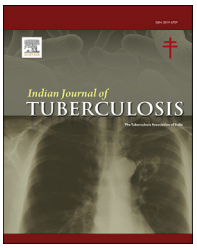
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Editorial

NATCON 2022: 77th National Conference of Tuberculosis & Chest Diseases

1. Presidential address

I consider it a great privilege and honour to present the Presidential address in this prestigious 77th National Conference of Tuberculosis Association of India. (NATCON 2022) organised by the Department of Tuberculosis and Respiratory Diseases, Sarojini Naidu Medical College, Agra in association with Uttar pradesh TB Association and The Union South East Asia Region under the aegis of Tuberculosis Association of India. I am immensely thankful to the Tuberculosis Association of India for the wonderful opportunity given to me to preside over this National Conference. I am extremely happy that the 77th NATCON is taking place at Agra where the thing of beauty, the great Taj Mahal exists. I congratulate all those who have been working hard to make this Conference a great success. I am sure all the delegates will have a fruitful time in the scientific sessions and the young Doctors will be benefitted with the latest knowledge and skills. I am indeed excited to acknowledge the presence of some of the eminent and renowned Professors of Tuberculosis and Pulmonologist and leaders in the field including International Delegates in this Conference. I also acknowledge the presence of the representatives from reputed Respiratory Institutions and International Organisations like World Health Organisations and the Union Against Tuberculosis and Lung Diseases, Senior Directors and Faculties from the top Healthcare Research and Teaching Institutions in India and abroad.

The problem of Tuberculosis is a global emergency and TB is no.1 killer disease. More number of people are dying of TB than any other disease. More women and even bread winners are dying with Tuberculosis which is indirectly affecting the Socio-economic development of the Community. I appeal to this august gathering in this prestigious Conference of the Tuberculosis Association of India to focus on the major concerns of reducing number of deaths due to TB, stopping TB and to bring an end to TB.

The Government of India has set a target of 2025 to End TB, 5 years ahead of sustainable development goals. The Government has started several programmes and interventions towards ending TB. The National strategic plan (2017–2025) for ending TB in India embraces these opportunities and proposes changes in Tuberculosis treatment, care and support and service delivery. The Government has made significant efforts in strengthening support structures, program and implementation of TB control. This includes mandatory notification of all TB cases, integration of programs with general health services. (National Health Mission), expansion of diagnostic services, expansion of programatic management of MDR TB, single window services for HIV/TB co-infection, drug resistance surveillance etc.

Tuberculosis is one of the most ancient disease of mankind. In spite of several innovative and newer modalities for treatment of TB, people are still suffering and it is among the top ten killer infectious diseases. According to WHO TB is a worldwide pandemic. Multi Drug Resistance TB (MDR TB) is another threat to TB eradication. Tuberculosis is a social disease which is closely associated with poverty and development. National TB Elimination Programme has been trying to meet the challenges posed by this disease.

The Stop TB partnership announced the theme for this year's World TB Day **"Yes! We can END TB"** The theme brings attention that it is our collective power to end TB by 2030 and therefore reach the SDG goals. The theme brings hope and builds on the amazing work done in the past two years by many of us (NGOs, Faith based Organisations, Government Organisations) to recover from COVID epidemic and ensures access to new diagnostic, new treatment regimens, digital technology and artificial intelligence (AI) for the TB response. We need to engage those affected communities and civil society who are leading the movement to end TB. This year is going to be critical for all of us engaged in TB work and should be declared as a year of hope to get full support, attention and energy for a collective "YES! WE CAN END TB". We have to focus on several key areas such as financial needs to scale up, speed up research and development of new TB vaccine, new rapid molecular diagnosis and new shorter and more efficient treatment regimens, TB prevention, TB in children etc. We should engage ourselves in an active CAMPAIGN TO END TUBERCULOSIS.

The pipeline report published by Treatment Action Group in 2021 has outlined the forward momentum observed in TB treatment, vaccines, and preventive treatment in the last 20 years. Advances in Science have made it possible to treat TB infection in as little as one month and most forms of Drug Resistant TB in 4–6 months. Despite these advances and breakthroughs in TB Research, scientific effort is still needed to implement and scale up the short drug regimens. The shorter regimens face several barriers such as lack of awareness, poor access to TB care, limited capacity to diagnose and treat TB, and lack of availability of newer drugs that constitute some of these shorter regimens. Efforts to scale up these innovations have also been undermined. The shorter regimens for TB can have great benefit for the people with TB and their families and even for health care providers and health care system. The shorter regimens can help people to complete the treatment for TB compared to the existing regimens and enables the patients to be cured faster and reduce the treatment cost. This is cost effective for the Government as shorter the period of treatment the Government can save more money. We should move to all oral treatment for drug resistant TB. The injectables needs to be stopped because they are very toxic and painful. TB disease is a long term disease. The process of healing is not only physical, it involves lot of social as well as mental and spiritual healing as it is a stigmatised disease especially in our Country. The health care workers in Sanatoria are trained to take care of the wholistic health of the patient. It takes a lot of commitment and a deep commitment to provide holistic healing to someone. We need to enable the patient to understand what is going on around them and bring them out of depression and social stigma. The civil society, the faith based organisations and the Government should come together and make it a priority. TB healing is not about the medication, it is also about the support system such as counselling. When we are doing campaign to end TB we should also address the stigma in the community and make them understand that TB is a normal disease like any other. We have a long history of very strict Directly Observed Treatment (DOT) It is just not the new commodities, new drugs and new technology, but also how we provide care. Patient centered care is to be included in our National policies. The new way of treatment adherence like providing more education and counselling and digital adherence tool is more beneficial which may provide better results.

The Sanatorium was central to Tuberculosis treatment in the 19th and the earliest 20th centuries. The drawn nature of treatment and highly infectious nature of the disease made the Sanatorium regimens effective and popular before the anti-biotics entered the scene. The Sanatorium was not just the Hospital, it was a social world – isolated from the rest of the society, it created its own definition of "Community". Now it is faded from public memory. The Union Mission Tubercuosis Sanatorium in Madanapalli, Chittoor District, Andhra Pradesh provides the site for this historical note. Any understanding of Tuberculosis without the engagement with the Sanatorium will be an incomplete one. The Sanatorium has been a key Institution that defined and dominated the treatment of the disease before the chemotherapy era of the 1940s. The more detail study of the TB is important as the disease was dreadful in the past, persists in being a major health problem in many parts of the world even today. Tuberculosis was considered as a hopeless disease by many physicians in the past and argued that we cannot prevent or resist this disease. As faith in medicine declined in finding speedy cure for TB, many 19th century physicians stressed the importance of hygiene, change of climate, sunshine, mental tranquillity, exercise, fresh air, avoidance of excessive passions, and proper diet in the prevention and treatment of TB. In the middle of 19th centuary the primary therapy for Tuberculosis was prolonged bed rest, nutritious food, fresh air and change of climate and for such therapies special Institutions called "Sanatoria" were set up. For many TB Patients the sanatorium was not just a Hospital, but also a home that these patients could hide in, away from the stigma and associated sufferings of the disease. The high level of poverty in India leads to increase incidence of TB and low access to the health care facilities in India legitimated for the establishment of Sanatoria. The Sanatorium in India provides many possibilities for doing research work because the patients are under daily observation for much longer period than they usually are in general Hospitals. The relevance of Sanatorium is greater in India than in the West due to higher poverty and poor hygienic conditions. The recent rise in TB incidence in India along with HIV infection pushes us to rethink the Sanaorium as a relic of the past. Not much has changed in India since independence – high poverty, poor health care, poor education, poor nutrition and lack of proper public healthcare in rural India. Slow curing disease lkeTuberculosis, the Sanatorium with a modified treatment regimens of "rest, diet – exercise and drugs" may provide more effective cure especially for the poor.The current excessive dependents on drugs and the condition of malnutrition will not be able to control the incidence of TB. Food insecurity is a growing concern for a large number of Indians and sanatoria never compromised and provided a rich diet. Today may be the appropriate time in India to resurrect the sanatoria to life in the hope of the better controlling the twin concerns of rising incidence of TB and poor nutrition. Active case finding with a door to door survey and providing nutritious food to all the infected should be made mandatory in our programme.

I propose in this prestigious National Conference (NATCON 22) that we should get back to basics for the Elimination of TB. We should engage ourselves in a campaign to end Tuberculosis, Treat TB with shorter drug regimens, supervisory nutrition for the Patients with bio-fortified food supplements. Consumption of bio-fortified foods improves innate and adaptive immune function and also cognitive function especially in children. Recent efforts have focused in developing improved vaccine to prevent MTb infection. There is a renewed interest in BCG Revaccination of young adults. (Research Study done at Arogyavarm Medical Centre, Madanapalli on Nursing Students of the age group of 18–28 in 2015.).

There is a potential Novel data to show that BCG revaccination in young adults in India enhance a MTb – specific CD4+ T cell immune signature potentially associated with controlled TB infection.

Hopeless and stigmatised lives should be lighted with a flame of hope, dignity and beauty for their lives. We should get

back to the basics and beyond in TB Elimination. Let us make a corporative effort …….. "Yes! We can end TB" and we need leaders to end TB.

Conflicts of interest

The authors have none to declare.

B. Wesley*
President, NATCON 2022
Director, Arogyavaram Medical Centre (Formerly Known as Union Mission Tuberculosis Sanatorium), Chittoor, Andhra Pradesh, 517326, India

*Corresponding author. Mobile: 9440893669
E-mail address: umts100@amc-arogyavaram.com

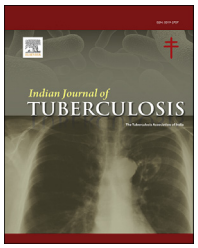
20 March 2023
Available online 30 March 2023

https://doi.org/10.1016/j.ijtb.2023.03.010

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Editorial

TB patients: Is sputum disinfection important?

Kiran A. Balani [a], *Tushar R. Sahasrabudhe* [a,*], *Kundan Mehta* [a], *Shahzad Mirza* [b]

[a] *Department of Respiratory Medicine, Dr. D.Y. Patil Medical College, Hospital & Research Centre, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune 411018, India*

[b] *Department of Microbiology, Dr. D.Y. Patil Medical College, Hospital & Research Centre, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune 411018, India*

ARTICLE INFO

Article history:
Received 7 March 2022
Accepted 29 March 2022
Available online 2 April 2022

Keywords:
Mycobacteria
Pulmonary tuberculosis
Disinfectants
Chloroxylenol
Phenol

ABSTRACT

Background: Patients with pulmonary tuberculosis (TB) may produce large amount of infectious sputum which needs to be handled carefully both in health care and household settings. As mycobacteria may survive for long duration in sputum; proper collection, disinfection and disposal is necessary to avoid potential disease transmission.

We aimed to assess the efficacy of bedside disinfectant treatment of sputum produced by TB patients using easily available disinfectants that can be used both in TB wards and household settings, to sterilize the infected sputum and compared it with sputum without disinfectant treatment.

Methods: It was a prospective case control study. Sputum of total 95 patients with sputum smear positive pulmonary tuberculosis was collected in sputum containers with lids. Patients on anti-tubercular treatment for more than 2 weeks were excluded. Each patient was given 3 sterile sputum containers to expectorate, Container A containing 5% Phenol solution, Container B containing 4.8% Chloroxylenol and Container C without any disinfectant, acting as a control. Thick sputum was liquified with Mucolytic agent N-acetyl cysteine (NAC). Aliquots of the sputum were sent for culture in Lowenstein-Jensen medium on day 0 (to confirm alive mycobacteria) and on day 1 i.e., after 24 hours (to evaluate effective sterilization). Drug resistance testing was done on all grown mycobacteria.

Results: If the samples on day 0 did not grow mycobacteria (indicating non-viable mycobacteria) or day 1 sample grew contaminants in any of the three containers, they were excluded from the analysis (15/95). In remaining 80 patients, bacilli were alive on day 0 and remained alive even after 24 hours (day 1) in control samples (without disinfectants). The sputum was effectively disinfected resulting in no growth after 24 hours (day 1) in 71/80 (88.75%) containing 5% Phenol and 72/80 (90%) with 4.8% Chloroxylenol. The efficacy of disinfection was 71/73 (97.2%) and 72/73 (98.6%) for drug sensitive mycobacteria respectively. The mycobacteria however remained alive with these disinfectants in all 7 samples of drug-resistant mycobacteria with an efficacy of 0%.

Conclusion: We recommend use of simple disinfectants like 5% Phenol or 4.8% Chloroxylenol for safe disposal of sputum of pulmonary tuberculosis patients. It is necessary as sputum collected without disinfection remained infectious after 24 hours. Resistance of all

* *Corresponding author.*
E-mail addresses: dr_tushar_s@yahoo.co.in, tushar.sahasrabudhe@dpu.edu.in (T.R. Sahasrabudhe).

https://doi.org/10.1016/j.ijtb.2022.03.027

drug resistant mycobacteria to disinfectants was a novel chance finding. This needs further confirmatory studies.

1. Introduction

Tuberculosis is caused by *Mycobacterium tuberculosis complex*. It continues to be a challenging public health problem for its significant morbidity and mortality worldwide, especially in countries like India.[1] National TB Elimination programme or NTEP, formerly known as Revised National TB Control Programme or RNTCP, effectively focused on early detection and free treatment of TB patients along with contact tracing.[2] We however need to give more emphasis on teaching cough etiquettes to the society in general, which has been shown to effectively break transmission chains.[3] Public spitting which is still highly prevalent in the country needs to stop. The prevent laws prohibiting public spitting need reinforcement.[4,5] Although mycobacteria typically transmit via airborne droplets, the sputum is equally infectious as it may become airborne after drying and during disposal.[6] National guidelines for infection control and prevention therefore do recommend wet mopping of wards with disinfectants.[7] Sputum handling at household level is usually poor and there is lack of adequate patient education on how to collect and dispose off sputum of TB patient. A study from New Delhi reported that only 18% of patients practiced safe sputum disposal.[8]

At ground level, we observe that TB patients collect sputum in open containers without any disinfectants and is often disposed off in wrong places such as washbasin, dustbins or public gutters which are open sanitation methods whereas sputum should ideally be disposed through close sanitation systems after sterilization. A study from northern India showed that safe sputum disposal was inversely proportionate to socioeconomic status and literacy.[9] Many patients often spit through the windows or in public spaces as & when they produce some, especially during working hours and there is a social dislike for carrying spittoons or spitting bags.[10] Similar apathy is also noted in health care setups where correct disposal techniques are neither taught to the patients nor are Health Care Workers (HCWs) aware of them. There is a general belief that the mycobacteria do not survive for long after getting spit out. One of the objectives of our study was to evaluate that too.

Various methods of sputum disinfection have been used by various researchers, such as bleach, acetic acid, boiling water etc.[11,12,13] Many good disinfectants such as glutaraldehyde and others[14] are not easily available or are costly. We therefore studied the efficacies of disinfectants, 5% phenol and 4.8% Chloroxylenol which are easily available, cheap and can be used by patients before disposing their sputum in hospital or home settings.

2. Methods

This was a prospective, case control study. Permission was obtained from Institutional ethics committee before the commencement of the study. Cooperation of Department of Microbiology was sought as the study involved 4 cultures for each patient and would occupy the incubator for up to 2 months.

The study participants were cases of sputum smear positive pulmonary tuberculosis above 18 years of age who were willing to give written and informed consent. Patients on antitubercular treatment for more than 2 weeks, those with scanty sputum and those having pulmonary infections other than tuberculosis were excluded. All participants were subjected to detailed history and examination to assess the study eligibility.

The sputum sample of each participant was sent for culture on Lowenstein-Jensen (LJ)[15] medium to confirm viability of the mycobacteria on day 0. Each subject was given 3 sterile sputum containers (with lid) to expectorate the sputum. Container A was containing 5% Phenol disinfectant solution, Container B was containing 4.8% Chloroxylenol solution and container C was empty, acting as a control. The sputum collection was started early morning and continued till patient could collect about 10 mL sputum in each container. Patients were explained that saliva and upper respiratory secretions is not sputum, and not to contaminate the containers with the saliva, water or food. N-acetyl cysteine was used as a mucolytic agent to liquify thick sputum and improve penetration of the disinfectant. The closed sputum containers remained bedside at room temperature to replicate the real-life scenario in health care or home settings.

From all the 3 containers containing sputum with/without disinfectants as mentioned above, aliquots of the sputum were sent after 24 hours to Microbiology laboratory for culture in Lowenstein-Jensen medium (to evaluate effectiveness of sterilization). The culture media were checked for growth till a maximum period of 8 weeks (2 months). The grown mycobacteria were identified and also subjected to Drug sensitivity Testing (DST) or Cartridge Based Nucleic Acid Amplification Test (CBNAAT) to identify drug resistance, if any.

3. Observation and results

Total 95 subjects were enrolled in the study. 15/95 patients with nonviable mycobacteria on day 0 sample (no growth on culture) and those with growth of contaminants on either day 0 or day 1 samples, were excluded from the analysis.

Out of remaining 80 patients, 73 (91.2%) turned out to be rifampicin sensitive on CBNAAT and 7 (8.8%) were rifampicin resistant.

In the sputum collected in plain containers (Container C) and kept for 24 hours; the mycobacteria remained alive in all the samples as confirmed by growth in LJ medium.

71/80 samples (88.75%) with 5% Phenol and 72/80 (90%) with 4.8% Chloroxylenol showed no growth in LJ medium

indicating effective sterilization of the sputum. The efficacy of disinfection was 71/73 (97.2%) and 72/73 (98.6%) for drug sensitive mycobacteria respectively (Fig. 1). All 7 samples (100%) containing Rifampicin Resistant mycobacteria as determined by CBNAAT later, showed growth in LJ medium even after 24 hours exposure to disinfectants in Container A and B, containing 5% Phenol and 4.8% Chloroxylenol respectively.

The data was analysed using SPSS statistical software. Test of significance was applied using Chi square test, and the results were highly significant ($p < 0.001$).

4. Discussion

Tuberculosis, a disease of great antiquity is still a communicable disease of concern and causes high mortality, especially in India (19.17%) as per Global TB report.[16] A total of 1.5 million people died from TB in 2020. Worldwide, TB is the 13th leading cause of death and the 2nd leading infectious killer disease after COVID-19.[16]

Spread of TB by infectious droplet nuclei is a widely accepted fact. It is therefore generally believed that only coughing is the method of transmission of TB. This belief needs to be revaluated in the light of existing evidence. Sputum by itself can also be an efficient vehicle in the spread of infection after being 'coughed' out.[17] Sputum can be re-aerosolized after drying because of wind, sweeping or numerous other processes and can potentially infect a susceptible host by inhalation or even ingestion.[18,19] Another prevalent belief is that mycobacteria do not survive long outside the body especially after drying or in sunlight.[20,21] There is however enough historical and scientific evidence that mycobacteria may survive outside the body or in soil for many weeks.[6,22,23]

There are very few studies to assess efficacy of sputum disinfection methods, in hospital and home scenario.[14,24] There are few studies to see if drug resistant and MDR bacteria respond differently to the common disinfectants.[25] With these above-mentioned facts in mind, we conducted this study, to find out cheap and effective means to disinfect sputum that can be applied both in hospital and home settings.

There are recommendations to suggest regular wet moping of floors with disinfectants for infected wards, no suggestions exist for disinfecting sputum or wet moping in home settings.[7]

Chloroxylenol, also known as para-chloro-meta-xylenol (PCMX), is widely available in the form of liquid, is thought to act by disrupting microbial cell walls and inactivating cellular enzymes.[26] It is readily available in India. Phenol too is a widely available disinfectant. We chose a product with active ingredient being p-Chloro-o-benzylphenol 5% which was one of the cheapest and easily available disinfectant to a common man. It works like any other phenolic disinfectant by denaturing the proteins and enzymes of the cells. In addition, it increases the permeability of the cell membrane and causes coagulation of the cell contents in microbes.

We added N-acetylcysteine[27] to the collected samples before culturing which is a standard method of preparing aliquots for the inoculation on culture media.

In our study, it was observed that both 5% Phenol and 4.8% Chloroxylenol are efficacious enough to disinfect the sputum of pulmonary tuberculosis patients as far as drug sensitive TB is concerned. Patients can use these simple disinfectants in wards or at their homes for sputum disposal.

It was a very important observation that the mycobacteria remained alive in all 80 sputum samples after keeping the sputum sample (Container C) for 24 hours at room temperature. This is consistent with the review done by Leonardo Martinez et al[6] on survival of mycobacteria in environment. This strongly supports the view that sputum of TB patients needs to be disinfected before disposal.

None of the disinfectants in our study killed the mycobacteria in sputum of patients with drug resistant pulmonary tuberculosis thus suggesting possible resistance to these

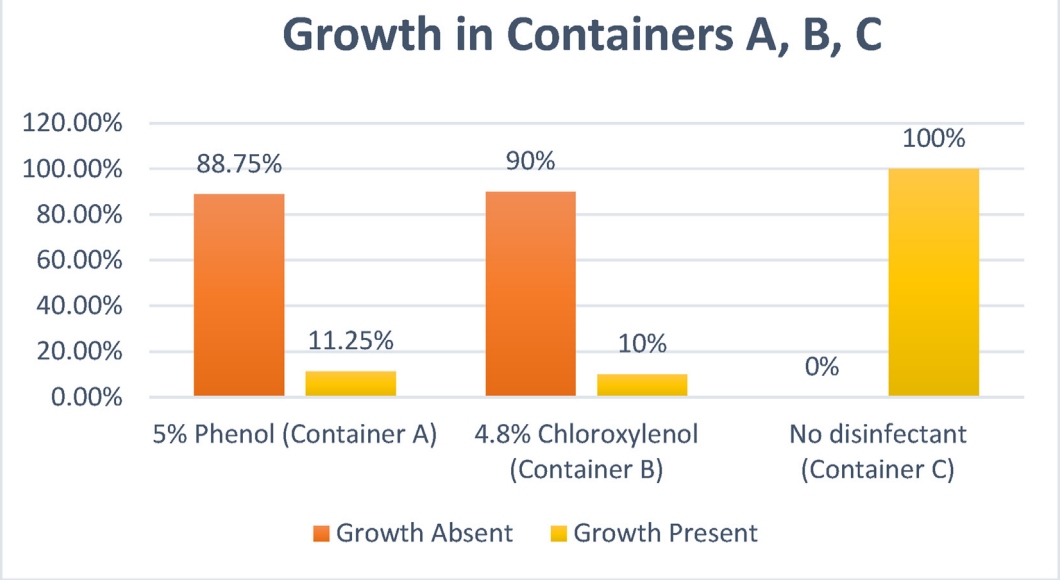


Fig. 1 – Growth in Containers A, B, C containing 5% Phenol, 4.8% Chloroxylenol and Plain (containing no disinfectant) respectively.

disinfectants too. Such cases may warrant adoption of different methods for sputum disinfection. However, this interpretation has limitations. Firstly, mycobacterial whole genome sequencing[28] was not done in our study and hence any probable common mutation pattern could not be identified. Secondly, this study had smaller number of Multi-Drug Resistant (MDR) cases as we randomly enrolled the patients in the study without considering the drug sensitive or drug resistance status. The reason being that such observation was not expected based on literature review[25] and was also not a defined objective of this study but just an incidental finding. Further research is however warranted with larger number of MDR-TB sputa to validate this finding and if proven true, other methods of sputum disinfection may need to be sought for the same.

5. Conclusion

Mycobacteria do remain alive for at least 24 hours in sputum in natural conditions and hence are potentially transmissible. Therefore, such sputum needs to be disinfected with appropriate disinfectants before its safe disposal. National guidelines should incorporate such disinfection methods and the HCWs as well as patients need to be educated about methods of safe sputum disinfection and disposal.

Finding that MDR mycobacteria are resistant to disinfectants too, is disturbing and needs confirmation of the same with larger sample size. New methods of disinfection may need to be adopted in such cases.

Contribution details

Kiran A. Balani, Concepts; Design; Definition of intellectual content; Literature search; Clinical studies; Experimental studies; Data acquisition; Data analysis; Statistical analysis; Manuscript preparation; Manuscript editing; Manuscript review; Guarantor. Tushar R. Sahasrabudhe, Concepts; Design; Definition of intellectual content; Literature search; Clinical studies; Experimental studies; Data acquisition; Data analysis; Statistical analysis; Manuscript preparation; Manuscript editing; Manuscript review. Kundan Mehta, Definition of intellectual content; Literature search; Manuscript editing; Manuscript review Shahzad Mirza, Concepts; Design; Data acquisition; Data analysis; Manuscript editing; Manuscript review.

Conflict of interest

All authors have none to declare.

REFERENCES

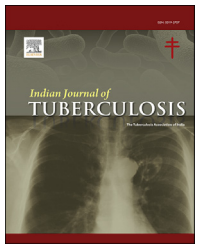
1. Huddart S, Svadzian A, Nafade V, et al. Tuberculosis case fatality in India: a systematic review and meta-analysis. *BMJ Global Health*. 2020;5, e002080.
2. Saini Varinder, Garg Kranti. Case finding strategies under national tuberculosis elimination programme (NTEP). *Indian J Tubercul*. 2020;67(4):101–106.
3. Zayas Gustavo, Chiang Ming C, Wong E, et al. Effectiveness of cough etiquette maneuvers in disrupting the chain of transmission of infectious respiratory diseases. *BMC Publ Health*. 2013;13:811.
4. Indian Express. Maharashtra govt approves anti-spitting law: offenders to pay fine, sweep govt offices. *Indian Express*; 2015, 18 June, Mumbai http://www.newindianexpress.com/nation/Maharashtra-Govt-Approves-Anti-Spitting-Law-Offenders-to-Pay-Fine-Sweep-Govt-Offices/.
5. Grace Chitra, Nayar Kesavan, Rajasekharan, Lekha Bhat, Anant Kumar, Ratheesh Babu, Muhammed Shaffi. The 'spittoon syndrome' how effective will be the anti-spitting initiatives in India. *Econ Polit Wkly*. 2016;26:27. https://doi.org/10.2139/ssrn.3113058.
6. Martinez Leonardo, Verma Renu, Croda Julio, et al. Detection, survival and infectious potential of *Mycobacterium tuberculosis* in the environment: a review of the evidence and epidemiological implications. *Eur Respir J*. 2019;53:1802302.
7. *National Guidelines for Infection Prevention and Control in Healthcare Facilities*. Ministry of Health and Family Welfare (MOHFW), Government of India; 2020.
8. Anthony Dzeyie Kevisetuo, Saurav Basu, Tanzin Dikid. The knowledge, attitude, and practices relating to tuberculosis among drug-resistant tuberculosis patients. *Indian J Med Specialities*. 2019;10(2):76–78.
9. Singh Abhishek, Goyal Vipin, Goel Shewtank. Sputum collection and disposal perceptions and practices among pulmonary tuberculosis patients from northern India. *J Clin Diagn Res*. 2016 Dec;10(12):16–18.
10. Changappa Cheriamane Deepu. Knowledge of cough Hygiene and Disposal of sputum in patients with pulmonary tuberculosis. *Eur Respir J*. 2018;52:PA4774.
11. Githui WA, Matu SW, Tunge N, et al. Biocidal effect of bleach on *Mycobacterium tuberculosis*: a safety measure. *Int J Tubercul Lung Dis*. 2007;11(7):798–802.
12. Cortesia Claudia, Vilchëze Catherine, Audrey Bernut, et al. Acetic acid, the active component of vinegar, is an effective tuberculocidal disinfectant. *mBio*. 2014;5(2):e00013–14.
13. Doig C, Seagar AL, Watt B, Forbes KJ. The efficacy of the heat killing of *Mycobacterium tuberculosis*. *J Clin Pathol*. 2002;55:778–779.
14. Best M, Sattar SA, Springthorpe VS, Kennedy ME. Efficacies of selected disinfectants against *Mycobacterium tuberculosis*. *J Clin Microbiol*. 1990;28(10):2234–2239.
15. Kennedy Kassaza, Orikiriza Patrick, Augusto Llosa, et al. Lowenstein-jensen selective medium for reducing contamination in *Mycobacterium tuberculosis* culture. *J Clin Microbiol*. 2014;52(7):2671–2673.
16. *Global Tuberculosis Report 2021*. Geneva: World Health Organisation; 2021. Licence: CC BY-NC-SA 3.0 IGO.
17. Robert G. Loudon, Sharon K. Spohn. Cough frequency and infectivity in patients with pulmonary tuberculosis, Am Rev Respir Dis, 99(1): 109-111.
18. Ghodbane R, Medie FM, Lepidi H, Nappez C, Drancourt M. Long-term survival of tuberculosis complex mycobacteria in soil. *Microbiology*. 2014;160(3):496–501.
19. Tshilombo, K. V., Mehtar, S., Sampson, S., Warren, R., & Steyn, N. L. Survival and re-aerosolization in dust of *Mycobacterium smegmatis*, a surrogate marker for *Mycobacterium tuberculosis*. Antimicrob Resist Infect Control, 4(S1), P100.
20. Mamahlodi Marang Tebogo. Potential benefits and harms of the use of UV radiation in transmission of tuberculosis in South African health facilities. *J Publ Health Afr*. 2019;10(1):742.

