
4.	NCC5	6.42 μg/ml
5.	NCV1	6.42 μg/ml

MIC_{99}values of AuNPs Vs *Mycobacterium tuberculosis* - H37Rv strain.

4. Conclusions

The biosynthesized gold nanoparticles were screened for effective anti-tuberculosis activity against the widely used *M. tuberculosis* H37Rv in this study. Among the 15 nanoparticles and 12 nanoconjugates tested, seven nanoparticles and seven nanoconjugates exhibited exceptional anti-TB activities against *M. tuberculosis*. The minimum inhibitory concentration demonstrating 99% inhibition and 80% inhibition was achieved at very low concentrations than the earlier reports. Various studies have reported on the anti-TB activity of the biosynthesized silver nanoparticles. But there were very few positive reports on the anti-TB activity of the biosynthesized gold nanoparticles. The present study reported on effective anti-tuberculosis activities of gold nanoparticles and nanoconjugates biosynthesized using medicinal plant extracts. Multi-drug resistance is one of the major challenges faced by the current medications in the treatment of Tuberculosis. The success of the study can pave way for a further extended research on gold nanoparticles as lead drug molecules in the treatment and management of Tuberculosis. The biosynthesized gold nanoparticles and nanoconjugates can be further exploited as novel therapeutics in the treatment of tuberculosis.

Conflicts of interest

The authors have none to declare.

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Original article

Prevalence, knowledge and practices towards tuberculosis prevention in the Bamenda III sub-division, Cameroon

Ndukong Nsanwe Ndi [a], *Hubert Shalanyuy Lukong* [b], *Moses Samje* [c,*]

[a] *Regional Technical Group for TB Control, North West Regional Delegation of Public Health, PO Box 452, Bamenda, Cameroon*

[b] *Institut Superieur des Sciences Appliquees a La Sante, Institut Universitaire de La Pointe, PO Box 1362, Bafoussam, West Region, Cameroon*

[c] *Department of Biomedical Sciences, University of Bamenda, PO Box 39, Bambili, North West Region, Cameroon*

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ABSTRACT

Background: Tuberculosis (TB) is a major public health problem in developing countries. WHO estimates the prevalence in Cameroon at 17.7% (179/100,000).

Objectives: To determine the prevalence of TB and assess the level of knowledge and practices towards TB prevention among residents of the Bamenda III sub-division of North West Cameroon.

Methods: A retrospective study design was used to generate data on the prevalence of TB from 2016 to 2020 while a descriptive cross-sectional study design was used to generate information on the level of knowledge and practices towards TB prevention.

Results: Out of 4950 presumptive cases of TB (all forms), 469 (9.5%) were placed on TB treatment. The highest prevalence was in 2016 (2.9%) and the lowest in 2019 (1.8%). The majority (65.3%, n = 186) of respondents had adequate knowledge of TB while 143 (57.1%) demonstrated adequate practices in TB prevention. There was a positive correlation between knowledge and practice towards TB prevention ($\chi 2 = 29.47$, $p = 0.001$).

Conclusion: The prevalence of TB (2016–2020) was 9.5%. About one-third of respondents had inadequate knowledge and practices in TB prevention. Sensitization and intensified case finding in favor of TB in this community is encouraged.

1. Background

TB remained one of the most common causes of death from a single infectious pathogen in 2019.[1] Globally an estimated 10 million people developed TB disease in 2019 and because of COVID 19 pandemic, TB control activities were affected as most resources were channeled towards the fight against this pandemic.[2]

* *Corresponding author.*
E-mail address: msamje@gmail.com (M. Samje).

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In Cameroon, just over 50% (24319 cases) of TB cases (all forms) were notified in 2019.[3] Country estimates from WHO, suggests a very significant gap between the numbers of notified TB cases and the incidence (approximately 47,000 cases) of TB in Cameroon.[1] The North West Region notified 831 TB cases (all forms) in 2019,[3] having a notification rate of 22%.

This significant burden of undetected TB cases if not addressed, Cameroon may fall short of its 2024 vision which is to reduce TB incidence by 30% and the WHOs sustainable development goal of eliminating TB by 2030.[4]

A survey in 2016 by Nolna et al in Cameroon found out that a vast population had insufficient understanding of TB, and erroneous information about the symptoms and mode of transmission of the disease.[5] Hence, an understanding of the level of knowledge and practices towards TB prevention in the community is essential.

The aim of this study was to generate relevant data on the prevalence of TB, and assess the level of knowledge and practices towards TB prevention among residents of the Bamenda III sub Division.

2. Material and methods

2.1. Study setting

This study was carried out in the Bamenda III sub division which has an estimated total surface area of about 22.9 Km^2 hectares and a population of about 150,000.[6] The population of Bamenda III municipality is cosmopolitan; made up of indigenous Nkwen and Ndzah people although migrants from other regions of Cameroon and Nigerians are also spread within these 2 villages. The most dominant population is the youth and serves as a pool of potential human resources for the sub division.

2.2. Study designs

A retrospective and descriptive cross-sectional study were respectively employed to generate data on prevalence of TB for a five-year period (2016–2020) and the level of knowledge and practices towards TB prevention. For prevalence, all the presumptive TB cases from Bamenda III sub division presenting with signs and symptoms suggestive of TB with complete data were included in the study. In evaluating the level of knowledge and practice, all adults aged 18 years and above who were permanent residents in the area and who accepted to participate in the study were considered eligible to participate in the study.

2.3. Study procedure

Data to determine the prevalence was extracted from TB screening registers, laboratory TB registers and the TB treatment registers. The prevalence was then determined by taking the total notified cases from 2016 to 2020 (469) divided by the total number suspects from the community (4950). The annual risk of TB infection (1.9%) was calculated by dividing the prevalence rate (9.5%) by the period of study (five years). Stratification was done based on age, sex, and address. The various forms of TB in circulation were also generated by classifying the TB types. They were classified as: Smear positive pulmonary TB, Smear Negative pulmonary TB, Extra pulmonary TB, Multi Drug resistant TB or a Retreatment.

2.4. Knowledge and practice

The population sample size was determined using a single population sample size range according to Charan and Biswas as follows:

$$\frac{(Z\alpha/2)2p(1-p)}{d2}$$

where, $Z\alpha/2$ is the critical value of the Normal distribution at $\alpha/2$ (for a confidence level of 95%, α is 0.05 and the critical value is 1.96), d^2 the margin of error, p the sample proportion. The p value for this survey was obtained based on result obtained from previous studies that indicated 73% of the study participants had knowledge about the main TB signs and symptoms[7] and at 95% confidence interval, 5% margin of error (MOE) and 10% non-respondent rate in our estimated sample, a total sample size of 285 was obtained.

$Z = 1.96$, $p = 0.73$, $d = 0.05$, $q = 1-p$.

Sample size = 285.

During sampling, 20 of the 46 quarters in Nkwen village and 5 of the 9 quarters in Nzah village were selected using random sampling. Households were selected from each quarter by systematic random sampling based on WHO survey method for Expanded Program on Immunization (EPI).[8] Eligible respondents in a house hold were selected for the interview if they gave consent. In the absence of eligible respondent, a neighboring house hold was chosen. A standardized questionnaire designed in English based on WHO guidelines[9] was used for data collection. A self-administered questionnaire with 20 closed ended questions divided into 3 sections was administered to generate information on; sociodemographic characteristics (5 questions), knowledge on TB (10 questions) and practices towards TB prevention (6 questions). A respondent was considered to have adequate knowledge if he/she scored $\geq$5 on a scale of 10 or inadequate knowledge (<5 on a scale of 10)[10] On preventive practices, a score of $\geq$3 on a scale of 6 was considered adequate while <3 on a scale of 6 was inadequate practice.

2.5. Data analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 20. Chi squared test was used to determine relationships between variables. The trend over time for the prevalence of TB from 2016 to 2020 was examined using the *Mantel Haezel Chi*^{2} test for Linear trend. Logistic regression technique was used to determine the association between communities' knowledge and practice towards TB prevention. Associations were considered significant at $p \leq 0.05$.

2.6. Ethical considerations

Ethical clearance to carry out the study was obtained from the Institutional Review Board (IRB) of the University of

Bamenda (2021/0330H/UBa/IRB). An administrative authorization was obtained from the Delegation of Health, North West Region (no.25/ATT/NWR/RDPH/BRIGAD). Informed consent was obtained from each study participants. Participation was absolutely voluntary. The information obtained was handled with utmost confidentially.

3. Results

3.1. Prevalence of TB (2016–2020)

There was a higher prevalence of TB (all forms) in males (57.8% n = 271) than females (42.2% n = 198) and the age group 21–40 years (55.9%, n = 262). A significant difference was observed in the prevalence of TB between gender and between age groups with most TB cases confirmed bacteriologically. Furthermore, there was a statistically significant association between prevalence of TB and gender ($p = 0.016$) and the prevalence of TB and age range ($p = 0.0001$) (Table 1).

3.2. Trend in TB incidence from 2016 to 2020 in Bamenda III sub division

A significant steady decline ($\rho = 0.032$) in the incidence of TB cases (all forms) was observed from 2016 to 2020 (Fig. 1).

3.3. Level of knowledge on TB in the study site

Out of the 305 respondents, 199 (65.2%) had adequate knowledge about TB. Male respondents and respondents with secondary level of education were more likely to be knowledgeable than their counterparts (p = 0.014 and p = 0.011 respectively). Details are shown in Table 2.

With regards to the source of information on TB, 32.8% of respondents got information on TB either from family or friends, while 30.5% was from the media.

3.3.1. Practices towards TB prevention

Only (57.1%; n = 143) demonstrated adequate practices at TB prevention and of these, with respondents below 30 years of age, males, and those with secondary education having better practices (Table 3).

3.3.1.1. *On the question "At what point do you usually go to check a health problem?"*. Unlike males who will go for check-up only when health condition is very severe, female regularly go for routine check-ups ($p = 0.0001$, $X^2 = 23.86$). In like manner, those in the 31–40 years age group will regularly go for health check-up as compared to their counterparts.

3.3.1.2. *On the question, "What action would you take if you found out that you have TB?"*. From the 50% who said they would seek health care, 69.8% (n = 107) would talk to a health personnel, 20% (n = 31) would talk to parents while 10.2% (n = 16) would talk to spouse. For management of TB, 80% (n = 244) would go to a health facility immediately, 16.7% (n = 51) would pursue other treatment options, while 3.3% (n = 10) would chose a pharmacy. For those who chose a health facility, 49.5% (n = 151) would seek medical help as soon as symptoms can be related to TB while 26.9% (n = 82) would only seek medical help when their symptoms lasts for 3–4weeks.

When respondents were asked why they would not go to the hospital if diagnosed with TB, the majority (43%, n = 131) said lack of finance, while 20% (n = 61) would not know the location of the Treatment Centers. A lower number (13.1%; n = 40) indicated the negative attitude of health workers would discourage them.

3.3.1.3. *Correlation between knowledge and practice*. There was a positive correlation between adequate knowledge and adequate practice ($r = 0.582$), inadequate knowledge and inadequate practice ($r = 0.261$) with the former being statistically significant ($p = 0.001$). A negative correlation was observed with inadequate knowledge *and* adequate practice ($r = -0.041$, $p = 0.406$).

4. Discussions

4.1. Prevalence of tuberculosis (2016–2020)

The prevalence of TB (all forms) for this study was found to be 9.5% (63/100,000) and of these, 74% was pulmonary TB. It highlights a high smear-positive TB case findings in this study population with males and those in the age group 21–40 years mostly affected. This is consistent with works by Ana-Anyangwe et al and Noeske et al[11,12] However, it is in contrast with studies by Ahmad et al[13] who observed that women are uniquely affected by TB and are more likely to have active disease when compared to men. Our findings

Table 1 – Prevalence of TB cases (2016–2020) by demographic characteristics.

		Tuberculosis cases		*P-value*
		Positive (bacteriological) N (%)	Negative (clinical) N (%)	
Gender	Male	210 (44.8)	61 (13.0)	**0.016***
	Female	135 (28.8)	63 (13.4)	
Age group	1 year or less	04 (0.900)	10 (02.1)	**0.0001***
	2–20yrs	31 (06.60)	24 (05.1)	
	21–40yrs	205 (43.7)	57 (12.2)	
	41–60yrs	94 (20.00)	25 (05.3)	
	61+ years	11 (02.30)	08 (01.7)	

* Statistically significant at 0.05 significance level.

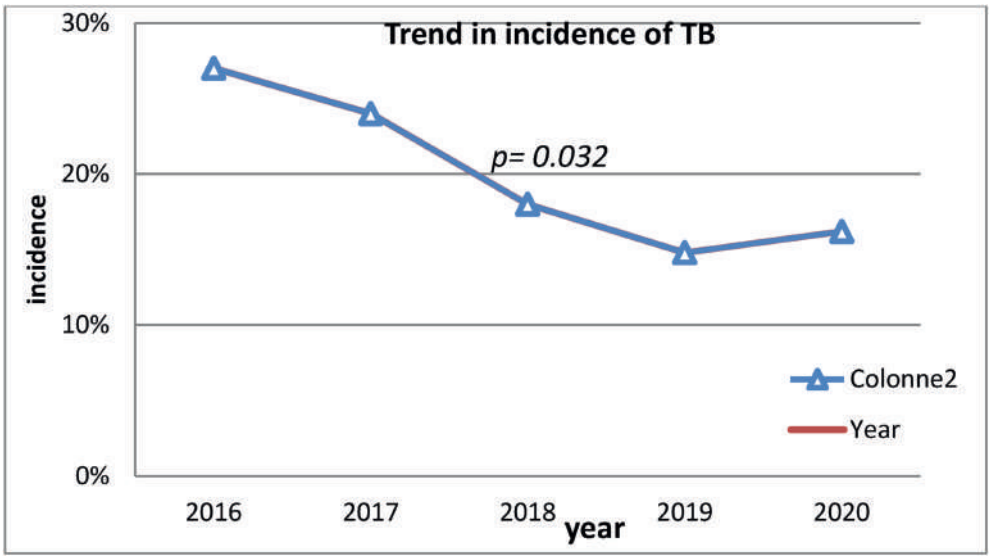

Fig. 1 – Trend in TB incidence from 2016 to 2020 in Bamenda III Sub Division.

Table 2 – Distribution of level of knowledge by demography.

Variable		Total N (%)	Knowledge about TB, N (%)		*Chi square (p-value)*
			Adequate	Inadequate	
Age range (years)	*<30*	*212 (69.5)*	*139 (45.6)*	*73 (23.9)*	*27.63 (0.014)**
	31–40	*63 (20.7)*	*40 (13.1)*	*23 (06.6)*	
	41–50	*20 (6.6)*	*13 (4.2)*	*07 (02.3)*	
	Over 50	*10 (3.3)*	*7 (2.30)*	*03 (1.00)*	
Gender	*Male*	*183 (60.0)*	*121(39.7)*	*62(20.30)*	*16.28 (1.29)*
	Female	*122 (40.0)*	*78 (25.6)*	*44 (14.3)*	
Educational level	*No formal education*	*52 (17.0)*	*33 (10.9)*	*19 (6.20)*	*29.74 (0.011)**
	Primary	*30 (9.8)*	*19 (6.20)*	*11 (3.60)*	
	secondary	*112 (36.7)*	*73(24.00)*	*39 (12.8)*	
	High school	*80 (26.2)*	*54 (17.7)*	*26 (11.2)*	
	Higher education	*21 (6.9)*	*13(4.30)*	*08 (2.60)*	
Nearness to a clinic (Km)	*0–10*	*91 (29.8)*	*51(24.20)*	*40 (5.6)*	*21.33 (0.091)*
	11–20	*81 (26.6)*	*41 (20.5)*	*40 (6.10)*	
	21–30	*92 (30.2)*	*34 (10.2)*	*02 (0.70)*	
	> 30	*41 (13.4)*	*24(10.60)*	*17 (2.8)*	

which revealed that prevalence is higher in males could be associated to their daily activities which expose them to infection considering the route of transmission. Given the limited geographical coverage of this survey, large scale data on the prevalence of TB in the country is recommended so as to ascertain the severity of TB in communities. The drop in TB trend from 2016 to 2019 could be attributed to the social unrest in this part of the country which has greatly affected health care services especially at the operational level as some facilities were forced to close.