21. David Hugo L, Jones Wilbur D, Newman Carol M. Ultraviolet light inactivation and photoreactivation in the mycobacteria. *Infect Immun*. 1971;4(3):318–319.
22. Rogers J. Studies on the viability of the tubercle bacillus. *Am J Publ Health*. 1920;10(4):345–347.
23. Twitchell DC. *The Vitality of Tubercle Bacilli in Sputum*. Paper Presented at: Trans. Nat. Assoc. For Study and Prevent. Tuberc. Ann. Meeting. 1905.
24. Prasad Myneedu Vithal, Aggarwal A. Disposal of the large volume of sputum positive for *Mycobacterium tuberculosis* by using microwave sterilisation technology as an alternative to traditional autoclaving in a tertiary respiratory care hospital in Delhi, India. *Infection Prevention in Practice*. 2020:100072.
25. Shinoda N, Mitarai S, Suzuki E, et al. Disinfectant susceptibility of multi-drug resistant *Mycobacterium tuberculosis* isolated in Japan. *Antimicrob Resist Infect Control*. 2016;5:3.
26. Guo Yi, Gao Jingfeng, Cui Yingchao, et al. Chloroxylenol at environmental concentrations can promote conjugate transfer of antibiotic resistance genes by multiple mechanisms. *Sci Total Environ*. 2022;816:151599.
27. Amaral Eduardo P, Conceição Elisabete L, Costa Diego L, et al. N-acetyl-cysteine exhibits potent anti-mycobacterial activity in addition to its known anti-oxidative functions. *BMC Microbiol*. 2016;16:251.
28. Witney AA, Cosgrove CA, Arnold A, et al. Clinical use of whole genome sequencing for *Mycobacterium tuberculosis*. *BMC Med*. 2016;14:46.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Viewpoint

Catastrophic "costs": A hindrance to eliminate tuberculosis

*Yogesh Kaurav, Aditi Bharti**

Directorate of Health Services, Bhopal, M.P., India

ARTICLE INFO

Article history:
Received 3 November 2022
Accepted 11 January 2023
Available online 20 January 2023

Keywords:
Tuberculosis
Catastrophic costs
Universal health coverage
End TB strategy

ABSTRACT

Globally, one quarter of the population is infected with TB; and only a small proportion of those infected will become sick. Tuberculosis along with poverty disproportionately affects the households causing a financial burden and catastrophic costs (*if the total costs incurred by a household's exceeds 20% of its annual income*), which could be direct or indirect and procuring detrimental effects on the effective strategic plans. Out of all diseases, India accounts for 18% of the catastrophic health expenditure including tuberculosis. Therefore, an utmost need for a national cost survey either separately or combined with other health surveys should be held for the comprehension of the baseline burden of Tuberculosis in the affected households, to identify the predictors of catastrophic costs, and simultaneously, intensive research and appropriate innovations are needed to assess the effectiveness of the measures undertaken for the reduction of the proportionate patients who overlook catastrophic costs.

Tuberculosis (TB), known as "THE CAPTAIN OF ALL THESE MEN OF DEATH" (Bunyan 1680), is one of the most ancient diseases. About one quarter of the world's population is infected with TB; and only a small proportion of those infected will become sick with TB.[1] According to the 2021 Global TB report, eight of the countries accounted for about two-thirds of the global total i.e., India (26%), China (8.5%), Indonesia (8.4%), the Philippines (6.0%), Pakistan (5.8%), Nigeria (4.6%), Bangladesh (3.6%) and South Africa (3.3%).[2]

1. Tuberculosis and catastrophic "costs"

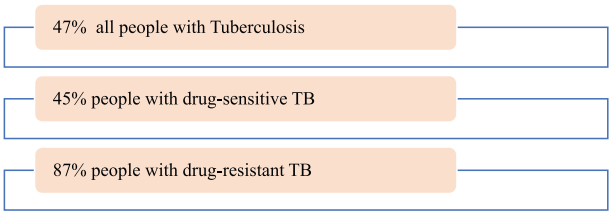
Tuberculosis and poverty had a bidirectional relationship, and it disproportionately affects the households causing a financial burden and catastrophic costs (*if the total costs incurred by a household exceeds 20% of that household's annual income*), which could be direct or indirect. Direct costs could either be the medical cost (consultation fees, diagnostic tests, and treatment) or nonmedical cost (transportation, accommodation, and increased food needs). Indirect costs include lost wages due to unemployment, time spent out of work, and associated loss of utility and productivity. These expenses there by result in detrimental effects like delayed care seeking, increased defaulters and poor treatment adherence and compliance leading to low treatment success rate, which has been even exaggerated by the COVID-19 pandemic.[3]

Globally, average percentage of TB affected people along with their households facing catastrophic costs in 23 national surveys[2].

Abbreviations: TB, Tuberculosis.

* *Corresponding author*. Directorate of Health Services, Bhopal, M.P., India.
E-mail address: draditi2019@gmail.com (A. Bharti).

https://doi.org/10.1016/j.ijtb.2023.01.003
0019-5707/



2. Strategy to combat

Nationally, as for the treatment of Tuberculosis, catastrophic healthcare expenditure accounted for about 20–30% in Drug Sensitive-TB and 60–70% of Drug resistant-TB. Thereafter, to address this, National Strategic Plan (NSP) and End TB Strategy of WHO for TB in India have set a target to eliminate the catastrophic cost due to TB by the year 2025 and 2035, respectively. End TB Strategy as well as the NSP measures the out-of-pocket expenditure through the concept of "catastrophic costs", an indicator that is used to measure progress towards universal health coverage (UHC).[3,4] UHC means that everyone can be provided with the health services they need without suffering from any financial hardship. However, Sustainable development goals (SDGs) marked to eliminate tuberculosis till 2030 through target 3.8, which stated that "*Achieve universal health coverage, including financial risk protection, access to quality essential healthcare services and access to safe, effective, quality and affordable essential medicines and vaccines for all*".[2]

Therefore, two indicators were fabricated to drive this target i.e., UHC service coverage index (SCI), and the population percentage experiencing household expenditures (indicators 3.8.1 and 3.8.2, respectively). In 2017 and 2015, latest published data for the two UHC indicators i.e., SCI and catastrophic expenditures was 66 (out of 100), and 12.7% of the world's population, belongs to out-of-pocket expenditures on health care. Although, post-2017 data for these two indicators are not yet obtained amid of COVID-19 pandemic.[2]

3. Way forward

To diminish the effect of catastrophic expenditure and to turn out to zero till 2030 (SDG) would not be possible without adequate planning, advocacy and implementing judicious interventions, which should be evidence-based. Meanwhile, there should be a need for a national cost survey either separately or combined with other health surveys conducted at national and state level for the comprehension of the baseline burden in the affected households, to identify the predictors of catastrophic costs to visualize the possible approaches, and also availing the tracking system to monitor the progress facilitating the goal of achieving zero catastrophic cost due to TB.

4. Conclusion

Constructive management of the predictors of catastrophic costs will ultimately added to a better clinical, and financial end-result for the community. Furthermore, intensive research and appropriate innovations are needed to assess the effectiveness of the measures undertaken for the reduction of the proportionate patients who overlook catastrophic costs, and the impact of the educational programs related to patients' compliance for the betterment of the treatment success rate.

Author contribution

Yogesh Kaurav: Concepts, Design, Definition of intellectual content, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor. **Aditi Bharti**: Concepts, Design, Definition of intellectual content, Literature search, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.

Conflicts of interest

The authors have none to declare.

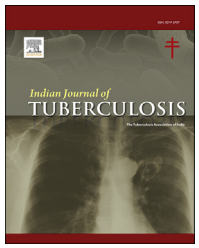
REFERENCES

1. World Health Organization. 10 facts on tuberculosis 2021 Oct 12. Available from: https://www.who.int/news-room/facts-in-pictures/detail/tuberculosis.
2. World Health Organization. Global tuberculosis report 2021. Available from https://www.who.int/publications/digital/global-tuberculosis-report-2021.
3. Central TB division. India TB report 2022. Ministry of Health and Family Welfare. Available from https://tbcindia.gov.in/WriteReadData/IndiaTBReport2022/TBAnnaulReport2022.pdf.
4. Ghazy RM, El Saeh HM, Abdulaziz S, et al. A systematic review and meta-analysis of the catastrophic costs incurred by tuberculosis patients. *Sci Rep*. 2022;12:558.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review article

Functional characterization of toxin-antitoxin system in *Mycobacterium tuberculosis*

Karthikeyan sundaram[*], *Leela Kagithakara Vajravelu*, *Alamu Juliana Paul*

Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattangulathur, Chennai, 603203, Tamilnadu, India

ARTICLE INFO

Article history:
Received 20 January 2022
Received in revised form
6 May 2022
Accepted 20 May 2022
Available online 27 May 2022

Keywords:
Mycobacterium toxin-antitoxin system
Microbial TA system
Toxin
Antitoxin

ABSTRACT

Toxin-Antitoxin (TA) system is abundant in the microbial genome, especially in bacteria and archaea. Its genetic elements and addiction modules with the role of bacterial persistence and virulence. The TA system consists of a toxin and most unstable antitoxin that could be a protein or non-encoded RNA, TA loci are chromosomally determined and their cellular functions are mostly unknown. Approximately 93 TA systems were demonstrated and more functionally available in *M. tuberculosis* (Mtb), the organism responsible for tuberculosis (TB). It is an airborne disease, which is causing ill-health to humans. *M. tuberculosis* possesses higher TA loci than other microbes and non-tubercle bacilli, the following TA types have been identified such as VapBC, MazEF, HigBA, RelBE, ParDE, DarTG, PemIK, MbcTA, and one tripartite type II TAC-Chaperone system. Toxin-antitoxin Database (TADB) brings a detailed update on Toxin-Antitoxin classification in the different pathogens such as *staphylococcus aureus, streptococcus pneumonia, Vibrio cholerae, Salmonella typhimurium, Shigella flexneri,* and *helicobacter pylori*, etc. So, this Toxin-Antitoxin system is a master regulator for bacterial growth, and an essential factor in analyzing the properties and function of disease persistence, biofilm formation, and pathogenicity. The TA system is an advanced tool to develop a new therapeutic agent against *M. tuberculosis*.

1. Introduction

Toxin-Antitoxin (TA) system abundantly occurs in bacteria and archaea, its genetic elements and addiction modules with a role in bacterial persistence and virulence. The TA system consists of a toxin and a more uncertain antagonistic antitoxin that could be a protein or non-encoded RNA. Approximately 93 TA systems have been exemplified so far and are more functionally available in *Mycobacterium tuberculosis* (Mtb), the organism responsible for tuberculosis (TB) in humans. That is an airborne disease. World Health Organization (WHO) global tuberculosis report 2021, shows the prevalence of the disease is quietly high and it's one of the top ten causing death and severe ill-health.[1,2] Drug-resistant tuberculosis (MDR-TB) is a global challenge. The drug-resistant strains frequently modify their genomic variation-like mutation; this mutation occurred due to persistent

* *Corresponding author*. Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattangulathur, Chennai-603203, Tamilnadu, India. Tel.: +91 9841665803.
E-mail address: karthikom85@gmail.com (K. sundaram).
https://doi.org/10.1016/j.ijtb.2022.05.010
0019-5707/

infection and it could be a reason for the following two important factors, the toxin-Antitoxin system, and the sigma factor. The TA loci are chromosomally determined and their cellular functions are mostly unknown, and the TA system comprises high in hypothetical proteins but has not been identified so far, and undergoing multiple functions, including stability of genomic regions, response in anti-addiction to mycobacteriophage infection, biofilm formation, and especially bacterial persistence of pathogenesis.[3] The TA system was distinguished in the seven classes based on the character of the action of the toxin type II systems. The presumed *M. tuberculosis* toxins studied thus significantly showed inhibition of bacterial growth. As *M. tuberculosis* possesses higher TA classes than other microbes and non-tubercle bacilli, more than 50 VapBC, 10 MazEF, 3 HipBA, 3 RelBE, 2 ParDE, DarTG and one tripartite type II Toxin-Antitoxin (TAC) Chaperone systems.[2] TA systems are categorized into six types, according to the concern of the functional analysis of the toxicity activity of toxin and antitoxin. Both are either proteins or RNA; it is narrated below; (i). The antisense non-encoded RNA antitoxin is to bind and interacts with toxin mRNA and inhibits toxin protein synthesis; (ii). The toxin and antitoxins are proteins and both are binding directly with each other and break down the toxin; (iii). The antitoxin is an RNA that interacts with the toxin protein and neutralizes the toxin; (iv). The toxin and antitoxin proteins are having the same target but do not bind with each other; (v). The toxin RNA was impaired by the antitoxin using endoribonuclease, which reduces the action of the toxin by cleaving the toxin RNA; (vi). The atypical TA family of TAC chaperone modules, and this module is helping to fold and protect the antitoxin from degradation.[2,3]

2. The family of VapBC

VapBC is part of the elements in TA loci. Hence, vapB acts as an antitoxin and vapC is a toxin that is associated with PIN (homologous PilT-N terminal domain) domain enzymatic activity against translation inhibition, which was first identified as plasmid domain in *Salmonella dublin*. M.tb is consisting of a high VapBC family, based on the TA system database shown the following vapBC family tested more including vapC1, vapC2, vapC5, vapC11, vapC20, vapC29 etc. The inhibition of translation via mRNA cleavage by vapBC families such as vapBC4, VapBC19, VapBC22, VapBC32, VapBC37, VapBC39, VapBC41, and the sequence selective of Rnase function were identified for VapC proteins.[4] Remarkably, the studies show that the specific tRNases are cleaving the initiator tRNA in the entero-bacteria *Shigella flexneri* and *Salmonella enterica*, Thus, toxin VapC seem to focus RNA by specific tools. The demonstration of VapC1 and VapC19 in *M.tb* have been analysed in the whole genome sequence of mRNA fragments by Mass-spectrometry. The protein toxin related to TA loci is bactericidal or bacteriostatic is still debatable; hence post-induction of VapC or $VapC_{LT2}$, ceased the growth of the cell rapidly when induced the $VapC^{D7A}_{LT2}$ encodes an assumed catalytically inactive VapC, and it wasn't induced the inhibition of cell growth. After 30 minutes, the cognate vapB genes were induced and vapC expression was aborted, as observed that the induction of $vapB_{LT2}$ in 30 mins and vapB in 90 mins is resumed in the cell growth. The slower recovery was observed when induction of VapB was associated with the comprehensive deficit and slowly coming back of the cellular amount of the $tRNA^{fMet}$. So, the VapC activity is bacteriostatic instead of bactericidal.[5] The short fragments of the VapB4 are crucial for the action of antitoxin by deleting the expression of the C and N terminal tag of VapB4-sfGFP in the existence of VapC4. So, there were 31 amino acids reduced in the C-terminal of VapB4 will terminate the action of antitoxin substance and omission of 54 amino acids N-terminal of VapB4 has minimal effect on the antitoxin action and VapB4 required amino acids side chain to bind with the VapC4 when induction of expression in wild type VapB4 deletion mutation.[6] The V26-SP-8 module has developed the structural and biochemical properties of the VapBC26 TA system from *M. tuberculosis* and can be used for novel antibacterial applications. The stapled peptides were synthesized and based on VapC26 α454–65 in vitro activity.[7]

3. The family of MazEF

The TA system consists of another family of MazEF in this family. Toxin and antitoxin reveal the mRNA and tRNA cleavages that happened in MazF mt-1 and mt-9 respectively, wherein the docking form of cognate antitoxin peptides, the MazF -mt9 toxin will be significantly inhibited by the engineered MazE-mt3 with the use of *M. tuberculosis* tRNALys(UUU) in tRNA cleavage analysis.[8] There are 80% of ORFs and non-encoded RNAs of *M. tuberculosis* is lacking the UCCUU split motif and these are assumed that it can resistant to cleave of MazF-mt3, and 14 genes are significantly over-representation of the cleavage motif and recommended these genes could be besieged by MazF-mt3 and this study concluded that the MazF-mt3 could cleave at 1537U↓CCUU1541 at the aSD sequence of 16S rRNA inside the setting of 70S ribosomes.[9] RNA sequences might alter protein expression by degrading mRNA; have a regulatory process to accommodate environmental factors by MazF-mt3 and MazF-mt7 or other mRNA and interferes with a sequence-related target, including the infection of *M. tuberculosis*, MazF-mt7 thus is an mRNA that intervenes that identifies a five-based UCGCU sequence; however, it seems to be less stern than MazF-mt3.[10] MazE3,6 mRNA expression phases were significantly diminished in the drug-resistant and drug-sensitive strains than H37Rv, Inversely, MazE3,9 expression was enhanced in drug-sensitive strains compared to drug-resistant strains.[11] The Asp-10, Arg-13, and Thr-36 of MazF-mt6 residues are most important in ribonuclease action to catalyse the proton–relay activity for RNA cleavage, which has been demonstrated by the structure-based mutagenesis and bacterial growth assay.[12]

4. The family of HigBA

The study analyzed the following crystal structure of HigA 2 in the three forms, it belongs to the $P2_12_12_1$ determined in the 2.0A° resolution, form II in $P4_32_12_1$ and determined in 3.2A° resolution, form III in $P3_121$ in 3.4A° resolution. When the

absence of electron density at the N-terminus is reflected those 25 residues are insignificant in each monomer despite the relatively high resolution, the N-terminal cleaved monomers contain the four consecutive α-helices and the other two antiparallel β-strands named α-helix bundle and β-Lid in the formerly determined HigB antitoxin. Docking analysis has shown the MtHigA2 binding with inclined and linear DNA on structural modification. Moreover, docking showed unfavourable interaction with DNA and HTH motifs in the solvent region were bent. MtHigA2 interact with linear DNA due to HTH motifs Lid distortion.[13] The different structural portions also present in the MtHigA3 in the monomer structure have two or three β-strands, four α-helices and one 3_{10}-helix and HigA3 antitoxin was amplified in H37Rv strain in the 34 residues shortened from the N- terminus of the mtHigA3 and crystal structure from *M. tuberculosis* was first demonstrated in the resolution of 1.97°A by single anomalous dispersion using selenomethionine-substituted protein and this HigA report to the *Coxiella Burnitti, Vibrio cholerae, Shigella Flexnari, E. Coli*, and *P. Vulgaris*.[14] The HigBA family of the TA system shows the translational inhibition in *M. tuberculosis* by HigB toxin expression when antitoxin HigA is absent by reducing the amount of IdeR and Zur controlled mRNAs and cleaving tmRNA in *M. tuberculosis*.[15]

5. The family of RelBE

RelBE is another module of the toxin-antitoxin system, which is consisting of three similar modules like RelFG, and RelIJK, in these three RelE interact with RelB-like protein physically, and it can control the binding with promotor DNA. Here the RelJ toxin has acted as a corepressor of protein and the other two RelB and RelF have acted as a transcriptional activator, the following study has successfully done interaction between the RelBE pairs in *M. tuberculosis* using GST-pull-down assays and observed that the assumed length of His-tagged Rel protein was readily pulled down the cognate GST-tagged Rel protein. Moreover, the RelJ antitoxin only can bind with its promotor region even low concentration of protein, but no binding activity was noted with the other two RelB and RelF like antitoxin proteins even with a high concentration of the proteins, and korch et al observed the *M. tuberculosis* toxins like RelE, RelK and one antitoxin RelF were expressed at the later stages of macrophage infection.[16]

6. The family of ParDE

The ParDE2 operon (Rv2142A; Rv2142c) is a type II TA system with a toxin gene and is connected to the 5-antitoxin gene. sharing a 4bp overlap, the sequence identity of the ParE and ParD of RK2 plasmid is 18% and 12% base pairs in MParE2 and MParD2 amino acid sequences in Blastp analysis, respectively. A single gene consisting of a 530bp product. The results revealed (i) The ParDE2 operon basal phase transcriptional activity will have functioned in exponential phase till the late stationary phase (ii) the parDE2 operon isn't a pseudogene and (iii) the mode of transcription of ParD2 and ParE2 genes will be single and bi-cistronic. Moreover, the parDE2 promotor was recognized as the mycobacterial sigma factor in the identical to −35 consensus sequence, which was induced specifically in the stationary phase, oxidative stress, and macrophage invasion, so possibilities of the parDE2 operon suggest that the regulation of the operon will be affecting the growth of *Mycobacterium smegmitis,* especially in the suitable environmental condition preferably in the oxidative stress form. The MParE2 protein is an inhibitor of DNA gyrase, which is to be a key enzyme in the process of DNA replication and the ideal target line to explore in *M. tuberculosis*. The activation of the parDE2 operon and release of the excess toxin under oxidative stress, the condition notorious to conquer in the host macrophage.[17]

7. The family of DarTG

The function of the DarT toxin domain is unidentified for DUF 4433, and the DarG antitoxin is coming under the macrodomain protein. The DarT has an enzymatic activity that alters the thymidines on a single-stranded DNA in a sequence-specific function through nucleotide modification, it's known as ADP-ribosylation. In the formation of de-ADP-ribosylation activity of DarG, the mutation starts to reduce the activity of macro domains, so it's a possible way to bind with the substrate. It was minimal or no reaction on the de-ADP-ribosylation activity that happened in TaqDarG after 21 minutes followed by a mutation in H82A and W83A this showed that the rest of N22A, K29E, G119E, and K80A have the significant action of inhibition. Interestingly, one of the mutations of K80A, which is equivalent to the main catalytic lysine residue in TARG1 showed that the crucial reaction of substrate tarts out of all the mutations assessed with static TaqDarG, so it will be preserved between TARG1 and DarG.[18] A recent study shows that $DarG_{Mtb}$ is essential even when $darT_{Mtb}$ was absent, as well the identified interactions between the enzymes associated with the DNA repair mechanism of mycobacterial replisome such as DNA polymerase (DnaE1, PolA), DNA helicase (DnaB), and DNA primase (DnaG) with $DarG_{Mtb}$ family, DarGMtb interactome is consisting the part of DNA-repair-associated proteins such as RecA, RecB, RecF, Lhr, and AlkA.[19]

8. The family of mt-PemIK

The recent studies identified the new toxin-antitoxin protein that is named mt-PemIK; the toxin is mt-PemK and the antitoxin is mt-PemI(RV3098A), which is demonstrated in neutralizing the toxin protein suppressed by the antitoxin protein in the appropriate His or Flag tag in E.Coli. the protein sequence of mt-PemK is 25.66% and the replacement of mutation with an mt-PemK mutant with the following three residues such as H19A, Q21A, or R25A loosen its toxicity capacity. This sequence resembles in nature the my-PemK and MazF/PemK proteins, characterized as endoribonucleases which are to be inhibiting bacterial growth. Likely mt-PemK has RNA cleavage properties and could be modified to regulate the pupylation stability, pup is the small prokaryotic ubiquitin-protein present in *Mycobacterium tubeculosis* and *M. smegmitis*.[20] According to TADB analysis showed that the

PemK and MazF toxin genes are identified in the following genes like Rv1103c-Rv1102c, Rv1494-Rv1495, Rv1943c-Rv1942c, and Rv0660c-Rv0659c, respectively, in domain2 (Fig. 1, Fig. 2, Fig. 3 and Table 1).

9. The family of the TAC chaperone

The bacterial TAC-chaperone is exported the unfolded protein in post-translation pathways from the cytoplasm. The fundamental of the TAC chaperone SEC pathway is made up of a hetero-trimetric membrane-embedded channel. Sec-YEG has two cytosolic elements, Sec-A and Sec-B. Sec-B can be functioned by balancing unfolded proteins and retaining them in an export competent state. Sec-B chaperones mostly originate in proteobacteria, and the same type of Sec-B protein Rv1957, which is controlling the stress-responsive toxin-antitoxin modules occurred in MtbRv1957. It could be functionally replaced the *E. coli* Sec B chaperone in vivo and in vitro.[21] The atypical TA family of TAC chaperone modules, and this module helps to fold and protect the antitoxin from degradation. As a molecular tool by which refined TA group become 'chaperone-addiction' TAC module from *M. tuberculosis* which the chaperone is targeting the antitoxin at a short carboxyl-terminal sequence (chaperone addiction sequence, ChAD) which is absent in chaperone-independent antitoxins. In these conditions, the ChAD sequence could enable uncertain antitoxin, thus the mechanism prevents the inhibition of toxins. The Chaperone–ChAD pairs are located in conventional toxin-antitoxin loci or unlinked proteins and can be

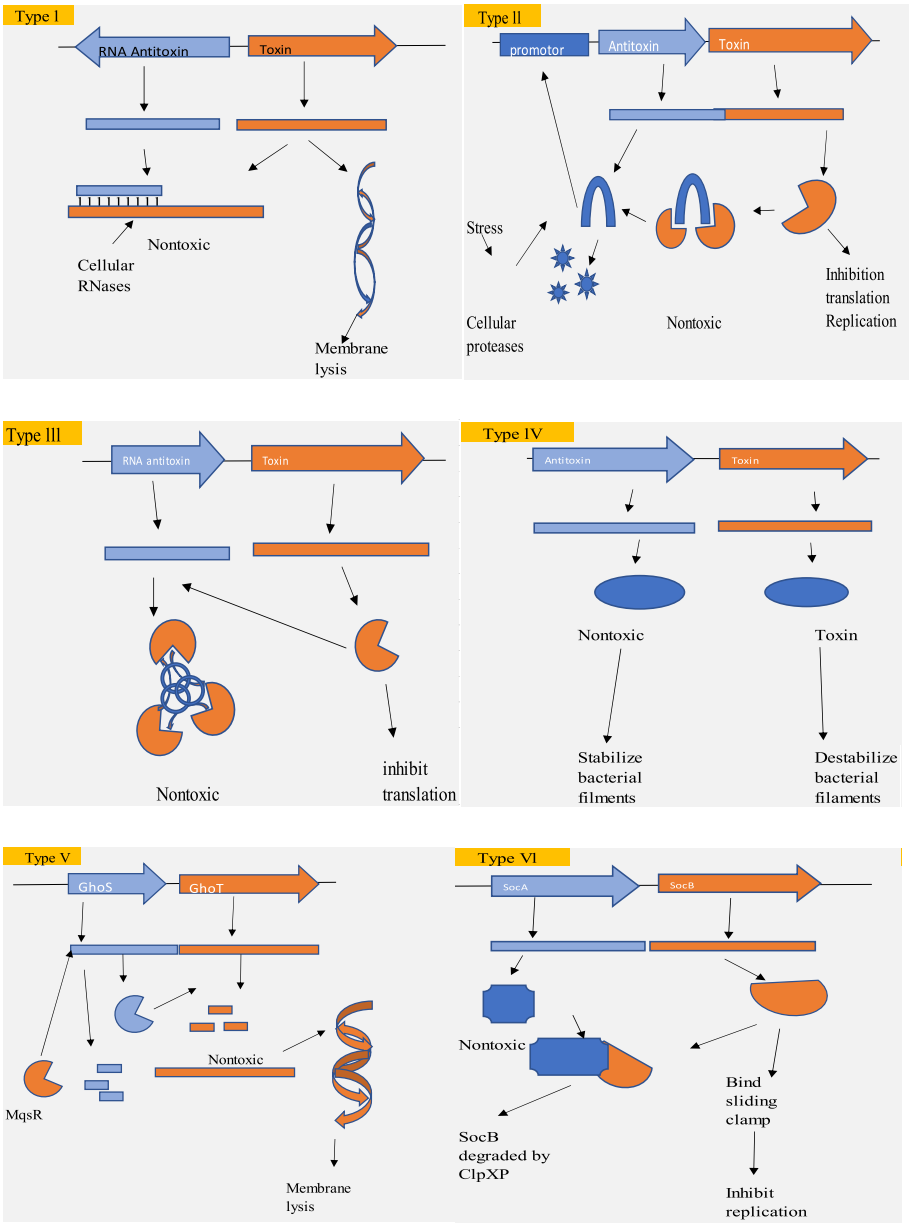


Fig. 1 – Different types of TA systems.

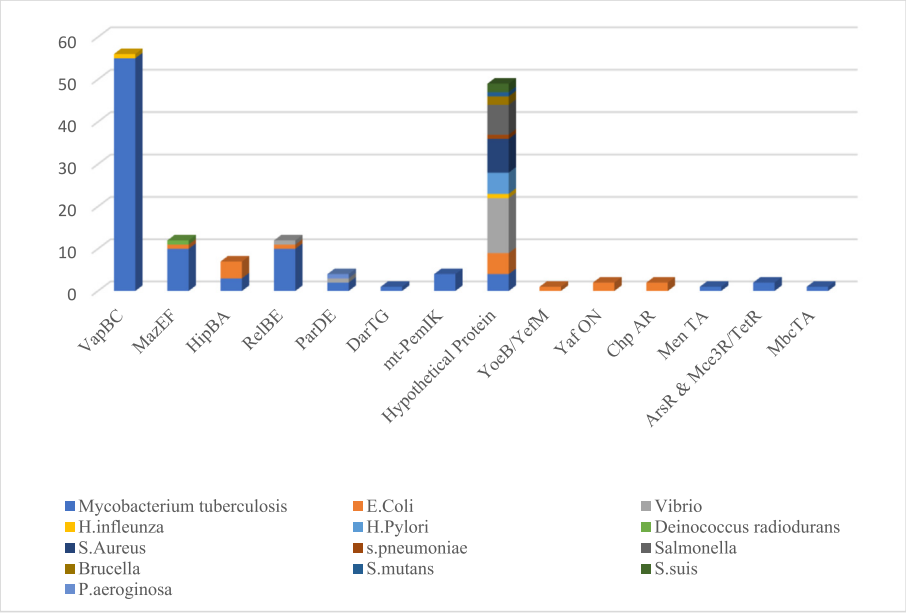


Fig. 2 – Graphical Diagram of TA Loci in different pathogens.[44,45]

provided in chaperone-dependent. It could optimize the expression and new strategy for biotechnological or medical purposes.[22]

10. Discussion

The genome of *M. tuberculosis* inscribed approximately higher TA Loci, which are mostly unidentified proteins than other pathogens, based on an earlier review of 79 TA loci with five types and six classes of TA family. This review analyzed 154 TA Loci in different pathogens and 93 are functionally available in *M. tuberculosis* with the recent contributions and determined the new TA families. These hypothetical proteins have significant roles to determine the master regulators in cell growth and pathogenesis of *M. tuberculosis*, and the study has also stated that the genome sequences are encoding in 4500 prokaryotic and 101 mycobacterial genomes have atypical PezAT TA loci and it is also preserved in other mycobacterial species and lineages classes of Actinobacteria.[23] When cognate antitoxin non-encoded RNA cleavages occur, the toxin proteins interact with the macromolecules and stimulating the inhibition of growth and producing the antibiotic tolerant persister daughter cells.[24] *M. tuberculosis* drug-resistant and toxicity were assessed in vivo in guinea pigs due to the significant number of MazF *M. tuberculosis* toxin proteins that play a combined expression.[25] Another study showed mycobacterial growth arrest occurred when the toxin MenT3 acts independently when not antagonist activity of the cognate antitoxin MenA3. The structural analysis demonstrated for MenT3 and MenT4 was done at 1.6 and 1.2A° resolution, to analyze the biochemical properties of MenT3. When MenT3 stopped in-vitro analysis of protein expression, then it spontaneously blocked the tRNA charging in-vivo. MenT3 expanded pyrimidines (C or U) to the 3′-CCA acceptor stems of uncharged tRNAs and demonstrated vital substrate specificity in-vitro.[26] There is the enzymatic activity in the toxin protein which degrades the tRNA into smaller fragments; it's reflecting the endonucleolytic cleavage. This cleavage was designated to specific anticodon stem-loop end.[5] Each different RNA is cleaved with structurally defined toxin protein of MazF by *M. tuberculosis* to cleave the mRNAs, for example, tRNAs cleft by MazF-mt9 toxin, 23S rRNA cleaves by MazF-mt6 toxin, and both 16S and 23S rRNA cleaves by MazF-mt3 toxin, and the inhibition of protein synthesis action occurred due to above enzyme related activity. Even though the entire tertiary fold of these MazF toxins is expected to be the same, it was undetermined, that how this toxin recognizes very structurally modified RNAs.[12] The experimental methods to construct the gene from presumed *M. tuberculosis*, the genome, have been inserted downstream of the inducible acetamidase promotor in an appropriate plasmid vector to assess the toxin and antitoxin activity. The Quantitative PCR (qPCR) Techniques found toxin-antitoxin focus on the identical sites of rRNA, proven this is a particular site of rRNA is likely refractory to the activity of single strand-specific endoribonucleases.[27,9,3] A recent study revealed that the peptide development with the antitoxin protein could be a bactericidal activity proven that it is inhibiting the growth of the H37Rv in-vitro, but preliminary results showed that in-vivo activities were poor, the antitoxin fragments are potential bactericidal properties and first tested on the *Bacillus anthracis* PemIK TA system. Structural and biochemical analyze the interaction between the TA system, PemK cleft the single-stranded RNA, Kang et al, designed the peptide of the HigBA TA system was proven to inhibit the growth of *Streptococcus pneumoniae*.[28] Type II toxin-antitoxin is unique in that the antitoxin protein binds with the toxin protein and neutralizes the toxin activity. In this study, the 23S rRNA of disassociated ribosome triggered the inhibition of protein

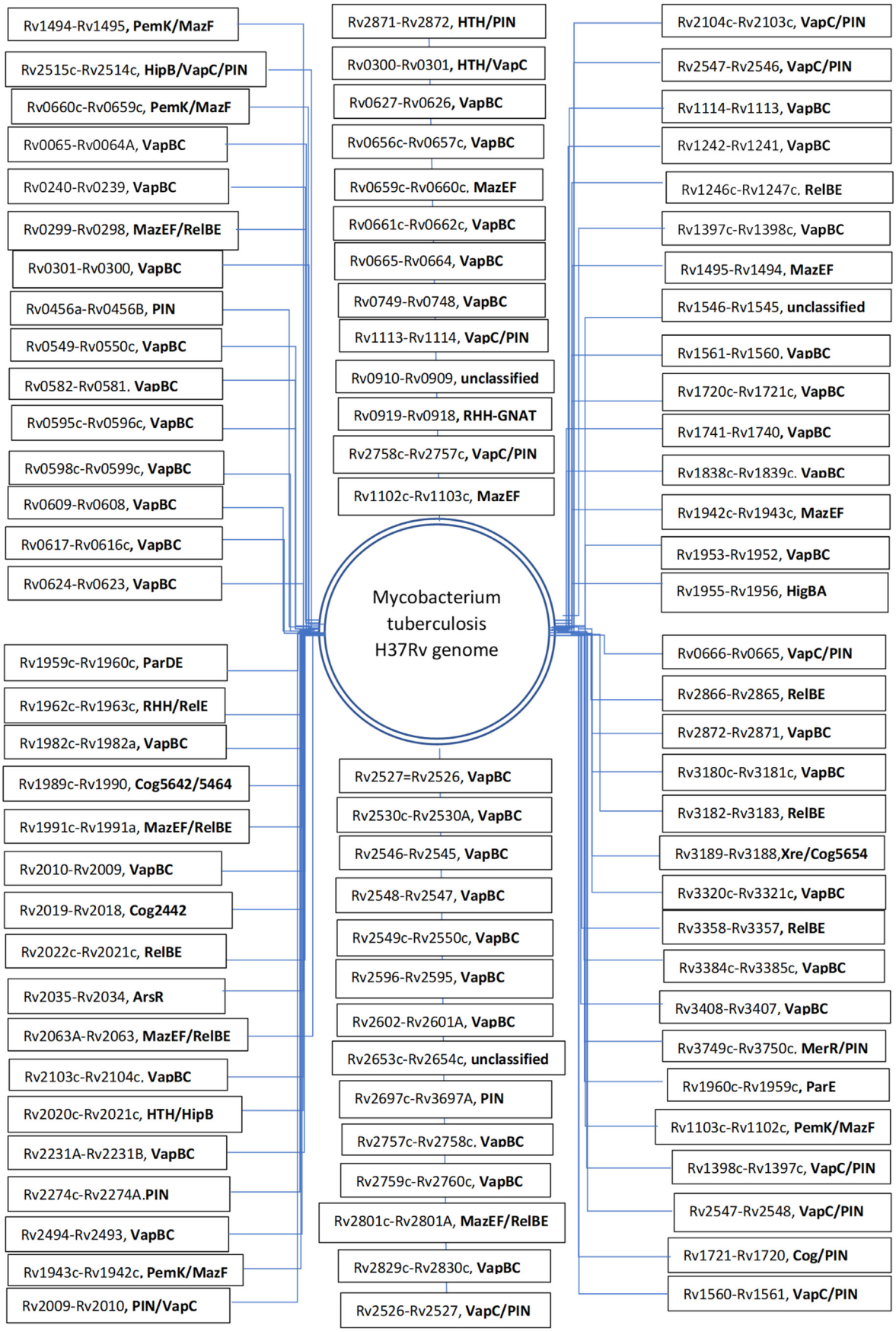


Fig. 3 – TA Loci available in *Mycobacterium tuberculosis* H37Rv genome.

Table 1 – TA family encoded among different pathogens.

	VapBC	MazEF	HipBA	RelBE	ParDE	DarTG	mt-PemIK	Hypothetical Protein	YoeB/YefM	Yaf ON	Chp AR	Men TA	ArsR & Mce3R/TetR	MbcTA
Mycobacterium tuberculosis	55	10	3	10	2	1	4	4				1	2	1
E.Coli		1	4	1				5	1	2	2			
Vibrio				1	1			13						
H.infleunza	1							1						
H.Pylori								5						
Deinococcus radiodurans		1												
S.Aureus								8						
s.pneumoniae								1						
Salmonella								7						
Brucella								2						
S.mutans								1						
S.suis								2						
P.aeroginosa					1									

synthesis due to toxin cleavage, Apart from RNA targets, the study showed that the inhibition occurs mutually with each other in Mycobacteria, because the MazF toxin and DNA topoisomerase I interact with each other's and maybe functioning stimulation, where the DNA topoisomerase I gene is an important enzyme of *M. tuberculosis*, this all studies showing that the MazF could express two alternative mechanisms to stimulate the bacterial cell death.[29] The *Mycobacterium Bovis BCG* and *M. tuberculosis* growth inhibition is bacteriostatic action because of the over-expression of VapC22, similarly seen in *Mycobacterium smegmatis*, the alteration of a well-preserved PIN domain residue (aspartic acid at a position of 8 with alanine) generated VapC22 inactive in *M. Bovis BCG* leading to abandoning the inhibition of growth.[30,31] The RNase, tRNase, and RNA sequence experiments showed notable impact on translational inhibition and toxicity-anti toxicity as a result of cleavage of RNA in mycobacteria.[32,33,30,25] Rv3098A is a new potential toxin protein that was tested and identified through PSI-BLAST searches in the *M. tuberculosis* H37Rv strain; it is called mt-PemK. In the open reading frame (ORF) located at the upstream and overlaps of mt-PemK by 4bp, the position of the upstream mt-PemK is called mt-PemI, which is coming under the protein sequence analysis of the MazF/PemK toxin family.[20] One of the studies states that Rv1044 and Rv1045 are antitoxins and toxins, respectively. Antitoxin neutralizes the toxin with the help of an enzyme that serine protein kinase to degrade the toxin activity, toxin acting as a tRNA nucleotidyltransferase action in this module.[34] One more study showed that the cholesterol-induced Rnase toxin (VapC21) can inhibit the translation of targeting the proT tRNA in *M. tuberculosis*, and this study revealed that cholesterol precise growth variation can be increased by the persistent daughter cells.[35] A transcriptomic study of antibiotic-induced persister daughter cells formation of *M. tuberculosis* showed that the TA system was up-regulated due to a general shutdown happening in the metabolic pathways, and this condition could be a potential outcome of future research on TA system in *M. tuberculosis* persistent cells.[36,37,38] One of the reviews analyzed that the PhoH2 gene in *M. tuberculosis* and *M. smegmitis* involved in the metabolism of fatty acids and implementing the response of hypoxia, this small protein resembled VapB and VapBC proteins in the TA system.[39] One of the studies showed the clinical and H37Rv strains in the demonstration of the MazE3 and MazE6 was no remarkable expression in the drug-resistant strain than standard strain, however, both were over-expressed in the drug-susceptible strain than standard strain.[40] The recent study evolved the stapled peptides from the TA system of VapBC26 in *M. tuberculosis* for the demonstration of V26-SP-8 peptides designed as a new antibacterial therapeutic tool.[41] A recent study analyzed the copper component interacts with the VapC4 TA system in *M. tuberculosis*, and it is high concentration whiles macrophages increases in the phagosomal concentration of Cu upon Mtb infection, hence it is up-regulated the CsoR and RicR in Cu resistant pathways while VapC4 expression.[42] One of the recent studies concluded that the HigB1 of *M. tuberculosis* is essential to identify the infection in guinea pigs, and a microarray assay analyzed that the removal of HigB1 leads to increasing the transcripts of ribosomal proteins and decreases in the expression of genes associated with virulence, detoxification, and adaptation.[43] The mechanism of the RNA cleavage with VapC in *M. tuberculosis* is the same compared with VapC of *Pyrobaculum aerophilum* and VapC1,19,27,29,39 is a magnesium-dependent

ribonuclease with identical sequence specificity and focusing UA*GG sequence.[46] The MbcAT were identified to monitor the bactericidal effect on fast-growing *M. smegmitis*, bringing the form of strong promotor to induce the MbcT will reiterate to bactericidal effect against Mtb while depletion of NAD + followed by the induction of P606-mbcT.[47] The study correlated with Xia Yu et al that Rv1044 is curiously binding with DNA in a sequence-specific manner and not upstream in DUF1814 TA systems in *M. tuberculosis*, so the potential properties of Rv1044 are acting outside of Rv1044-Rv1045 operon.[48,34]

11. Concluding remarks

The above reviews show the significance of the module to control the disease pathogenesis, persistence of daughter cell formation, drug-resistant strains, biofilm formation, cell growth as well cell death. where the above reviews underlined the TA genome encoded in prokaryotes and the fact of Sec-B in *E.coli* for two chaperone analyzes shown that the Sec-B chaperone is capable to perform generic chaperone function in the natural host as well as interacting with Sec translocon, so further studies are required for analysing about Sec-B chaperone and one of the studies encouraged that Rel protein can induce the growth inhibition and quorum sensing peptides roles are needed to elucidate the action on further analysis. So many bacterial species are encoding TA families such as *streptococci, staphylococci, Vibrio, salmonella, E. coli, helicobacter, pseudomonas* etc, but the genome correlates with *M. tuberculosis* is vast and consisting rich hypothetical protein in TA loci. Two factors are determining the microbial surveillance and the TA system comes in one of the factors after the sigma factor, where the functional characterization of the TA system is bringing the hope of demonstrating the new therapeutic tool against tuberculosis. Peptide-based mode of binding in the target mt-MezEF-TA will be a future therapeutic tool against *M.* tuberculosis.[28] The disease is a global alarming challenge, especially since MDR and XDR strains are increasing drastically due to mutants. So, the control of tuberculosis is in the detailed analysis required and the whole-genome sequence for TA loci identification is to be essential because this is the master regulator for the entire process of the microbial population.

Conflicts of interest

The authors have none to declare.

REFERENCES

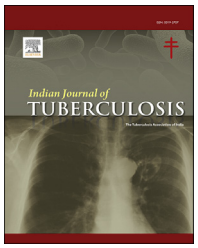
1. *Global Tuberculosis Report 2021*. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. Sala A, Bordes P, Genevaux P. Multitasking SecB chaperones in bacteria. *Front Microbiol*. 2014;5(5):666.
3. Ramage RH, Connolly LE, Cox JS. Comprehensive functional analysis of Mycobacterium tuberculosis toxin-antitoxin systems: implications for pathogenesis, stress responses, and evolution. *PLoS Genet*. 2009;5(12), e1000767.
4. Alberthsen SJ, Agner J, Sander RP, et al. Proteomic profiling of Mycobacterium tuberculosis identifies nutrient-starvation responsive toxin–antitoxin systems. The American society for biochemistry and molecular biology. *Mol Cell Proteomics*. 2013;12(5):1180–1191.
5. Winther SK, Brodersen DE, Brown AK, Gerdes K. VapC20 of Mycobacterium tuberculosis cleaves the sarcin–ricin loop of 23S rRNA. *Nat Commun*. 2013;4:2796.
6. Jin G, Pavelka Jr MS, Butler JS. Structure-function analysis of VapB4 antitoxin identifies critical features of a minimal VapC4 toxin-binding module. *J Bacteriol*. 2015;197(7):1197–1207.
7. Kang SM, Kim DH, Lee KY, et al. Functional details of the Mycobacterium tuberculosis VapBC26 toxin-antitoxin system based on a structural study: insights into unique binding and antibiotic peptides. *Nucleic Acids Res*. 2017;45(14):8564–8580.
8. Barth VC, Woychik NA. The sole Mycobacterium smegmatis MazF toxin targets tRNALys to impart highly selective, codon-dependent proteome reprogramming. *Front Genet*. 2020;14(10):1356.
9. Schifano JM, Irino OV, Knoblauch JG, et al. An RNA-seq method for defining endoribonuclease cleavage specificity identifies dual rRNA substrates for toxin MazF-mt3. *Nat Commun*. 2014;5:3538.
10. Zhu L, Sangita P, Hirofumi N, et al. The mRNA interferes, MazF-mt3 and MazF-mt7 from Mycobacterium tuberculosis target unique pentad sequences in single-stranded RNA. *Mol Microbiol*. 2008;69(3):559–569.
11. Zhao JL, Wei L, Xie WY, Cao XD, Yuan L. Viability, biofilm formation, and MazEF expression in drug-sensitive and drug-resistant Mycobacterium tuberculosis strains. *Infect Drug Resist*. 2018;6(11):345–358.
12. Hoffer ED, Miles SJ, Dunham CM. The structure and function of Mycobacterium tuberculosis MazF-mt6 toxin provide insights into conserved features of MazF endonucleases. *J Biol Chem*. 2017;292(19):7718–7726.
13. Richardson W, Kang GW, Lee HJ, et al. Chasing the structural diversity of the transcription regulator Mycobacterium tuberculosis $HigA_2$. *IUCrJ*. 2021;8:823–832.
14. Park JY, Kim HJ, Pathak C, et al. Induced DNA bending by unique dimerization of HigA antitoxin. *IUCrJ*. 2020;7:748–760.
15. Schuessler DL, Cortes T, Amanda SFH, et al. Induced ectopic expression of HigB toxin in Mycobacterium tuberculosis results in growth inhibition, reduced abundance of a subset of mRNAs and cleavage of tmRNA. *Mol Microbiol*. 2013;90(1):195–207.
16. Min Y, Gao C, Wang Y, Zhang H, He ZG. Characterization of the interaction and cross-regulation of three Mycobacterium tuberculosis RelBE modules. *PLoS One*. 2010;5(5), e10672.
17. Gupta M, Nishtha N, Meenakshi C, et al. The chromosomal parDE2 toxin–antitoxin system of Mycobacterium tuberculosis H37Rv: genetic and functional characterization. *Front Microbiol*. 2016;7:886.
18. Jankevicius G, Antonio A, Marijan A, Ivan A. The toxin-antitoxin system DarTG catalyzes reversible ADP-ribosylation of DNA. *Mol Cell*. 2016;64:1109–1116.
19. Anisha Z, Wang R, Laure B, et al. Depletion of the DarG antitoxin in Mycobacterium tuberculosis triggers the DNA-damage response and leads to cell death. *Mol Microbiol*. 2020;114:641–652.
20. Chi X, Chang Y, Li M, et al. Biochemical characterization of mt-PemIK, a novel toxin-antitoxin system in Mycobacterium tuberculosis. *FEBS (Fed Eur Biochem Soc) Lett*. 2018;592:4039–4050.
21. Lu Z, Wang H, TingTing Y. The SecB-like chaperone Rv1957 from Mycobacterium tuberculosis: crystallization and X-ray

crystallographic analysis. *Acta Crystallogr F Struct Biol Commun*. 2016;72:457–461.
22. Bordes P, Cirinesi AM, Roy U, et al. SecB-like chaperone controls a toxin-antitoxin stress-responsive system in Mycobacterium tuberculosis. *Proc Natl Acad Sci Unit States Am*. 2011;108(20):8438–8443.
23. Tandon H, Melarkode Akhila, Sandhya S, Srinivasan N. Molecular and structural basis of cross-reactivity in M. tuberculosis toxin–antitoxin systems. *Toxins*. 2020;12:481.
24. Germain E, Castro -RD, Zenkin N, Gerdes K. Molecular mechanism of bacterial persistence by HipA. *Mol Cell*. 2013;52, 248 –25.
25. Prabhakar T, Garima A, Singh Mamta, et al. MazF ribonucleases promote Mycobacterium tuberculosis drug tolerance and virulence in Guinea pigs. *Nat Commun*. 2015;6:6059.
26. Cai Y, Ben U, Gutierrez C, et al. A nucleotidyltransferase toxin inhibits growth of Mycobacterium tuberculosis through inactivation of tRNA acceptor stems. *Sci Adv*. 2020;6(31), eabb6651.
27. Cintron M, Zeng JM, Barth VC, et al. Accurate target identification for Mycobacterium tuberculosis endoribonuclease toxins requires expression in their native host. *Sci Rep*. 2019;9:5949.
28. Chen R, Zhou J, Xie W. Mechanistic insight into the peptide binding modes to two M.tb MazF Toxins. *Toxins*. 2021;13:319.
29. Lee IG, Sang JL, Susanna C, et al. Structural and functional studies of the Mycobacterium tuberculosis VapBC30 toxin-antitoxin system: implications for the design of novel antimicrobial peptides. *Nucleic Acids Res*. 2015;43(15):7624–7637.
30. Arun S, Gopinath C, Pankaj C, et al. VapC21 toxin contributes to drug-tolerance and interacts with non-cognate VapB32 antitoxin in Mycobacterium tuberculosis. *Front Microbiol*. 2020;11:2037.
31. Sakshi A, Prabhakar T, Amar Deep, et al. System-wide analysis unravels the differential regulation and in vivo essentiality of virulence-associated proteins B and C toxin-antitoxin systems of Mycobacterium tuberculosis. *J Infect Dis*. 2018;217:1809–1820.
32. Amar deep, Prabhakar T, Sakshi A, et al. Structural, functional and biological insights into the role of Mycobacterium tuberculosis VapBC11 toxin-antitoxin system: targeting a tRNase to tackle mycobacterial adaptation. *Nucleic Acids Res*. 2018;46(21):11639–11655.
33. Zaychikova MV, Zakharevich NV, Sagaidak MO, et al. Mycobacterium tuberculosis type II toxin-antitoxin systems: genetic polymorphisms and functional properties and the possibility of their use for genotyping. *PLoS One*. 2015;10(12), e0143682.
34. Yu X, Xiaopan G, Zhu K, et al. Characterization of a toxin-antitoxin system in Mycobacterium tuberculosis suggests neutralization by phosphorylation as the anti-toxicity mechanism. *Communication biology*. 2020;3:216.
35. Sakshi T, Manitosh P, Chandresh S, et al. Role of VapBC12 toxin-antitoxin locus in cholesterol-induced mycobacterial persistence. *mSystems*. 2020;5. e00855-20.
36. Sharp JD, Cruz JW, Sahadevan R, et al. Growth and translation inhibition through sequence-specific RNA binding by Mycobacterium tuberculosis VapC toxin. *J Biol Chem*. 2012;287(16):12835–12847.
37. Cruz JW, Sharp JD, Hoffer ED, et al. Growth-regulating Mycobacterium tuberculosis VapC-mt4 toxin is an isoacceptor-specific tRNase. *Nat Commun*. 2015;6:7480.
38. Bordes P, Genevaux P. Control of toxin-antitoxin systems by proteases in Mycobacterium tuberculosis. *Front Mol Biosci*. 2021;8:691399.
39. Andrews SVE, Arcus VL. PhoH2 proteins couple RNA helicase and RNAse activities. *Protein Sci*. 2020;29:883–892.
40. Hossein K, Hamid H, Jalil YK, et al. Comparison of toxin-antitoxin expression among drug-susceptible and drug-resistant clinical isolates of Mycobacterium tuberculosis. *Adv Respir Med*. 2021;89:110–114.
41. Kang SM, Moon H, Han SW, et al. Toxin-Activating stapled peptides discovered by the structural analysis were identified as new therapeutic candidates that trigger antibacterial activity against mycobacterium tuberculosis in the Mycobacterium smegmatis model. *Microorganisms*. 2021;9:568.
42. Barth VC, Chauhan U, Zeng J, et al. Mycobacterium tuberculosis VapC4 toxin engages small ORFs to initiate an integrated oxidative and copper stress response. *Proc Natl Acad Sci Unit States Am*. 2021;118(32), e2022136118.
43. Arun S, Sagar K, Chauhan NK, et al. HigB1 Toxin in Mycobacterium tuberculosis is upregulated during stress and required to establish infection on Guinea Pigs. *Front Microbiol*. 2021;12:748890.
44. Xie Y, Wei Y, Shen Y, et al. Tadb 2.0: an updated database of bacterial type II toxin-antitoxin Loci. *Nucleic Acids Res*. 2018;46:D749–D753.
45. Shao Y, Harrison EM, Bi D, et al. TADB: a web-based resource for Type 2 toxin-antitoxin loci in Bacteria and Archaea. *Nucleic Acids Res*. 2011;39:D606–D611.
46. Sharrock A, Ruthe A, Andrews ESV, Arcus VA, Hicks JL. VapC proteins from Mycobacterium tuberculosis share ribonuclease sequence specificity but differ in regulation and toxicity. *PLoS One*. 2018;13(8), e0203412.
47. Ariyachaokun K, Grabowska AD, Claude G, Olivier N. Multi-stress induction of the Mycobacterium tuberculosis MbcTA bactericidal toxin-antitoxin system. *Toxins*. 2020;16(5):329, 12.
48. Beck NI, Ben U, Hannah GH, Peter CF, Tim RB. Antitoxin autoregulation of M. tuberculosis toxin-antitoxin expression through negative cooperativity arising from multiple inverted repeat sequences. *Biochem J*. 2020;477:2401–2419.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review article

Infection control and preventing the transmission of tuberculosis in high-risk centres - recovery shelter for homeless people

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

ARTICLE INFO

Article history:
Received 22 October 2022
Accepted 24 November 2022
Available online 26 November 2022

Keywords:
Airborne precautions
Health care workers
Infection prevention control
Tuberculosis

ABSTRACT

The term "infection control" refers to the policies and practices used in hospitals and other healthcare facilities to limit the spread of illnesses with the primary goal of lowering infection rates. The objective is to reduce the chance of infection in patients and Healthcare workers (HCWs). This may be achieved by making all the HCWs to follow and practice the infection prevention and control (IPC) guidelines and by providing safe and quality healthcare. Because of more exposure to TB patients and insufficient TB infection prevention and control (TBIPC) procedures in a healthcare facility, healthcare workers (HCWs) working in TB centers are at an elevated risk of contracting TB. Although there are a number of TBIPC guidelines, there is limited knowledge of their contents, if they are applicable in the given situation, and whether they are being properly applied in TB centers. The purpose of this study was to observe the TBIPC guidelines' implementation in CES (Centre of equity studies) recovery shelters well as the elements that affect it. The percentage of public health care personnel who used proper TBIPC practices was low. The execution of TBIPC guidelines in tuberculosis (TB) centers was poor. It was impacted because TB treatment institutions and centers have unique health systems and TB disease burdens.

1. Introduction

Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis*, and in high TB burden countries, it is an infectious disease that has a high burden of morbidity and mortality. Different tactics such as surveillance, isolation, outbreak investigation and management and education[1] are used in TBIPC to lessen the risk of *M. tuberculosis* transmission. The hierarchy of control is a method for reducing workplace risks. The hierarchy of control is a method for systematically eliminating or reducing risks, and it lists risk controls in order to decrease the amount of protection and reliability from highest to lowest. The hierarchy of controls places eliminating

* *Corresponding author*. Indian Institute of Public Health-Delhi, Plot No. 47, Sector-44, Gurugram- 122002, Haryana, India.
E-mail address: surabhi.pandey@phfi.org (S. Pandey).
https://doi.org/10.1016/j.ijtb.2022.11.002
0019-5707/

hazards and risks at the top, followed by reducing risks through engineering, isolation, and substitution controls, and finally through administrative controls. The lowest level of control is lowering the risk through the use of protective personal equipment (PPE).[2] The hierarchy of control has three layers, which have been found to lessen and prevent the danger of exposure and transmission to *M. tuberculosis*: administrative control, environmental control, and respiratory protection.[3]

The policy places a strong emphasis on the necessity of infection prevention and control (IPC) in healthcare facilities and other facilities such as CES(Centre of equity studies) recovery shelters for homeless people where there is an elevated possibility of *M. tuberculosis* spread.[4] IPC procedures are crucial for lowering the chance of *M. tuberculosis* transmission because they lessen the amount of exposure to infectious droplet nuclei present in the air for those who are vulnerable to them.

1.1. *Transmission*

The risk of transmission in healthcare settings is increased when healthcare professionals and patients interact with people who:

- have pulmonary TB that is not yet suspected;
- are not receiving proper treatment;
- have not been segregated from other people,[5]

By inhaling minute particle aerosols (airborne route), TB is disseminated.*M. tuberculosis* is produced and transported by particles in droplet nuclei which are roughly 1–5μm in size[6] when a pulmonary TB patient coughs, sings, laughs, or sneezes. Depending on the environment, tubercle bacilli can float in the air for a lengthy span of time and can be carried by air currents across a space. The amount of live organisms present in respiratory secretions has a direct correlation with infectiousness. The length of exposure increases the probability of transmission. The greatest risk of contracting TB from an index case of pulmonary tuberculosis is among household members. Studies suggest after continuous eight hours of exposure, the duration is frequently seen as significant in healthcare settings, but this is not a rigid requirement. Smear positivity intensity, mechanical parameters (such as aerosol-generating techniques), and host susceptibility must all be taken into consideration. Only airborne micro droplets of TB germs can transmit *M. tuberculosis*. Shaking hands, using the restroom, or touching surfaces like bed sheets or toilet seats will not spread it.[5]

1.2. *Infection*

Infection is caused by bacteria that enter into the body and multiply and cause harm or disease. The incubation, prodromal, decline, and convalescence periods are the five stages of disease (sometimes known as stages or phases). A successful colonization of a host by a microbe is referred to as an infection. And it can cause illness.