4.2. *Knowledge on TB*

Knowledge on TB was based on respondents' ability to recognize the cause, modes of transmission, and the methods of prevention. More than half of the study population (65.2% n = 199) were knowledgeable about TB. Nonetheless, only 50.5% of study participants knew the etiology of the diseases as bacteria. Knowing the exact cause of the disease is the baseline for a positive attitude towards seeking health as well as applying effective preventive measures.[14] This low percentage on the awareness of the causative agent could be partly attributed to poor sensitization in communities. Although 83.3% (n = 254) of participants' from this study agreed TB transmission is preventable, only 69.8% could identify the right mode of transmission (airborne). This corroborates the lone available survey in Cameroon by Nolna et al[5] whereby, 67.9% of respondents said TB is transmitted by air. Adequate level of knowledge was observed among males, secondary school leavers, younger age group and those residing closer to a health facility in this study and this is consistent with other studies carried out.[15,16] Most respondents heard about TB from various sources including the median and family members. This is consistent with other studies conducted in different settings[17–19] and according to Sreeramareddy et al not being aware of the signs and

Table 3 – Practice towards TB prevention according to socio demographic characteristics.

Variable		Total n (%)	Practice towards TB prevention n (%)		Chi square (p-value)
			Adequate	Inadequate	
Age range (years)	Under 30	212 (69.5)	120(39.3)	92 (30.2)	28.21 (0.01)*
	31–40	63 (20.7)	36 (11.8)	27 (8.8)	
	41–50	20 (06.6)	12 (10.30)	08 (2.60)	
	Over 50	10 (03.3)	06 (1.90)	04 (1.30)	
Gender	Male	183 (60.0)	106 (34.4)	77 (25.2)	19.23 (1.01)
	Female	122 (40.0)	68 (22.3)	54 (17.7)	
Educational level	No formal school	52 (17.0)	29 (9.5)	23 (7.50)	30.17 (0.001)*
	Primary	30 (09.8)	16 (5.30)	14 (4.60)	
	Secondary	112 (36.7)	65 (21.3)	47 (15.5)	
	High school	80 (26.2)	46 (15.10)	34 (11.1)	
	Higher education	21 (06.9)	12 (4.00)	09 (3.00)	
Nearness to a clinic (Km)	0–10	91 (29.8)	51 (16.80)	40 (13.10)	26.46 (0.05)*
	11–20	81 (26.6)	47 (15.40)	34 (11.20)	
	21–30	92 (30.2)	54 (17.70)	38 (12.5)	
	>30	41(13.4)	22 (7.20)	19 (6.20)	

* Statistically significant at 0.05 significance level.

symptoms of TB, may lead to delay in seeking treatment.[20] Though the national TB control program is recording some successes through its use of mass media to achieve its objective, more still needs to be done.

The conclusion from this study highlights the need to develop targeted interventions to improve communication and information dissemination on tuberculosis to the general public. Owing to the multiethnic nature of the country and considering the sharp cultural and religious divide, TB messages must be developed in a way that is culturally sensitive and acceptable. The messages must address misconceptions about TB and disseminated widely in this community.

4.3. *Practices towards TB prevention*

About 42.9% of the study participants demonstrated inadequate practices towards TB prevention. This may be as a result of the low level of knowledge on TB recorded in this community. Respondents <30 years of age, male gender, high school leavers and nearness to clinic, were predictive of more favorable health seeking behavior for this study. The association of the male gender with a good health-seeking behavior when they have TB-like symptoms is in line with the Gambian TB prevalence survey[21] and in concordance with global TB epidemiological data which reports that the majority of TB cases are males.[22] This study also indicated that being knowledgeable was associated with good practice towards TB. Hence, the study proposes more health education programs to promote the awareness and early prevention of TB.

5. Strength

- This is the first study in the Bamenda III sub division of the North West Region. It has therefore set the benchmark for further studies in the same locality.

6. Limitations to the study

- Data on prevalence was collected from registers in Diagnostic and Treatment Centers within the North West Region for residents of Bamenda III who consulted with signs and symptoms for TB. Sample collection from the community for diagnosis and analyses would have given a better picture on the prevalence of TB in the study area.

7. Conclusions

The prevalence of TB in this community is 9.5% which we consider to be high considering the efforts made by the Government and Partners in reducing the incidence and burden of the disease. Although a decline in the trend was observed, an upsurge was noticed in 2020 which could be attributed to the introduction of new strategies which are more sensitive. Adequate practice correlated with adequate knowledge suggesting that more sensitization on the disease need to be done as a measure to combating TB. We recommend that health education tailored towards TB prevention practices should be implemented within the TB control program.

Conflicts of interest

The authors have none to declare.

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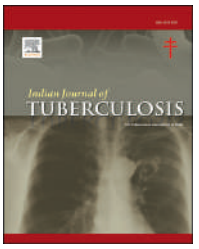

Original article

Frequency of rifampicin-resistant mycobacterium tuberculosis by GeneXpert MTB/RIF assay and its correlates among 2605 probable tuberculosis patients in upper Egypt

Ebtisam S.A. Hassanin [a], *Aliae AR. Mohamed Hussein* [b,*], *Shaimaa S. Abdelrheem* [c], *Ebtehal Dongol* [d], *Ahmed Hamdy Ahmed Mhsb* [e], *Asmaa M. Zahran* [f], *Mohamed Zein* [g], *Islam G. Sayed* [h]

[a] *Ministry of Health, Assiut, 71515, Egypt*
[b] *Chest Department, Assiut University Hospitals, Assiut, 71515, Egypt*
[c] *Department of Public Health and Community Medicine, Faculty of Medicine, Aswan University, Egypt and Armed Forces College of Medicine (AFCM), Cairo, Egypt*
[d] *South Valley University, Qena, Egypt*
[e] *Aswan University, Egypt*
[f] *Clinical Pathology Department, South Egypt Cancer Institute Assiut University, Egypt*
[g] *Faculty of Medicine, Assiut University, Egypt*
[h] *Aswan Faculty of Medicine, Aswan, Egypt*

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ABSTRACT

Rationale: GeneXpert MTB/RIF (*Mycobacterium tuberculosis*/rifampicin) assay is a method for detecting rifampicin resistance (RR-MTB) in suspected samples in less than 2 hours with high sensitivity and specificity yield. **This study aimed** to use the GeneXpert MTB/RIF assay to determine the frequency of RR-MTB and to study the possible influencing correlates associated with positive results.

Subjects and methods: This is a retrospective cross-sectional study of patients who visited TB clinic in 5 years (2016–2021). According to the data sheet of the patients, all the collected specimens were divided into 2 parts one for diagnosis by Ziehl–Neelsen stain and the other part for GeneXpert analysis. GeneXpert was also used to look for evidence of RR.

Results: Out of the 2605 total samples screened, 718 (27.6%) tested positive for MTB on GeneXpert assay; of them 633 (88.4%) were sensitive to Rifampicin, 83 (11.6%) were resistant to Rifampicin and 2 cases were undetermined. Factors contributing to RR-MTB were: smoker/ex-smoker, with 2.5 times more risk ($p = 0.013.0$, $p = 0.001$); recurrence cases had a 4-fold increased risk ($p < 0.001$); patients with very low *M. tuberculosis* detected on the GeneXpert MTB/RIF test were 8 times more likely to have RR-TB ($P = 0.004$).

* *Corresponding author*.
E-mail addresses: aliaehussein@gmail.com, aliaehussein@aun.edu.eg (A.AR. Mohamed Hussein).
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Conclusion: This study disclosed a high-rate MTB in Egyptian probable TB cases. Smoking, recurrence and cases with a very low *M. tuberculosis* burden noticed on the GeneXpert MTB/RIF test had augmented risk of RR-TB.

1. Introduction

Tuberculosis (TB) is a global health challenge with millions of new cases and deaths annually world-wide. According to the 2020 WHO report, about 6 million new and relapsed cases were diagnosed with about 160,000 cases laboratory-confirmed cases with multidrug-resistant tuberculosis (MDR-TB)/relapsing TB (/RR-TB).[1] As for Egypt in 2020, about 7000 new and relapsed cases were diagnosed with 85% of the bacteriologically confirmed new pulmonary TB cases tested for rifampicin resistance were resistant.[2]

Rifampicin is an efficient drug for treatment of tuberculosis. It has been prescribed as the first-line treatment in drug-susceptible TB patients as well as in isoniazid resistant TB patients.[3] It acts on the RNA polymerase and the *rpoB* gene is the encoding gene.[4] About 25 out of 34 mutations in that gene have been found linked to the rifampicin resistance.[5] Multidrug-resistant tuberculosis is defined as resistance to rifampicin and isoniazid with many contributing reasons.[6–8]

Prompt rapid diagnosis of *Mycobacterium tuberculosis* (MTB) and rifampicin resistance is warranted to ensure proper management in order to decrease the risk of transmission and spread of rifampicin resistant mycobacterium tuberculosis. Drug susceptibility testing (DST) on culture isolate is the standard for detection of the drug resistant tuberculosis.[9] However, it is time consuming, of limited availability in many regions and requires well-trained personnel to do, therefore, the need for more rapid, reliable, cost effective and safer testing arose.[9]

GeneXpert MTB/RIF (*M. tuberculosis*/rifampicin) assay is a method that uses real-time polymerase chain reaction (PCR) in a closed automated system which detects rifampicin resistance in suspected samples in less than 2 hours.[10] This test showed high sensitivity and specificity in several regions either in Egypt[11–13] or globally[14–19] compared to the DST or culture with a high and accurate detection rate. Also, Gene Xpert MTB/Rif testing has reliable results in diagnosis of MTB both pulmonary[20] and extra pulmonary[21], adult and pediatric population.[22,23]

There is a recommendation of WHO about the possibility to use the Gene Xpert MTB/Rif testing initially to diagnose drug resistance in adults with high sensitivity and specificity.[24] Several reports on drug resistance TB were performed employing small sample size, and consequently the results may not be expanded to a larger people. Also, there is lack of knowledge of the possible factors that may correlate to the MTB diagnosed using GeneXpert MTB/RIF with emphasis on the rifampicin resistant tuberculosis infection.

So, we aimed in this study to use the GeneXpert MTB/RIF assay to determine the frequency of MTB among probable tuberculosis patients in Upper Egypt, and further to detect the RR-MTB and the possible influencing correlates associated with GeneXpert MTB/RIF positive results.

2. Subjects and methods

2.1. Study design and setting

This was a retrospective cross-sectional study where clinical and laboratory data of patients who visited the TB clinic at Assiut Chest Hospital, Upper Egypt between January 2016 and December 2021 were retrieved after receiving endorsement from the Ethics Committee. Assiut Chest Hospital is a referral governmental hospital where patients are referred from diverse health care settings across Upper Egypt governorates for disease diagnosis and management.

2.2. Study population and selection

The study included all the probable tuberculosis patients who visited the Tuberculosis Clinic at Assiut Chest Hospital for diagnosis and management of the disease. All age groups enrolled in the study had clinical and radiological findings consistent with tuberculosis to be eligible. Patients with incomplete data were excluded from the study.

2.3. Case definition

A probable TB patient has symptoms/signs suggestive of tuberculosis, a chest radiograph consistent with tuberculosis, immunologic evidence of MTB infection, and a positive response to TB treatment but no bacteriological confirmation.[25]

2.4. Data collection

Demographic, clinical, laboratory, and radiological data findings of 2686 consecutive probable TB patients (pulmonary and extra pulmonary) were retrieved retrospectively from the patient's medical and laboratory charts during the study period including age, sex, residence, smoking status, associated comorbidities, TB treatment history, and chest radiography results. In addition, to results of Ziehl–Neelsen (ZN) smear, and GeneXpert findings.

2.5. Laboratory investigations

According to the data sheet of the patients, all the specimens (sputum-tissue and aspirate, CSF, pleural fluid, gastric lavage aspirate, or other tissue samples) were collected according to their sites. Each sample was divided into 2 parts one for pathological diagnosis by acid fast bacilli microscopy by

Ziehl–Neelsen stain and the other part for GeneXpert analysis. If the presence of MTB was detected, GeneXpert was also used to look for evidence of rifampicin resistance. Internal quality controls (sample processing control and probe check control) were used during the assay.

GeneXpert analysis (Assay version 5) was performed in harmony to the manufacturer protocol (Cepheid, Cepheid Inc. Sunnyvale, California, USA), module GX-IV-4.[26]

2.6. *Data processing and statistical analysis*

Data were analyzed using a statistical package of the Social Sciences; SPSS statistics for windows, Version 26.0 software program (IBM Corporation, Armonk, NY, USA). The following statistics were applied: (a) Descriptive statistics as number, percentage were used for categorical variables. Median and IQR were used for quantitative variables with non-parametric data. (b) Analytic statistics as Chi-square $\chi 2$ -test and where applicable Monte Carlo exact test were used to find association between the outcome variable test status and two or more categorical independent variables. Mann–Whitney test was used for comparison between two groups having quantitative variables with non-parametric data. The relation between the GeneXpert MTB or GeneXpert RR-MTB positive results and diverse study variables was investigated using binary logistic regression analyses and adjusted odds ratios (AOR) were calculated. For all tests, the confidence interval was set at 95% and P values less than 0.05 were considered statistically significant.

2.7. *Ethical considerations*

The research proposal was approved by the ethical committee of the Faculty of South Valley University (IRB number 247/21).

3. Results

In the current study, GeneXpert testing was performed on a total of 2686 patients who were clinically suspected of having tuberculosis, after excluding 81 cases with invalid or error results, data of 2605 patients were analyzed. A total of 718 cases out of 2605 (27.6%) tested positive for *Mycobacterium tuberculosis* (MTB) on GeneXpert assay. Amongst the GeneXpert MTB positive cases, 633 (88.4%) were sensitive to Rifampicin and 2 cases were undetermined, whereas 83 cases (11.6%) were resistant to Rifampicin. The prevalence of Rifampicin-resistant *Mycobacterium tuberculosis* (RR-MTB) in TB-confirmed patients was reported to be 11.6% in this study (83 cases) (Fig. 1).

Out of the 2605 total screened samples, 1849 were pulmonary samples: 1720 sputum samples, 66 bronchoalveolar lavage fluids, and 63 lung abscess samples. Whereas, 756 samples were extra-pulmonary origin: 397 pleural fluid, urine and stool samples were 102 and 72, respectively.

Fig. 2 illustrated the GeneXpet MTB bacterial burden results for pulmonary and extra-pulmonary samples (718 positive cases; 646 pulmonary, 72 extra pulmonary). Most of pulmonary samples showed high to medium MTB bacterial burden, 263 (40.7%) and 238 (36.8%) pulmonary samples, while most of extra pulmonary samples (72 cases) showed low and very low MTB burden (17, 23.6% and 32 cases, 44.4%).

Fig. 3 disclosed the number of investigated cases in each year of the study period, as well as the estimated number of MTB positive cases and RRMTB in TB-confirmed patients. The highest frequencies were recorded in 2019 then noticeable decrease in case detection was recorded in subsequent years 2020, 2021.