The normal body vital function: Temperature: 37 °C [98.6 °F], Heart Rate: 60–100/min, Breathing Rate: 12–18/min, Blood pressure: 90/60 and 120/80 mm Hg. And when the infection occurs, normal body vital functions get interrupted.[7]

Some infections are spread when an infected person talks, cough or sneeze, and the droplet they produce contain germs. Depending on the size of the droplets and the intensity of expulsion, droplets can be pushed up to 6–12 feet. Larger droplets have a higher likelihood of falling to the ground or floor quickly.[8] The tiny droplet may be breathed in by people who are near or may fall and contaminate an object or surface. When a person with TB coughs, tiny droplets of tubercle bacilli are released from the infected lungs into the air, where they are inhaled by an uninfected person.[9] In the built environment, objects like doorknobs, countertops, medical equipment, handrails, clothing, and mobile phones that people routinely come into direct contact are likely to be the most significant vectors for contamination and transmission.[10]

Infection management considers elements that affect how an infection spreads within the healthcare environment, whether it occurs between patients, among patients and among staff. This includes preventative actions like washing, sanitizing, sterilizing, and disinfecting hands.

1.3. *Risk*

A risk is a possibility or likelihood that something will negatively impact your health in some other way. Risk does not guarantee that something negative will occur. It's only conceivable. Your health risks are either high or low depending on a number of traits or risk factors.[11] In the case of tuberculosis, anyone who comes in contact of people who have active tuberculosis is susceptible to contracting the disease. People living in areas where tuberculosis cases are more prevalent puts one at a higher risk of contracting the disease. Employee or resident of long-term care facilities. People who assist tuberculosis patients or come in contact with them have risk of getting TB infection. Individuals infected with active TB and who are not diagnosed on-time and thus are not put on treatment when they are infected. People who inject illegal narcotics or medications run the risk of contracting TB. People who have diabetes, end-stage renal disease, or HIV infection are also at a higher risk of developing TB.

1.4. *IPC interventions*

The World Health Organization (WHO) describes infection prevention and control (IPC) as a scientific strategy and workable solution intended to reduce the risk of infection-related harm to patients and medical personnel. It is a subset of epidemiology but also plays a crucial role in social sciences, global health, and infectious diseases.[12] A crucial public health concern for improving patient safety and the health system is effective IPC. Some of the IPC interventions which are necessary for the recovery shelter for homeless people are mentioned below:

1.5. *Hierarchy of infection prevention and control measures*

The purpose of TB prevention and control is to reduce the likelihood that *M. tuberculosis* will spread throughout people. It has been demonstrated that the danger of exposure and transmission to *M. tuberculosis* can be decreased and prevented by implementing a three-level hierarchy of controls

consisting of administrative controls, environmental controls, and respiratory protection.[13]

1.5.1. *Administrative controls*

It is the first and crucial level of the hierarchy is administrative controls. These are management strategies meant to lessen the chance of coming into contact with TB patients.[13] The strategies is to reduce the time it takes from when a patient with undiagnosed respiratory TB enters a healthcare facility until their condition is presumptively diagnosed, the patient is put on airborne precautions, the diagnosis is made, and antimicrobial treatment is started; provides respiratory protection for the HCW; and assesses the success of its IPC strategies and interventions, and collaborating with the state or local health agency.[14]

1.5.2. *Environmental controls*

Utilizing environmental management to stop the spread of infectious droplet nuclei and lower their concentration is the second rung of the hierarchy. Controlling the infection source by local exhaust ventilation and eliminating contaminated air through general ventilation make up primary environmental control. Controlling airflow to prevent air contamination in an area close to the source is secondary environmental management.[13]

1.5.3. *Respiratory protection controls*

The control of respiratory protection is used at the hierarchy's third tier. It entails wearing personal preventative gadgets in circumstances where there is a significant risk of *M. tuberculosis* exposure. Utilizing a few straightforward safety measures can lower the chance of infection.[13]

1.5.4. *Respiratory hygiene or cough etiquette*

The method of preventing the spread of respiratory secretions that could contain infectious particles by covering the mouth and nose while breathing, coughing, or sneezing (e.g., by donning a surgical mask or a cloth mask, or by covering the mouth with tissues or a sleeve, flexed elbow, or hand).[13]

1.5.5. *Mechanical ventilation*

An air supply or exhaust fan (or both) is used to drive air into or out of a room to produce ventilation.

1.5.6. *Mixed-mode ventilation*

One can select the ideal ventilation mode based on the situation using a ventilation setup that combines natural and mechanical ventilation.[13]

1.5.7. *Natural ventilation*

Introducing and dispersing external the entry or exit of air structure using natural intensity. These pressures may come from the wind or the difference in air density between interior and outdoor spaces.[13]

1.6. *IPC equipments*

The fundamental components of an infection prevention programme are created to stop the transmission of infections in medical facilities. The risk of infection among patients and healthcare workers is decreased when these components are present and continuously applied.

1.6.1. *Air purifier or air cleaner*

A convenient electrical indoor device designed to filter out, neutralize, or put an end to potentially dangerous airborne particles.[13]

1.6.2. *Germicidal UV light (GUV)*

Modern terminology for UVGI is GUV. To allay end-user's anxieties of ionizing radiation, which GUV does not contain, the word “irradiation” is eliminate from the acronym.[13]

1.6.3. *GUV fixture or luminaire*

A device that disperses the GUV radiation coming from a single source or a number of sources. Although the sources themselves are not included, it does contain all the components required for safe and reliable operation, as well as the mechanisms for attaching the references to the power source.[13]

1.6.4. *Ultraviolet germicidal irradiation (UVGI)*

Using ultraviolet C (UVC) light to destroy or inactivate bacteria. Germicidal lamps produce UVGI, which has the power to destroy or inactivate microorganisms in the air or on surfaces that have been exposed to it directly. UVC is produced by low-pressure mercury-vapor lamps.[13]

1.6.5. *Upper-room GUV*

GUV systems are made to provide high amounts of UVC irradiance above the heads of room occupants while reducing UVC exposure in the lower or inhabited part of the space.[13]

2. Conclusion and recommendation

Administrative controls are crucial for infection management since they attempt to lower *M. tuberculosis* exposure and transmission. They oversee patient care and recognize and categories suspected TB cases.

The use of protective masks, ventilation systems, separating patients who may be contagious from other patients, and routine TB screenings of healthcare personnel all help to minimize the spread of TB in the medical field. UV radiation kills the TB bacteria, thus there should be enough of natural light present. Good hygiene prevents the spread of TB bacteria by covering the face when someone coughs or sneezes. The wall where fresh air enters should not be where the exhaust is located. Proper cross-ventilation is required. Using high-efficiency particulate air (HEPA) filtration or ultraviolet germicidal irradiation to clean the air.

Conflicts of interest

The authors have none to declare.

Acknowledgements

The authors would like to acknowledge the staff of the NGO (Centre for Equity Studies) for allowing us to visit there

recovery shelter for homeless people. We would also like to thank New Delhi Tuberculosis Centre for their support.

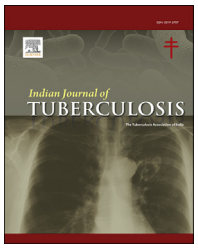
REFERENCES

1. Habboush Y, Yarrarpu S, Guzman N. Infection Control - StatPearls - NCBI Bookshelf. StatePearls Publication, Treasure Island (FL). Published 2022. Accessed September 11, 2022. https://www.ncbi.nlm.nih.gov/books/NBK519017/.
2. Victoria. The hierarchy of control - WorkSafe. Work Victoria. Published online 2020:1. Accessed September 11, 2022. https://www.worksafe.vic.gov.au/hierarchy-control.
3. Islam MS, Chughtai AA, Banu S, Seale H. Context matters: Examining the factors impacting the implementation of tuberculosis infection prevention and control guidelines in health settings in seven high tuberculosis burden countries. *J Infect Public Health*. 2021;14(5):588–597. https://doi.org/10.1016/J.JIPH.2021.01.014.
4. Labena F, Kassa Y, Gambura E. Tuberculosis and public health care workers: infection prevention and control practices and associated factors among governmental health centers and hospitals in wolaita zone, Southern Ethiopia. *J Multidiscip Healthc*. 2021;14:2111–2122. https://doi.org/10.2147/JMDH.S321592.
5. Coulter C. Infection control guidelines for the management of patients with suspected or confirmed pulmonary tuberculosis in healthcare settings. *Commun Dis Intell Q Rep*. 2016;40(3):E360–E366.
6. (PDF) tuberculosis: infection control/exposure control issues for oral healthcare workers. Accessed September 11, 2022. https://www.researchgate.net/publication/5672911_Tuberculosis_Infection_ControlExposure_Control_Issues_for_Oral_Healthcare_Workers.
7. University Of Rochester Medical Center. Vital Signs (Body Temperature, Pulse Rate, Respiration Rate, Blood Pressure) - Health Encyclopedia - University of Rochester Medical Center. U of R. Published 2016. Accessed September 11, 2022. https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=85&ContentID=P00866.
8. LaMorte WW. *Indirect Person-To Person Transmission*. Boston University School of Public Health. Published; 2016. https://sphweb.bumc.bu.edu/otlt/mph-modules/ph/ph709_transmission/PH709_Transmission4.html. Accessed September 11, 2022.
9. Müller A. TB Online - how TB is spread. Published 2016. Accessed September 11, 2022. https://www.tbonline.info/posts/2016/3/31/how-tb-spread-1/.
10. Zoppi L. What are fomites? News medical and life sciences. Published 2021. Accessed September 11, 2022. https://www.news-medical.net/health/What-are-Fomites.aspx.
11. Understanding health risks | NIH news in health. Published online https://newsinhealth.nih.gov/2016/10/understanding-health-risks; 2016. Accessed September 11, 2022.
12. Infection Prevention and Control - Physiopedia. Accessed September 11, 2022. https://www.physio-pedia.com/Infection_Prevention_and_Control.
13. Christof C, Nußbaumer-Streit B, Gartlehner G. *WHO Guidelines on Tuberculosis Infection Prevention and Control*. 82. 2020. https://doi.org/10.1055/a-1241-4321.
14. Jensen PA, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. *MMWR Recomm Rep*. 2005;54(17):1–141. https://doi.org/10.1128/9781555815684.app5.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

A retrospective analysis of 1019 cases of tuberculous cervical lymphadenitis in a rural setup in 20 years

Supreet R. Prabhu [a,*,c], *Enosh Steward N* [b], *Sharon Enosh Steward* [b]

[a] *Dr. N. D. Desai Medical College & Hospital, Nadiad, Gujarat, India*
[b] *Kumar's Dental, Maxillofacial & Facial Aesthetic Clinique, Vadodara, Gujarat, India*

ARTICLE INFO

Article history:
Received 5 October 2020
Received in revised form
5 March 2022
Accepted 26 March 2022
Available online 6 April 2022

Keywords:
Cervical lymph nodes
Anti-Tuberculosis treatment
Intensive phase
Continuation phase
Rifampicin

ABSTRACT

Background: This article is to review cervical lymphadenitis due to tuberculosis (TB), their presentation, their aetiology, the methods used to diagnose them, the treatment modalities offered and the response to treatment.
Methods: 1019 patients were diagnosed and treated for TB of the lymph nodes of the neck from 1st November 2001 to 31st August 2020 at a tertiary ENT hospital, Nadiad, Gujarat, India. Study consisted about 61% males and 39% females with the mean age being 37.3 years.
Result: Commonest factor or habit among those diagnosed for tuberculous cervical lymphadenitis was consumption of unpasteurized milk. HIV and diabetes were the most common co-morbid conditions found with this disease. Swelling in the neck was most common clinical feature followed by loss of weight, formation of abscess, fever and fistula. Rifampicin resistance was found in 1.5% of patients among those tested for the same.
Conclusion: The most commonly affected site for extra pulmonary TB is posterior triangle of neck than the anterior triangle. Patients with HIV and diabetes are at higher risk for the same. Testing for drug susceptibility has to be done due to increased resistant of drugs for extra pulmonary TB. GeneXpert and histopathological examination are important for its confirmation.

1. Introduction

Tuberculosis is the most commonly found chronic infectious disease in developing countries like India which can cause a lot of morbidity and even mortality if not treated on time but if detected and treated in a timely manner, results in an uneventful recovery. It is caused by various strains of mycobacteria like *Mycobacterium Tuberculosis*.[1] The pulmonary form of tuberculosis is most common but we encounter a lot of

* *Corresponding author*. A1/134 Aashiyana Siddharth bunglows, Opp. Urmi School, Sama savli road, Karelibaug, Vadodara, Gujarat 390018, India.
E-mail address: sansup@rediffmail.com (S.R. Prabhu).
[c] **Address:** Dr. Hasmukh Shah's ENT hospital, Kirtikunj Society, Vitthal Kanya Vidhyalaya Road, Nadiad – 387001, Gujarat, India. (M) +91 78744 40069, +91 98254 98524.
https://doi.org/10.1016/j.ijtb.2022.03.023

extra-pulmonary cases too with the most common being the affection of lymph nodes and amongst the group of lymph nodes, the most commonly affected ones is the cervical group of lymph nodes classically called 'Scrofula'. Even where it is controlled to a large extent in the developed countries, it is again posing a new health challenge due to the migration of people from developing countries from Asia & Africa with a high prevalence of tuberculosis and the increasing high incidence of HIV infection in those countries.[2] Few types of extra-pulmonary TB like tuberculous meningitis and military TB have a higher morbidity and mortality rate.[3] The commonest sites affected are lymph nodes, pleura, bones, joints, urogenital tract and meninges.[4] Thus it requires the clinician to be extra suspicious when any patient presents with cervical lymphadenopathy and investigate accordingly so that no case is missed. This study reviews the experience of this tertiary E.N.T. centre at a rural/semi urban center in Nadiad, Gujarat, India as regards the management of 1019 cases of tuberculous cervical lymphadenopathy who presented to us in a 20 year period.

2. Patients and methods

Medical records of 1019 patients admitted at a tertiary hospital in Nadiad, Gujarat, India period from 1st November 2001 to 31st October 2020 were examined. We included all the patients who presented with cervical lymphadenopathy irrespective of the age and were diagnosed as tuberculosis either by FNABC, lymph node biopsy or geneXpert cartridge-based nucleic acid amplification test (CBNAAT) study. There was no exclusion criteria once the patient was diagnosed as having tuberculous lymphadenopathy irrespective of age, gender, predisposing factor or associated comorbid conditions. There were 621 (61%) males and 398 (39%) females with age ranging from 3 years to 96 years having the mean age of 37.3 years. All the patients were admitted and treated in the same hospital. Investigations done in every case were in form of routine Haemogram, S. HIV, R.B.S., S.G.P.T. and S. Creatinine. X-ray chest was done in all cases and only 1.5% patients were found to have an associated pulmonary Koch's lesion along with tuberculous cervical lymphadenitis. FNABC was done in all patients of solitary and multiple matted lymph nodes above 2 cm in size without caseation or cold abscess formation (Fig. 1), excision biopsy of the lymph node was done only in case where FNABC was not conclusive, where there was caseation and cold abscess formation they were subjected to drainage of the abscess and the necrotic lymph nodes or granulation from the floor of the abscess was subjected to histopathological examination. GeneXpert study and resistance to rifampicin was seen only in cases which were not responding to treatment. This was because of the prohibitive cost of the geneXpert (CBNAAT) study. However, since the Government of India has offered free of cost geneXpert study in the district hospitals we are subjecting every specimen of suspected tuberculous cervical lymphadenopathy for the same. It helps to diagnose tuberculosis within about three hours and even give information about rifampicin resistance. Those with rifampicin resistance are subjected to line probe assay, culture & drug susceptibility testing and treatment is initiated according to the report obtained. Of the patients subjected to geneXpert study, rifampicin resistance was found in five cases and those patients were referred to higher center for treatment of drug resistance tuberculosis. Once confirmed as TB on F.N.A.B.C. or lymph node biopsy (Fig. 2) the patient was subjected to two month of intensive phase in form of rifampicin, INH, pyrazinamide and ethambutol followed by four months of continuation phase in form of rifampicin and INH (in cases diagnosed before 2017) & rifampicin, INH and ethambutol (in cases diagnosed from 2017 onwards). The dose of the drugs was given as per kg body weight and was fixed as per the Table 1. Pyridoxine (Vitamin B6) was given in the intensive as well as the continuation phase in the dose of 0.4 mg/kg body weight once daily to prevent the side effect of INH in form of peripheral neuropathy.

3. Results

The factor or habit most common among all the patients diagnosed with cervical lymphadenopathy due to tuberculosis was consumption of unpasteurized milk as 967 patients (94.93%) affected gave history of drinking unpasteurized milk which is a very common practice in the rural set up of India (Chart 1). Moreover nearly 64% of these patients gave a history of not even boiling the milk before drinking. Only 51 patients (5.03%) gave a history of drinking pasteurized milk when they presented with the disease but even among them 66% had a history of consuming unpasteurized milk for some years in the past. Diabetes was the most common co-morbid condition found in 61 (5.99%) patients along with HIV in 20 (2%) patients. Swelling in the neck was the most common presenting symptom found in all 1019 (100%) patients as shown in graph 1; of these, loss of weight was found in 152 (14.97%) patients followed by low grade fever in 122 (12%) patients, abscess formation in 112 (11.05%) patients and sinus formation in nearly 2% patients.

Regarding the sensitivity of FNABC, histopathology and bacterial study of all the cervical lymphadenopathy subsequently diagnosed as TB, FNABC was positive in 82% of

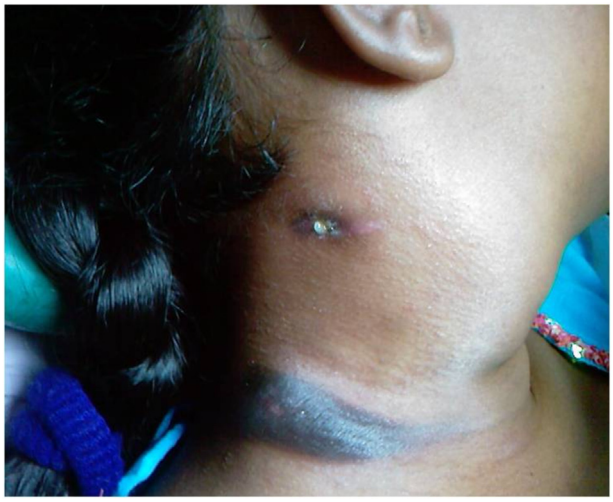


Fig. 1 – shows cold abscess formation at the cervical region.

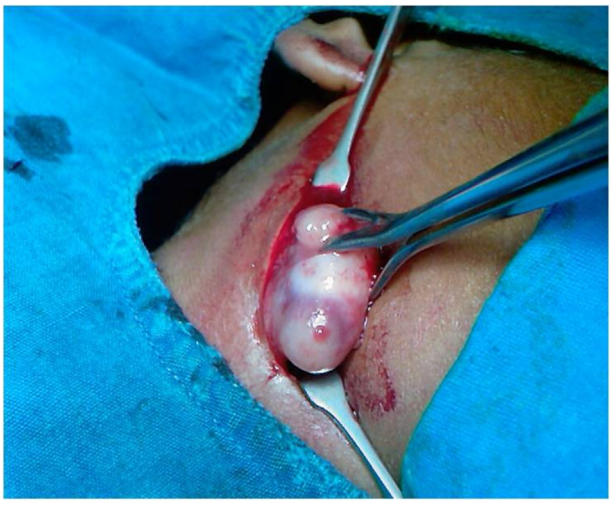


Fig. 2 – shows lymph node biopsy performed under general anaesthesia.

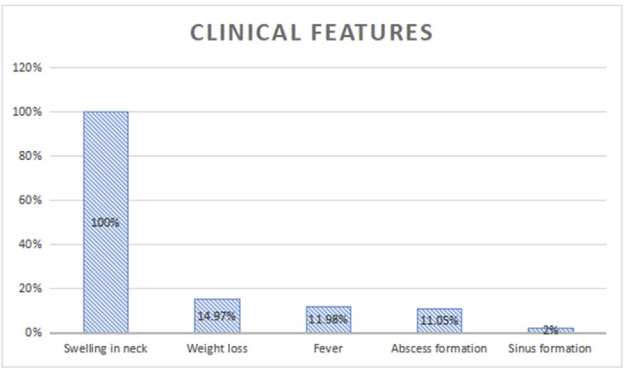


Fig. 3 – shows various clinical features and the percentage of patients having the clinical features.

patients while HPE and CBNAAT were positive in 100% patients in whom the tests were performed. Table 2 gives the account of the diagnostic method used and result of diagnosis. In some cases, even when FNABC was suggestive of tuberculosis, excisional biopsy was done due to the large size of the node and presence of caseation necrosis. Of the 611 patients in whom FNABC was done, 42% cases showed epithelioid histiocytes, 26% cases showed giant cell granulomas and few showed caseation necrosis. Solid nodes were found in 489 cases and they contained giant cells, granulomas, areas of necrosis and epithelioid histiocytes; in these cases AFB was not done. Cold abscesses were seen in 122 cases wherein AFB was done and was positive and extensive areas of necrosis and caseation. The commonest site of the cervical lymphadenopathy/cold abscess was in the posterior triangle of the neck in 743 (73.04%) patients of which 668 (90%) patients presented with a swelling in the supraclavicular triangle. Various clinical features associated with tuberculous lymphadenitis are presented in Fig. 3. Of the 276 patients who presented with cervical lymphadenopathy in the anterior triangle, the commonest group of lymph nodes enlarged were the level II or the jugulo-digastric nodes in 231 (83.76%) patients, followed by the level I B or submandibular group of lymph nodes in 33 (11.96%) patients and 12 (4.28%) patients presented with level VI cervical nodes (prelaryngeal/pretracheal) involvement which were mostly caseated. 173 (17.05%) patients presented with solitary lymph nodes of more than two cm in size, 529 (51.95%) patients presented with multiple discrete nodes of more than two cm in size, 183 (17.97%) patients presented with matted lymph nodes, 112 (11.05%) patients presented with caseation of lymph nodes with cold abscess formation and 22 (2.15%) patients presented with sinus formation with pus discharge (Fig. 4). Average size of nodes which diagnosed as tuberculosis on presentation were 3*2 cm. The largest nodal mass was 8*6 cm and nodes as small as 1.5*1 cm too were diagnosed as TB on biopsy or investigations to diagnose the bacteria like CBNAAT. The patients were divided into four groups. A) Those who presented with single lymph node enlargement (43%). B) Those who had multiple lymph node enlargement in a single group unilaterally (32%). C) Those who had multiple group of lymph node enlargement unilaterally (20%). D)

Table 1 – Table showing doses of drugs as per recommendation of the national body for Tuberculosis control.

Weight Category	Number of tablets		Inj Streptomycin
	Intensive phase HRZE	Continuation phase HRE	
Kg	75/150/400/275	75/150/275	Gm
25–39	2	2	0.5
40–54	3	3	0.75
55–69	4	4	1
>=70	5	5	1

Table 2 – Table shows the account of the diagnostic method used and result of diagnosis.

Diagnostic methods for TB used	Number of patients tested by methods	Number of patients detected positive	Percentage of positivity rate
FNABC	611	501	82%
Lymph Node Biopsy	800	800	100%
GeneXpert	300	300	100%

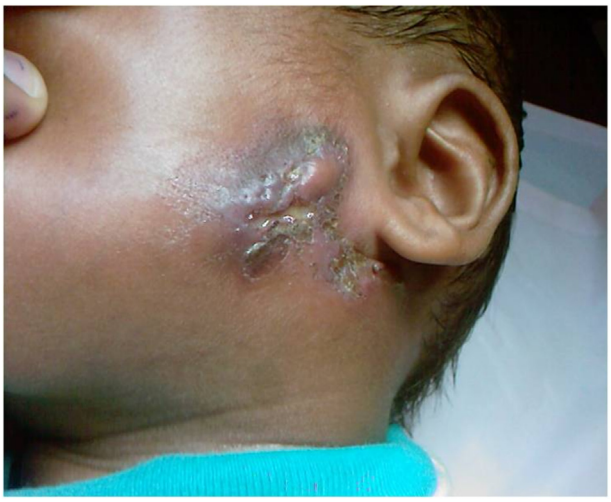


Fig. 4 – shows patient presenting with sinus formation and pus discharge.

Those having bilateral enlargement of lymph nodes which is 5%. 35 patients defaulted on the treatment and the treatment was initiated again and they responded well to treatment. For patients who had discontinued the treatment for less than a month continuously, treatment was given for that many additional number of days and for those who had discontinued the treatment for more than a month at a stretch then the treatment was initiated as if it was a fresh case and the treatment period before the time of default was not considered. Five patients who did not respond to the treatment were found to have rifampicin resistance. Paradoxical reaction in form of appearance of new nodes, increase in size of existing nodes and formation of new cold abscesses occurred in 50 (4.95%) patients and of these cases, we had to operate the ones having development of new cold abscesses and they accounted for 1% patients. Paradoxical reactions do not mean recurrence of disease. They subsequently responded well to the standard anti-tuberculous treatment mentioned above. We observed several drug related complications as pyrazinamide, INH and rifampicin altered the liver enzymes and caused hepatitis. Only those patients who presented with persistent vomiting, anorexia or icterus were subjected to liver functions tests. Liver enzymes were found to be raised in 10 patients but it was raised more than five times in five patients and those patients were referred to a higher center for treatment. Hyperuricemia which is a side effect of Pyrazinamide was found in 3 patients. Complete resolution of lymph nodes was seen in 70.6% patients, Average time taken for complete resolution was 63 days. Enlargement of new nodes or necrosis of the existing nodes probably due to resistance or paradoxical reaction was seen in 9.6% patients. Lymph nodes which did not regress but on repeat biopsy, were found to be negative on histopathology or bacteriological study (CBNAAT) constituted 17.5% patients. Lymph nodes which did not regress that were positive on repeat histopathology and bacteriological examination constituted 2.3% patients and they were referred to higher government TB treatment center for culture and to find out resistance to the commonly used first line anti-tuberculous medicines.

4. Discussion

In this article we describe our extensive experience over 20 years in managing patients affected with cervical lymphadenopathy due to tuberculosis. The common clinical presentations which make us suspect that the patients are suffering from tuberculous lymphadenitis, the investigations which we use to confirm our diagnosis, our experience in treating these patients and the complications we encountered while doing the same are explained in detail.

Tuberculosis has been affecting mankind since time immemorial. John Bunyan in 18th century referred to tuberculosis as the 'captain of all these men of death'. Though in the western and developed world countries this disease has become a rarity, it is still a big public health program in developing countries like ours. It has been documented that 1.5% of the population of India is suffering from tuberculosis.[5] What we have discussed here is the commonest form of extrapulmonary tuberculosis which presents in form of cervical lymphadenopathy and passes through different phases from a solitary lymph node, to getting matted due to periadenitis and forming a large lymph node mass which ultimately undergoes caseous necrosis with abscess and fistula formation. This was famously called as 'Scrofula' or King's evil in the early part of the last century when it was rampant in the western world.

TB lymphadenitis may occur due to:

- Reactivation of healed focus involved during primary infection
- Progressive primary tuberculosis i.e. spread from lung into mediastinal lymph node
- Spread from tonsil and
- Hematogenous spread due to military TB.

The disease progresses as an early exudative lesion to a caseous lesion and ultimately to late fibro-calcific lesion.[6] Cervical lymphadenopathy may be due to other causes like reactive lymphadenitis due to some non-specific infection in the Head and neck area, infection by other mycobacteria, cat-scratch disease, sarcoidosis, fungal disease, toxoplasmosis, blood dyscariasis and primary or metastatic neoplasms. Excisional biopsy has been supported classically as the definitive diagnostic procedure for diagnosis of nodal TB.[7] The diagnosis of TB is supported by identification of caseating granulomatous inflammation with Langhans and foreign body giant cells.[8] Even though it has been thought of as one of the causes for generalized lymphadenopathy, the involvement of inguinal or axillary group of lymph node was not common in this study which was similar to the finding by few other author.[9–11] 43%–63% of all cases of cervical lymphadenitis are diagnosed as tuberculous cervical lymphadenopathy.[2,9] Tuberculous lymphadenitis is considered a local manifestation of the systemic disease, whereas lymphadenitis due to non tuberculous *Mycobacteria* is truly a localized disease.[12] In our study of all the cases of cervical

lymphadenopathy that we encountered, more than half (i.e. 54%) were due to Tuberculosis.

The commonest age group of affection was in the 2nd and 3rd decade of life. In our study we had a male preponderance with approximately only 1/3rd of the patients being females unlike what was found in studies conducted by a few other authors.[2,11,13–15] The correlation of neck node enlargement in association with tuberculosis has been established by many authors[13,16–18] and can be due to infection of adenoids, Waldeyer's ring and tonsils.

In this study, the diagnosis of extra pulmonary tuberculosis was done mainly by the presence of granuloma with extensive caseous necrosis. Granulomatous conditions of various diverse aetiologies apparently have related histologic features, although the aetiologic agent is not always identifiable. Although various infectious granulomas showing histopathologic patterns be sufficiently different to prevent an accurate diagnosis, atypical presentations may necessitate identification of the specific aetiologic agent by direct microscopic examination, culture, serology, or molecular detection.[19] There are a number of reasons for diagnosis of EPTB to be challenging which are as follows: the paucibacillary nature of the specimens, the lack of adequate sample amounts, or volumes, the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, microbiology, and PCR), resulting in a non-uniform distribution of microorganisms.[20] Most of the available techniques are low either in sensitivity or specificity as compared to culture and thus the definitive diagnosis of tuberculous lymphadenitis is often difficult.[21] F.N.A.B.C. & excisional biopsy for histopathology examination and CBNAAT were the most commonly used investigation to prove Tuberculosis and start anti-tuberculous treatment. They were very reliable in F.N.A.B.C. had a positivity rate of 82% and excision biopsy specimen sent for histopathology had a positivity rate of 100%. Presently we follow the gold standard of diagnosing tuberculosis in the cervical lymph nodes through microbiologic detection by Cartridge Based Nucleic Acid Amplification Test (CBNAAT) or GeneXpert and by histopathological examination.

Matting and fixity of lymph nodes to surrounding structures were present in 17.97% of cases while other observers have observed in 66–79% of cases.[14,16,22] Matting is due to periadenitis and is an important diagnostic criterion. In late and untreated patients abscess formation and discharging sinuses were also observed with weight loss, fever and night sweats. Patients having HIV in our study were very only 1.95% unlike other studies where the rate is higher.[23] Because of the challenges of diagnosing and obtaining positive cultures for extra-pulmonary TB, treating patients for this disease has been mainly empirical rather than based on drug susceptibility patterns of infecting strains. Such empirical treatment for patients with extra-pulmonary TB no doubt delays effective treatment and may too often lead to a poor prognosis. GeneXpert, an automatic molecular assay, has been endorsed by the WHO based on a systematic review demonstrating its excellent performance for detecting *M. tuberculosis* and rifampicin resistance in various types of specimens.[24]

Patients were treated with drugs and doses as per the recommendations of the national body for Tuberculosis control (Table 1). Complete resolution of lymph nodes was seen in 70.6% patients, average time taken for complete resolution was about 9 weeks. Paradoxical reactions in form of appearance of new nodes (and not recurrence) or increase in size and necrosis of the existing nodes occurred in nearly 1 in 10 patients in our study. Similar type of paradoxical reactions were found in 13.4% patients in a study of 501 patients at Rabla hospital, Tunisia.[25] Various studies have shown the occurrence of paradoxical reactions in nearly 5–35% patients. The reason for paradoxical reaction in a cervical lymph node affected by tuberculosis is not very clear but is probably due to an altered response of the body's immunological system. The first theory of paradoxical reaction is that it is probably due to delay in the immune activation and the alternative hypothesis is that it is a hypersensitivity reaction to the antigen released from dying mycobacteria. 2.3% of patients were diagnosed as having tuberculous lymphadenopathy resistant to the routine recommended treatment. They did not regress in spite of 2 months of intensive phase and were found to contain active bacteria on C.B.N.A.A.T. Second highest total number of estimated MDR TB cases were in India 2008 while after China (100,000 cases) according to WHO in 2010). Drug resistance surveys in several states have indicated that the prevalence of MDR TB in India is 2–3% among new cases and 12–17% among reinfection cases.[26]

5. Conclusion

In conclusion, of all the cases for cervical lymphadenopathy, about 65% of cases were of tuberculous cervical lymphadenopathy. Neck swelling, loss of weight, low grade fever, abscess formation and sinus formation were the most common clinical features seen. The most frequent site of cervical lymphadenopathy for extra-pulmonary TB was the posterior triangle of neck followed by the anterior triangle. Males, young persons (<30 years of age), and persons living in rural regions are at high risk for developing extra-pulmonary TB especially those consuming unpasteurized milk. Patients with diabetes and HIV have risk for extra-pulmonary TB. It also was observed by us that most extra-pulmonary TB are diagnosed from clinical symptoms, which suggests a high likelihood of misdiagnosis and diagnostic delays of extra-pulmonary TB cases. The role of drug susceptibility testing is important in successful development of effective treatment regimens for patients with extra-pulmonary TB due to increased trend of drug resistant TB in India. All patients of cervical lymphadenopathy should not be put on anti tuberculous treatment without microbiological or pathological diagnosis which is unfortunately still the case in our setup.

Financial support and sponsorship

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests

All authors have none to declare.

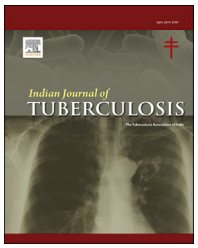
REFERENCES

1. Kumar V, Abbas AK, Fausto N, Mitchell RN. *Robbins Basic Pathology*. 8th ed. Saunders Elsevier; 2007:516–522.
2. Jha BC, Dass A, Nagarkar NM, Gupta R, Singhal S. Cervical tuberculous lymphadenopathy: changing clinical pattern and concepts in management. *Postgrad Med*. 2001;77:185–187.
3. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis*. 2010;10:803–812.
4. Solovic I, Jonsson J, Korzeniewska-Kosełа M, et al. Challenges in diagnosing extra-pulmonary tuberculosis in the European Union, 2011. *Euro Surveill*. 2013;18:20432.
5. Mukherjee AK. Tuberculosis control programme in India: progress and prospects. *Indian J Tubercul*. 1995;42:75.
6. Sarwar A, Haque AU, Aftab S, et al. Spectrum of morphological changes in tuberculous lymphadenitis. *Int J Pathol*. 2004;2(2):85–89.
7. Geldmacher H, Taube C, Kroeger C, Magnussen H, Kirsten D. Assessment of lymph node tuberculosis in northern Germany. *Chest*. 2002;121:1177–1182.
8. Handa U, Mundi I, Mohan S. Nodal tuberculosis revisited: a review. *J Infect Dev Ctries*. 2012;6(1):6–12.
9. Dandapat MC, Mishra BM, Dash SP, et al. Peripheral lymph node tuberculosis: a review of 80 cases. *Br J Surg*. 1990;77:911–912.
10. Kent DC. Tuberculous lymphadenitis: not a localised disease process. *Am J Med Sci*. 1967;254:866–873.
11. Challapalli M, Varnado SC, Cunningham DG. Tuberculous inguinal lymphadenitis. *Pediatr Infect Dis J*. 1995;14:723–724.
12. Gandhare A, Mahashur A. Tuberculosis of the lymph nodes: many facets, many hues. *Astrocyte*. 2017;4:80–86.
13. Krishnaswamy H, Koshi G, Kulkarni KG, Job CK. Tuberculous lymphadenitis in South India - a histopathological and bacteriological study. *Tubercle*. 1972;53:215–220.
14. Dandapat MC, Padhi NC, Nanda BP. Peripheral lymph node tuberculosis - a comparison of various methods of management. *Indian J Tubercul*. 1986;33:20–23.
15. Patel RV, Mehta RT. Short term chemotherapy in tuberculous lymphadenitis. *Indian J Surg*. 1987;49:33641.
16. Pamra SP, Mathur GP. A cooperative study of tubercular cervical adenitis. *Indian J Mad Res*. 1974;62:1641.
17. Fraser HS. Peripheral tuberculous lymphadenitis. *Br J Dis Chest*. 1965;59:164.
18. Hooper AA. Tuberculous peripheral lymphadenitis. *Br J Surg*. 1972;59:353–359.
19. Zumla A, James DG. Granulomatous infections: etiology and classification. *Clin Infect Dis*. 1996;23(1):146–158.
20. Gautam H, Agrawal SK, Verma SK, Singh UB. Cervical tuberculous lymphadenitis: clinical profile and diagnostic modalities. *Int J Mycobacteriol*. 2018;7(3):212–216.
21. Zewdie O, Abebe T, Mihret A, et al. Concentration of fine needle aspirates similar to molecular method improves sensitivity of the diagnosis of tuberculous lymphadenitis in Addis Ababa, Ethiopia. *BMC Infect Dis*. 2017;17:77.
22. Murty Madhusuden TV. Tuberculous lymphadenitis in children. *Indian Pediatr*. 1976;XIII:533–538.
23. Peto HM, Pratt RH, Harrington TA, Lobue PA, Armstrong LR. Epidemiology of extra-pulmonary tuberculosis in the United States, 1993–2006. *Clin Infect Dis*. 2009;49:1350–1357.
24. World Health Organization. *Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System Policy Statement*. Geneva: The Organization; 2011. WHO/HTM/TB/2011.4.
25. Chahed H, Hachicha H, Berriche A, et al. Paradoxical reaction associated with cervical lymph node tuberculosis: predictive factors and therapeutic management. *Int J Infect Dis*. 2017;54:4–7.
26. Institute of Medicine (US). *Facing the Reality of Drug-Resistant Tuberculosis in India: Challenges and Potential Solutions: Summary of a Joint Workshop by the Institute of Medicine, the Indian National Science Academy, and the Indian Council of Medical Research*. Washington (DC): National Academies Press (US); 2012. https://doi.org/10.17226/13243.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

TB related stigma and gender disparity among unaffected population in central Kerala, a survey

K.S. Kumari Indira[*,1], *Nisha Mathew*[1]

Department of Pulmonary Medicine, Sree Narayana Institute of Medical Sciences, Kochi, Kerala, 683594, India

ARTICLE INFO

Article history:
Received 14 November 2021
Accepted 30 March 2022
Available online 6 April 2022

Keywords:
Enacted
Internalized
Knowledge
Perceived
Social

ABSTRACT

Background: TB continues to ravage high burden countries despite aggressive TB control measures. Poverty and adverse socioeconomic and cultural factors play a significant role in stigmatization, causing delayed health care seeking, non-compliance to treatment and spread of disease in the community. Women are more vulnerable to stigmatization, posing the risk of gender inequality in health care. The objectives of this study were to ascertain the degree of stigmatization and gender disparity in TB related stigma in the community.
Methods: Study was conducted among TB unaffected persons, using consecutive sampling from bystanders of patients attending the hospital for diseases other than TB. Closed structured questionnaire was used for measuring socio-demographic, knowledge and stigma variables. Stigma scoring was done using TB vignette.
Results: Majority subjects (119 males and 102 females) were from rural area and low socioeconomic status; more than 60% of males and females having college education. Half the subjects answered more than half the TB knowledge questions correctly. Knowledge score was significantly lower among females compared with males ($p < 0.002$) despite high literacy. Overall stigma scoring was low (mean score = 15.9; total 75). Stigma was higher among females compared with males ($p < 0.002$); more profound among females receiving female vignettes (Chi-square = 14.1, $p < 0.0001$). The association was significant even after adjusting for co-variables (OR = 3.323, P = 0.005). Low knowledge showed minimal (statistically insignificant) association with stigma.
Conclusions: Perceived stigma though low, was more among females and much higher with female vignette, indicating significant gender disparity in stigma towards TB.

1. Introduction

Effective chemotherapy and robust TB control programs have proved its results in high economy nations, where tuberculosis is no longer a public health problem. These countries are now heading towards TB elimination. However, in most of the low economy nations TB continues to be a disease of high morbidity and mortality. The economic, social, cultural and geographical diversities had always been the challenges for effective implementation of public health programs in these countries.

* *Corresponding author*. Mobile: 9349252112.
E-mail addresses: indiraks.dr@gmail.com (K.S. Kumari Indira), nishalovin05@gmail.com (N. Mathew).
[1] *Present address:* Consultant Pulmonologist, C. M. Hospital, Pandalam, Kerala PIN 689501.
https://doi.org/10.1016/j.ijtb.2022.03.028
0019-5707/

India had strengthened its National TB control program in 1990s with strong political back up and sustainable budget allocation. Since then, the country had achieved steadfast progress in achieving TB control bringing down prevalence and TB deaths. However, there are concerns about under reporting of TB cases and increasing drug resistant tuberculosis in the country indicating major gaps in achieving goals of TB elimination.[1]

The major barriers of TB control have been identified as the diverse socioeconomic and cultural characteristics of the high burden countries. Despite the absolutely free TB services, there is significant delay in health care seeking and non-compliance to treatment facilitating disease transmission in the community. The negative impact on TB services is attributed to poverty and stigma related with TB.[2–10] Reports also suggest that TB stigma is variable between the gender, females being most affected than males.[11,12] Community awareness programs has shown its effectiveness in reducing stigma and facilitating early health care seeking and compliance to treatment.[13,14]

This study intends to determine the prevalence of perceived stigma towards TB among subjects unaffected with TB and to detect if the stigma is gender specific. The study is expected to yield data that would be useful for designing strategies for strengthening the TB control programs by incorporation of suitable strategies for minimizing stigma in the community.

2. Methodology

This was a cross-sectional survey conducted among bystanders (respondents) of patients attending the out-patient department of a tertiary care center in Ernakulam district of Kerala. The primary objective of the study was to determine the prevalence of perceived TB related stigma among subjects who were not affected with the disease and to detect if there is gender disparity in perceived stigma.

Stigma scoring was done using a questionnaire based on male and female vignettes, describing a scenario where patients are diagnosed with TB. Each respondent was given one vignette. Recruitment was made in such a way to include equal numbers of males and females and one half of either gender received male vignette and the other half received female vignette. Thus, there were four groups of respondents: male receiving male vignette (group 1), male receiving female vignette (group 2), female receiving male vignette (group 3) and female receiving female vignette (group 4).

Sample size was calculated for detecting minimum 10% higher prevalence in stigma towards female vignette compared with male vignette. Statistical assumptions for calculating the sample size were based on a pilot study in which 30% of study subjects were detected to have stigma towards tuberculosis. To detect 10% difference in prevalence of stigma between groups (with α-error less than 5% and β more than 80%), sample size was estimated as 48 for each group (total 196 for four groups). Consecutive sampling was done till sample size of 48 was met for each group. Respondents were identified from out-patient departments of medical and allied specialties of the hospital. Subjects who had tuberculosis or whose relatives had tuberculosis anytime in the past were excluded. Respondents aged more than 18 years were included. From each family only one respondent was recruited. Respondents of patients having severe illness, non-natives of Kerala who had been residing in Kerala State for less than one year, those having poor cognitive powers or who cannot communicate effectively, who cannot communicate in Malayalam or English and those not willing to give written informed consent were excluded.

Structured self-administered questionnaire was given to eligible subjects after obtaining signed informed consent. The questionnaire was prepared in English, translated to Malayalam (local language) and back translated to English for maintaining the equivalence of the tool. All questions were having objective answers only. Standardization and pilot testing of the questionnaire were done in the same setting. Subjects enrolled for pilot testing were excluded from study. Subjects who were unable to comprehend the questionnaire themselves were assisted by the research assistant to fill up the data.

The questionnaire had three sessions, one about socio-demographic details, second about knowledge and awareness about tuberculosis and third about their perceived stigma towards tuberculosis. Socioeconomic status of the subjects was assessed using revised Kuppuswamy's socioeconomic status scale 2012.[15,16]

In second session for knowledge assessment, there were questions about the etiology of the disease, how the disease spreads, symptoms and about the availability and access to services for diagnosing and treating the disease (total score of 36).

The session on stigma included questions enquiring if patients diagnosed with tuberculosis will be secluded and ill-treated by their spouse, family members, friends, co-workers and employers. It also included questions seeking their perception and beliefs on marital disruption and difficulty of the affected person or their siblings for getting marriage alliances. The stigma tool was adopted from Explanatory Model Interview catalogue (EMIC) instrument. EMIC is a tool for assessing the perspectives of patients or public regarding diseases that have significant socio-cultural association. This tool is widely used for assessing stigma associated with chronic disease like tuberculosis and HIV.[5]

Male and female vignettes were used for getting data on prevalence and gender disparity in stigmatization. Items for scoring stigma were similar for male and female vignettes, total score being 75. Ethical clearance was obtained from the Institutional Ethics committee before the start of the study.

3. Results

221 subjects were recruited, 119 males (53.8%) and 102 females (46.2%). Mean age of the study subjects was 39.4 (SD 12.7). 57 males (group 1) and 62 females (group 3) received male vignettes. 49 males (group 2) and 53 females (group 4) received female vignettes.

There was no significant difference in the sociodemographic features between the four groups (Table 1). Males and

females had same level of literacy, 61.3% (n = 73) and 64.7% (n = 66) respectively having college education.

Total score for knowledge (and awareness) on TB was 36 and mean score for all respondents was 19.92 (SD 4.9). 50% of the subjects answered more than half the questions correctly (median = 18). Respondents were categorized into high vs low knowledge groups using cut off at median score for knowledge (score 18). It was observed that knowledge score was higher among males (significant $p < 0.002$) and respondents from higher SES strata, statistically insignificant, $p < 0.055$ (Table 2). This was ascertained in Mann–Whitney U analysis where male gender and high socioeconomic status showed significant positive association with high knowledge score ($p < 0.002$ and .032 respectively). Age and residency location (urban vs. rural) did not show significant difference in knowledge scoring (Table 2).

Stigma scoring was done using male and female vignettes, total score being 75. Overall stigma score among the respondents ranged from 0 to 52, with mean, median and mode of 15.9, 14.0 and 3.0 respectively. Subjects were categorized into two groups for analytical purpose as high stigma versus low stigma group with cut off at score 19.

Prevalence of high perceived stigma (score ≥19) was observed among 31.7% (n = 70) of study subjects, 22.7% (n = 27) and 42.2% (n = 43) among males and females respectively. Univariate analysis was done to determine the association of sociodemographic variables, knowledge status and type of vignette with stigma. It was observed that age and residency location (urban vs rural) showed no relationship with stigma. High stigma was observed among respondents with low knowledge score (OR = 1.71) but was not statistically significant. Among all variables, female gender showed highest association with stigma (statistically significant OR = 2.48, $p < 0.002$; Table 3). Study subjects who received female vignette reported perceived stigma more frequently than male vignette, but the difference was not statistically significant (OR = 1.35, $p = 0.32$).

In univariate analysis of four categories of respondents (group 1 to 4) with stigma, significant difference in stigma reporting was observed between groups (Chi-square = 16.1, P = 0.001). The difference was more profound in stigma reporting by female respondents towards female compared with male vignettes, 52.8% (n = 28) and 19.4% (n = 12) respectively. In the case of male respondents, the response was similar across both vignettes (Table 4 & Fig. 1). In stratified analysis (Table 4), it was observed that variability in stigma response towards male and female vignettes was highly significant for female respondents (Chi-square = 14.1, $p < 0.0001$), whereas for male respondents the response was not different (Chi-square = .24, $p = 0.62$).

Multivariate logistic regression analysis using enter method was done to identify the relative strength of association of variables with stigma. The variables included were age, socioeconomic status, urban/rural residence, knowledge status and the gender and vignette group (group 1–4). Group 1 (male receiving male vignette) was the reference category for 'group'. The only variable that retained significant association with high stigma was group 4 (i.e., female subjects responding to female vignette; OR = 3.323, P = 0.005). Socioeconomic status and knowledge were observed to have minimal relationship with stigma and was not statistically significant (Table 5).

4. Discussion

Stigma is a state where a person is devalued due to the presence of a particular social or medical condition. This leads to altered behavior of the affected person or the community leading to exclusion, rejection, blame, or discrimination.[17]

Stigma is a major barrier for TB control as it is associated with delayed health seeking and non-compliance to treatment favouring disease transmission in the community.[4,6]

This study was done to determine the gender disparity and extent of stigmatisation among unaffected individuals. It was conducted among bystanders of patients attending outpatient department of a tertiary care centre for diseases other than TB. The questionnaire included three parts, one for data on socio-demographic characteristics, second for assessing knowledge and awareness on TB and third for assessing perceived stigma towards TB. We excluded subjects who themselves or their immediate relatives had TB any time. Participants were mostly middle-aged subjects (mean age 39.4) hailing from rural area (79.2%) and belonging

Table 1 – Baseline characteristics of study population.

Variables	Total 221	Male		Female	
		Group 1 (n = 57)	Group 2 (n = 49)	Group 3 (n = 62)	Group 4 (n = 53)
Age-mean (SD)	39.4 (12.7)	41.0 (14.7)	38.5 (10.8)	39.2 (13.2)	38.7 (12.7)
Urban: n, %	46 (20.8)	10 (17.5%)	10 (20.4%)	16 (25.8%)	10 (18.9%)
Rural: n, %	175 (79.2)	47 (82.5%)	39 (79.6%)	46 (74.2%)	43 (81.1%)
Upper SES[a]	7 (3.2)	4 (7.0%)	1 (2.1%)	1 (1.6%)	1 (1.9%)
Upper middle SES[a]	33 (15.0)	10 (17.5%)	2 (4.2%)	13 (19.4%)	8 (15.4%)
Lower middle SES[a]	73 (33.0)	23 (40.4%)	18 (37.5%)	20 (32.3%)	12 (23.1%)
Upper lower SES[a]	105 (47.5)	20 (35.1%)	25 (52.1%)	29 (46.8%)	31 (57.7%)
Lower SES[a]	3 (1.4)	0 (0%)	2 (4.2%)	0	1 (1.9%)

Group 1 = Male receiving Male vignette, Group 2 = Male receiving Female vignette.
Group 3 = Female receiving Male vignette, Group 4 = Female receiving Female vignette.
[a] Socioeconomic status.

Table 2 – Relationship of knowledge score with sociodemographic variables.

Variables	Knowledge Mean, Median, SD	Knowledge score ≥18 n (%)	Knowledge score <18 n (%)	Chi-square P value
Age <40	20.2, 19, 5.3	62 (54.4%)	52 (45.6%)	.9
Age >39	19.6, 19, 4.7	59 (55.1%)	48 (44.9%)	P = 1.0
Male	20.8, 20, 4.8	77 (64.7%)	42 (35.3%)	10.3
Female	18.9, 18, 5.0	44 (43.1%)	58 (56.9%)	P = 0.002
Urban	18.5, 19, 5.1	24 (52.2%)	22 (47.8%)	.156
Rural	20.3, 19, 4.9	97 (55.4%)	78 (44.6%)	P = 0.741
SES[a] High	20.7, 20.5, 5.1	54 (62.8%)	32 (37.2%)	3.67
SES[a] Low	19.4, 18, 4.8	67 (49.6%)	68 (50.4%)	P = 0.055

[a] Socioeconomic status.

Table 3 – Relationship of Stigma with type of vignette, knowledge and socio-demographic variables.

Variables	Low stigma (score <19) n = 151	High stigma (score ≥19) n = 70	OR P value
Age <40	79 (69.3%)	35 (30.7%)	1.097 (.622, 1.934)
Age >39	72 (67.3%)	35 (32.7%)	P = 0.77
Male	92 (77.3%)	27 (22.7%)	2.48 (1.38, 4.44)
Female	59 (57.8%)	43 (42.2%)	P = 0.002
Urban	29 (63.1%)	17 (36.9%)	1.13 (.58, 2.22)
Rural	105 (60.0%)	70 (40.0%)	P = 0.73
High SES[a]	55 (64.0%)	31 (36.0%)	1.44 (.78, 2.47)
Low SES[a]	96 (71.1%)	39 (28.9%)	P = 0.27
High knowledge	89 (73.6%)	32 (26.45%)	1.71 (.96, 3.02)
Low knowledge	62 (62.0%)	38 (38.0%)	P = 0.081
Male vignette	76 (71.7%)	30 (28.3%)	1.35 (.76, 2.4)
Female vignette	75 (65.2%)	40 (34.8%)	P = 0.32

[a] Socioeconomic status.

to low socioeconomic status (80%). As expected for Kerala, the literacy level of the respondents was found to be high, more than 60%of males and females having college education. Overall, knowledge on TB was found to be good, more than half the respondents had answered more than 18 questions (out of 36) correctly. Despite the high literacy rate, knowledge score among females was found to be significantly lower than male respondents ($p < 0.002$). Subjects from high SES strata and urban setting had higher knowledge score compared with their counterparts, but not statistically significant.

Poverty, poor literacy, misconceptions and poor public health awareness are factors associated with high stigma in a community. In this study, neither the study subjects, nor their immediate relatives were having TB. Probing was done to identify their perceptions on how much discrimination a TB affected individual would have to face from the family and society. Thus, this study aimed to measure the *perceived stigma* (perception of an unaffected person) in contrast to the stigma experience of affected persons (*anticipated, internalised and enacted* stigma).

The questions included were: whether they would feel ashamed or disrespectful in the society, their perceptions on rejection by family, society and employer and about marital disharmony and difficulty in getting marriage alliances. When compared with studies within the country and other nations, it was observed that the overall stigma score for the study population was considerably low.[3,18,19]

Table 4 – Table showing univariate analysis of Stigma with Study Groups and Gender.

Group	Low stigma (score <19)	High stigma (score ≥19)		Chi-square for gender strata
Group 1[a]	42 (73.7%)	15 (26.3%)	Chi-square = 16.1	Male strata
Group 2[a]	34 (69.4%)	15 (30.6%)	P = 0.001	Chi-square = .24
				P = 0.62
Group 3[a]	50 (80.6%)	12 (19.4%)		Female strata
Group 4[a]	25 (47.2%)	28 (52.8%)		Chi-square = 14.1
				P < 0.0001

[a] Group 1 = Male receiving Male vignette, Group 2 = Male receiving Female vignette, Group 3 = Female receiving Male vignette, Group 4 = Female receiving Female vignette.

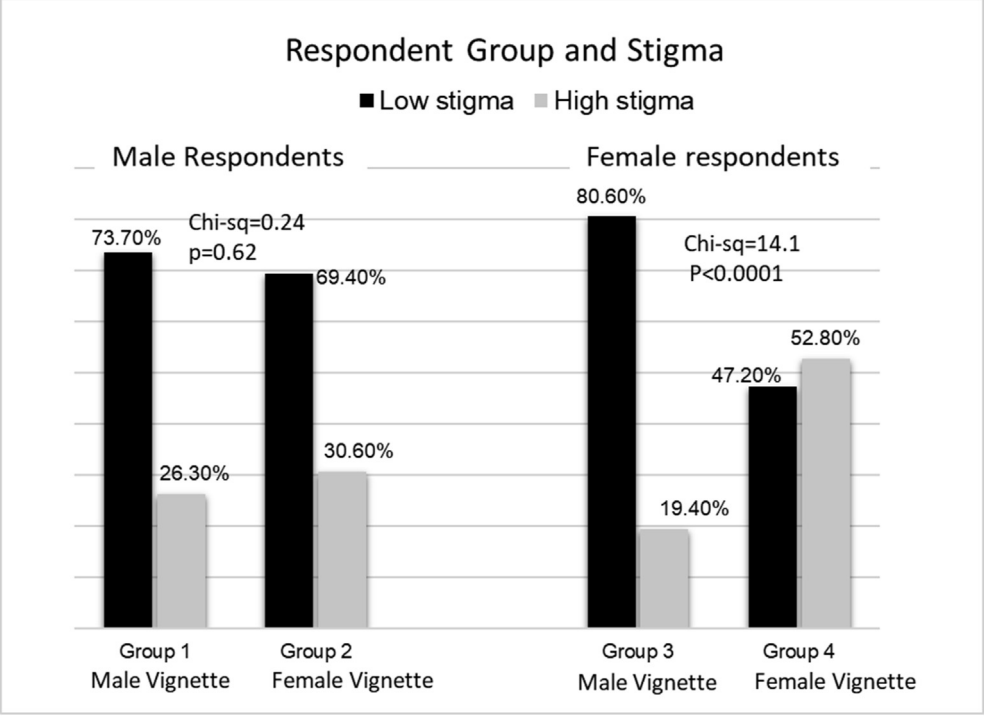


Fig. 1 – Relationship between stigma, gender and study groups. Footnote: Group 1 = Male receiving Male vignette, Group 2 = Male receiving Female vignette. Group 3 = Female receiving Male vignette, Group 4 = Female receiving Female vignette. Legend: It can be seen that stigma perception among male respondents was not different between male vs. female vignettes ($p = 0.62$), whereas female respondents reported more stigma towards female vignette compared with male vignette ($p < 0.0001$).

More than half the participants had stigma score less than 14 (out of total score 75) and less than one third subjects had high perceived stigma (score >19). It was observed that female gender (OR = 2.5, $p < 0.002$), those with low knowledge (OR = 1.7, $p = 0.08$) and low SES (OR = 1.4, $p = 0.27$) had higher odds for TB related stigma in the order of statistical significance. Overall, no significant difference was observed in reported stigma towards male versus female vignettes. However, the gender disparity in stigma reporting was evident in stratified analysis. More than 70% of male participants reported not having stigmatisation to TB and it was almost equal towards the male and female vignettes. On the other hand, more than half the females reported having perceived stigma towards female vignette, in contrast to less than 20% females reporting perceived stigma towards male vignette.

There are many socio-economic and cultural factors that contribute to stigmatisation of a disease. It is observed that TB related stigma has an indirect relationship with knowledge and awareness on TB and is associated with female gender, poor literacy, rural residency and low socio-economic status.[3,19,20]

Table 5 – Multivariate Logistic regression analysis showing Association between stigma and variables.

Variables in equation	B	S.E.	Wald	df	Sig.	Exp(B)
AGE	.007	.013	.294	1	.588	1.007
Urban/Rural	-.306	.367	.696	1	.404	.737
SES[1]	-.633	.346	3.347	1	.067	.531
KNOW[2]	.367	.314	1.365	1	.243	1.443
Group 1[a,¶]			14.777	3	.002	
Group 2[a]	.311	.449	.481	1	.488	1.365
Group 3[a]	-.413	.449	.847	1	.357	.661
Group 4[a]	1.201	.430	7.782	1	.005	3.323
Constant	-.902	.657	1.885	1	.170	.406

3: Group 1 = male receiving male vignette, group, 2 = male receiving female vignette, group 3 = – female receiving male vignette and group 4 = female receiving female vignette.