The median age of patients was 42^{26-58} years with a male predominance (60.5%). The study included two groups of patients based on GeneXpert MTB/RIF assay results: i) Patients with MTB were 718 cases; ii) patients with non-tuberculosis (n = 1887). The age distribution of MTB+ cases was 24.3% for those under 15 years old, and 26.5% for those over 65 years old and the remaining proportion for those patients between 15 and 64 years old. The median age of MTB+ patients was low compared to the median age of MTB− patients, and the variance was statistically important ($P < 0.05$). The highest positive finding for MTB was observed in the age group 15–34 years (33.1%). The positive MTB results were slightly higher in males (29.4%) than females (24.8%) patients. Smoker patients appeared to have higher positive findings of MTB+ cases (35.1%). Comorbid subjects had the highest rate of MTB. Categorical analysis for significance of association of MTB towards radiographic findings was null ($P > 0.05$). Out of the total 1849 pulmonary samples, 646 (34.9%) were positive for detection of *M. tuberculosis*. Whereas, out of the 756 extra pulmonary samples, 72 (9.5%) were positive for detection of MTB and the variance was statistically considerable ($P < 0.001$) (Table 1).

Table 2, illustrated that 606 cases had positive results by routine ZN Smear microscopy out of a total of 718 positive samples subjected to a confirmatory GeneXpert MTB assay. ZN Smear microscopy revealed no samples that were positive on ZN microscopy but negative on GeneXpert.

Additionally, from the patients detected with MTB on the GeneXpert MTB/RIF assay, 16.9% belonged to the newly diagnosed cases. In contrast, 80.6% of MTB+ patients reported a history of TB treatment in the past, defined as recurrence cases, and 55.2% were re-treated after treatment failure. Both recurrence and failure of treatment patients had considerably high proportions of MTB+ (80.6% and 55.2%, respectively) compared to new patients (16.9%) ($P < 0.001$), and the odds of developing MTB were significantly higher in recurrence patients; OR: 17.36 (95%CI: 9.51–31.68, $P < 0.001$).

Association between RR-MTB and patient's baseline characteristics were illustrated in Table 3. The proportions of rifampicin-resistant tuberculosis in diverse age groups did not show any significant variance. Patients belonging to the rural regions had higher rate RR-MTB (14.9%), compared to urban regions (4.4%) and the difference was statistically significant ($P < 0.001$). Significance of association of RR-MTB towards smoking, comorbid disorders were also observed (**P<0.001**). Regarding the type of cases, when compared to the new and failure of treatment cases, the frequency of RR-MTB was predominantly higher in recurrent cases who had a history of anti-tuberculosis treatment in the past[43]; out of the total 83 cases.

Table 3 also displayed the possible correlates of RR-TB among the patients with tuberculosis detected on the

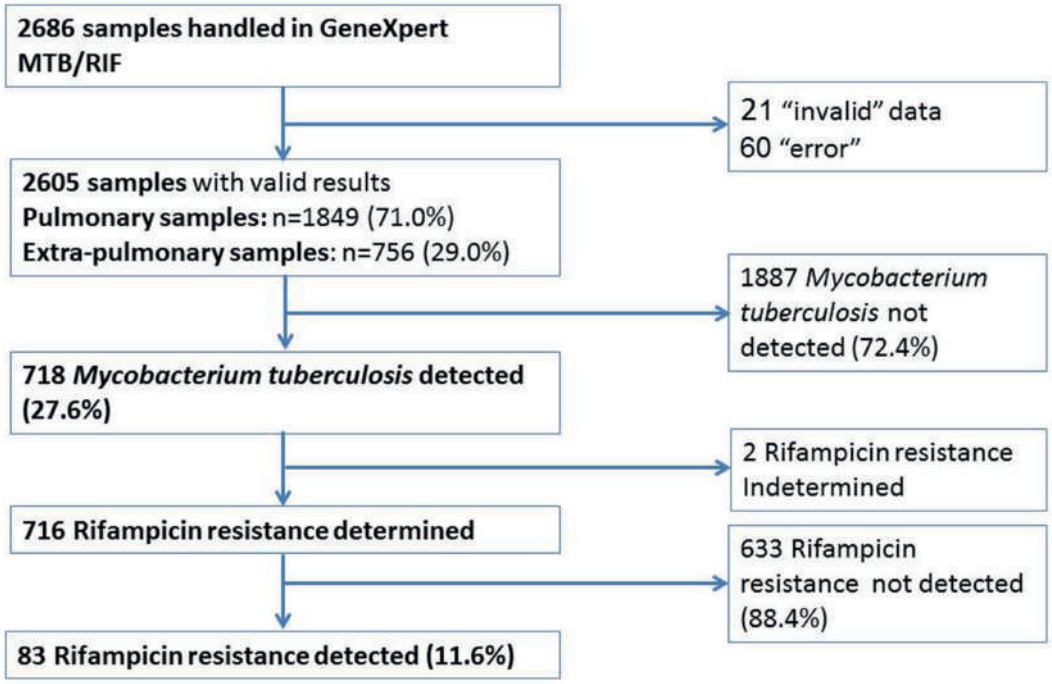


Fig. 1 – Flowchart of sample results using the GeneXpert MTB/RIF assay

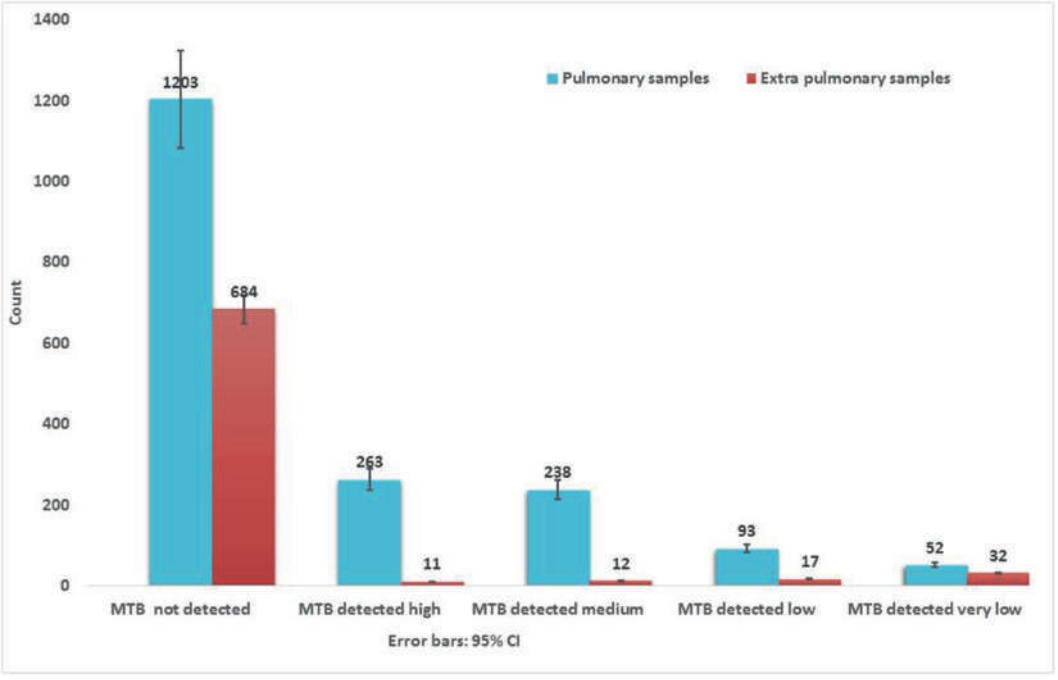
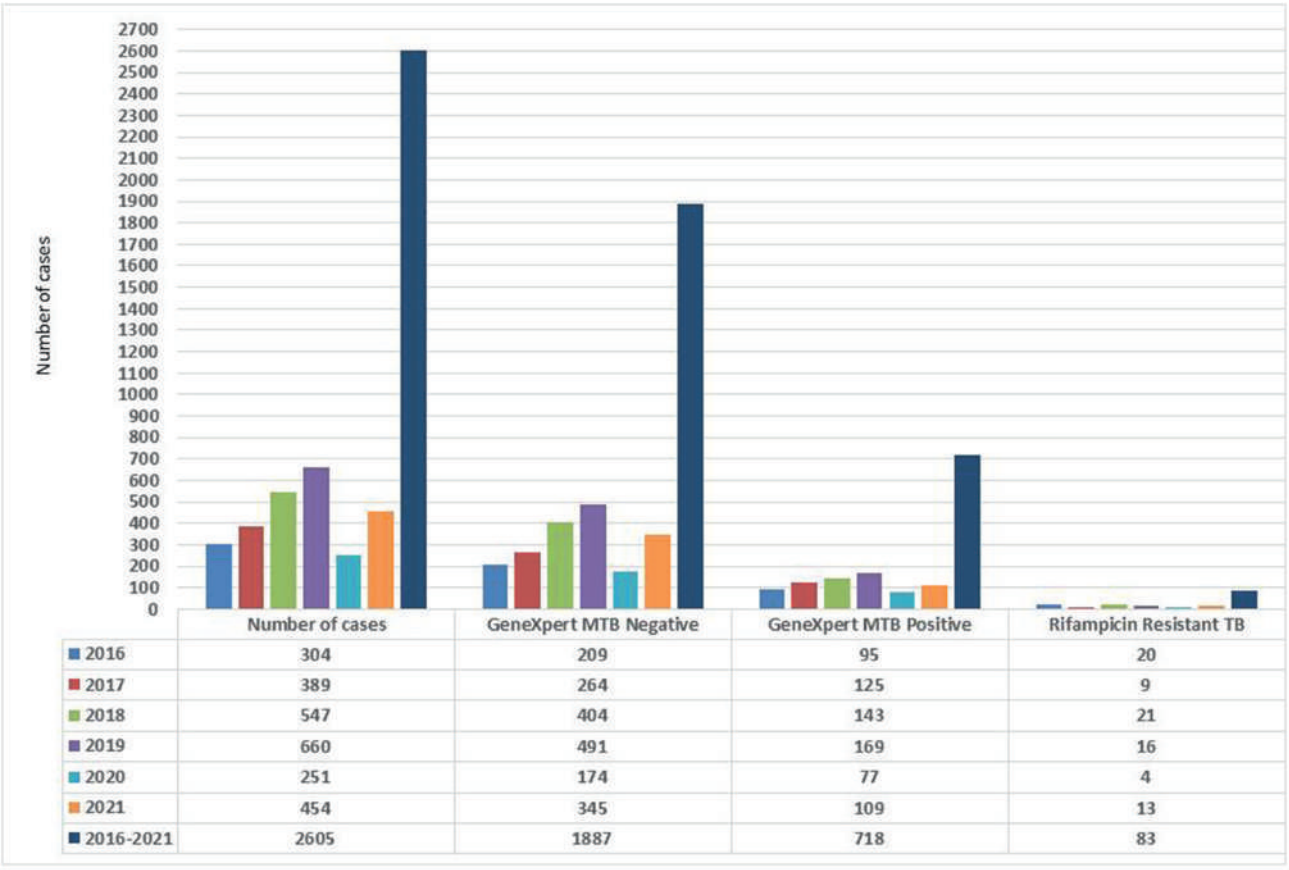


Fig. 2 – GeneXpert results for pulmonary and extra-pulmonary samples

GeneXpert MTB/RIF test in Upper Egypt. After controlling for each independent factor during multivariate analysis, we observed that smoker and ex-smoker patients had almost 2.5 times more likely to contract RR-MTB compared to non-smokers and the difference was significant (AOR = 2.54, 95% CI = 1.21–5.10, p = 0.013.0, AOR = 2.61, 95% CI = 1.94–4.81, p = 0.001 respectively). Recurrence cases had a 4-fold increased risk of RR-TB compared to other positive MTB cases and the variance was statistically significant (AOR-= 3.91, 95% CI = 2.05–7.32, p < 0.001). Patients with very low *M. tuberculosis* detected on the GeneXpert MTB/RIF test were 8 times more likely to have RR-TB (AOR, 7.90; 95% CI, 1.94–32.14;



	Number of cases	GeneXpert MTB Negative	GeneXpert MTB Positive	Rifampicin Resistant TB
2016	304	209	95	20
2017	389	264	125	9
2018	547	404	143	21
2019	660	491	169	16
2020	251	174	77	4
2021	454	345	109	13
2016-2021	2605	1887	718	83

Fig. 3 – Tabulation and graph of GeneXpert *mychobacterium tuberculosis*/rifampicin assay tests, 2016–2021

P = 0.004). Additionally, having low bacillary load was associated with decrease in MTB+/RR+ Rifampicin resistant results by GeneXpert MTB/RIF assay test.

4. Discussion

The main results of the current study is that, out of the 2605 total samples screened, 718 (27.6%) tested positive for MTB on GeneXpert assay; of them 633 (88.4%) were sensitive to Rifampicin, 83 (11.6%) were resistant to Rifampicin and 2 cases were indetermined. Factors contributing to RR-MTB were: smoker and ex-smoker, with almost 2.5 times more likely to contract RR-MTB compared to nonsmokers (p = 0.013.0, p = 0.001); recurrence cases had a 4-fold increased risk of RR-TB compared to other positive MTB cases and the variance was statistically significant (p < 0.001); patients with very low *M. tuberculosis* detected on the GeneXpert MTB/RIF test were 8 times more likely to have RR-TB (P = 0.004); and, having low bacillary load was associated with decrease in MTB+/RR+ Rifampicin resistant results by GeneXpert MTB/RIF assay test.

The reported prevalence of MTB was (27.6%) in the current study and RR-MTB among TB-confirmed cases was (11.6%). Prior diverse studies stated that the prevalence of MTB, ranging from over (15–25%).[27–30] Compared to low-mid income countries, the prevalence of *M. tuberculosis* in this study was low compared to a previous report in Ghana (31.4%)[31] and Ethiopia (32.2%).[32] However, similar frequency was recorded in Ethiopia with a prevalence rate over (13%),[33,34] In Nepal, **Sah et al. (2020)** disclosed a prevalence rate (13.8%),[35] and **Admassu et al. (2022)** illustrated that the total incidence of MTB was (9.3%).[36] This discrepancy could be elucidated by the variance in diagnosis approaches, study location, study population, geographical distinction, or TB control strategy and the sample heterogeneity.

The present work documented that the age distribution of MTB+ cases was 24.3% for those <15 years old, and 26.5% for those over 65 years old and the remaining proportion for those cases between 15 and 64 years old. The median age of MTB+ patients was low in comparison with the median age of MTB– patients (P = 0.005). However, there was no considerable alliance between age categories and MTB (P = 0.055). **Jaleta et al. (2017)** established that age category (<10, 10–16, 17–23, 24–30, 31 to 37, 38–44, 45–51, 52–58 and >58 years) was considerably allied with MTB.[29] Similarly, **Mulu et al. (2017)** confirmed the same result. These disparities across the studies could be associated with the sample size variation and alteration in cut-off values for age categories.[30] Moreover, in agreement with our study where the highest positive finding for MTB was observed in the age group 15–34 years (33.1%), **Sah et al. (2020)** demonstrated a high frequency of MTB, in age group (15–39 years).[35] This could be ascribed to the widespread range of motion of this age sub-category subjects to get infected by the TB bacilli.

Table 1 – Baseline characteristics of the 2605 included patients and possible correlates for positive Mycobacterium Tuberculosis (MTB).