¶ Group 1 was entered as the reference category for variable 'Group'.

[a] SES- socioeconomic status, 2: KNOW- knowledge status on TB.

In this study, although participants were mainly from rural areas, high literacy rate was noted which was consistent with the high literary status of the State. On the other hand, gender equivalence in literacy rate did not reflect in the knowledge score and prevalence of perceived stigma. It was observed that significantly fewer proportion of females had sufficient knowledge and awareness about TB compared with males (43.1% vs. 64.7%, $p < 0.002$), indicating discordance between literacy status and disease related knowledge. The data indicates that that the knowledge and awareness on TB is lower among females and those from low SES, contributing to stigmatisation. These observations are consistent with previous publications, indicating the role of socio-cultural factors for stigmatisation towards diseases like TB.[3,21,22]

Differential scoring for perceived stigma towards male and female vignettes by females (and not males) was indicative of gender discrepancy in perceived stigma towards TB. Gender discrimination is not uncommon in our community and it is possible that females would have experienced more enacted and internalised stigma or discrimination in their lifetime towards other medical or social issues.

Negative perceptions of females towards TB tempt them to keep their illness a secret. In a study conducted in Peru, the authors reported that men are unconcerned about opinions of others about their illness, in contrast to women who felt fear of prejudice and rejection from family and society, causing delayed health seeking.[18]

Stigma is classified into four categories: *perceived stigma* (perception of an unaffected person about affected persons), *anticipated stigma* (expectations of an affected person on being devalued or prejudiced post disclosure), *enacted stigma* (experiences of discrimination, prejudice) and *internalized stigma* (endorsing negative feelings and beliefs associated with the stigmatized condition).[23,24]

Comprehensive data on stigma should assess all four categories of stigma: perceived stigma, anticipated stigma, enacted stigma, and internalized stigma. Hence appraising stigma would require measurements at different levels: patient level, family members, co-workers, health workers and the public.[3,23–26]

Further, stigma data is a subjective feeling and requires qualitative tools for its measurements, adding to the complexity of stigma studies. It is recommended to have a combination of qualitative and quantitative tools for stigma studies. The lone use of quantitative tools is not sufficient to expose all aspects of stigmatization.[27]

As the objective of this study was to identify stigma perceptions among people who are not affected with the disease (*perceived* stigma), the study was designed to survey subjects not affected with TB. We carried out the study among bystanders of patients attending a tertiary care hospital for illnesses other than TB, using closed structured questionnaire. Qualitative study was not possible because of resource limitations and time constraints. These are major setbacks of this study; limiting comparison with other studies conducted among TB affected individuals using qualitative methods.

It can be presumed that the extremely low perceived stigma among the participants could be due to these methodological differences. The attitude and response of people not affected with disease would be different from that of affected persons and it quite possible the subjects in this study would have answered the questions in an unconcerned manner. On the other hand, the low stigma observed can be real as Kerala has the top rank in literacy and health indicators among all the states in India.

Most of the publications are based on studies for ascertaining *enacted* or *internalized* stigma among TB patients and few studies included perceptions of the public and care givers. Studies from TB high burden countries indicate existence of high degree of enacted and internalized stigma in the community, adversely affecting effective implementation of TB control programs.[28–31]

TB related stigma is reported to be very high in India.[32]

In a nation-wide community survey conducted in 30 cities in India, it was estimated that 73% of the population had perceived stigma towards TB. Significant knowledge gap was observed in this study which showed high degree of variability between study sites.[33] It was also observed that TB related stigma is closely related with poverty and poor socio-economic status.[3,34,35] An international study reported that among the four countries studied, stigma index was highest in India, discouraging people from health care seeking and treatment completion.[7] Yet another study reported that people who believe that TB is curable are less vulnerable to stigma and have better adherence to treatment.[36]

In our study it was observed that the negative feelings about TB were more commonly reported by female respondents, despite that the literacy level of women was found to be equal to men. However, it was observed that women had significantly lower level of TB knowledge and awareness, probably indicating that women were not exposed to health education programs. The stereotypical roles assigned to either gender in the community could have resulted in fewer opportunities for women to get educated on health issues, emphasizing the role of health education programs even among literate groups. It is also possible that the experience of enacted stigma due to any social issues would be more among women than men, prompting them to disclose their negative feelings. Previous studies have reported such gender disparity in stigma, associated with delayed health seeking and non-compliance to treatment by women compared with men.[12,18,23,34,37]

It has been identified that socioeconomic inequalities prevailing in a community cause delay in diagnosis and non-adherence to treatment, adversely affecting the fate of current TB control programs. There is a growing consensus that TB control programs should include socio-economic and community educational interventions to overcome these social barriers.[38–40]

Evidence on impact of social interventions on outcome of TB control programs are yet to be available as there are not many studies on this perspective. Recent studies in few high burden countries indicate that the treatment compliance, cure rate and contact reporting for screening have considerably improved with schemes providing educational modules and social and financial support to the patients and families.[41–43] Government of India is taking rigorous measures for eliminating TB from the country by 2025. Despite the huge investment for TB control in India over the past few decades, significant lag is observed in achieving the goals of End TB strategy. Experts are of the opinion that stigma and its social

determinants (poverty and lack of knowledge) are the major barriers of TB control in high burden countries. It is highlighted that unless the nations take prudent steps for social interventions for alleviating poverty and stigma, investments for TB control would go futile.[44,45] Gender differences in TB related stigma existing in high burden countries warrants the need for gender specific research and interventions.[37]

Most nations have not yet seriously considered social intervention as a part of National TB control programs. Being a complex issue with wide regional diversity it is extremely challenging to execute social interventions appropriate to each location and nations would have to boost the budgeting towards TB control. The advocacy is to develop a comprehensive patient centered model for addressing poverty and social determinants of TB with the partnership of different disciplines. We need to have clear understanding of the extent and nature of socio-economic barriers pertinent for each community to formulate such initiatives. It is envisaged that research oriented social interventions incorporated with current TB control programs would be more cost effective and yield immediate results in containing the disease.

5. Summary

Perceived stigma towards TB was observed to be very low among the population studied, possibly attributable to the high literacy and high health indices of Kerala State. However methodological drawbacks of this project limit comparison with other studies as it could also be an underestimate of the true state. Nevertheless, the high stigma reported by females particularly towards female vignette indicates significant gender disparity towards TB related stigma. Experts are of the opinion that unless social interventions addressing the socio-economic barriers are integrated with the TB control programs, the disease will continue to thrive as the major public health problem of low economy nations. More research is warranted for understanding the root cause of the social problem and its impact on TB control. TB control programs need to be strengthened by incorporating research oriented social interventions with special focus on underprivileged groups and females. Such a strategy is envisaged to be more cost-effective, yielding early results towards achieving goals of end TB strategy.

Conflicts of interest

The study was funded by Tuberculosis Association of India, New Delhi (2017-18). The authors have no conflict of interest in the conduct of study or in the manuscript preparation.

Acknowledgments

We acknowledge the support of staff of all medical departments of the Institute for providing support for the conduct of the study. The project was funded by TB Association of India. The funding agency did not have any role in the study design, analysis or manuscript writing.

REFERENCES

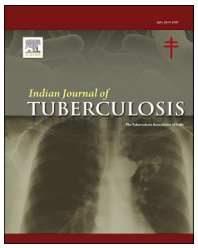
1. Padayatchi N, Daftary A, Naidu N, Naidoo K, Pai M. Tuberculosis: treatment failure, or failure to treat? Lessons from India and South Africa. *BMJ Glob Health*. 2019;4(1) [PMC free article] [PubMed] [Google Scholar].
2. Mathew AS, Takalkar AM. Living with tuberculosis: the myths and the stigma from the Indian perspective. *Clin Infect Dis*. 2007 Nov 1;45(9):1247. https://doi.org/10.1086/522312. PMID: 17918097.
3. Shah RB, Shah AM, Patel PM, Thakker RM. Stigma associated with tuberculosis – an Indian perspective. *Natl J Physiol Pharm Pharmacol*. 2020;10(7):579–585.
4. Aryal S, Badhu A, Pandey S, et al. Stigma related to Tuberculosis among patients attending DOTS clinics of Dharan Municipality. *Kathmandu Univ Med J*. 2012;37(1):48–52.
5. Atre S, Kudale A, Morankar S, Gosoniu D, Weiss MG. Gender and community views of stigma and tuberculosis in rural Maharashtra, India. *Glob Publ Health*. 2011;6(1):56–71. https://doi.org/10.1080/17441690903334240. PMID: 21509994.
6. Courtwright A, Turner AN. Tuberculosis and stigmatization: pathways and interventions. *Publ Health Rep*. 2010;125:34–42.
7. Somma D, Thomas BE, Karim F, et al. Gender and socio-cultural determinants of TB-related stigma in Bangladesh, India, Malawi and Colombia. *Int J Tubercul Lung Dis*. 2008 Jul;12(7):856–866. PMID: 18544216.
8. FatiregunA. A., & EjeckamC. C. Determinants of patient delay in seeking treatment among pulmonary tuberculosis cases in a government specialist hospital in Ibadan, Nigeria. Tanzan J Health Res, 12(2), 113-120. https://doi.org/10.4314/thrb.v12i2.56398
9. Cambanis A, Ramsay A, Yassin MA, Cuevas LE. Duration and associated factors of patient delay during tuberculosis screening in rural Cameroon. *Trop Med Int Health*. 2007 Nov;12(11):1309–1314. https://doi.org/10.1111/j.1365-3156.2007.01925.x. Epub 2007 Oct 22. PMID: 17949400.
10. Bawankule S, Quazi SZ, Gaidhane AM, Khatib N. Delay in DOTS for new pulmonary tuberculosis patient from rural area of Wardha District, India. *Online J Health Allied Sci*. 2010;9(1):5. http://www.ojhas.org/issue33/2010-1-5.htm.
11. Atre SR, Kudale AM, Morankar SN, Rangan SG, Weiss MG. Cultural concepts of tuberculosis and gender among the general population without tuberculosis in rural Maharashtra, India. *Trop Med Int Health*. 2004 Nov;9(11):1228–1238. https://doi.org/10.1111/j.1365-3156.2004.01321.x. PMID: 15548321.
12. Ganapathy S, Thomas BE, Jawahar MS, Selvi KJ, Sivasubramaniam, Weiss M. Perceptions of gender and tuberculosis in a south Indian urban community. *Indian J Tubercul*. 2008 Jan;55(1):9–14. PMID: 18361305.
13. Narayan B. *Tuberculosis in India: A Need of Public Awareness & Education University of Mauritius Research Journal*. vol. 21. 2015. Available at: https://www.ajol.info/index.php/umrj/article/view/122074.
14. Thakur G, Thakur S, Thakur H. Status and challenges for tuberculosis control in India -Stakeholders' perspective. *Indian J Tubercul*. 2021;68(3):334–339. https://doi.org/10.1016/j.ijtb.2020.10.0013.
15. Mishra D, Singh HP. Kuppuswamy's socioeconomic status scale—a revision. *Indian J Pediatr*. 2003;70:273–274.
16. Patro BK, Jeyashree K, Gupta PK. Kuppuswamy's socioeconomic status scale 2010-the need for periodic revision. *Indian J Pediatr*. 2012 Mar;79(3):395–396. https://doi.org/10.1007/s12098-011-0517-7. Epub 2011 Jul 15. PMID: 21761123.
17. Weiss MG, Ramakrishna J. Stigma interventions and research for international health. *Lancet*. 2006 Feb

11;367(9509):536–538. https://doi.org/10.1016/S0140-6736(06)68189-0. PMID: 16473134.
18. Onifade DA, Bayer AM, Montoya R, et al. Gender-related factors influencing tuberculosis control in shantytowns: a qualitative study. *BMC Publ Health*. 2010;10:381. https://doi.org/10.1186/1471-2458-10-381. . Accessed September 28, 2021.
19. Gelaw SM. Socioeconomic factors associated with knowledge on tuberculosis among adults in Ethiopia. *Tuberc Res Treat*. 2016;2016:6207457. https://doi.org/10.1155/2016/6207457. Epub 2016 Feb 1. PMID: 26949546; PMCID: PMC4753341.
20. Hargreaves JR, Boccia D, Evans CA, Adato M, Petticrew M, Porter JD. The social determinants of tuberculosis: from evidence to action. *Am J Publ Health*. 2011;101(4):654–662. https://doi.org/10.2105/AJPH.2010.199505.
21. Dodor EA, Neal K, Kelly S. An exploration of the causes of tuberculosis stigma in an urban district in Ghana. *Int J Tubercul Lung Dis*. 2008;12:1048–1054.
22. Macq J, Solis A, Martinez G, Martiny P, Dujardin B. An exploration of the social stigma of tuberculosis in five "municipios" of Nicaragua to reflect on local interventions. *Health Pol*. 2005 Oct;74(2):205–217. https://doi.org/10.1016/j.healthpol.2005.01.003. PMID: 16153480.
23. Mukerji R, Turan JM. Exploring manifestations of TB-related stigma experienced by women in Kolkata, India. *Ann Glob Health*. 2018 Nov 5;84(4):727–735. https://doi.org/10.9204/aogh.2383. PMID: 30779523; PMCID: PMC6748300.
24. Mukerji R, de Laat MM, Kapata N, Gerrets R, Klipstein-Grobusch K, Grobusch MP. Assessing the consequences of stigma for tuberculosis patients in urban Zambia. *PLoS One*. 2015 Mar 25;10(3), e0119861. https://doi.org/10.1371/journal.pone.0119861. PMID: 25806955; PMCID: PMC4373828.
25. Bresenham D, Kipp AM, Medina-Marino A. Quantification and correlates of tuberculosis stigma along the tuberculosis testing and treatment cascades in South Africa: a cross-sectional study. *Infect Dis Poverty*. 2020 Oct 22;9(1):145. https://doi.org/10.1186/s40249-020-00762-8. PMID: 33092636; PMCID: PMC7579945.
26. Datiko DG, Jerene D, Suarez P. Stigma matters in ending tuberculosis: nationwide survey of stigma in Ethiopia. *BMC Publ Health*. 2020;20:190. https://doi.org/10.1186/s12889-019-7915.
27. Mitchell Ellen MH, Susan van den Hof, eds. *TB Stigma Measurement Guide. Challenge TB*; 2018. https://www.challengetb.org/publications/tools/ua/TB_Stigma_Measurement_Guidance.pdf.
28. Gautam N, Karki RR, Khanam R. Knowledge on tuberculosis and utilization of DOTS service by tuberculosis patients in Lalitpur District, Nepal. *PLoS One*. 2021 Jan 25;16(1), e0245686. https://doi.org/10.1371/journal.pone.0245686. PMID: 33493188; PMCID: PMC7833137.
29. Yin X, Yan S, Tong Y, et al. Status of tuberculosis-related stigma and associated factors: a cross-sectional study in central China. *Trop Med Int Health*. 2018 Feb;23(2):199–205. https://doi.org/10.1111/tmi.13017. Epub 2017 Dec 29. PMID: 29178244.
30. Teo AKJ, Tan RKJ, Smyth C, et al. Characterizing and measuring tuberculosis stigma in the community: a mixed-methods study in Cambodia. *Open Forum Infect Dis*. 2020 Sep 16;7(10):ofaa422. https://doi.org/10.1093/ofid/ofaa422. PMID: 33134412; PMCID: PMC7585330.
31. Bifftu BB, Dachew BA. Perceived stigma and associated factors among people with Schizophrenia at amanuel mental specialized hospital, Addis Ababa, Ethiopia: a cross-sectional Institution based study. *Psychiatr J*. 2014;2014:694565. https://doi.org/10.1155/2014/694565. Epub 2014 May 21. PMID: 24967300; PMCID: PMC4055492.
32. Dhingra VK, Khan Shadab. A sociological study on stigma among TB patients in Delhi. *Indian J Tubercul*. 2010;57:12–18.
33. Sagili KD, Satyanarayana S, Chadha SS. Is knowledge regarding tuberculosis associated with stigmatising and discriminating attitudes of general population towards tuberculosis patients? Findings from a community based survey in 30 districts of India. *PLoS One*. 2016 Feb 1;11(2), e0147274. https://doi.org/10.1371/journal.pone.0147274. PMID: 26829713; PMCID: PMC4734597.
34. McArthur E, Bali S, Khan AA. Socio-cultural and knowledge-based barriers to tuberculosis diagnosis for women in Bhopal, India. *Indian J Commun Med*. 2016 Jan-Mar;41(1):62–64. https://doi.org/10.4103/0970-0218.170990. PMID: 26917876; PMCID: PMC4746957.
35. Das P, Basu M, Dutta S, Das D. Perception of tuberculosis among general patients of tertiary care hospitals of Bengal. *Lung India*. 2012;29(4):319–324.
36. Atre S, Kudale A, Morankar S, Gosoniu D, Weiss MG. Gender and community views of stigma and tuberculosis in rural Maharashtra, India. *Glob Publ Health*. 2011;6(1):56–71.
37. Krishnan L, Akande T, Shankar AV, et al. Gender-related barriers and delays in accessing tuberculosis diagnostic and treatment services: a systematic review of qualitative studies. *Tuberc Res Treat*. 2014;2014:215059. https://doi.org/10.1155/2014/215059. Epub 2014 May 11. PMID: 24900921; PMCID: PMC4037602.
38. Bhargava A, Benedetti A, Oxlade O, Pai M, Menzies D. Undernutrition and the incidence of tuberculosis in India: national and subnational estimates of the population-attributable fraction related to undernutrition. *Natl Med J India*. 2014;27(3):128–133. PMID: 25668081.
39. Pai M, Bhaumik S, Bhuyan SS. India's plan to eliminate tuberculosis by 2025: converting rhetoric into reality. *BMJ Glob Health*. 2017;2, e000326.
40. Oxlade O, Murray M. Tuberculosis and poverty: why are the poor at greater risk in India? *PLoS One*. 2012;7, e47533. https://doi.org/10.1371/journal.pone.0047533.
41. Klein K, Bernachea MP, Irribarren S, Gibbons L, Chirico C, Rubinstein F. Evaluation of a social protection policy on tuberculosis treatment outcomes: a prospective cohort study. *PLoS Med*. 2019;16(4), e1002788. https://doi.org/10.1371/journal.pmed.1002788.
42. Rocha C, Montoya R, Zevallos K, et al. The innovative socio-economic interventions against tuberculosis (ISIAT) project: an operational assessment. Suppl 2(Suppl 2) *Int J Tubercul Lung Dis*. 2011 Jun;15:50–57. https://doi.org/10.5588/ijtld.10.0447. PMID: 21740659; PMCID: PMC3160483.
43. Wingfield T, Tovar MA, Huff D, et al. A randomized controlled study of socioeconomic support to enhance tuberculosis prevention and treatment, Peru. *Bull World Health Organ*. 2017 Apr 1;95(4):270–280. https://doi.org/10.2471/BLT.16.170167. Epub 2017 Feb 9. PMID: 28479622; PMCID: PMC5407248.
44. Thomas Beena E, Stephen A. Tuberculosis related stigma in India: roadblocks and the way forward. *Expet Rev Respir Med*. 2021;15(7):859–861. https://doi.org/10.1080/17476348.2020.1826314.
45. Alema HB, Hailemariam SA, Misgina KH, et al. Health care seeking delay among pulmonary tuberculosis patients in North West zone of Tigrai region, North Ethiopia. *BMC Infect Dis*. 2019 Apr 5;19(1):309. https://doi.org/10.1186/s12879-019-3893-7. PMID: 30953459; PMCID: PMC6451246.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Experiences of community tuberculosis volunteers in Ibadan north local government: A qualitative study

Oluwadamilare Akingbade [a,b,*], *Funmilayo A. Okanlawon* [c], *Inioluwa O. Aderemi* [b,c], *Yetunde O. Tola* [a,b]

[a] *The Nethersole School of Nursing, Faculty of Medicine, Chinese University of Hong Kong, Sha Tin, New Territories, Hong Kong (SAR), China*

[b] *Institute of Nursing Research Nigeria, Osogbo, Osun State, Nigeria*

[c] *Department of Nursing, Faculty of Clinical Science, College of Medicine, University of Ibadan, Nigeria*

ARTICLE INFO

Article history:
Received 7 January 2022
Accepted 16 April 2022
Available online 22 April 2022

Keywords:
Community-based
Infectious
Diseases
Care

ABSTRACT

Background: Although several efforts have gone into combating tuberculosis (TB) in Nigeria, the country remains one of the worst hit with TB globally. Community Tuberculosis Care (CTBC) which refers to community TB efforts taken beyond the confines of the hospital facilities, has been suggested as a means to reach TB cases not reported or diagnosed. However, CTBC is in the formative stage in Nigeria, and the experiences of Community Tuberculosis Volunteers (CTVs) remain unclear. Hence, the study was conducted to explore the experiences of CTVs in Ibadan North Local Government.

Methods: A qualitative descriptive design with focus group discussion was adopted. CTVs in the Ibadan-north Local Government were recruited, and data were collected using a semi-structured interview guide. The discussions were audio-recorded. Qualitative content analysis method was used for data analysis.

Results: All the CTVs (10) in the local government were interviewed. Four themes emerged which include: CTVs' activities, the need of the patient living with TB, success stories, and challenges faced by CTVs. CTBC activities by the CTVs include case finding, awareness rallies, community education. The needs of the patient living with TB include finances, love, attention and support. Challenges encountered by them include myths, poor family and governmental support.

Conclusion: CTBC was progressing well in this community as the CTVs have many success stories to share. However, the CTVs needed more support from the government in terms of finances, free and adequate drug supply, and assistance with media advert.

* *Corresponding author*. The Nethersole School of Nursing, Faculty of Medicine, The Chinese University of Hong Kong, 601, Esther Lee Building, China. Tel.: +(852) 3943 9917.
E-mail address: callednurse@gmail.com (O. Akingbade).

https://doi.org/10.1016/j.ijtb.2022.04.002

1. Introduction

According to the Global Tuberculosis Report of 2020, Tuberculosis (TB) has continued to be a major source of health problem worldwide, over the years.[1] It remains the leading cause of death from a single infectious agent, and one of the top 10 causes of death worldwide.[1,2] TB, a communicable disease caused by the bacillus *Mycobacterium tuberculosis* typically affects the lungs (known as pulmonary TB) but can also affect other sites (known as extra-pulmonary TB).[3]

WHO developed the Stop TB strategy in 2006.[3] One of the components of the End TB Strategy is to empower the community to take charge in the prevention and control of TB.[4] In 2008, the World Health Organization gave a list of national guidelines for the prevention and control of TB, Community-Based Tuberculosis Care being one.[5]

> *"Community-Based Tuberculosis Care (CTBC) activities refers to activities that extend beyond the premises of formal health facilities to community-based structures (for example, schools and places of worship) and households."*[6]

CBTC has been piloted in six East-African and two South-African countries that were severely affected by TB/HIV in Africa. The review project concluded that these CTBC projects were effective, affordable and acceptable.[7] The report recommended that National Tuberculosis Control Programs should consider harnessing community contribution into TB care, especially in places where there is a need to increase the accessibility of effective TB care. Similar results were obtained in a community-based project in the Republic of Indonesia in 2001.[7] CTBC has been said to reduce the risk of TB infection in a community while reducing stigma about TB in the community.[4] CTBC could aid case finding, default tracing, community awareness campaigns, prevention of transmission of TB and treatment support.[8–10]

Although Nigeria is making progress in its fight to reduce the burden of the TB disease majorly through the expansion and enhancement of Directly Observed Treatment Short Course (DOTS) since 2002, the progress is still considered slow.[8] Therefore, CTBC has been proffered as the solution to the estimated one-third of TB cases in the world that are still either not reported or not diagnosed. However, CTBC in Nigeria is still immature, and its level in Nigeria is yet to be fully developed.[7] The Center for Disease Control and Prevention in 2013 has explained the need for Community TB Volunteers (CTVs) as grass-root personnel with specified roles for the CTBC.[9]

To become a CTV, The National Tuberculosis and Leprosy Control Programme specified that such person must be living in the community, recognized and recommended by the community, able to speak the local languages, and willing to be part of the program. This is believed to help improve effective communication between the CTV and the community they serve .[10]The use of trained CTVs to spread information on TB was found to have improved the overall knowledge and attitudes of patients with TB[11]; and made access to treatment less stressful, because it required less effort and the patient could negotiate a more flexible and convenient time schedule with the CTV.[12] Previous studies reported that CTVs are faced with challenges like lack of family support, poor remuneration, shortage of staff, poor information system, poor drug supply and lack of incentives.[13–15] Also, another study reported that the effectiveness of the CTVs was diluted as they were overworked from attending to other duties such as malaria case finding and treatment, treatment management of pneumonia and diarrhea.[16]

In Oyo State, to the best of our knowledge, this is the first study that will document the experiences of CTVs. It is hoped that this study will help to identify the extent to which CTBC is practised in the LGA and the challenges that are militating against the operationalization will also be identified. All these will help policymakers, health professionals, and the community at large to know the proper steps to take in eradicating TB in the local government and by extension, align this state with the global target of ending TB by the year 2035, as stated in the third target of the End TB strategy.

2. Methods

Design: Owing to the knowledge gap in understanding the experiences of CTVs, a qualitative descriptive design[17] using a Focus Group Discussion-where the facilitator takes a peripheral role to guide the discussion while exploring individual and shared perspectives-[18] was deemed appropriate for this study. The Consolidated criteria for reporting qualitative research (COREQ) guidelines[19] were used for reporting this study.

Ethical Considerations: Ethical approval was obtained from the ethical committee of the Department of Planning, Research and Statistics, Ministry of Health, Oyo State, with approval no: AD13/479/306. Consent was sought from all the participants after the principal investigator had fully explained the purpose of the study and the method of data collection. Participants were informed that they could withdraw from the study whenever they wished without facing any consequence. Anonymity and confidentiality of the participants were upheld as each participant was identified with a unique identification code that could not be traced to the participant. Study files were stored in a locked cabinet.

Procedure and Sample: Total population sampling, a type of purposive sampling that involves selection of the entire population[20] was used to select all ten CVs in the LGA for the focus group discussion. Data collection spanned for one hour 15 minutes. Participants converged at Idi-Ogungun Primary Health Care centre for the sessions. The discussions were audio-recorded, while the observer took notes.

Analysis: Qualitative content analysis, which is a method of choice for descriptive qualitative studies[17] was used for data analysis. The principal investigator transcribed the audio recording verbatim and compared the transcript with the notes that were taken. Three levels of coding were used. Level 1 entailed coding the participant's responses with as many

keywords as possible. In level 2, keywords from level 1 were organized into clusters; while in level 3, broad themes that formed an umbrella for more than one theme were identified from the clusters.

2.1. *Rigour*

To accurately present the experiences and conclusions of the participants, some steps were taken to ensure rigour throughout data collection and analysis. To ensure credibility, open-ended questions were utilized. Dependability and transferability were ensured through audit trails by the last author, who was not involved in the data collection process. Confirmability was ensured by reviewing the themes, participants' quotes and interpretations to ascertain congruence and validate findings.[21,22]

3. Results

3.1. *Socio-demographic characteristics*

The ten participants were community TB workers at Ibadan North Local Government. Each of them had been allocated to different slums. Two of them were males and the rest, females. They were members of the community where they lived and worked. Most of them had secondary certificate qualifications. They were all from the Yoruba tribe, and they spoke the language very fluently. The findings of this study represent the respondents' CTBC experiences and challenges with CTBC activities. Four themes with subthemes were derived and are explained further below.

3.1.1. *Themes*

Concepts identified from the qualitative data were categorised into four major themes. The elements of these themes were further discussed in sub-themes.

1. Community tuberculosis volunteers' activities

These sum up the CTBC activities reported by the participants. They are further explained in the following three subthemes.

i) *Case-finding*

Participants explained that they had to move into the neighbourhood searching for new cases. This they sometimes do en-masse through public address systems like megaphones. Sometimes, the patients find the CTVs at their homes, even late into the night.

> *"It is not that we sit down at a point, and we wait for the patients to come and meet us. We go around on case-finding missions. Also, there are times that all the community TB workers gather together for advocacy. We do it slum by slum …" (P 4)*

> *"In fact, we have megaphones that we use for awareness programs when we move around" (P 7).*

> *"One of the patients I am treating presently was brought to my house 11pm in the night!… In fact, I did not want to open the door because I was not sure of who was at the door until I picked the voice of the community PRO. Instantly, I gave him the specimen bottle for the sample and first thing the next morning, we went to the facility for laboratory test"* (P2).

ii) *Awareness rallies in the community*

The participants explained that they create awareness by holding rallies frequently. In order to draw the attention of community members, self-composed songs related to TB, its side effects and treatments are sung in the local language. Participants explained that they often access more slums in the rally since they must go on the rallies on foot.

> *"We hold rallies frequently, and we sing different songs to catch the awareness of the people while at the same time passing the important information to them"* (P 10).

> *"In fact, we go beyond our slums. There are just eight slums in this local government, but we make sure we even cover the slums that are not shortlisted for this program in this local government. Similarly, we even go beyond this local government regarding our advocacy trips"* (P 8).

iii) *Community education*

Participants in this study expressed that since they have been equipped with training about CBTC, they go about the participating communities and more to educate community policymakers and those who influence the day to day activities of community dwellers like religious leaders and traders.

> *"As you know that this is the community where we dwell, we gather the gate-keepers, community members, traders, pastors, imams, artisans, Landlord associations, etc. together and by this, all the community members are aware that CTBC services are available in the community" (P 9).*

2. The need of the patient living with TB

Three subthemes emerged under this theme. The needs of the patients were documented as financial, psychological and medications.

i *Finances*

Participants expressed that in their experience dealing with patients living with TB or undergoing TB treatment, financial constraint has been the main challenge of the patients. They reported that this challenge transcends all areas of the patient's life including feeding, transportation to the treatment centre, and the purchase of medication.

> *"In Nigeria, a severe problem that we have is economic recession. These patients will need to take drugs, but they do not have the food to eat; some, not even garri. They are not able to take a*

balanced diet. These drugs require that the patients feed very well" (P 3).

"Also, some patients do not have the transport fare to reach the health facility. In this case, we help them to get the drugs and take it to them at home" (P 2).

ii *Love, attention and support*

Another major challenge that patients faced based on the participants' experience in this study is unmet psychological needs such as family or social support. Support from family members was the most commonly mentioned unmet needs of patients living with TB.

"In the case of one of my patients I am treating, it was from a heart-to-heart talk that I discovered that he is not using his drugs very well. After discussing with him, I also talked with his family members, and when all these were not bringing the needed change, I brought the boy to the Tuberculosis and Leprosy Control Officer in the Local Government (P 7)".

"They must not be stigmatized. There is one of the patients I take care of, I am the one that feeds him morning, afternoon, and night. Stigmatization against them can even make them depressed leading to their death" (P 8).

"As the patients living with TB are very weak and they do not have the strength to do many things, their family members need to really support them in their activities of daily living" (P 6).

iii *The need for multivitamins and blood-building drugs*

Aside from medication for TB, most of the participants stressed that patients need multivitamins in order to improve and maintain a balance in their health. While the patients were on the anti-TB drugs, the need for other drugs was stressed.

"Many of these patients do not even have the money to eat let alone buying blood-building drugs. If the government can provide these drugs, it will go a very long way in helping the patient. For a patient of mine, I am the one that bought Vitamin B Co. for him with my money. It is Astymin that is usually prescribed for them and many of them don't even have the money" (P4).

"Multivitamins were prescribed for one of my patients. On getting to his place before coming here this morning, he told me that he could not afford it since he just had 400 naira and the total amount was 900 naira" (P 3).

3. Success stories

The participants commented on the level of success they have recorded so far. In all, they had attended to 70 patients. The success stories they shared appeared to be a consolation that they were doing the right thing for their community.

"I saw a patient in July that was positive for TB and HIV. He had become a walking skeleton! If you see him today, you will not know that he has had TB. He is now looking very healthy and strong. God has helped me so far with all my patients as none of them died and all of them have been complying with their medications" (P 8).

"I have made a lot of successes. One boy was brought to me from Lagos. When the boy came, his body was as white as paper. They thought that it was a spiritual attack. This is because the child fought with another child before the sickness started, and they were thinking it was the child's mother that was behind the predicament, and needed to be appeased and they have bought five sheep to offer as sacrifice. Within just two weeks that the boy started the drugs, he had seen considerable changes. Anytime the boy or his parents call me, they are always showering prayers like I did something special" (P2).

"I have a patient with me. In fact, He had been declared 'dead' at the Central Mosque around our area, and he was taken to UCH and was resuscitated, and they couldn't really establish a diagnosis. He was however discovered to be coughing. I collected the sample it was negative. I now went ahead to do the x-ray, and the results revealed that he had TB. Since then, he has been on the TB treatment and is making marked progress.

Now, you can't even see him and say he has had TB before" (P 6).

4. Challenges faced by CTVs

Four categories of challenges were identified as explained in the following subthemes:

i *Myths and misconceptions*

While the CTVs have organised health education and awareness campaigns, some misconceptions among the community members were still noticed.

"The first challenge is that people are not well informed about this disease. Anytime we want to get sputum from our clients, some think we will be using it for fetish purposes. Their orientation is not enough" (P 5).

"In fact, there was a time we went to take the sputum in a herbalist's house and you can't believe that the herbalist said we must return the saliva we collected earlier on. It was the person that went with me that bailed me out"(P 4).

ii *Governmental support*

The CTVs lamented inadequate governmental support.

"Even the government is not making things easy! Imagine the way they advertise immunisation and other health jingles on radio and television. Why can't they do the same thing for TB as this will go a very long way" (P 5)?

"Government should be doing jingles, and they should also fund outreach programmes all around the place. For example, during

the World Tuberculosis Day, we were able to visit different radio stations and TV stations and even some other newspaper houses." (P 6).

iii *Poor Reception from family members*

The CTVs lamented the harsh treatment they experienced in some of the communities.

"When we get to a house, for example, and we greet, 'Good afternoon house, is there anybody coughing in this house?', We receive abusive responses like: 'It is you and your family that will be coughing, God forbid bad thing!" (P 2).

iv *Poor Remuneration*

The CTVs reported that they needed more financial support.

"Haaa. The money is very small. Risk of life is involved here. It is the protection of God that we are counting on. In fact, we are not given any drug to build our immunity" (P 9).

4. Discussion

This study gives an insight into the experiences of CTVs in Ibadan North Local Government Area (LGA). We found that case detection was one of the activities by CTVs, similar to a study conducted in Ghana which also identified case detection through house-to-house visits as the method employed to create awareness and encourage diagnosis and treatment.[23] Similarly, the findings of a review on empowerment of CTVs showed that CTVs act as educators in the community, and they provide closer health services to the community, to improve diagnosis and treatment.[13] This shows that CTBC in the LGA had been effective and the purpose of establishing CTBC was achieved to a reasonable extent. Through case detection, new cases were discovered, diagnosed and treated. Through awareness rallies and education, the spread of TB was reduced.

According to the characteristics of a CTV as described by the National Tuberculosis and Leprosy Control Programme of the Federal Ministry of Health Nigeria,[10] the respondents of this study were found to be residents of the community who knew the nooks and crannies of the community and understood the language of the community members. They also displayed their willingness to participate in the program. Thus, it can be inferred that the criteria for selecting the CTVs were met in the community.

One of the challenges identified by participants in this study was a lack of family support. Having to deal with family members who were unwelcoming, or who thought the sample collection method was fetish, made the case detection process difficult. CTVs in Indonesia[24] and Nepal[25] also had similar challenges. Poor governmental support which CTVs identified in this study happened to be a general challenge in most community TB programs as well. Poor drug supply,[24] poor information system,[24] lack of incentives,[23] were terms used to describe how little the support from the government was. CTVs in this study spent their money to transport themselves and even the patients to DOTS centres. The CTVs in Ghana described this challenge as poor remuneration.[23] CTVs in this study wished the government would take the announcement of TB detection and treatment on social media seriously as it did with others such as childhood immunization scheme.

CTVs in our study shared stories of their success and the effectiveness of CTBC in Ibadan North LGA. They were able to debunk myths, detect hidden TB patients, encourage them to get tested and treated. They reported a good number of patients they had detected and had treated or were still treating, with no record of death. This serves as a form of encouragement to the CTVs and a proof to the members of the community and the world at large that TB can be treated and cured. Respondents in Indonesia also stated that the success of their services encouraged them to do more and serve their community[24] which boosted their self-esteem and morale.

Strengths and Limitations: This study is the first documentation of experiences of CTVs in this LGA. However, this study focused only on one LGA and thus might not be the overall picture of CTBC in Oyo State. Further studies that will provide an understanding of events in other LGAs in the state are hereby recommended.

5. Conclusion

The findings of this study show that CTBC in Ibadan North LGA was being progressive and effective. However, the CTVs needed more support from the government in terms of finances, free and adequate drug supply, and assistance with social media advert.

Authors' contributions

Study Conception and Design: OA and FO.
Data collection: OA.
Data analysis and interpretation: OA and FO.
Drafting of the article: IA and OA.
Critical revision of the article: YOT.

Conflicts of interest

All authors have none to declare.

Acknowledgement

None.

REFERENCES

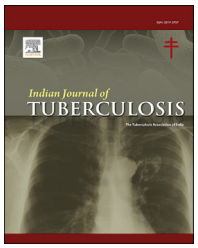
1. World Health Organization. *Global Tuberculosis Report*; 2020. https://www.who.int/teams/global-tuberculosis-programme/tb-reports.

2. Chakya J, Khan M, Ntoumi F, et al. Global tuberculosis report 2020 – reflections on the global TB burden, treatment and prevention efforts. *Int J Infect Dis*. 2021. https://doi.org/10.1016/j.ijid.2021.02.107.
3. World Health Organization. *Global Tuberculosis Report*; 2015. https://www.who.int/teams/global-tuberculosis-programme/tb-reports.
4. Stop Tb Partnership & World Health Organization. *The Global Plan to Stop TB, 2006-2015/Stop TB Partnership*. World Health Organization; 2006. https://apps.who.int/iris/handle/10665/43404.
5. World Health Organization. *Community Involvement in Tuberculosis Care and Prevention*; 2008. https://www.ncbi.nlm.nih.gov/books/NBK143698/pdf/Bookshelf_NBK143698.pdf.
6. World Health Organization. *The ENGAGE-TB Approach: integrating community-based tuberculosis activities into the work of non-governmental and other civil society organizations*; 2012. https://apps.who.int/iris/bitstream/handle/10665/75997/9789241504508_eng.pdf?sequence=1.
7. Ochei KCA. Review of community TB care intervention in Nigeria – a care study of NGO intervention – a capstone project. *S Am J Publ Health*. 2014;2(1). ISSN: 2309-6470.
8. World Health Organization. *Community Engagement in Tuberculosis*; 2014. https://www.who.int/tb/publications/Community_Engagement_factsheet_2014corr_29may15.pdf?ua=1.
9. Center for Disease Control and Prevention. *Core Curriculum on Tuberculosis: What the Clinician Should Know*; 2013. http://www.cdc.gov/tb/education/corecurr/pdf/chapter8.pdf.
10. Federal Ministry of Health Nigeria. *National Tuberculosis and Leprosy Control Programme Workers Manual 2012*; 2012. Available at: http://www.who.int/hiv/pub/guidelines/nigeria_tb.pdf.
11. Balogun M, Sekoni A, Meloni ST, Odukoya O, Onajole A, Longe-Peters O, Ogunsola F, Kanki PJ. Trained community volunteers improve tuberculosis knowledge and attitudes among adults in a Periurban community in southwest Nigeria. *Am J Trop Med Hyg*. 2015;92(3):625–632. https://doi.org/10.4269/ajtmh.14-0527.
12. Erah P, Ojieabu W. Success of the control of tuberculosis in Nigeria. *African Journal Online*; 2009. www.ajol.info/index.php/ijhr/article/view/55382.
13. Jauhar M, Rohana GAPD, Rachmawati U, Kusumawardani LH, Rasdiyanah. Empowering community health volunteer in community-based tuberculosis case management programs in lower-income countries: a systematic review. *JCOEMPH*. 2019;2(2):172–180. https://doi.org/10.22146/jcoemph.47148.
14. Han WW, Saw S, Isaakidis P, et al. Different challenges, different approaches and related expenditures of community-based tuberculosis activities by international non-governmental organizations in Myanmar. *Infect Dis Poverty*. 2017;6(1):59. https://doi.org/10.1186/s40249-017-0263-9.
15. Sichangi LP. *Influence of Community Health Volunteers on Implementation of Community Based Tuberculosis Care*. Bungoma County, Kenya: University of Nairobi; 2016.
16. Rahedi Ong'ang'o J, Mwachari C, Kipruto H, Karanja S. The effects on tuberculosis treatment adherence from utilising community health workers: a comparison of selected rural and urban settings in Kenya. *PLoS One*. 2014;9(2), e88937.
17. Sandelowski M. Whatever happened to qualitative description? *Res Nurs Health*. 2000;23(4):334–340. https://doi.org/10.1002/1098-240x (200008)23:4 PMID: 10940958.
18. Bloor M, Frankland J, Thomas M, Robson K. *Focus Groups in Social Research*. Thousand Oaks, CA: Sage Publications Inc; 2001.
19. Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *Int J Qual Health Care*. 2007;19(6):349–357.
20. Dissertation Laerd. *Total Population Sampling*; 2012. https://dissertation.laerd.com/total-population-sampling.php.
21. Polit DF, Beck CT. *Essentials of Nursing Research*. 8th ed. Lippincott: Williams& Wilkins; 2012.
22. Cypress BS. Rigor or reliability and validity in qualitative research: perspectives, strategies, reconceptualization, and recommendations. *Dimens Crit Care Nurs*. 2017;36(4):253–263. https://doi.org/10.1097/DCC.0000000000000253. PMID: 28570380.
23. Ngrugma JI. *Factors Contributing to Low Tuberculosis Case Detection in Bawku West District in the Upper East Region of Ghana*. Legon: School Of Public Health, College of Health Sciences, University Of Ghana; 2018 (Master's Thesis).
24. Lukman M, Ibrahim K, Yani DI, sari SP, Juniarti N. Exploring strategies to improve the performance of community health volunteers for tuberculosis care and prevention: a qualitative study. *Int J Community Based Nurs Midwifery*. 2019;7(4):270–278. https://doi.org/10.30476/IJCBNM.2019.81353.0.
25. Biermann O, Dixit K, Rai B, Caws M, Lonnroth K, Viney K. Building on facilitators and overcoming barriers to implement active tuberculosis case-finding in Nepal, experiences of community health workers and people with tuberculosis. *BMC Health Serv Res*. 2021;21:295. https://doi.org/10.1186/s12913-021-06290-x.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Comparison of conventional diagnostic methods with molecular method for the diagnosis of pulmonary tuberculosis

Monika Sharma [a,*], *Shobha Broor* [b], *Megha Maheshwari* [c], *Dharam Pal Singh Sudan* [d]

[a] *Department of Microbiology, SGT Medical College, Hospital and Research Institute, SGT University, Budhera, Gurugram, India*
[b] *All India Institute of Medical Sciences, India*
[c] *Department of Microbiology, B Block, Dr Babasaheb Ambedkar Medical College, Rohini, Sector 6, New Delhi, India*
[d] *Department of Pulmonary Medicine, SGT Medical College, Hospital and Research Institute, SGT University, Budhera, Gurugram, India*

ARTICLE INFO

Article history:
Received 19 October 2021
Accepted 24 April 2022
Available online 4 May 2022

Keywords:
Ziehl neelsen staining
Auramine O Staining
CBNAAT
Mycobacterium tuberculosis

ABSTRACT

Background: Tuberculosis remains one of the deadliest communicable diseases. Prompt diagnosis of active tuberculosis cases facilitates timely therapeutic intervention and minimizes the community transmission. Although conventional microscopy has low sensitivity, still it remains the corner stone for the diagnosis of pulmonary tuberculosis in high burden countries like India. On the other hand, Nucleic acid amplification techniques due to their rapidity and sensitivity, not only help in early diagnosis and management of tuberculosis but also curtail the transmission of the disease. This study therefore was aimed at assessing the diagnostic performance of Microscopy by Ziehl Neelsen (ZN) and Auramine Staining (AO) with Gene Xpert/CBNAAT (Cartridge based nucleic acid amplification test) in the diagnosis of Pulmonary Tuberculosis.

Methods: A prospective comparative study was done on the sputum samples of 1583 adult patients from November 2018 to May 2020 suspected of having pulmonary tuberculosis as per NTEP criteria visiting the Designated Microscopic Centre of SGT Medical College, Budhera, Gurugram. Each sample was subjected to ZN staining, AO staining, and was run on CBNAAT as per National Tuberculosis Elimination Program (NTEP) guidelines. The sensitivity, specificity, PPV and NPV and Area under the curve of ZN microscopy and Fluorescent Microscopy were calculated taking CBNAAT as reference in absence of culture.

Results: Out of the 1583 samples studied, 145 (9.15%) and 197 (12.44%) were positive by ZN and AO staining methods respectively. By CBNAAT 246 (15.54%) samples were positive for *M. tuberculosis*. AO was also able to detect more pauci-bacillary cases than ZN. While CBNAAT detected *M. tuberculosis* in 49 sputum samples which were missed by both methods of microscopy. On the other hand there were 9 samples which were positive for AFB by both the smear microscopy techniques but *M. tuberculosis* was not detected by

* *Corresponding author*. Tel: 9958782917.
E-mail addresses: monica303s@gmail.com (M. Sharma), shobha.broor@gmail.com (S. Broor), megha0gera51@gmail.com (M. Maheshwari), hod.tbchest@sgtuniversity.org (D.P.S. Sudan).

https://doi.org/10.1016/j.ijtb.2022.04.006

CBNAAT, these were considered as Non-Tuberculous Mycobacteria. Seventeen samples were resistant to rifampicin.

Conclusion: Auramine Staining technique is more sensitive and less time consuming for the diagnosis of pulmonary tuberculosis as compared to the conventional ZN Staining. CBNAAT can be a useful tool for early diagnosis of patients with high clinical suspicion of pulmonary tuberculosis and detecting rifampicin resistance.

1. Introduction

Mycobacterium tuberculosis (MTB) has affected human health for at least 70,000 years.[1] World Health Organization (WHO) has estimated that around 10 million people globally, equivalent to 130 cases per 100,000 population were infected with the disease in 2019. The mortality of tuberculosis (TB) in 2019 was 1.2 million worldwide. India alone contributed about 27% of world's TB cases. The estimated incidence of new TB cases in India was 193 per 100,000 population in 2019.[2]

The major obstacle in global control of the disease is the inadequate ability to rapidly and accurately diagnose active pulmonary tuberculosis in developing countries.[3] Millions of pulmonary tuberculosis (PTB) cases therefore go undiagnosed each year and unknowingly spread the disease. Accurate and rapid detection of PTB and drug resistance facilitates therapeutic interventions and minimizes community transmission.[4]

In most of the countries including India, conventional microscopy (Ziehl Neelsen Technique) is the corner stone of tuberculosis diagnosis because of its low cost, rapidity, simplicity of procedure and high specificity. However, it lacks sensitivity, as the detection limit is 10,000 bacilli per ml of sputum therefore it can only detect 10–75% of PTB cases.[5] An alternative technique to Ziehl Neelsen staining is Auramine Staining (AO) by Fluorescent Microscopy (FM) which is 8–10% more sensitive than ZN because Acid Fast Bacteria (AFB) can be seen at lower magnification (40x). The time required to examine the smears in FM is less (25%) as compared to ZN Technique.[6–8] Culture is a very sensitive diagnostic technique for tubercle bacilli, detecting as few as 10 to 100 bacilli per ml of sputum.[9] However, the bacilli grow slowly on solid media and colonies appear in about two weeks and sometimes it may take up to eight weeks.

The rapid detection of MTB and drug resistance in infected patients is essential for disease management, because of risk of transmission in community and emergence of MDR-TB (Multidrug resistant tuberculosis) and XDR-TB (Extensively drug resistant tuberculosis).

There are number of Nucleic Acid Amplification (NAA) methods that have been developed for rapid detection and identification of MTB in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases.[9–11] These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.

In December 2010, WHO endorsed the Gene Xpert (Xpert MTB/Rif assay) for the diagnosis of TB. The Gene Xpert utilizes a DNA-PCR technique for the simultaneous detection of *M. tuberculosis* and Rifampicin resistance related mutations. It is the first fully automated bench top cartridge based nucleic acid amplification (CBNAAT) assay for TB detection that includes all necessary steps of DNA-PCR. It gives results within 2 hours. Diagnostic accuracy of Gene Xpert/CBNAAT has been reported to be high for pulmonary TB.[12,13] Extrapulmonary cases in whom AFB smear examination is usually negative, patients with high risk of tuberculosis like HIV-TB coinfected patients and pediatric patients are most benefitted from GeneXpert.[13,14]

This study was carried out with the objective to determine the diagnostic accuracy of CBNAAT in comparison to staining methods for the diagnosis of pulmonary TB. This study will help us in better understanding and the usefulness of ZN Staining and Auramine Staining as compared to CBNAAT as a diagnostic tool for the diagnosis of pulmonary tuberculosis.

2. Methodology

2.1. Study design and study area

This prospective comparative study was done in the Department of Microbiology and Department of Pulmonary Medicine, Faculty of Medicine and Health Sciences, SGT University, Budhera, Gurugram (Haryana) from November 2018 to May 2020. The study was approved by the Institutional Ethical Committee (IEC) and written informed consent was obtained from all participants for use of their sputum for TB diagnostics and research. Samples from both outpatients and inpatients were included in the study.

2.2. Inclusion criteria

Study subjects included patients with clinical suspicion of pulmonary tuberculosis having symptoms of cough with or without expectoration for >2 weeks with evening rise in temperature and/or/weight loss, fatigue, hemoptysis, loss of appetite. Patients with or without previous history of tuberculosis and patients who were referred from ICTC (Integrated Counseling and Testing Centre) were included in the study.

2.3. Exclusion criteria

1. Patients ≤14 years of age
2. Patients on Anti Tubercular Treatment (ATT)

2.4. Sample collection

Two early morning sputum samples were collected from 1583 consecutive patients during the period of study (spot sample if morning sample was unavailable), one in clean, sterile, heat proof, wide - mouth container for staining and another in clean, sterile 50 ml falcon tube for the CBNAAT. From the wide mouth container, two sputum smears were made on glass slides one each for Ziehl Neelsen (ZN) Stain and Auramine Stain (AO). At the time of sample collection, a proforma/questionnaire was used to collect data about the patients. The processing of samples was carried out in Type II A2 Bio Safety Cabinet. ZN staining was done by Light Microscope. The AFB appeared as red rods against blue background in ZN Staining (Fig. 1). Fluorescent staining by Auramine dye was done as per the technique recommended by World Health Organization (WHO) and visualized under Fluorescent Microscope (Labomed LED Lx400 eFL). The tubercle bacilli were seen as yellow luminous slightly curved rods on a green background (Fig. 2).

2.4.1. CBNAAT

CBNAAT is a recently introduced real time polymerase chain reaction based method for detection of TB. It is a MTB complex specific, automated, cartridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real time PCR, providing results within 100 minutes.[16] It has a highly specific primer and five unique molecular probes to target the rpoB gene associated with rifampicin resistance.[17] As it detects only *M. tuberculosis* complex and no other mycobacteria so no cross reactions have been observed.[18]

For CBNAAT, sputum sample was poured into a single use disposable cartridge that is placed into the Xpert Dx module with results produced in less than 2 hours. Each PCR run comprised of an internal control for sample processing (DNA Extraction) and PCR validity (presence of inhibitors) with positive and negative control tested every day. The system automatically interprets all results from measured fluorescent signals with embedded calculations algorithms, into the following categories positive, negative or invalid if PCR inhibitors are detected with amplification failure. The strain was categorized as susceptible or resistant to rifampicin.[18]

Statistical Analysis was performed using SPSS software 21 version. The correlation between the diagnostic tests was determined by Kappa Test. To calculate the sensitivity and specificity of the tests the receiver operator curve was used. CBNAAT was taken as reference to compare ZN and AO Staining. The sputum samples positive and negative by CBNAAT were considered as true positive and true negative. In the absence of Culture, CBNAAT which amplifies and detects specific gene target of *M. tuberculosis*, was used as a reference assay.

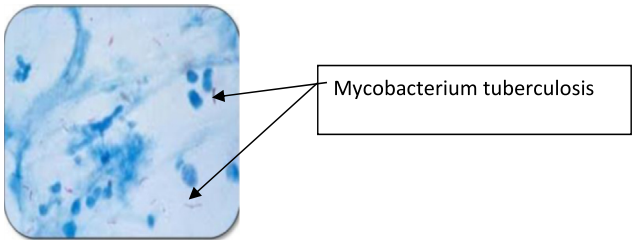


Fig. 1 – Sputum smear stained with (Ziehl Neelsen Staining) showing red colored bacilli with blue background.

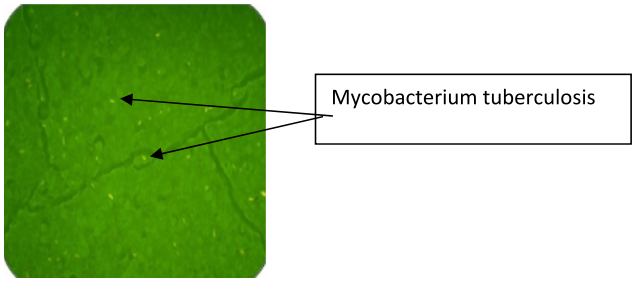


Fig. 2 – Sputum smear stained with (Auramine Staining) showing yellow curved rods on green background.

3. Results

A total of 1583 sputum samples were collected from presumptive TB patients who met the inclusion criterion.

3.1. Demographic profile

Among the total enrolled population, the proportion of males, 1045 (66%) were higher than females 538 (34%). Maximum number of patients were from 21 to 40 years age group followed by 41–60 years age group (Table 1). By ZN Staining AFB were detected in 109 (10.4%) of 1045 male patients and 36 (6.6%) of 538 female patients. By AO Staining AFB were seen in 149 (14.2%) male patients and 48 (8.9%) of female patients while 181 (17.3%) male patients and 65 (12.08%) female patients were found positive by CBNAAT. Thus the rate of diagnosis of tuberculosis was also higher in males as compared to females by all the three methods. The distribution of *M. tuberculosis* infection among different age groups is shown in Table 1 which reflected that the population more prone to tuberculosis concentrated in age range from 21 to 40 years followed by age group from 41 to 60 years (Fig. 3, Fig. 4).

3.2. Detection of M. tuberculosis by various methods

Of the 1583 sputum samples studied, 145 (9.15%) showed the presence of AFB by ZN Staining under oil immersion objective (100x). By Auramine Staining, Acid Fast Bacilli were observed in 197 (12.44%) samples. CBNAAT was done on 1583 samples and 246 (15.54%) samples were positive for *M. tuberculosis* complex (Table 2).

On comparison of three methods it was observed that one hundred and forty five samples were positive by both ZN and AO Staining, whereas additional 52 samples showed the presence of AFB by AO Staining only. None of the sample positive by ZN was negative by AO staining. Additionally 58 samples were positive for *M. tuberculosis* complex by CBNAAT but negative by both the staining methods. There were nine samples which were found positive for AFB by ZN and AO

Table 1 – Age and gender wise distribution of patients enrolled and AFB positive by ziehl Neelsen (ZN), Auramine (AO) staining and CBNAAT.

Age group	Enrolled	Gender-wise Enrollment		Number of AFB positive cases by ZN		Number of AFB positive cases by AO		Number of *Mtb* detected cases by CBNAAT	
		Male	Female	Male	Female	Male	Female	Male	Female
14–20 yrs	201	114	87	13	4	17	7	16	11
21–40 yrs	565	360	205	47	17	59	21	75	29
41–60 yrs	439	325	114	38	8	48	11	61	15
61–80 yrs	342	224	118	11	6	25	8	29	9
>80 yrs	36	22	14	0	1	0	1	0	1
Total	1583	1045	538	109 (10.4%)	36 (6.6%)	149 (14.2%)	48 (8.9%)	181 (17.3%)	65 (12.08%)
Chi-Square value	535.1	21.78		5.51		4.6		7.64	
(p-value)	(0.00***)	(0.00***)		0.24		0.33		0.10	

Data represents Age group and Gender wise distribution of enrolled cases and AFB positive cases.

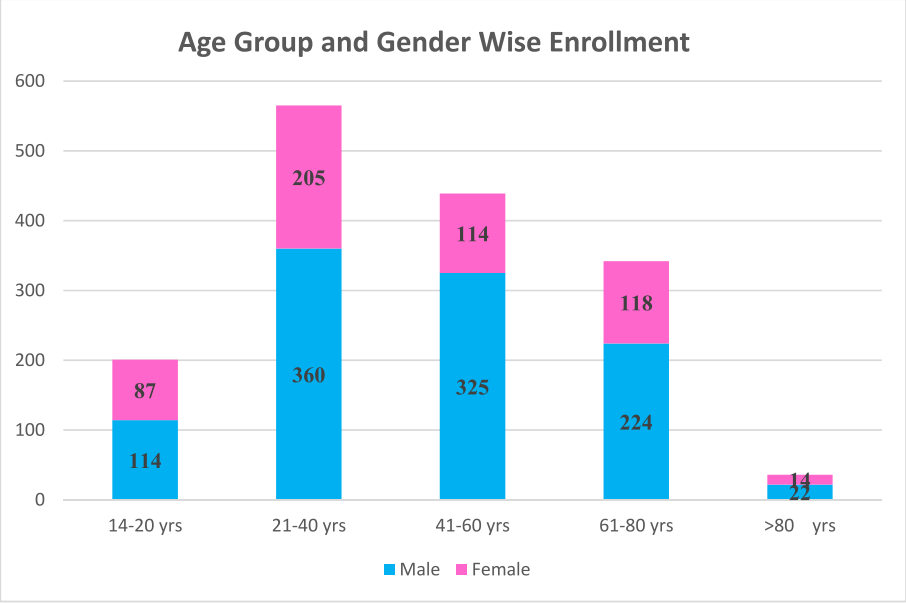


Fig. 3 – Age group and gender wise enrollment of patients.