Parameters	GeneXpert MTB assay result		Total n = 2605	P value	AOR (95% CI)	P value
	MTB+ Cases n (%) n = 718	MTB– Cases n (%) n = 1887				
Age group classification						
<15	55 (24.3)	171 (75.7)	226	0.055	1.14 (0.48–4.5)	0.092
15–34	257 (33.1)	519 (66.9)	776		1.43 (0.56–2.00)	0.083
35 = 49	141 (25.8)	406 (74.2)	547		1.11 (0.19–1.90)	0.062
50–64	161 (24.3)	502 (75.7)	663		1.04 (0.44–2.55)	0.088
≥65	104 (26.5)	289 (73.5)	393			
Median [25th; 75th *percentile*]	38 [25; 57]	44 [25; 57]	42 [26; 58]	**0.005∞**	1.013 (0.99–1.63)	**0.051**
Gender						
Male	463 (29.4)	1113 (70.6)	1576	**0.010**	1.22 0).73–2.03 (	0.451
Female	255 (24.8)	774 (75.2)	1029			
Residence						
Rural	492 (26.2)	1384 (73.8)	1876	0.054	1.46 (0.91–2.33)	0.118
Urban	226 (31.0)	503 (69.0)	729			
Special habits						
Smoker	235 (35.1)	435 (64.9)	670	**<0.001**	1.39 (0.79–2.45)	0.250
Ex-smoker	21 (17.2)	101 (82.8)	122		0.23 (0.05–1.09)	0.065
Nonsmoker	462 (25.5)	1351 (74.5)	1813			
Comorbidities						
Yes	45 (9.2)	446 (90.8)	491	**<0.001**	0.73 (0.22–2.12)	0.098
No	673 (31.8)	1441 (68.2)	2114			
ZN smear microscopy status						
Negative	112 (5.6)	1887 (94.4)	1999	**<0.001**?	0.0 (0.0–)	0.988
1+	229 (100.0)	0 (0.0)	229		2.14 (0.0–)	1.000
2+	191 (100.0)	0 (0.0)	191		1.21 (0.0–)	1.000
3+	186 (100.0)	0 (0.0)	186			
Main radiographic findings						
Nodule	120 (30.2)	277 (69.8)	397	0.062	–	–
Micro-Nodule	97 (33.8)	190 (66.2)	287			
Cavitary lesion	95 (30.0)	222 (70.0)	317			
Consolidation	112 (35.9)	200 (64.1)	312			
Pleural effusion	133 (38.8)	210 (61.2)	343			
Lymphadenopathy	89 (46.1)	104 (53.9)	193			
Extra-pulmonary lesion	72 (9.5)	684 (90.5)	756			
Site of lesion in radiographic findings						
Bilateral lesion	492 (26.2)	1384 (73.8)	1876	0.054	2.19 (0.90–1.10)	0.099
Unilateral lesion	226 (31.0)	503 (69.0)	729			
Anatomical site of TB						
Extra- pulmonary	72 (9.5)	684 (90.5)	756	**<0.001**	3.29 (0.97–1.18)	0.098
Pulmonary	646 (34.9)	1203 (65.1)	1849			
Type of case						
Failure of treatment	144 (55.2)	117 (44.8)	261	**<0.001**	2.28 (0.72–3.29)	0.061
Recurrence	224 (80.6)	54 (19.4)	278		17.36 (9.51–31.68)	**<0.001**
New	350 (16.9)	1716 (83.1)	2066			

We found that the positive MTB results was slightly higher in males (29.4%) than female's patients (24.8%) (p = 0.010). Compatible result were recorded from the **WHO (2016)**[38] and previous studies.[32,35–37] Nevertheless, **Jaleta et al. (2017)** established that the illness was somewhat predominant among females: 188 (25.3%). This variance might be attributed to behavior change, numerous environmental elements, and the higher male exposure to diverse elements such as smoking and alcoholism that constitute a hazard of getting infected by TB bacilli.[29]

This study demonstrated that the smoker cases appeared to have higher positive findings of MTB+ cases (35.1%). Accordingly, **Amere et al. (2018)** displayed that smoking attributed for over one of every six cases of TB illness in the high-TB-prevalent nations, also they disclosed that smoking reported for more than one of every seven TB deceases.[39]

This study confirmed that comorbid study subjects had a higher rate of MTB. Similarly, **Bhattacharya et al. (2020)** found that comorbid disorders principally diabetes mellitus, existed in 50% of their cases, frequently in pulmonary than extra-pulmonary TB cases.[40]

We found that out of the total 1849 pulmonary samples, 646 (34.9%) were positive for detection of *M. tuberculosis*. Whereas, out of the 756 extra pulmonary samples, 72 (9.5%) were positive for detection of MTB with a considerable variance (P < 0.001). Compatible to our results, **Admassu et al.**

Table 2 – Comparison of results of GeneXpert MTB assay and Ziehl–Neelsen (ZN) examination results (n = 2605).

ZN smear results		GeneXpert result, n (%)					Total
		Negative (n = 1887)	Positive (n = 718)				(N = 2605)
		Negative	Positive Very low	Positive Low	Positive Medium	Positive High	
Negative ZN smear	**Negative = 1999**	1887 (100.0)	53 (63.1)	34 (30.9)	19 (7.6)	6 (2.2)	1999 (76.7)
Positive ZN smear (scanty)	**Positive = 606**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Positive ZN smear (+)		0 (0.0)	31 (36.9)	68 (61.8)	88 (35.2)	42 (15.3)	229 (8.8)
Positive ZN smear (++)		0 (0.0)	0 (0.0)	8 (7.3)	103 (41.2)	80 (29.2)	191 (7.3)
***Positive* ZN smear (+++)**		0 (0.0)	0 (0.0)	0 (0.0)	40 (16.0)	146 (53.3)	186 (7.1)
Total	**2605**	**1887**	**84**	**110**	**250**	**274**	2605 (100.0)

MTB, *Mycobacterium tuberculosis*; TB, tuberculosis. ZN, Ziehl–Neelsen.

(2022) found that there was a statistically considerable relation between GeneXpert positivity results and types of samples (P value <0.05).[37]

The present study disclosed that, both recurrence and failure of treatment cases had considerably high proportions of MTB+ (80.6% and 55.2%, respectively) compared to new cases (16.9%) (P < 0.001). In lines with this result, former studies found that history of prior treatment non-success was ominously allied with TB incidence (P < 0.05).[35,37,41] However, **Jaleta et al. (2017)** found opposite results regarding the history of prior non-successful treatment. Moreover, we found that the odds of developing MTB were significantly higher in recurrence patients; OR: 17.36 (95% CI: 9.51–31.68, P < 0.001).[29] The possible explanation is that the treatment failure cases failed to eradicate organism from affected personnel, possible development of drug resistance, or continuing spread of TB bacilli.[11,30]

This study summarized that the prevalence of RR-MTB among TB-confirmed patients was reported to be 11.6% in this study. In harmony with this result, previous studies for example: two previous studies in Egypt found that the prevalence of RR-MTB was (10.1%), (10.2%) respectively,[11,13] Nepal (10.2%),[35] Ethiopia (10.3%),[30] Nigeria (13.9%),[42] this figure is lower than previous studies in Ethiopia (15.8–33.3%) ([29,43,44]). However, the universally reported frequency of RR-TB was <1%,[45,46] in Ghana (4.5%),[47] and Swaziland (7%).[48] We may attribute this variance in prevalence of RR/MTB to several factors including differences in prevalence to anthropometric elements, ecological factors, variation in the study population, sample size variability, variances in alertness of study populations concerning resistance to drugs, and unfortunate patient compliance.

This study found that the proportions of RR-TB in diverse age groups did not show any considerable variance (p = 0.353), previous studies supported our finding.[35,49] In contrast a previous report in Nigeria, found that patients above 45 years was unconventionally allied with RR-MTB.[50] In India, when age stratified as follow (less than 20, 20–40, 40–60, above 60 years), it was considerably allied with RR-MTB.[41] Also, similar results were reported in Ethiopia[44] and Egypt.[51]

Farghaly et al. (2021), found that (59.5%) of non-DR-MTB cases and (55.6%) of MTB-RR cases were males. However, we did not find any considerable variance between the 2 groups regarding the gender.[13]

We found that cases belonging to the rural regions had higher rate RR-MTB (14.9%), compared to urban regions (4.4%) and the difference was statistically significant (P < 0.001). **Ali et al, (2019)** confirmed similar result.[52] **Sah et al. (2020)** displayed that topographical variety of the patients was not among the contributive elements for the incidence of RR-MTB.[35]

A recent systematic review summarized that smoking was definitely and considerably allied with DR-MTB. Subcategory analysis also displayed that smoking was an independent hazard element for MDR-TB[53]. The present study displayed similar result (P < 0.001).

This study found that the frequency of RR-MTB was predominantly higher in recurrent cases that had a history of antituberculous treatment in the past; 43 out of the total 83 cases, previous studies confirmed similar results.[3,37,54] Nevertheless, **Sah et al. (2020)** found that although the frequency of RR-TB among cases with history of prior TB treatment were higher (13.79%) in comparison with new cases (5.75%), there was absence of any relation between these variables (p = 0.45). These conclusions specify that improper experience with anti-TB agents might have augmented DR-MTB development and it may advocate TB prevention program weakness.[35]

Comorbid disorders are persistently being acknowledged as a dynamic element in TB control. This study found that the frequency of RR-MTB was predominantly higher among the cases with comorbid disorders, in harmony a recent study in China found that tobacco-smoking, cavitation on chest radiology and comorbid disorders were hazard elements for the development of drug resistance among retreated TB cases.[55] Moreover, **Alemu et al. (2021)** stated that the hazard of decease in DR-MTB cases with any type of comorbid disorder was 2 times in comparison with their corresponding subjects.[56]

This study summarized that after controlling for each independent factor during multivariate analysis, we detected that smoker and ex-smoker cases had almost 2.5 times more probable to contract RR-TB compared to non-smokers, recurrence cases had a 4-fold increased risk of RR-TB compared to other positive MTB cases and patients with very low *M. tuberculosis* detected on the GeneXpert MTB/RIF test were 8 times more probable to have RR-TB.

As regards smoking, **Ali et al. (2019)** stated that previous smoking history was a prognosticator of DR-TB (OR (95%

Table 3 – Association between Baseline Characteristics and Rifampicin Resistant MTB among 716 TB Confirmed Patients, and possible correlates for Positive Rifampicin Resistance (RR-MTB).

Parameters	GeneXpert MTB/RIF assay		Total n = 716[a]	P value (χ2)	AOR (95%CI)	P value
	MTB+/RR+ Rifampicin resistant n = 83 n (%)	MTB+/RR− Rifampicin sensitive n = 633 n (%)				
Age groups classification						
<15	11 (20.0)	44 (80.0)	55	0.353∘	0.43 (0.41–1.4)	0.132
15–34	30 (11.7)	227 (88.3)	257		1.14 (0.48–4.53)	0.452
35 = 49	15 (10.6)	126 (89.4)	141		1.05 (0.66–1.66)	0.832
50–64	16 (9.9)	145 (90.1)	161		1.16 (0.17–1.95)	0.522
≥65	11 (10.8)	91 (89.2)	102			
Median [25th; 75th *percentile*]	35 [24; 50]	38 [25; 57]	38 [25; 57]	0.305∞	0.98 (0.97–1.005)	0.170
Gender						
Male	58 (12.6)	404 (87.4)	462	0.278	0..83 (0.41–1.66)	0.608
Female	25 (9.8)	229 (90.2)	254			
Residence						
Rural	73 (14.9)	417 (85.1)	490	**<0.001**	1.94 (0.32–2.97)	0.071
Urban	10 (4.4)	216 (95.6)	226			
Special habits						
Smoker	39 (16.7)	195 (83.3)	234	**<0.001**	2.54 (1.21–5.10)	**0.013**
Ex-smoker	8 (38.1)	13 (61.9)	21		2.61 (1.94–4.81)	**0.001**
Non-smoker	36 (7.8)	425 (92.2)	461			
Comorbidities						
Yes	16 (35.6)	29 (64.4)	45	**<0.001**	1.65 (0.88–2.48)	0.091
No	67 (10.0)	604 (90.0)	671			
Main radiographic findings						
Nodule	20 (16.4)	102 (83.6)	122	0.061		
Micro-Nodule	11 (10.8)	91 (89.2)	102			
Cavitary lesion	10 (9.3)	98 (90.7)	108			
Consolidation	20 (20.0)	80 (80.0)	100			
Pleural effusion	5 (4.8)	99 (95.2)	104			
Lymphadenopathy	5 (4.6)	103 (95.4)	108			
Extra-pulmonary lesion	12 (16.7)	60 (83.3)	72			
Site of lesion in radiographic findings						
Bilateral lesion	58 (12.6)	404 (87.4)	462	0.080	1.73 (0.95–2.57)	0.937
Unilateral lesion	25 (9.8)	229 (90.2)	254			
ZN smear microscopy status						
Negative	11 (10.0)	99 (90.0)	110	0.397	0.18 (0.05–0.64 (	**0.009**
1+	21 (9.2)	208 (90.8)	229		0.23 (0.95–0.57)	**0.001**
2+	26 (13.6)	165 (86.4)	191		0.74 (0.37–1.52)	**0.416**
3+	25 (13.4)	161 (86.6)	186			
Xpert MTB/RIF bacterial burden						
Very low	9 (11.0)	73 (89.0)	82	0.785	7.90 (1.94–32.14)	**0.004**
Low	12 (10.9)	98 (89.1)	110		1.24 (0.35–4.32)	0.091
Medium	26 (10.4)	224 (89.6)	250		2.48 (0.22–5.03)	0.071
High	36 (13.1)	238 (86.9)	274			
Anatomical site of TB						
Extra-pulmonary	12 (16.7)	60 (83.3)	72	0.156	0.59 (0.25–1.55)	0.287
Pulmonary	71 (11.0)	573 (89.0)	644			
Type of case						
Failure of treatment	13 (9.0)	131 (91.0)	144	**<0.001**	2.04 (0.92–4.49)	0.071
Recurrence	43 (19.4)	179 (80.6)	222		3.91 (2.05–7.32)	**<0.001**
New	27 (7.7)	323 (92.3)	350			

∘Pearson Chi-Square; ∞ Mann–Whitney U; ? Monte Carlo exact test p value < 0.05 is Statistically significant.
Abbreviations: IQR, interquartile range; AOR, adjusted odds ratio; RR, rifampicin resistance; TB, tuberculosis; ZN, ZN, Ziehl–Neelsen.
[a] 2 cases indetermined; Non-married: (Single/Widowed/divorced)

CI) = 4 (1.2–13.2)),[52] In contrast, others, summarized that prior tobacco smoking, was not ominously statistically allied with the incidence of RR/TB.[44]

Chuchottaworn et al, (2015) disclosed that the essential hazard aspects for expansion of drug resistance were untreated TB cases, high TB bacilli load 3+, and existence of lung cavities on chest radiography.[57]

Lastly, a surprising result that patients with very low *M. tuberculosis* detected on the GeneXpert MTB/RIF test were 8 times more likely to have RR-TB (P = 0.004). This may not be

fully understood, however, evidence suggest an interplay of several mechanisms may explain this finding: first, drug-resistant M.tb adapts differently than drug-susceptible strains to the lung environment at the cellular level.[58] Second, during *M.tb* infection certain bacterial sub-populations, known as persisters, can become phenotypically tolerant to antimycobacterial drugs without acquiring genetic mutations. This is associated with a metabolic inactive-non-replicating status of the persistent undetected bacilli.[59] Third, exposure of M.tb to drugs induces a bacterial stress response; thus, only those M.tb strains able to adapt will prevail, initiating a competitive selection process between M.tb clones that may acquire different beneficial mutations to survive (clonal interference).[60] Finally, *M.tb* has evolved to exploit AM resources as a strategy for host immune evasion like *M.tb* ability to escape macrophage killing that may dictate the outcome of the infection.[61]

The strength of our conclusions comprises the fact that the study population was employed at a largest TB management center in Egypt (Assiut Chest Hospital) which is a referral governmental hospital where patients are referred from the diverse health care settings across Upper Egypt governorates.

This study had limitations: First: although it is a large sample size study, it was limited to one referral center in Egypt. Thus, a larger national study using the GeneXpert test will yield a better estimation. Second: a large number of excluded cases due to invalid or error results. Third: the lower number of RR-MTB in this study could interrupt the results of the logistic regression evaluation for determining the significant correlates. Fourth: we did not use a confirmatory test for TB diagnosis such as conventional cultures as GeneXpert test may subject to have some fallacies. Fourth: its retrospective nature. Fifth: The Xpert test cannot discriminate among viable and non-viable organisms during the recognition of DNA of MTB, and thus we cannot use it to either follow up the cases or efficiency of the treatment. Finally: The advent of coronavirus disease 2019 outbreak, with its implications, throughout the study period hindered patients, healthcare supervisors, and investigators attending TB management amenities with its influence on such studies.

5. Conclusion

This study disclosed a high-rate MTB in Egyptian probable TB cases. Smoking, recurrence and cases with a very low M. *tuberculosis* burden noticed on the GeneXpert MTB/RIF test had augmented risk of RR-TB.

Author contribution

All authors have read and approved the final version of the manuscript.

Ethical

The study was approved by the ethical committee of South valley Faculty of Medicine IRB number: 247/21.

Conflicts of interest

The authors have none to declare.

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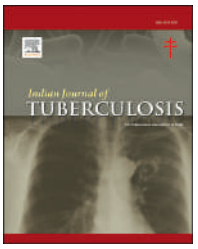

Original article

To study the occurrence of risk factors for pulmonary tuberculosis in the homeless population in areas of Delhi, India

Kamal K. Chopra [a], *Alveena Malik* [b], *Abha Indora* [b], *Praveen Pandey* [b], *Surabhi Pandey* [c,*]

[a] *New Delhi Tuberculosis Centre, New Delhi, India*
[b] *MPH in Indian Institute of Public Health, Delhi, India*
[c] *Indian Institute of Public Health Delhi, India*

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ABSTRACT

Background: In India, there are only a few studies done in the area of assessing the risk factors of Tuberculosis (TB) among the homeless population. The homeless population has quite a higher chance of developing Pulmonary Tuberculosis (PTB) as compared with the general population due to the presence of an inappropriate environment and high prevalence of risk factors.

Methods: This study was done among the homeless population in both males and females aged 18 years and above in areas of Delhi (Yamuna Pusta and Mansarovar Park). The participants were screened for TB symptoms and risk factors to diagnose active PTB in them.

Results: Out of 200 participants, 17 were diagnosed with active PTB. The overall occurrence of Tuberculosis among the studied homeless population was found to be 85 cases per 1000 population. The occurrence of behavioral habits such as smoking was found to be 41.2% (7/17), tobacco chewing at 47.1% (8/17), and alcohol at 47.1% (8/17) among the cases. The occurrence of HIV coinfection was 5.9% (1/17) and diabetes was 5.9% (1/17). The prevalence of TB among homeless females was 1.5 times higher than homeless males but out of 17 diagnosed patients, males had a higher prevalence of TB as compared to females.

Conclusion: The occurrence of PTB in the homeless population is quite high as it is also reported in a study in the United States that the national incidence of tuberculosis in the homeless population was 36 cases/100,000 and it needs to be addressed to eliminate tuberculosis.[7] Moreover, the risk factors such as tobacco, smoking, alcohol, coinfections, etc. might have played a major role in the development of PTB. Also, there is a need for larger studies with large sample sizes to provide evidence against the same.

* *Corresponding author.*
E-mail address: surabhi.pandey@phfi.org (S. Pandey).
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1. Introduction

Homelessness is one of the major issues faced by India. About 4.5 lakh families having a total population of 17.73 lakh are living without any kind of support roof cover. The sex ratio of the homeless population of India is about 694 females per 1000 males.[1] According to India's 2011 Census, five metropolitan cities such as Mumbai, Kolkata, Bangalore, Delhi, and Chennai have a high contribution of 26% of India's homeless population.[2] People who experience homelessness are at higher risk of developing tuberculosis disease. The increased chances of getting tuberculosis infection are because of various factors that accelerate a person's risk of getting Tuberculosis disease. These factors include substance abuse, HIV infection, diabetes, and the homeless shelter surroundings or environment such as overcrowding and the poor state of the ventilation system.[3] Hence, a combination of such factors becomes favorable for spreading tuberculosis. Apart from this, the homeless population often lacks access to the medical facilities which are required to make an early diagnosis of tuberculosis disease.

According to a study in Germany, the highest risk for having latent tuberculosis infection was found in patients who are from high-incidence countries.[4] Hence, it becomes important to either treat latent tuberculosis infection or monitoring of active TB disease is needed at an early stage. A study in Chennai city, South India on tuberculosis among the homeless shows a high prevalence of tuberculosis among the vulnerable population, and treating it becomes a big challenge. Also, there is a need for more studies from India to provide evidence of the high prevalence of tuberculosis among the homeless population.[5]

A comprehensive review of the homeless population in India having PTB is needed but remains unknown. To understand it better and to address the occurrence of tuberculosis disease and associated risk factors in this key population, we studied PTB among the homeless population in areas of Delhi during the months of June and July 2022. The objective of this study was to find the occurrence of risk factors for PTB among the homeless population.

2. Methods

2.1. Definition of homeless population

The *homeless population* is defined as persons who are not living in "*census houses*", but are living in buildings or live in the open on roadsides, pavements, in Hume pipes, under flyovers and staircases, or in the open in places of worship, railway platforms, etc.[6]

2.2. Study area

This study was conducted in the months of June and July 2022 where homeless people are living in areas of Delhi (Yamuna Pusta and Mansarovar Park in the coastal area and east Delhi respectively).

2.3. Study population

All individuals were male and female, aged 18 years and above who were living and continue to live, in the selected areas of Delhi, forming the study population. This study was a part of a homeless project named *Hausla: National resource team for urban homeless,* which is operated under an NGO (Centre of equity studies). Recovery shelter is the program that runs under *Hausla* to deliver shelter-based care and support to homeless people with acute and chronic disease conditions who have received primary treatment in a hospital. The sample size for this study was taken as 200 according to the number of participants screened under the project *Hausla*.

2.4. Tuberculosis screening procedure

All the individuals in the selected areas of Delhi, aged 18yrs and above were questioned about their chest symptoms, suggestive of TB disease like having cough for more than 2 weeks, blood in cough, weight loss, night sweats, loss of appetite, and status of risk factors and coinfection such as smoking, tobacco, alcohol consumption, I.V drug use, HIV and diabetes.

After screening the individuals through a questionnaire that was prepared with the help of a questionnaire from 'Hausla Project', a Chest X-ray was done for all the individuals, and the radiograph was read immediately by a doctor. For those people who had abnormal chest radiographs, sputum samples were collected. The samples were then transported to the lab and examined by trained laboratory technicians. The results were then graded as per NTEP guidelines. The individuals with abnormal chest X-rays or suggestive of Tuberculosis disease started the treatment on the same day.

2.5. Data collection

Data about demographics (e.g., age, sex, etc.), current location, past history of TB treatment, comorbidities, alcohol & tobacco consumption, and smoking were collected using a pre-coded interview schedule (Questionnaire).

2.6. Data management and analysis

Data were checked for errors and were analyzed using Excel and STATA/SE 15.1 software. The occurrence of PTB among the homeless and associated risk factors was estimated per 1000 population.

2.7. Ethics approval

Informed written consent for screening of the homeless population was taken by the NGO (Centre for Equity studies), the contents of which were verbally read and explained to the participants in their local language. The trained field investigators approached eligible individuals and explained the procedures, risks, and benefits of the study. Individuals below 18 years were not included in the study. All patients who were found positive for PTB were referred to NTEP and were followed through with their treatment and free counseling was provided to them to encourage completion of the entire course of treatment.

3. Results

Out of 200 homeless patients, 17 patients were reported with pulmonary tuberculosis and were found to have abnormalities in X-ray suggestive of tuberculosis. The Tuberculosis prevalence among homeless people was 85 cases per 1000 population during the months of June and July. Table 1 is the descriptive table of all the participants among the homeless population in areas of Delhi. Among 17 tuberculosis patients, 5 were sleeping on the streets while 12 were sleeping at other locations (workplace or in jhuggi). Out of 200 people, the TB cases were found to be slightly higher in females 8.8% (7/80) as compared to males 8.3% (10/120) among the age group 18–70 years. Fig. 1 shows the prevalence of males and females amongst the diagnosed tuberculosis patients. The occurrence of behavioral habits such as smoking was found to be 41.2% (7/17) and tobacco at 47.1% (8/17). None were found to be substance abuse. However, 47.1% (8/17) were alcohol users. The occurrence of HIV coinfection was 5.9% (1/17) and diabetes was also 5.9% (1/17). Fig. 2 is the graphical representation of the risk factors among the diagnosed tuberculosis patients in the homeless population.

The estimated occurrence is quite high i.e., 85 cases per 1000 population which suggest that the homeless population has a higher chance of getting active PTB. Similar findings were reported from other countries and from a pilot study conducted in Chennai, India.[5] There is a requirement for some large-scale studies to estimate the prevalence of Pulmonary tuberculosis as there was a small sample size in this study. Moreover, the occurrence of behavioral habits seems quite high in this population which could be a possible reason for the development of tuberculosis in such patients. Likewise, the occurrence of coinfection such as HIV and Diabetes along with behavioral risk factors have possibly increased the risk of development of PTB. Therefore, it becomes necessary to address TB control among the homeless population for achieving WHO's goal to end TB by 2030.

All the PTB cases which were confirmed were referred for treatment to NTEP and all were started with treatment as per the guidelines. However, due to some vulnerable situations, the homeless population at times, is unable to continue with the treatment and this leads to treatment failure, thus 1 PTB diagnosed patient when moved to some other place, could not be traced.

Table 1 – Descriptive table of all participants among the homeless population in areas of Delhi.

Participants (n)	200
Age in years (mean, SD)	35.3 (14.3)
Sex (%)	
Male	120 (60%)
Female	80 (40%)
History of Tuberculosis (%)	
Yes	31 (15.5%)
No	169 (84.5)
Smoking (%)	
Yes	58 (29%)
No	142 (71%)
Alcohol (%)	
Yes	73 (36.5%)
No	127 (63.5%)
Tobacco (%)	
Yes	108 (54%)
No	92 (46%)
I.V Drug use (%)	
Yes	1 (0.5%)
No	199 (99.5%)
Diabetes (%)	
Yes	12 (6%)
No	40 (20%)
Unknown	148 (74%)
HIV (%)	
Reactive	4 (2%)
Non-reactive	61 (30.5%)
Unknown	135 (67.5%)
Status of Tuberculosis (%)	
Male (n = 120)	10 (8.3%)
Female (n = 80)	7 (8.8%)

n-number of participants, SD–standard deviation, %- percentage.

4. Discussion

Tuberculosis is one of the diseases to which the homeless population is prone as the factors which are associated with homelessness provide an ideal environment for *Mycobacterium tuberculosis* to thrive. Many factors are responsible for the progression of disease in this population such as poor nutrition, smoking, alcohol, tobacco, and co-infections (HIV and Diabetes). The occurrence of PTB in the homeless population is quite high and a study in the United States have reported that the national incidence of tuberculosis in the this population was 36 cases/100,000 and it needs to be addressed to eliminate tuberculosis.[7] One of the studies by the UK charity Homeless Link found out that about a third of the homeless population regularly eat less than two meals a day which can lead to a weak immune response and hence can become a risk factor for tuberculosis. In the case of alcohol, it was found that about 20% of those surveyed admitted to drinking alcohol more than four times per week, hence, excessive consumption of alcohol can increase the risk of progression of tuberculosis. In tobacco smoking, it was found that the smoking rate was 77% among those who were surveyed, compared with 21% in the wider population. "There's a clear correlation between smoking and tuberculosis", says Mario Raviglione (WHO, Geneva, Switzerland).[8] Hence, this shows that the homeless population has a higher risk of developing PTB and it needs to be addressed as early as possible as homeless people tend to present themselves for their treatment late after they have potentially infected others. Also, apart from early screening, ensuring that these people comply with their treatment presents a difficult challenge. This key population usually adheres to their regimen for a few weeks before disappearing and may come to resume it afterward which can be challenging as tuberculosis treatment entails 6 months of daily ingestion of three to four drugs. Hence, this study was done to provide evidence regarding the occurrence of risk factors that leads to the progression of PTB in the homeless population.

5. Limitations

As this study is on a small sample size, hence we have interpreted the results with some limitations. This study on the prevalence of tuberculosis is from a single city only that is

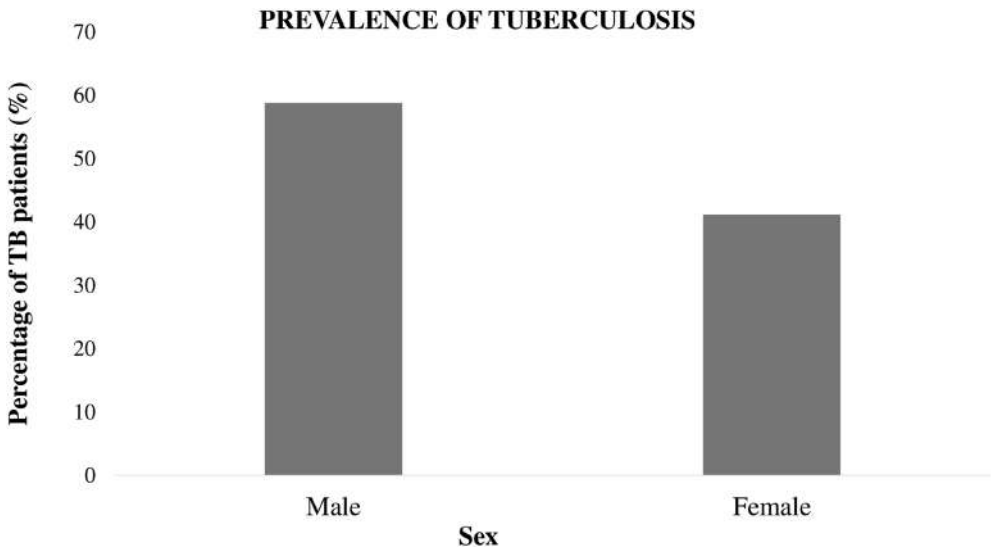

Fig. 1 – Shows the occurrence of tuberculosis among males and females in the study population. Out of 17 diagnosed patients, 10 were males and 7 were females.

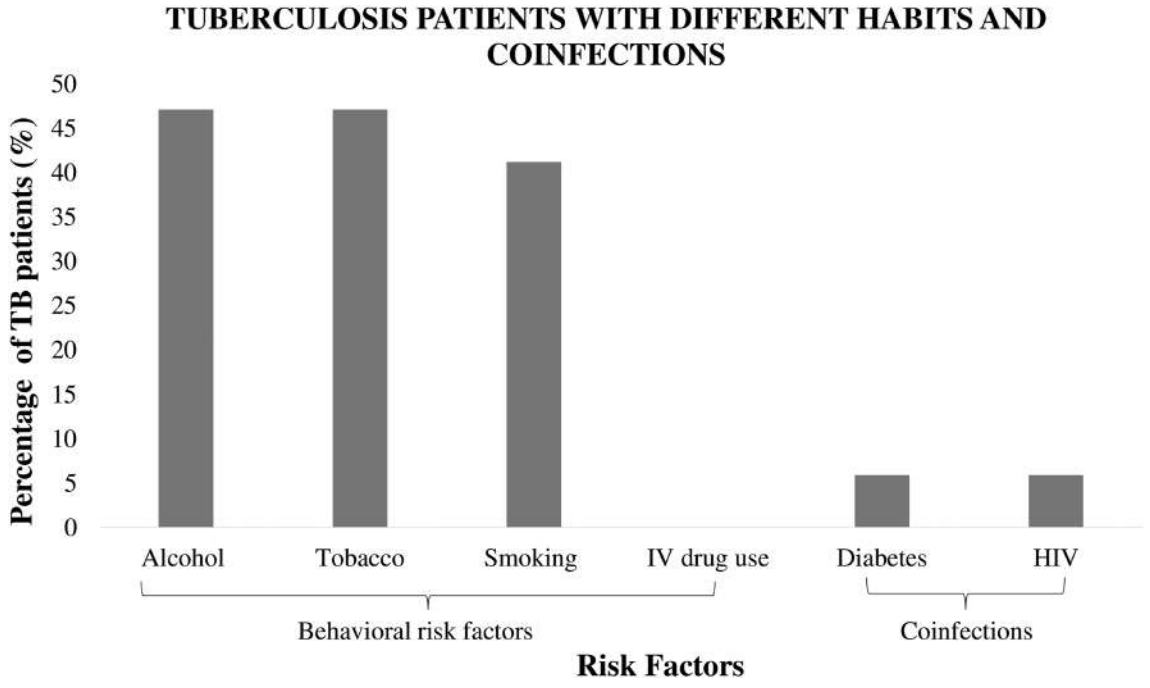

Fig. 2 – Shows the prevalence of different behavioral habits and coinfection among diagnosed tuberculosis patients. It was seen that consumption of alcohol and tobacco is the highest. Also, there were few cases of coinfections as well. Therefore, these risk factors might have contributed in the development of PTB.

Delhi, India and we have only included 2 areas of Delhi, Yamuna Pusta and Mansarovar park. Therefore, results may vary in other areas in Delhi or other parts of India. Another limitation is the small sample size. To analyze the national scenario, a large sample size is needed to draw implications for the program. The third limitation is as this was a cross-sectional study, it lacks sufficient information to conclude that the risk factors and coinfection were present before the development of TB and that these risk factors have led to the development of active PTB in the population.

6. Conclusion

This study's findings conclude that there was a high occurrence of tuberculosis among the homeless population and risk factors play a

major role in the development of PTB. The treatment success in such a key population becomes a challenge as this population is deprived of basic necessities of life which makes them prioritize those above the treatment. Thus, it becomes essential to bridge the gap between medical care and the homeless population to lower their risk of developing TB. Also, there is a need to learn from other countries about their strategy for dealing with this key population to achieve goal of National TB elimination by 2025.

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Conflicts of interest

The authors have none to declare.

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Original article

Correlating clinical breakpoint concentration of moxifloxacin with gyrA mutations using the *GenoType MTBDRsl* assay Version 2.0

Zeeshan Sidiq [a], *M. Hanif* [a,*], *Kaushal Kumar Dwivedi* [a], *Kamal Kishore Chopra* [a], *Ashwani Khanna* [b], *B.K. Vashishat* [b]

[a] *New Delhi Tuberculosis Centre, Jawaharlal Nehru Marg, Delhi Gate, New Delhi, 110002, India*
[b] *State TB Cell, NTEP Delhi, Health Centre Building, Delhi Government Colony, Gulabi Bagh, Delhi, 110007, India*

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MDRTB

ABSTRACT

Introduction: Widespread use of Fluoroquinolones (FQs) has led to the development of its resistance in clinical isolates of *Mycobacterium tuberculosis*. However, in *Mycobacterium tuberculosis*, phenotypic resistance to FQs has been shown to be heterogeneous, ranging from low-level resistance to high-level resistance. This stratification in resistance has important implications for the inclusion of moxifloxacin (Mfx) in the treatment regimen. The World Health Organization recommends the use of GenoType MTBDRsl assay as the initial test for detecting resistance conferring mutations (both high and low) to FQs in patients with confirmed MDR-RR TB. The present study was conducted to explore the relationship of MTBDRsl Version 2.0 detected mutations in gyrA gene and genotypic DST of Mfx at WHO defined Clinical Breakpoint (CB).

Materials and methods: A total of 200 sputum samples from Confirmed MDR/RR TB patients were included in this study. All of these samples had mutations conferring resistance to FQ confirmed by GenoType MTBDRsl assay. These samples were further subjected to Phenotypic DST against moxifloxacin using the Bactec MGIT-960 system.

Results: All of the 200 representative FQ resistant isolates had mutations in gyrA gene only with no detectable mutation in gyrB gene. 109 (54.5%) of the isolates had mutations associated with high-level increase in MIC while 91 (45.5%) isolates had mutations associated with low-level increase in MIC. Phenotypic DST of these 200 isolates against Mfx at CB (1.0μg/ml) revealed that of the 109 isolates with mutations associated with high-level increase in MIC and expected to be resistant at CB, only 34 (31.2%) were resistant and the remaining 75 (68.8%) were sensitive.

Conclusion: Moxifloxacin is an important drug in the regimen for treating Drug-resistant TB and the decision to exclude this drug from the regimen should not be taken merely on the basis of mutational patterns. It should rather be taken after considering the combined results of mutational analysis and phenotypic DST.

* *Corresponding author*. New Delhi tuberculosis Centre, JLN Marg, Delhi Gate, New Delhi 110002, India. Tel.: +9810979064.
E-mail address: irldlndc@rntcp.org (M. Hanif).

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1. Introduction

Fluoroquinolones (FQs) are considered critical components of MDR-TB regimens as they have been shown to be associated with better outcomes.[1,2] World Health Organization (WHO) treatment guidelines acknowledged the superior bactericidal activity of the later-generation fluoroquinolones such as levofloxacin, moxifloxacin and gatifloxacin over ofloxacin and ciprofloxacin.[3] Unfortunately, the wide spread use of FQs for treatment of various infections has led to the development of its resistance in clinical isolates of *Mycobacterium tuberculosis* (MTB). However, phenotypic resistance to FQs has been shown to be heterogeneous in MTB isolates, varying from low level resistance (suggesting that infections might still be treated effectively with increased concentrations of, or alternative, fluoroquinolones), to high-level resistance (where MTB cannot be cleared with any fluoroquinolones).[4] Phenotypic resistance to FQ is associated with mutation in the quinolone resistance-determining region (QRDR) of DNA subunits A (gyrA) and B (gyrB), which encode a type II DNA topoisomerase. Mutations in subunit A confer both high-level and low-level resistance, whereas those in subunit B confer low-level resistance only.[5] Previous studies have confirmed that the presence of different mutations in QRDR correlates with different levels of resistance.[6]

WHO Guidelines for the programmatic management of drug-resistant tuberculosis 2019 update, recommends the use of *GenoType MTBDRsl* LPA to detect resistance-conferring mutations to fluoroquinolones in patients with confirmed MDR/RR-TB. *GenoType MTBDRsl* includes the QRDR of gyrA (from codon 85 to 96) and of gyrB (from codon 536 to 541) genes for detection of resistance to fluoroquinolones and the rrs (nucleic acid position 1401, 1402 and 1484) and the eis promoter region (from −37 to −2 nucleotides upstream) for detection of resistance to SLI drugs.[7] Mutations conferring resistance to Moxifloxacin (Mfx) are stratified into mutations associated with a low-level increase in MIC and high-level increase in MIC depending on their associated MIC distribution. This stratification has important implications for the inclusion of Mfx in the treatment regimen. According to the available literature, if mutations associated with MIC increase above the critical concentration (CC) but below the clinical breakpoint (CB) (defined as mutations associated with a low-level increase in MIC), are detected, high dose Mfx is likely to be effective and if mutations associated with MIC increases above the clinical breakpoint (CB) (defined as mutations associated with a high-level increase in MIC), are detected, Mfx even at high dose is likely to be ineffective.[8–10] The present study was conducted to explore the relationship of MTBDR*sl* detected mutations in gyrA gene and genotypic DST of Mfx at WHO defined clinical breakpoint of 1.0μg/ml.

2. Materials and methods

2.1. Study population

The study was conducted in the laboratory of New Delhi Tuberculosis Centre (NDTBC). NDTBC serves as an Intermediate Reference Laboratory providing diagnostic and follow-up support to 17 National Tuberculosis Elimination Program (NTEP) designated Tuberculosis districts. The laboratory is accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL) and is undergoing regular rounds of annual proficiency testing for culture, phenotypic drug susceptibility testing (pDST) and genotypic drug resistance testing (gDRT) of *M. tuberculosis* conducted by National Institute of Tuberculosis and Respiratory diseases (NITRD), New Delhi, India. A total of 200 sputum samples obtained from patients identified as MDR/RR TB in the 17 districts showing resistance conferring mutations to Mfx on *GenoType MTBDRsl* assay were included in this study for further testing. *GenoType MTBDRsl* and DST at WHO approved Clinical Breakpoint for Mfx were performed.

2.2. Sample processing

Sputum samples were decontaminated using the standard N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) method,[11] and smear-positive samples were directly processed for DNA extraction. Smear-negative samples were primarily cultured on MGIT Bactec 960 medium. DNA was extracted using GenoLyse kits (Hain Lifescience, Germany). After extraction, the supernatant was collected, transferred into a fresh tube, and stored at −20 °C for further processing.

GenoType MTBDRsl was performed on a portion of this supernatant to determine phenotypic resistance to Mfx as per the manufacturer's Instructions (Hain Lifescience, Nehren, Germany).[12] Briefly, the procedure consists of three steps: DNA extraction, amplification with biotinylated primers, and reverse hybridization. Resistance was interpreted according to the presence and absence of wild-type and mutant probes. The presence of all wild-types was interpreted to indicate no detectable mutation. The absence of any wild-type probe indicated that the probe could not bind the respective amplicon and was considered a detectable mutation.

The DST of GenoType MTBDR*sl* identified FQ resistant isolates was performed by 1% proportionate method using the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) following the standard protocol.[13] Briefly, culture suspensions for inoculation were first well dispersed with no large visible clumps. After thorough mixing and homogenization of the culture suspensions, the tubes were allowed to rest for at least 15 min, and the supernatant was used to inoculate the drug-containing and drug-naïve MGIT with OADC supplement. The tubes were then placed in the DST set carrier and entered into the MGIT 960 instrument using the DST entry feature. The test drug i.e. Mfx was obtained in a chemically pure form M/s Sigma Aldrich. The Stock solution was filtered in a sterile manner with a 0.22 μm pore-size polycarbonate filter. The stock solution was stored at −80 °C in small aliquots. Frozen drug solutions were thawed once before use and the leftover was then discarded.

3. Results

All of the 200 representative FQ resistant isolates had mutations in gyrA gene only, with no detectable mutation in gyrB

gene. The most common mutations observed were D94G (MUT3C+ΔWT3 or MUT3C and all Wild types) in 93 (46.5%) isolates followed by A90V (MUT1+ ΔWT2 or MUT1 and all Wild types) in 57 (28.5%), D94A (MUT3A+ ΔWT3 or MUT3A and all Wild types) in 23 (11.5%), D94N/Y (MUT3B+ΔWT3) in 16 (8.0%) isolates and S91P (MUT2+ ΔWT2 or MUT2 and all Wild types) in 5 (2.5%) isolates. 6 (3.0%) isolates had WT2 and WT3 missing with no MUT band present. The results are detailed in Table 1.

The mutational pattern revealed that 109 (54.5%) of the isolates had mutations associated with high-level increase in MIC (MUT3B+ΔWT3 and MUT3C+ΔWT3 or MUT3C and all Wild types) and dose adjustment for Mfx is not possible and hence Mfx even at high dose will not be effective. While 91 (45.5%) isolates had mutations associated with a low-level increase in MIC which may be overcome by a higher dose of Mfx.

Phenotypic DST of these 200 isolates against Mfx at CB (1.0μg/ml) revealed that of the 91 isolates with mutations associated with Low-level increase in MIC and expected to be sensitive at CB, 80 (88%) were sensitive while 11 (12%) were resistant. Of the 109 isolates with mutations associated with high-level increase in MIC and expected to be resistant at CB, only 34 (31.2%) were resistant and the remaining 75 (68.8%) were sensitive. To further break it down, of the 16 isolates with MUT3B band present, 9 (56.3%) were resistant and 7 (43.7%) were sensitive and of the 93 isolates with MUT3C band present, only 25 (26.9%) were resistant while the remaining 68 (73.1%) isolates were sensitive to Mfx at CB. The results are detailed in Tables 2 and 3.

4. Discussion

Effective treatment for MDR-TB depends on concocting a regimen consisting of multiple agents to which the infecting organism is susceptible and that are bactericidal. This aim is bridled by the dearth of agents that are both highly bactericidal and nontoxic. The World Health Organization (WHO) in their 2011 treatment guidelines acknowledged the higher bactericidal activity of the later-generation fluoroquinolones such as levofloxacin, moxifloxacin and gatifloxacin over ofloxacin and ciprofloxacin.[14] Later generation fluoroquinolones are now critical components of MDR-TB regimens as they have been shown to be associated with better outcomes.[15,16] Moxifloxacin in particular has an important role to play in the treatment of MDR-TB and in patients who are not able to tolerate the standard regimen.

In 2016, WHO recommended the use of MTBDR*sl* V1.0 as an initial diagnostic test for detection of FQ and Second-line Injectable (SLI) class of drug resistance directly from smear positive clinical specimens and indirectly from culture isolates in patients with confirmed MDR/RR TB.[17] Later, this version of MTBDR*sl* was upgraded to MTBDRsl V2.0 in order to enhance the sensitivity of the test.[18] Several studies have reported performance of Genotype MTBDR*sl* V2.0 with enhanced sensitivity ranging from 83.6 to 100% and 94.4–100% and specificity of 89.2–100% and 91.4–98.5% for FQ and SLI drugs, respectively.[18–20] All the isolates tested in this study had mutations in gyrA gene only. This is in consistence with the previous studies that have demonstrated that FQ resistance is mainly attributed to gyrA mutations.[21,22] In our study, D94G (46.5%) was the most frequently observed mutation, followed by D94A (28.5%). The observations are in agreement with various previously published studies.[23–26] The results of this study also indicated that 54.5% of the isolates had mutations associated with High-level increase in MIC and that dose adjustment for Mfx may not be possible. Similar observations have been reported in the earlier studies.[24,25]

A clear association between different gyr mutations and the corresponding levels of resistance to FQs has been demonstrated in earlier studies, which suggests that a low-level resistance to FQ can be treated with higher doses of the respective drug, but for isolates with high-level resistance this may not be possible.[27,28] In this study, however, phenotypic DST revealed that of the 54.5% isolates with mutations associated with high-level increase in MIC, only 31.2% were resistant to high dose of Mfx (1.0 μg/ml) while the remaining 68.8% were sensitive. Out of the 91 isolates with mutations associated with low-level increase in MIC, only 88% were sensitive and the remaining 12% were resistant. This implies that the mutational pattern revealed by GenoType MTBDR*sl* in this study does not correspond well with the results of Phenotypic DST, especially in the case of high-level resistance mutations.

Table 1 – Frequency and mutations conferring genotypic resistance to fluoroquinolones.

gyrA mutation probe	Missing wild type probe	Mutation	Frequency n=200
gyrA Mut1	WT2	A90V	54 (27%)
gyrA Mut1	NIL	A90V	3 (1.5%)
gyrA Mut2	WT2	S91P	4 (2.0%)
gyrA Mut2	NIL	S91P	1 (0.5%)
gyrA Mut3A	WT3	D94A	19 (9.5%)
gyrA Mut3A	NIL	D94A	4 (2.0%)
gyrA Mut3B	WT3	D94N/Y	16 (8.0%)
gyrA Mut3C	WT3	D94G	88 (44.0%)
gyrA Mut3C	NIL	D94G	5 (2.5%)
NIL	WT2	NIL	3 (1.5%)
NIL	WT3	NIL	3 (1.5%)

Table 2 – Genotypic susceptibility against moxifloxacin at 1.0 μg/ml.

gyrA mutation probe	Total	Number susceptible	Number resistant
gyrA Mut1	57	49 (86.0%)	8 (several.0%)
gyrA Mut2	5	5 (100%)	0 (0.0%)
gyrA Mut3A	23	21 (91.3%)	2 (8.7%)
gyrA Mut3B	16	7 (43.7%)	9 (56.3%)
gyrA Mut3C	93	68 (73.1%)	25 (26.9%)
NIL	6	5 (83.3%)	1 (16.7%)

5. Conclusion

The present study explored the concordance between high-level mutations in Mfx identified using Genotype MTBDRsl

Table 3 – Comparison of the susceptibility patterns (expected vs. actual) and concordance with higher dose of moxifloxacin.

gyrA mutation probe	Number	Expected Resistant results	Actual Resistant Results	Expected Sensitive results	Actual Sensitive Results	Concordance with high dose moxifloxacin
gyrA MUT 1	57	0 (0.0%)	8 (14.0%)	57 (100%)	49 (86.0%)	86%
gyrA MUT 2	5	0 (0.0%)	0 (0.0%)	5 (100%)	5 (100%)	100%
gyrA MUT3A	23	0 (0.0%)	2 (8.7%)	23 (100%)	21 (91.3%)	91.30%
NIL	6	0 (0.0%)	1 (16.7%)	6 (100%)	5 (83.3%)	83.3%
gyr MUT1,2 and 3A combined	**91**	**0 (0.0%)**	**11 (12.0%)**	**91 (100%)**	**80 (88.0%)**	**88.00%**
gyrA Mut3B	16	16 (100%)	9 (56.3%)	0 (0.0%)	7 (43.7%)	56.30%
gyrA Mut 3C	93	93 (100%)	25 (26.9%)	0 (0.0%)	68 (73.1%)	26.90%
gyrA Mut3B and 3C combined	**109**	**109 (100%)**	**34 (31.2%)**	**0 (0.0%)**	**75 (68.8%)**	**31.20%**

Version 2.0 and phenotypic DST at WHO defined Clinical Break point. The results of the current study strongly suggest that acquisition of genetic mutations leading to resistance to a particular drug does not in veritably exclude it from the treatment regimen. Moxifloxacin is an important drug and its inclusion in the regimen for treating Drug-resistant TB is associated with better outcomes. Hence, the decision to exclude this drug from the regimen should not be taken merely on the basis of mutational pattern. Rather, the results of mutational analysis and phenotypic DST combined should be considered for decision making.

Conflicts of interest

The authors have none to declare.

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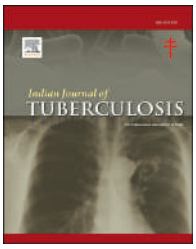

Short communication

NATCON 2022 Panel discussion: "TB vaccination - Going Forward"

V.V. Banurekha [a], *C. Padmapriyadarsini* [a,*], *Salial Bhargava* [b], *K. Sachdeva* [c]

[a] *ICMR – National Institute for Research in Tuberculosis, Chennai, India*
[b] *MGM Medical College, Indore, Madhya Pradesh, India*
[c] *International Union Against Tuberculosis and Lung Disease (The Union), New Delhi, India*

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ABSTRACT

Vaccination is important tuberculosis (TB) preventive strategy that is essential to achieve the goals of the End TB strategy. The BCG vaccination at birth offers protection against TB in young children but not in adolescents and adults. New TB vaccines are the need of the hour. The TB vaccine development pipeline in the past years is encouraging with newer TB vaccines in clinical trials in humans. The focus of the newer TB vaccine is the prevention of infection, disease, and recurrence of TB disease. Therapeutic vaccines focus on better treatment outcomes and prevention of TB recurrence. BCG revaccination is of current interest. Novel, safe, and efficient TB vaccines that prevent TB infection and disease if introduced in 2025 could drastically reduce the rate of TB incidence. However, the development of an effective vaccine for TB is challenging. Engagement of stakeholders, mobilizing funding, and advocacy could accelerate the newer TB vaccine development process.

1. Introduction

Despite ongoing attempts to combat the disease, tuberculosis (TB) still has a significant global impact. India accounts for one-fourth of the global TB burden. The end TB strategy with a vision of a world free of TB envisages a 95% reduction in TB mortality and a 90% reduction in TB incidence rate in 2035 compared to 2015.[1] The development of reliable and efficient vaccines is one of the key interventions in the eradication of communicable infectious diseases. A safe and effective TB vaccine is a felt need to strengthen the existing TB preventive strategies of cough hygiene, airborne infection control strategies, and TB preventive therapy to accelerate the decline in TB incidence. Bacillus Calmette Guerin (BCG) is the only vaccine that is approved for use against TB since its discovery over 100 years. Systematic review and meta-analysis have documented that BCG vaccination at birth offers protection against TB in young children but not in adolescents and adults.[2] Adult population accounts for nearly 90% of TB transmission globally. Hence, there is a need for newer TB vaccines to reduce the burden of TB (see Table 1).

* Corresponding author. ICMR-National Institute for Research in Tuberculosis, No.1, Mayor Sathyamoorthy Road, Chetpet, Chennai, 600031, India. Phone: +91-44-2836 9600
E-mail address: padmapriyadarsi.nic@icmr.gov.in (C. Padmapriyadarsini).
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Table 1 – Details of newer TB vaccines currently in Phase II/III clinical trials in humans.

Clinical trial phase	Name of vaccine	Developer	Vaccine type	Route of administration	Intended effect
III	Immuvac (Mw) or MIP	Cadila	Whole-cell *M. indicus pranii (heat killed)*,	Intradermal	POD
III	VPM1002	Serum Institute/Vaccine Projekt Management (VPM)/Max Planck Institute	Live rBCG	Intradermal	POI POD POR
III	M72/AS01E	GSK/Aeras	Protein/adjuvant subunit vaccine	Intramuscular	POD
III	MTBVAC	Biofabri/Bharat Biotech	Live, genetically attenuated MTB	Intradermal	POD
III	GamTBvac	Russia	Protein/adjuvant subunit	Subcutaneous	POD
II	DAR-901	Dartmouth University, USA	Inactivated whole-cell *M. obuense*	Intradermal	POI
II	H56:IC31	Staten Serum Institute	Protein/adjuvant subunit vaccine	Intramuscular	POR
II	ID93/GLA-SE	Sponsored by Quratis/NIH	Protein/adjuvant subunit vaccine	Intramuscular	POI Therapeutic
II	RUTI	Archivel Pharma	Fragmented MTB	Subcutaneous	Therapeutic
II	AEC/BC02	Anhui Zhifei Longcom Biopharmaceutical Co., Lt	Protein/adjuvant subunit vaccine	Intramuscular	POI

POI: Prevention of infection; POD: Prevention of disease (POD).
Prevention of recurrence (POR).

Attributes of an ideal TB vaccine include being efficacious and safe in at-risk infants, children, and adults, effective against all forms of TB including drug-resistant TB, easy formulation to be able to manufacture at a large scale, and logistically feasible to transport, store under low technology conditions and administer in field settings at the point of care. The TB vaccine development pipeline in the past years is encouraging with more than a dozen newer TB vaccines in clinical trials in humans.[3]

2. Vaccination strategies with newer TB vaccines

The vaccines developed for TB are designed for the prevention of infection (POI), prevention of disease (POD), prevention of recurrence (POR), or therapeutic vaccines. *Mtb*-uninfected individuals are the target population for POI and POD for pre-exposure vaccination strategies. Post-exposure strategies include vaccination for POD in *Mtb*-infected individuals, in treated TB patients for POR, and therapeutic vaccination of TB patients to improve treatment outcomes and POR.[4]

The prospects envisaged for a new TB vaccine include the following[5]

- Priming vaccine: Replace the primary vaccination of BCG
- Booster vaccine following BCG vaccination: Addressing the shortcomings of BCG long-term effects.
- Latent infection preventive vaccine: Inhibit endogenous reactivation of infected individuals while preventing exogenous re-infection
- Therapeutic vaccine: Effective supplement to conventional chemotherapy in improving treatment outcomes

3. Newer TB vaccines in clinical trials in humans

Depending on the platform or method of development, TB vaccines can be categorised as live attenuated vaccines, inactivated vaccines, sub-unit vaccines, recombinant live vaccines, DNA vaccines, etc. Various routes of administration of new TB vaccines which include intradermal, subcutaneous, intramuscular, and intranasal are being explored for safety, immunogenicity, and efficacy.

The Table below provides a description of the TB vaccines that are now undergoing human clinical trials.[3] The vaccines in Phase III clinical trials include Immuvac (Mw), VPM1002, M72/AS01E, MTBVAC, and GamTBvac. Phase II clinical trials are in progress for the following vaccines: DAR-901, H56:IC3, ID93/GLA-SE, RUTI, and AEC/BC02. In addition, vaccines namely AdHu5Ag85A (aerosol), BNT164, TB/FLU-05E (aerosol), TB/FLU-01L, TB/FLU-04L, and ChAdOx1 85A + MVA85A are being evaluated in Phase I clinical trials.[3]

BCG revaccination is of interest in the context of new emerging findings. Recent studies have documented the possible benefits of BCG revaccination which include a reduction in the rate of sustained Quantiferon TB (QFT) conversion with an efficacy of 45.4%, immunological response by

significantly boosting antimycobacterial Th1/Th17, and innate effectors in Interferon-gamma release assay (IGRA) positive and negative individuals and 36% efficacy in TB disease prevention at the end of 15 years.[6–8] BCG revaccination is currently being evaluated for the prevention of infection in phase III clinical trials in countries including South Africa and Unites States.[3] Preclinical animal studies are also evaluating the intravenous route as a different method of administering BCG.[9] The efficacy of BCG in the prevention of TB is reduced by prior TB infection sensitization with environmental mycobacteria. In this context, BCG vaccination following treatment of latent TB infection by preventive therapy is a strategy that needs to be explored in future studies.[10]

4. Challenges and the way forward

The development of newer TB vaccines is a challenge. There is a lack of specific protective antigens in TB due to the gaps in the knowledge of the immune responses in TB. Hence, there is constraints in the selection of the appropriate antigen including the number required for maximum vaccine efficacy.[5] There is no appropriate biomarker that is a good correlate for protection against TB that can be used in the early stage of vaccine development. The lack of suitable vaccine evaluation animal models affects the predictive ability during the pre-clinical evaluation of vaccines in animals. This limitation could be attributed to the variation in the modality of TB infection and disease among the species and the host genetic heterogeneity in susceptibility to TB.[5] Moreover, there is variation in the endpoint criteria for evaluation of vaccine effectiveness in animal and human studies which further delays the development of an effective vaccine that can offer an optimum clinical impact.

Conducting clinical trials to determine vaccine efficacy/effectiveness is time-consuming and requires adequate funds. Many TB-endemic countries lack the infrastructure and funding for clinical trials for TB vaccine development. Funding support is vital for the progression of TB vaccine candidates through all stages of development. The average annual investment in TB vaccine development in the past years has been US$ 115 million against the required US$ 790 million per year.[11] The inherent high level of attrition in vaccine development and insufficient investment in late-stage clinical research impedes vaccine development progress. The majority of the doses of TB vaccines will be required in developing countries at a low cost. This is a hurdle for commercial vaccine developers in the context of demand, market uncertainty, and profit. Delays in the new drug/vaccine development could also be attributed to the stringent regulatory environment and approval procedures prevailing in the country.

The World Health Organisation (WHO) has been the forerunner in promoting vaccine candidates for policy recommendations based on prequalification as per preferred product characteristics. The learnings from SARS-CoV2 vaccine development has opened avenues for new approaches and technologies to be adapted for progress in newer TB vaccines. Contrary, to the COVID-19 scenario, a significant impact of an effective vaccine in TB will be apparent after many years. The health and economic impact of new TB vaccine that is 50% effective in preventing disease among adolescents and adults which if probably introduced in 2025, could avert up to 37.2 to 76 million new TB cases, 4.6 to 8.5 million deaths, 21.9 to 42.3 million treatment and US$ 36.6 to 41.5 billion in costs faced by TB affected households, especially for the poorest and this will be evident over a period of 25 years (2025–2050).[11] There is a significant market for TB vaccines with 6.6 billion population requiring the vaccine with substantial return on investment.

Novel, safe and efficient TB vaccines that prevent TB infection and disease is the need of the hour and if introduced in 2025 could drastically reduce the rate of TB incidence. Strong political commitment and engagement of various stakeholders from industry, research, academia, biotechnology firms and manufacturers would hasten the TB vaccine development process. Mobilizing funding from partners is crucial to assist in various stages of vaccine development. Capacity building for clinical trials in TB vaccine is essential. Conducive regulatory environment and quick approval processes could avoid the delay in vaccine development. Community engagement and advocacy would support, accelerate and generate demand for newer TB vaccines.

Conflicts of interest

The authors have none to declare.

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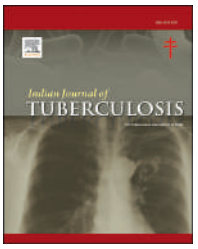

Short communication

Basic spirometry and advanced pulmonary function tests – NATCON 2022

Rupak Singla [a,*], *Amitesh Gupta* [b], *Dipti Gothi* [c], *Saurabh Mittal* [d], *Saroj Meena* [e], *Neeraj Gupta* [f]

[a] *Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Disease, New Delhi, India*
[b] *Department of Pulmonary Medicine, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi, India*
[c] *Department of Pulmonary and Critical Care Medicine, ESI PGIMSR, Basaidarapur, New Delhi, India*
[d] *Department of Pulmonary, Sleep and Critical Care Medicine, AIIMS, New Delhi, India*
[e] *Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi, India*
[f] *Sir Ganga Ram Hospital, New Delhi, India*

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ABSTRACT

At the 77th National Conference on Tuberculosis and Chest Diseases, which took place on February 27, 2023, a pre-conference workshop on Basic Spirometry and Advanced Pulmonary Function Tests was held under the auspices of NATCON-2022.

With the assistance of highly experienced faculty who are national and international level experts in their fields, the workshop covered all important aspects of basic spirometry and advanced Pulmonary Function Tests.

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With the assistance of highly experienced faculty who are national and international level experts in their fields, the workshop covered all important aspects of basic spirometry and advanced Pulmonary Function Tests.

The faculty for this workshop were Dr Rupak Singla, Dr Amitesh Gupta, Dr Dipti Gothi, Dr Neeraj Gupta, Dr Saurabh Mittal and Dr Saroj Meena.

Students, medical college teachers, and private practitioners were among those who took part. The workshop

* Corresponding author. Tel: 91-9891168908.
E-mail address: drrupaksingla@yahoo.com (R. Singla).
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educated the participants on the most recent global and national guidelines for various pulmonary function tests. The participants were appraised with the latest ERS/ATS guidelines 2021 on technical standard on interpretive strategies for routine lung function tests. The detailed academic lectures, as well as the hands-on sessions, were extremely beneficial to the participants in their everyday clinical practise. The main highlights of the workshop are listed below.

The session on basics of spirometry covered the types of spirometers available in market along with basic measurements, reference values, repeatability and acceptability, and how to perform a quality procedure. The session was followed by live demonstration of spirometry procedure and how to differentiate between poor and good effort. This was followed by the session on interpretation of spirometry where the participants learned to differentiate obstructive and restrictive disease processes. They also learnt to grade the severity of the obstructive and restrictive diseases as per latest guidelines.

The participants learned about the principles behind advanced pulmonary function tests which included sessions on lung volumes and diffusion capacity. Indications, contraindications, factors which increase or decrease the lung volumes and diffusion capacity and how to interpret the values were covered. Hands on demonstration was also given to the participants where they actively participated and had a healthy discussion with the faculty.

Any PFT workshop is incomplete without academic sessions on FENO and Impulse Oscillometry (IOS). Sessions on FENO and Impulse Oscillometry were taken by experienced faculty having vast experience in their respective fields. In session on FENO participants were appraised about the utility of FENO in clinical practice. They had a hands-on demonstration on FENO machine where they interacted with faculty and cleared their doubts. In session on Oscillometry the participants were appraised about the basics of the technique and other indications. It was demonstrated that the Oscillometry can differentiate small airway obstruction from large airway obstruction and is more sensitive than spirometry for peripheral airway disease.

Conclusion

The one-day workshop on ‘Basic spirometry and advanced Pulmonary Function Tests’ significantly improved the knowledge of pulmonary functions. Testing for pulmonary function enables precise, repeatable evaluation of the respiratory system's functioning status. It is important to stress that pulmonary function tests, if done and interpreted properly, they may assist in evaluation and management of several respiratory disorders.

Conflicts of interest

The authors have none to declare.

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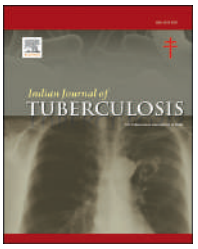

Short communication

Newer TB diagnostics: An update

Priya Rajendran [a], *C. Padmapriyadarsini* [a,*], *Sanjeev Nair* [b], *S. Sivakumar* [a]

[a] *ICMR-National Institute for Research in Tuberculosis, Chetpet, Chennai, India*
[b] *Government Medical College, Thrissur, India*

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ABSTRACT

In recent years, nucleic-acid amplification tests (NAATs), which are highly specific and sensitive, have helped to transform the TB diagnostic landscape. According to the WHO 2021 Guidelines on Diagnostics, the NAATs used in TB diagnosis at the point of care (POC) include Xpert MTB/RIF a cartridge-based test manufactured by Cepheid, and Truenat a chip-based test manufactured by Molbio. Other POC tests that are expected to be implemented in near future include Xpert Omni and Xpert MTB/XDR. The use of line probe assay is involved at the level of reference labs for the detection of MTB and its resistance to first-line (Isoniazid and Rifampicin) and second-line (fluoroquinolones and second-line injectables) drugs. When the currently available NAATs detect mutations for drug resistance at a particular region of MTB sequence, the Whole genome sequencing (WGS) platform demonstrates the exceptional potential for reliable and comprehensive resistance prediction for MTB isolates, by multiple gene regions or whole genome sequence analysis allowing for accurate clinical decisions.

1. Introduction

Of the estimated 10.6 million people who fell ill with tuberculosis (TB) caused by *M. tuberculosis* (MTB) in 2021, only 6.4 million were detected and notified, leading to a gap of 4.2 million cases. As we envisage TB elimination by 2025, five years ahead of the Sustainable Development Goal, an essential step in attaining this goal is early diagnosis of TB. Incorporating an effective, accurate, and rapid TB diagnosis in turn results in an early and effective treatment output in addition to filling the diagnostic gap. In addition to the TB epidemic, drug-resistant TB (DR-TB) serves as a major threat and hindrance to the TB elimination goal. The DR-TB definition varies depending on MTB's resistance to different drugs involved in the treatment regimen. Multidrug-resistant TB (MDR-TB) is resistant to two common drugs rifampicin (RIF) and isoniazid (INH) whereas if the resistance is conferred to RIF alone, it is termed RIF-resistant TB (RR-TB). Pre extensively resistant TB (Pre -XDR-TB), is MDR-TB or RR-TB that is also resistant to any fluoroquinolone (FLQ). An MDR-TB/RR-TB strain that is also resistant to one of the group A drugs (levofloxacin (LFX), moxifloxacin (MFX), linezolid (LZD) and Bedaquiline (BDQ)) is termed XDR TB. This updated definition of PreXDR TB and

* Corresponding author. ICMR-National Institute for Research in Tuberculosis, No.1, Mayor Sathyamoorthy Road, Chetpet, Chennai 600031
E-mail address: padmapriyadarsi.nic@icmr.gov.in (C. Padmapriyadarsini).
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XDR-TB was made by WHO in 2021 mainly to scale up rapid molecular tests for the detection of resistance to recently developed or repurposed drugs comprising oral TB regimen. This communication focuses on currently available phenotypic and Genotypic tests for MTB diagnosis and its drug resistance.

2. Phenotypic tests

Smear microscopy of acid-fast bacilli (AFB) is the simple and most widely available diagnostic method for the diagnosis of pulmonary TB (PTB). Despite its advantages like cost effectiveness, ease of use, and utility in resource-limited settings, the major drawback of smear microscopy includes its lower sensitivity (50%–60%) which has reduced its use in recent years. Culture is considered the 'gold' standard for TB diagnosis and can detect more PTB cases than smear microscopy with the advantage of further drug susceptibility testing (DST) for positive isolates. Since solid culture (LJ medium) takes four to eight weeks for MTB growth to become detectable, this is currently being replaced by liquid culture. The liquid culture system endorsed by WHO in 2007 is the Mycobacterial growth indicator tube (MGIT) system for MTB detection and DST with a rapid turnaround time (TAT) ranging from 10 to 42 days.

3. Genotypic tests

With the recent advancements in molecular diagnostics, various Nucleic acid-based amplification tests (NAATS) endorsed by WHO are available both at the point-of-care level and at the reference laboratory level for the detection of MTB and DR-TB (Fig. 1). With the given limitations of longer TAT of phenotypic culture-based DST, these molecular tests are gaining significance in recent years.

(i) Truenat MTB and MTB –RIF DX test:

Truenat assay, a product developed in India is recommended as an initial diagnostic test for MTB detection in pulmonary TB patients by WHO in 2020. The assay is a chip-based test and works on the principle of micro real-time PCR. While Truenat MTB chips are used for MTB detection, Truenat MTB-Rif Dx chips are used for RIF resistance detection. Truenat assays are carried out as a two-step procedure where initial DNA extraction from the sputum sample is carried out using a Trueprep device followed by MTB and RIF resistance detection in a True lab device. The portable true lab devices are available in Uno-, Duo-, and Quattro-throughput formats, and are designed for Point of Care (POC) setup with power backup.[1] The pooled sensitivity and specificity of the assays are found to be 80% and 96% for MTB detection and 84% and 97% for RIF resistance detection respectively.[2] Due to its cost-effectiveness, and robustness, Truenat is widely used in programmatic settings at present.

(ii) Xpert MTB/RIF and Xpert MTB/RIF Ultra

The Xpert testing (MTB/RIF or MTB/RIF Ultra) is currently recommended as another initial diagnostic test to overcome the limitation of smear microscopy. Both are cartridge-based tests that could simultaneously detect the presence of MTB and mutations associated with RIF resistance.[3] However, Xpert Ultra differs from Xpert in targets for detection (IS1081 and IS6110), improved assay chemistry, cartridge design, and lesser turnaround time (TAT).[4] Although Xpert assays have demonstrated higher sensitivity (89%) and specificity (99%) in diagnosing adult pulmonary TB, they have reduced sensitivity in vulnerable populations like children and HIV coinfected patients, and in extrapulmonary TB.[5,6] In case of Xpert Ultra, the specificity of the assay is lower; and in view of the possibility of higher numbers of false positives, is currently not used in the national program in India. Both assays have limitations like the requirement of continuous power supply and higher cost.

(iii) Xpert MTB/Omni

To overcome the obstacle of power supply requirement in resource-limited settings, and other limitations in Xpert testing, GeneXpert Omni (Omni; Cepheid), was developed

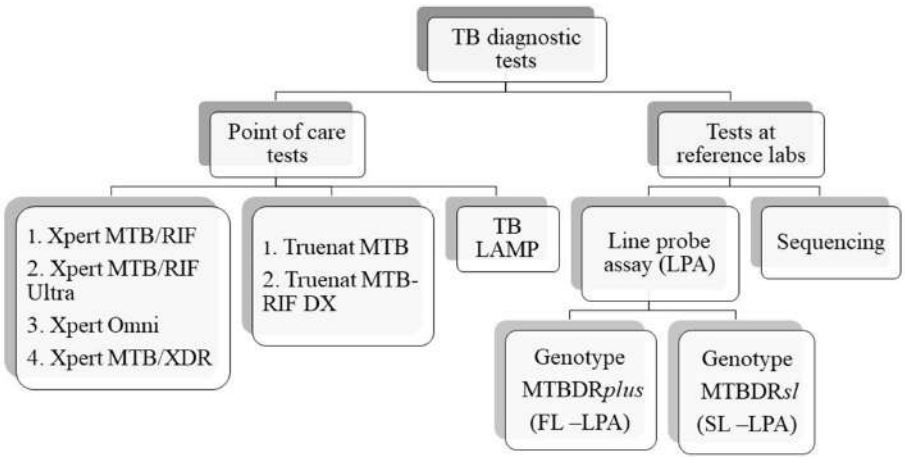

Fig. 1 – Currently available WHO-endorsed molecular diagnostic tests for TB.

with battery-operated technology. It has the advantages of automatic connectivity and a two-day battery life with higher durability.[7] The system is shown to be cost-effective in peripheral healthcare settings resulting in the avoidance of further delays and costs in transporting samples to reference centres.

(iv) Xpert MTB/XDR

Xpert MTB/XDR assay, another NAAT developed by Cepheid, detects resistance to INH, FLQ, ethionamide (ETH), and second-line injectables (SLID). The 16 associated mutations to these drugs are detected with a TAT of 90 min and demonstrate 94% sensitivity and 100% specificity.[8] Multicentric studies are being conducted at a large scale to determine its feasibility as a follow-up test to Xpert assays at the POC level. The assay is expected to be recommended by WHO based on the results obtained and will be of top importance since it could help in the early recognition of drug resistance, particularly in the scenario of recommendation for newer shorter (6-month) regimes for MDR TB.

(v) Loop-mediated isothermal amplification technology (LAMP)

LAMP technology works on the principle of amplification using four different sets of primers followed by strand displacement reaction at 65 °C for 15–60 min. Its operational feasibility has been evaluated in highly endemic countries in peripheral settings and based on the results obtained, and it was recommended by WHO in 2016 for TB diagnosis. The sensitivity and specificity of this test in various settings ranged between 76-80% and 97–98%, respectively.[9]

(vi) Line probe assays (LPA)

Line probe assay (LPA) is recommended by WHO for over a decade for molecular detection of MTB and its resistance to first-line (Genotype MTBDR*plus*) and second-line (Genotype MTBDR*sl*) drugs (Hain LifeScience Germany) with a TAT of <6 h. The assay works on the principle of PCR and reverses hybridization and the interpretation is based on the absence of wild-type or mutation bands. Genotype MTBDR*plus* is used to detect mutations in *rpoB* region for RIF resistance and *inhA* for high-level Isoniazid (INH) resistance, with 78% sensitivity and 100% specificity. The low level of INH resistance is detected by the presence of *inhA* promoter genes that are also known to be associated with Ethionamide and prothionamide resistance. Genotype MTBDR*sl* 2.0 assay is used to detect resistance to second-line drugs that include fluoroquinolones (FLQ) conferring mutations in the genes *gyrA* and *gyrB* and second-line injectables (SLID) conferring mutations in the genes *rrs* and *eis*.[10] The sensitivity and specificity of LPA are reported to be 100% and 98.9% for FLQ, and 89.2% and 98.5% for SLID. The major advantage of LPA is the provision of prompt results on drug resistance for the patients to be started on appropriate treatment regimens instead of waiting for phenotypic DST.

4. Whole genome sequencing (WGS)

Although the NAATs and LPA are known to be rapid and accessible diagnostics, they can detect mutations for drug resistance only at the particular region of the MTB sequence. Another limitation of these molecular tests is the lack of differentiation between silent mutations from the mutations that hamper drug efficacy, resulting in an increased rate of false resistance results.[11] In such instances, whole genome sequencing (WGS) affords a complete analysis of the whole MTB genome with a 96% agreement with culture-based DST.[12] Currently, genotypic sensitivity to most drugs required for the treatment of MDR-TB, and validation for newer drugs including Bedaquiline, Delamanid, and Pretomanid is being carried out using WGS. With ongoing technological advancements, it is likely to be available as POC worldwide, as currently sputum-based sequencing techniques and kits (nanopore) in place of isolates are available and are being used in research mode. Moreover, in the future, with improved knowledge of the genomics involved in TB resistance, WGS is likely to be used in tailoring individual-based TB treatment.

5. Conclusion

Appropriate and effective TB treatment depends on the prompt diagnosis of TB and its drug resistance. Efforts are being made worldwide to develop new technologies in such a way that individual-based tailored regimens can be formulated for treatment. If we continue to diagnose TB with these recent advancements, we will succeed in TB control and reduce morbidity at a global level.

Conflicts of interest

The authors have none to declare.

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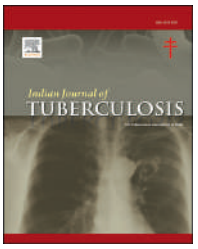

Short communication

Pre-conference workshop on pulmonary rehabilitation and smoking cessation – NATCON 2022

Raj Kumar [a,*], *Parul Mrigpuri* [b], *Vishal Bansal* [c], *Balakrishnan Menon* [b], *Sonam Spalgais* [b], *Sidharth Raj Yadav* [b], *Rajendra Prasad* [d], *Nitesh Gupta* [e]

[a] *Vallabhbhai Patel Chest Institute, New Delhi, India*
[b] *Department of Pulmonary Medicine, Vallabhbhai Patel Chest Institute, New Delhi, India*
[c] *Department of Physiology, Vallabhbhai Patel Chest Institute, New Delhi, India*
[d] *Director Medical Education and Professor & Head of Department, Pulmonary Medicine of Era University's Era's Lucknow Medical College, India*
[e] *Department of Pulmonary, Critical Care and Sleep Medicine, Vardhman Mahavir Medical College and Safdarjang Hospital, New Delhi, India*

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ABSTRACT

The 77th National Conference of Tuberculosis and Chest Diseases was held on 27th February 2023. The workshop on Pulmonary rehabilitation and smoking cessation was conducted as a part of the various pre-conference workshops being conducted on the occasion. It helped the participants to know regarding the role, efficacy and benefits of pulmonary rehabilitation and smoking cessation for the management of Chronic respiratory diseases.

The 77th National Conference of Tuberculosis and Chest Diseases was held on 27th February 2023. The workshop on Pulmonary rehabilitation and smoking cessation was conducted as a part of the various pre-conference workshops being conducted on the occasion. The workshop covered the basics of Pulmonary rehabilitation and gave an insight into smoking cessation with specialists in the field with many years of experience forming part of workshop faculty.

The faculty for this workshop were Dr. Raj Kumar, Dr. Parul Mrigpuri, Dr. Vishal Bansal, Dr. Balakrishnan Menon, Dr. Sonam Spalgais, Dr. Sidharth Raj Yadav, Dr. Rajendra Prasad and Dr Nitesh Gupta.

Post graduate students from all over the country formed the major percentage of participants however, some private practitioners and faculties from medical colleges also took out time from their busy schedule to join the workshop and

* Corresponding author. Vallabhbhai Patel Chest Institute, New Delhi, India. Tel: +91 9810146835.
E-mail address: rajkumarvpci@gmail.com (R. Kumar).
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refresh their knowledge on the subject. The workshop was divided into two parts, the pre-lunch session covered Pulmonary rehabilitation and post-lunch session concentrated on the smoking cessation part.

In the pre-lunch session the participants were sensitized with the concept, definition and mechanism of pulmonary rehabilitation and its importance in chronic lung diseases. A detailed description of the components of pulmonary rehabilitation was given and nutritional and psychological aspects of pulmonary rehabilitation were also taken care off. The participants were trained in patient assessment with respect to pulmonary rehabilitation and exercise advice. The British Thoracic guidelines for home oxygen use in adults and European society guidelines on long term home non-invasive ventilation for management of Chronic obstructive pulmonary disease were discussed in detail. The participants were given an insight into Tele and home rehabilitation and were familiarized with setting up of pulmonary rehabilitation clinic. Video demonstration of oximetry, six-minute walk test and various exercises was given and nutritional assessment using Harpenden caliper was demonstrated.

In the post-lunch session, the participants learned about the epidemiology, forms and hazards of tobacco use. The National tobacco control program was covered in detail, following which the participants learned the art of assessing the smoking status. The particulars of non-pharmacological and pharmacological means of smoking cessation were taught in detail. Lastly the session concluded with the demonstration of various forms of tobacco, breath carbon monoxide analyser and a live call to National tobacco quit line services to understand it's working.

Conclusion

This workshop on 'Pulmonary rehabilitation and smoking cessation' helped the participants to know regarding the role, efficacy and benefits of Pulmonary Rehabilitation and Smoking Cessation for the management of Chronic Respiratory Diseases. It is important to integrate pulmonary Rehabilitation and smoking Cessation as essential components into clinical respiratory practice and such workshops are a step towards the same.

Conflicts of interest

The authors have none to declare.