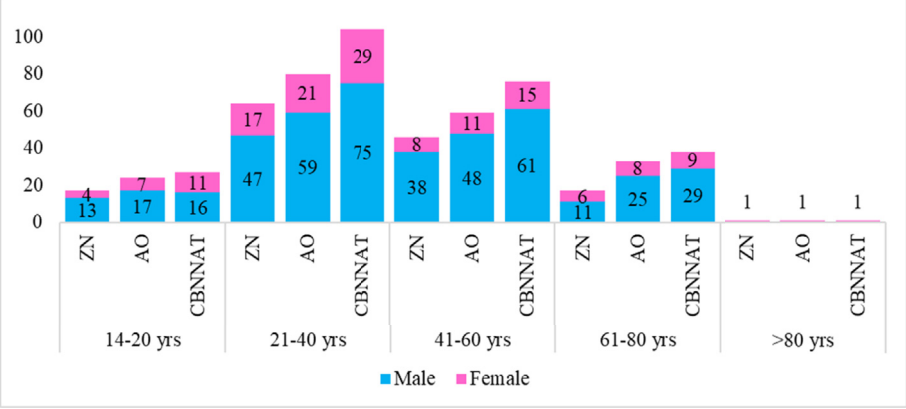


Fig. 4 – Age Group and Gender Wise Distribution of AFB Positive and *Mycobacterium tuberculosis* detected Patients by ZN Staining AO Staining and CBNAAT.

Table 2 – Detection of *Mycobacterium tuberculosis* by various methods. (No. of samples tested = 1583).

Methods Used	Positive Samples (%)
ZN Staining	145 (9.15%)
Auramine Staining	197 (12.44%)
CBNAAT (Gene Xpert)	246 (15.54%)

staining were however negative for *M. tuberculosis* complex by CBNAAT (Table 3).

The present study showed the sensitivity, specificity, positive predictive value, negative predictive value of ZN Staining as 55.28%, 62.50%, 93.79%, and 12.00% and of AO Staining was 76.42%, 62.50%, 95.43%, 20.55% respectively, with reference to CBNAAT in the diagnosis of pulmonary tuberculosis.

The ROC analysis (Fig. 5) was done to compare ZN and AO staining techniques. It shows that Auramine staining has (AUC = 0.777) while ZN Staining (AUC = 0.626). AO staining shows (kappa = 0.822, $p < 0.001$) compared to ZN Staining (kappa = 0.565, $p < 0.001$). CBNAAT was used as reference.

Of the total 246 samples found positive by CBNAAT, 173 (70.32%) were newly infected TB cases, out of which 166 (95.95%) showed rifampicin sensitivity and 7 (4.04%) were rifampicin resistant. Among, 73 (29.67%) previously treated cases infected with TB, 63 (86.30%) showed sensitivity to rifampicin while 10 (13.69%) were resistant to rifampicin (Table 4).

4. Discussion

Tuberculosis is a major public health problem worldwide since ages. For the control of tuberculosis, current recommendations emphasize on early case detection so as to allow treatment of patients and there by limit the transmission of the bacilli. The mainstay for its control is the rapid and accurate identification of the infected individuals.[19] The laboratory plays a critical role in diagnosis of pulmonary tuberculosis. The simplest rapid method is the detection of acid fast bacilli by microscopy which is considered as the evidence of infective stage. In developing countries, microscopy of sputum is by far the fastest, cheapest and reliable method for the diagnosis of pulmonary tuberculosis. The estimated detection limit of microscopy is as high as 10^4 bacilli/ml of sputum. In the early stages of HIV infection, when CMI is only partially compromised, pulmonary tuberculosis presents typically as upper lobe infiltrates and cavitations with diffuse infiltrates and little or no cavitation resulting in paucibacillary picture of sputum. The reduced load of bacilli in the sputum makes it even more difficult to diagnose these cases on sputum microscopy.[20]

The conventional culture is time consuming and it requires a Bio safety laboratory (BSL-3) and trained laboratory personnel. CBNAAT is a rapid nucleic acid amplification-based test and less technical expertise is required. Other advantage is that in a single setting the MTB as well as Rifampicin resistance can be detected through CBNAAT. That is why for such cases CBNAAT is recommended.

A total of 1583 patients were enrolled in the study out of which there were 1045 (66%) males and 538 (34%) females. Out of the 1045 males, 109 (75.17%) were found AFB positive by ZN Staining, 149 (75.63%) by AO Staining while 181 (73.5%) by CBNAAT. Among 538 females, 36 (24.82%), 48 (24.36%) and 65 (26.4%) were positive by ZN, AO and CBNAAT respectively. The incidence of Pulmonary TB in our study was higher in males than females. Our study showed that male:female ratio of incident TB cases for all ages was 3:1 while it was found to be 2:1 as per Global TB Report 2020. In a study by Gelacha et al[21] majority of presumptive TB patients 56.6% were males while 45.8% were females. Similar to our study, in a study by Ahmed et al,[22] out of total 241 patients included in the study, majority i.e. 141 (58.5%) were males where as only 100 (41.5%) were females. Further in the present study, highest occurrence of TB was found in the age group 21–40 followed by 41–60 years. In a study done in India by Dewan R et al,[23] 76% patients were males and the mean age of patients was 35 ± 9 years i.e. 69% patients were in 20–40 years age group. Similarly in a study by Bhavanarushi et al,[24] mean age of pulmonary TB patients was 45 ± 18.30 years (mean ± SD) with male preponderance (76%). Study by Ahmed et al[22] also concluded that more prone population to tuberculosis were found in age ranging from 21 to 40 years. In the productive age group individuals have more chances of exposure may be at their work places, public transport etc than the pediatrics or elderly population. The result agrees with the findings obtained in the study conducted by US Government "Tuberculosis in the United States: National Tuberculosis Surveillance System" in the manner that TB is primarily a disease that affects young adults in their productive years.[25]

Results of this study demonstrated that of the 1583 samples, CBNAAT detected approximately 3% more TB cases than AO and 6% more than ZN. It has been established in many studies that CBNAAT has superior performance over Fluorescent and ZN microscopy.[22,26–28] Auramine Staining produced a higher diagnostic yield compared to that of ZN staining technique among our study samples. This finding confirms the previously reported high yielding performance of Auramine Staining over the conventional ZN staining technique for AFB detection.[8,29,30] The main advantage of

Table 3 – Comparison of CBNAAT results With ZN Staining and AO Staining.

	CBNAAT (Gene Xpert)	ZN Staining		Kappa value	Auramine Staining		Kappa value
		Positive	Negative		Positive	Negative	
Positive	246	136	110	0.565**	188	58	0.822**
Negative	1337	9	1328		9	1328	
Total	1583	145	1438		197	1386	

**$p < 0.001$.

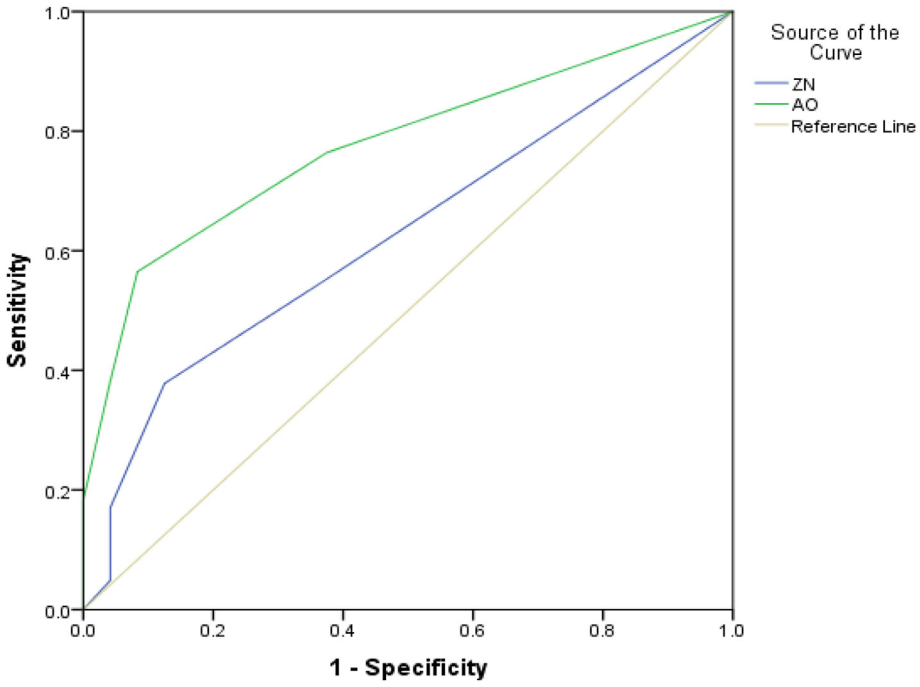


Fig. 5 – Receiver Operator Characteristic Curve. Area under the curve (AUC) = 0.626 (ZN Staining), AUC = 0.777 (AO Staining).

Table 4 – Rifampicin resistance by CBNAAT according to treatment history.

Clinical History	MTB Positive		Chi-Square value (p-value)
	Rifampicin Sensitive	Rifampicin Resistant	
New cases (n = 173)	166 (95.95%)	7 (4.04%)	7.43 (0.01**)
Previously Treated (n = 73)	63 (86.30%)	10 (13.69%)	
Total (246)	229 (93.08%)	17 (6.91%)	

**p < 0.01.

fluorescence microscopy is that it uses a low-power (40x) objective. The field seen in fluorescence microscopy is about 0.34mm,[2] whereas that seen with an oil-immersion objective is only about 0.02mm[2] thus the field is many times larger than that seen in conventional bright-field microscopy through an oil-immersion objective. Fluorescence microscopy allows the same area of a smear to be scanned in a much shorter time than can be achieved by conventional microscopy after staining with the Ziehl Neelsen technique.[15] As compared to ZN Staining, rate of reading is approximately three times faster in Auramine staining, thus helps in saving nearly 2 minutes per slide which corroborate with the findings of study conducted by Marais Brittle.[31] But a real disadvantage of the fluorochrome method is that fluorescence fades with time and the slides cannot be preserved for future and must be read within 24 hours.[32]

In the present study out of 1583 patients total 246 cases were found to be positive for tuberculosis out of which 173 (69.91%) were new cases and 73 (30.08%) were previously treated cases. Out of 173 new cases, 7 (4.06%) were rifampicin resistant whereas among 74 previously treated cases 10 (13.51%) came out to be rifampicin resistant.

The results of our study revealed that sensitivity and specificity of ZN staining was 55.28% and 62.50% and that of AO staining was 76.42% and 62.50% respectively. As compared to a study by Ahmed et al[22] in Sudan higher sensitivity of ZN and AO was reported i.e. 74.6% and 86.6% as well as the specificity of ZN and AO was 98.8% and 97.7% respectively. In a study by Thapa et al[33] the sensitivity of ZN and AO staining was 39.53% and 48.84% which was low in comparison with our study but the specificity of both the methods were higher i.e. 98.94% and 96.84% respectively in their study. A study conducted by Hooja et al[34] found similar sensitivity of 55.55% and 71.85% of ZN and AO staining but the specificity as 99.19% was much higher than our study. In a comparative study of Fluorescent microscopy, ZN staining and culture for the diagnosis of pulmonary tuberculosis by Laifangbam[8] et al in Manipur the sensitivity and specificity of ZN was 59.72% and 93.33% and that of Fluorescent microscopy was 97.22% and 90%.The sensitivity and specificity of fluorescent staining was 95.45% and 99.45% respectively and that of ZN staining was 63.64% and 100% respectively in a study by Kumar et al[35] when the conventional staining method was compared with fluorescent staining in diagnosis of pulmonary tuberculosis among HIV seropositive individuals. The specificity of ZN and AO staining in our study was found to be low as compared to other studies, due to nine samples (3.6%) in which *Mycobacterium tuberculosis* Complex was not detected by CBNAAT but these samples

were positive for AFB by both the staining methods. This may be attributed to Non-Tuberculous Mycobacteria (NTM) or saprophytes. Paul W. Wright concluded in a study that ZN and AO cannot differentiate between NTM and MTB, it can only be confirmed using culture.[36] Culture and identification was beyond the scope of this study so we could not confirm the presence of NTM in these samples. Pulmonary infections due to NTM represent an emerging issue, particularly in Western countries where NTM prevalence may surpass TB.[37] The recovery rates of NTM from AFB smear positive clinical specimens vary widely, from 7.3 to 10.6% in Korea[38] to 21.1% in Spain,[39] and 24.8–48.5% in the United States.[36] Further study is contemplated using culture and identification. All the nine cases suspected with NTM were initiated on Anti Tuberculosis Drugs. Seven of them responded and reported to have no clinical signs and symptoms at the end of treatment. One of these patients relapsed after 6 months of the completion of ATT. Outcome of one patient remains unknown. Many studies have been done on molecular techniques for the detection of MTBC, indicating that PCR is a useful and convenient method for rapid diagnosis of tuberculosis from clinical specimens. It is also to be noted that CBNAAT also have its own limitations, including the yield of extraction and PCR inhibitors in some clinical specimens.[40]

5. Conclusion

The study concluded that Auramine Staining is more efficient over ZN Staining in detecting *M. tuberculosis* in sputum and less time consuming as compared to ZN Staining, CBNAAT has a high capacity for detecting MTB and for predicting multidrug resistance in the smear positive and smear negative sputum samples. It also helps in the detection of paucibacillary MTB. This study highlights the importance of CBNAAT for early and accurate detection and prompt treatment of TB. This study also highlights the fact that for optimum diagnosis of MTB infection a combination of tests as ZN, AO, and CBNAAT is ideal.

The main limitation of our study was false positive samples diagnosed by AFB. These samples were not detected by CBNAAT so further evaluation was needed. Since AFB were detected in these samples, they could be either Non-Tuberculous Mycobacteria or Saprophytic Mycobacteria.

Declaration of competing interest

The authors have none to declare.

Acknowledgement

We extend our thanks and gratitude to the Professor and Heads of Pulmonary Medicine as well as Microbiology for providing us all the facilities available in the Departments while carrying out this work successfully. We are also thankful to the Lab team of the Designated Microscopic Centre of SGT Medical College and Hospital, Budhera, Gurugram for their help and support.

REFERENCES

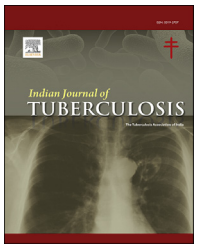
1. Comas I, Coscolla M, Luo T, et al. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. *Nat Genet*. 2013;45(10):1176–1182.
2. *Global Tuberculosis Report 2020*. Geneva: World Health Organization; 2020. License: CC BY-NC-SA 3.0 IGO. (Last Accessed on September'2021).
3. Walis RS, Pai M, Menzies D, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet*. 2010;375:1920–1937.
4. McNerey R, Daley P. Towards a point-of-care test for active tuberculosis: obstacles and opportunities. *Nat Rev Microbiol*. 2011;9:204–213.
5. Darwish M, Magda AEW, Hazem A. Diagnostic assessment of Xpert MTB/RIF in sample of Mycobacterium tuberculosis. Egyptian patients. *Afr J Microbiol Res*. 2013;7:5107–5113.
6. Cuevas LE, Al-Sonoboli N, Lawson L, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross- sectional evaluation. *PLoS Med*. 2011;8(7):1001–1057.
7. Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis a systematic review. *Lancet Infect Dis*. 2006;6:570–581.
8. Laifangbam S, Singh HL, Singh NB, Devki KM, Singh NT. A comparative study of fluorescent microscopy with ziehl-neelsen staining and cuture for the diagnosis of pulmonary tuberculosis. *KUMJ*. 2009;7(27):226–230.
9. *International Standard for Tuberculosis Care*. 3rd ed.; 2014. www.who.int/tb/publications/standards-tb-care-2014.
10. Piersimoni C, Scarparo C, Piccoli P, et al. Performance assessment of two commercial amplification assays for direct detection of Mycobacterium tuberculosis complex from respiratory and extrapulmonary specimens. *J Clin Microbiol*. 2002;40:4138–4142.
11. Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fibreoptic bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. *J Int Med Res*. 2005;33:260–265.
12. Shah I, Gupta Y. Role of molecular tests for diagnosis of tuberculosis in children. *Pediatric On Call J*. 2015;12(1).
13. WHO Policy Xpert MTB/RIF. *World Health Organisation :Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistant: Xpert Mtb/rif System*. Policy statement; 2011.
14. Annual status report. TB India. http://webcache.googleusercontent.com/search?q=cache:fkstKIE98oJ:www.tbcindia.nic/index1.php%3Flang%3D1%26level%3D1%26sublinkid%3D4160%3D2807+&cd=1&hl=en&ct=cink&gl=in; 2015.
15. Frieden T. *Toman's Tuberculosis: Case Detection, Treatment and Monitoring-Question and Answers*. 2nd ed. Geneva, Switzerland: World Health Organization; 2004:31.
16. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol*. 2011;6:1067–1082.
17. Helb D, Jones M, Story E, et al. Rapid detection of mycobacterium tuberculosis and rifampicin resistance by use of on demand, near-patient technology. *J Clin Microbiol*. 2010;48:229–237.
18. Padmaja GV, Srijana K, Sadhna C. Comparison of Ziehl-Neelsen's stain, fluorescent stain with CBNAAT of sputum for the diagnosis of pulmonary tuberculosis. *J NTR Univ Health Sci*. 2019;8:238–243.
19. Prasanthi K, Kumari AR. Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. *Indian J Med Microbial*. 2005;23:179–185.

20. Mamilla R, Suhasini S. Sensitivity of FM staining versus Z-N staining in diagnosing sputum smear positive PTB. *IOSR J Dent Med Sci*. Mar. 2015;14(3):29–33. Ver. III (23).
21. Gealcha AG, Kebede A, Mamo H. Light Emitting diode fluorescent microscopy and Xppert MTB/RIF assay for diagnosis of pulmonary tuberculosis among patients attending Ambo hospital, west central Ethiopia. *BMC Infect Dis*. 2017;17:613.
22. Mohammed Ahmed AE, Yousif N, ELhassan M, Edris AI, Mudasir Z. Diagnostic performance of ziehl-neelsen, fluorescent auramine O stains and genexpert for the detection of Mycobacterium tuberculosis. *IOSR J Dent Med Sci*. 2018;17(2).
23. Dewan R, Anuradha S, Khanna A, et al. Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. *J Indian Acad Clin Med*. 2015;16(2):114–117.
24. Sreekanth B, Amarendra G, Dhanalaxmi A, Rajini M. Effectiveness of CBNAAT in the diagnosis of sputum negative tuberculosis. *Natl J Lab Med*. 2020;9(1):MO01–MO02.
25. Shrestha P, Khanal H, Dahal P, Dongol P. Programmatic impact of implementing gene Xpert MTB/RIF assay for the detection of Mycobacterium tuberculosis in respiratory specimens from pulmonary tuberculosis suspected patients in resource limited laboratory settings of eastern Nepal. *Open Microbiol J*. 2018;12:9–17.
26. Munir MK, Rehman S, Aasim M, Iqbal R, Saeed S. Comparison of ziehl neelson microscopy with GeneXpert for detection of Mycobacterium tuberculosis. *IOSR J Dent Med Sci*. 2015;14(11):56–60.
27. Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Comparison of Genexpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. *J of Infect Dev Ctries*. 2016;10(4):418–422.
28. Alvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R. *Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIFassay in a District Hospital in India*. vol. 2012. Tuberculosis Research and Treatment; 2012. Article ID 932862, 4 pages.
29. Nazar AO, Mustafa AM, Mona KH, Mohamed MT. Comparative study of auramine-O staining and Ziehl-Neelsen for diagnosis of pulmonary tuberculosis. *Nat Sci*. 2014;12(11):59–63.
30. Hung NV, Sy DN, Anthony RM, Cobelens FG, Soolingen DV. Fluorescence microscopy for tuberculosis diagnosis. *Lancet Infect Dis*. 2007;7(4):238–239.
31. Marais BJ, Brittle W, Painczyk K, et al. Use of light –emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. *Clin Infect Dis*. 2008;l47(2):203–207.
32. Kulkarani HK, Jao Wiseman MP, Jayaprakash T, Banur A. Evaluation of different staining methods for the detection of acid fast bacilli in sputum samples. *Int. J.Curr.Microbiol.App.Sci*. 2015;4(12):536–540.
33. Thapa A, Gurung P, Ghimire GR. Evaluation of Gene Xpert MTB/RIF assay for tha detection of Mycobacterium tuberculosis in sputum of patients suspected of pulmonary tuberculosis visiting National Tuberculosis Centre, Thimi, Bhaktapur, Nepal. *SAARC J Tuberc Lung Dis HIV/AIDS*. 2016;XIII(1).
34. Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L. Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. *Indian J Tubercul*. 2011;58(2):72–76.
35. Suria Kumar J, Chandrasekar C, Rajasekaran S. Comparison of conventional and fluorescent staining methods in diagnosis of pulmonary tuberculosis among HIV seropositive individuals. *J Evol Med Dent Sci*. 2012;1(4):463.
36. Paul WW, Richard J, Wallace JR, Brown BA, Griffith DE. Sensitivity of fluorochrome microscopy for detection of Mycobacterium tuberculosis versus non-tuberculous mycobacteria. *J Clin Microbiol*. 1998;36:1046–1049.
37. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. *Clin Chest Med*. 2015;36:1–11. PMID: 25676515.
38. Jeon K, Koh WJ, Kwon OJ, et al. Recovery rate of NTM from AFB smear positive sputum specimens at a medical centre in South Korea. *Int J Tubercul Lung Dis*. 2005;9:1046–1051.
39. Coll P, Garrigo M, Moreno C, Marti N. Routine use of Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) test for detection of Mycobacterium tuberculosis with smear –positive and smear –negative specimens. *Int J Tubercul Lung Dis*. 2003;7:886–891.
40. Bajrani R, Mulliqi G, Kurti A, Lila G, Raka L. Assessment of diagnostic accuracy of GeneXpert Mycobacterium tuberculosis/rifampicin in diagnosis of pulmonary tuberculosis in kosovo. *Biomed Biotechnol Res J*. 2018;2:191–195.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Effect of time, temperature and pH on *Mycobacterium tuberculosis* culture positivity of gastric aspirate: An experimental study

Rajeev Sharma [a], *Suruchi Shukla* [a], *Urmila Singh* [a], *Sarika Gupta* [b], *Amita Jain* [a,*]

[a] *Department of Microbiology, King George's Medical University, Lucknow, UP, 226003, India*
[b] *Department of Pediatrics, King George's Medical University, Lucknow, UP, 226003, India*

ARTICLE INFO

Article history:
Received 8 December 2021
Accepted 28 April 2022
Available online 10 May 2022

Keywords:
Mycobacterium tuberculosis
Gastric aspirate
CBNAAT
Neutralization
Liquid culture
Pediatric tuberculosis

ABSTRACT

Background: The culture of gastric aspirate (GA) has been used for bacteriological confirmation of pulmonary tuberculosis in children and patients who are unable to expectorate. Sodium bicarbonate neutralization of gastric aspirates is commonly recommended to increase culture positivity. We aim to study *Mycobacterium tuberculosis* (MTB) culture positivity of GA collected from confirmed case of pulmonary tuberculosis after storing it at different temperature, pH & time.

Methods: GA specimens from 865 patients of either sex predominately non-expectorating children/adults with suspected pulmonary TB were collected. Gastric lavage was performed in the morning after an overnight fasting (at least 6hrs fasting). The GA specimens were tested by CBNAAT (GeneXpert) and AFB microscopy & those who were positive on CBNAAT were further processed with MTB culture on Growth Indicator Tube (MGIT™) culture. pH neutralized and non-neutralized CBNAAT positive GA specimens were culture within 2 hours of collection and 24 hours after storage at 4 °C & room temperature.

Results: MTB was detected in 6.8% of collected GA specimens by CBNAAT. Culture positivity of neutralized GA specimens when processed within 2 hours of collection, was higher compared to paired non-neutralized GA specimens. Neutralized GA specimens had higher contamination rate than non-neutralized GA specimens. Storage of GA specimens at $Deg C had better culture yield than those stored at room temperature.

Conclusion: Early neutralization of acid in Gastric aspirate (GA) is essential for better culture positivity of *M. tuberculosis* (MTB). If there is a delay in processing GA, it should be kept at 4 °C after neutralization; however, positivity decreases with time.

Abbreviations: MTB, *Mycobacterium tuberculosis*; GA, Gastric aspirate; CBNAAT, Cartridge based nucleic acid amplification test; MGIT Culture, Mycobacterium growth indicator tube culture; AFB, Acid fast bacilli.

* *Corresponding author*. Department of Microbiology, King George's Medical University, Lucknow, U.P, 226003, India.
Tel: +91-9415023928.
E-mail addresses: virology.net@gmail.com, amita602002@yahoo.com (A. Jain).

https://doi.org/10.1016/j.ijtb.2022.04.009
0019-5707/

1. Introduction

Globally, TB illness developed in an estimated 10 million persons in 2019 (130 per 100,000 population. An estimated 1.4 million persons died from TB in 2019. In 2019, 1.2 million children fell ill with TB globally. TB in children and adolescents is frequently neglected by healthcare providers, and it can be difficult to detect and cure.[16]

In India TB affects people of all sex and age groups, men accounted for 61.7% of all TB cases in 2020, and Children (0–4yrs) formed 5.65% of total cases. India has the highest burden of TB, DR-TB, and TB deaths in the world, with an estimated 2.7 million incident cases (27% of the global incidence), 131, 000 cases of DR-TB annually (approx 27% of global incidence). There are almost 440,000 (37% of global figures) TB deaths annually.[19]

Tuberculosis remains a major health problem in India, even today. Millions of people continue to be affected with tuberculosis every year. Incidence of drug resistant TB (MDR-TB) is increasing worldwide and the emerging multidrug/extensively-drug resistant TB (MR/XDR-TB) strains are leading to high mortality and financial burden.[1]

Despite advancements in detection and treatment, tuberculosis (TB) remains one of the world's most serious public health problems. The microbiology of the disease has immense potential to be studied for better diagnosis of the disease. Tuberculosis (TB) can manifest in many ways, from asymptomatic infection to life-threatening disease. Latent tuberculosis (LTB) is an asymptomatic and non-transmissible condition, while active tuberculosis is transmissible (in active pulmonary TB) and may be diagnosed by culture-based or molecular diagnostics. Rising trend in incidence of tuberculosis (TB) as well as development of resistance to anti-TB drugs has needed much attention and has turned out the need for early confirmatory diagnosis of TB.[2] It was estimated that approximately 50% of patients with suspicious active TB are unable to produce or express out sputum. Sputum may demonstrate a negative sputum smear for acid fast bacillus (AFB) for microscopy.[3]

A major challenge in the management of suspected pulmonary tuberculosis (TB) is confirmation of the diagnosis in the pediatric or adult patients who are not able to expectorate.[4] Diagnosis in such cases is generally made on the basis of clinical history given by patient or patient's attendants, clinical examination findings, tuberculin skin test and chest radiograph.[4–6] Microbiological confirmation is difficult in children because of the paucibacillary nature of disease. Common methods to obtain required samples are Sputum induction (SI), bronchoalveolar lavage (BAL), gastric lavage (GL) and gastric aspirate (GA). Choice of procedure depends on the health center's facilities, cost and cooperation of patient. GA is preferred sample for diagnosis of TB in children/patient who swallow their sputum and cannot expectorate.[7–9] The bacteriological confirmation has relied on the culture of specimens from sequential gastric lavages. Early morning gastric aspirate is a preferred sample/specimen for most young children with presumptive tuberculosis & it needs overnight fasting.

Neutralization of gastric aspirates with sodium bicarbonate is conventionally recommended to improve yield on culture. There have been few studies that evaluated the efficacy of neutralization of gastric aspirates with sodium bicarbonate or sodium carbonate.[10,11] Often samples for diagnosis of TB is not able to reach laboratory immediately after collection. There is delay in transport and processing. The temperature of transport is also often not controlled and in our conditions maintaining cold chain is also difficult and cumbersome.

Therefore, present study was planned to study effect of acid neutralization, delay in sample processing and transport temperature on MTB culture positivity in gastric aspirates of confirmed TB cases.

2. Methods

Present experimental study was conducted during January 2020 to July 2021 at Intermediate Tuberculosis Reference laboratory, Department Of Microbiology, King George's Medical University (KGMU), Lucknow, Uttar Pradesh (U.P.), India. GA sample from 865 (777/865 children & 88/865 adults) suspected pulmonary tuberculosis cases were consecutively collected in the morning after an overnight fasting; at least after 6 hours of fasting for adults and children and 4 hours of fasting for infants.[12] Those patients who were able to expectorate the sputum and recently fed were excluded from the study. As per our laboratory data, the MTB positivity in the GA specimen was around 3–4% positivity. Thus, sample size according to Formula of Dennis was 59.

Gastric aspirate collection procedure[13]

It was performed as an inpatient procedure in the morning when the child wakes up, at the child's bedside or in the ward procedure room, or as an outpatient procedure in the procedure room. The child must be fasting for at least 6 hours (infants for 3 hours) before the procedure. All equipment were prepared before starting the procedure. The patient was positioned on his or her back or side with the help of an assistant. Distance between nose and stomach was measured, to estimate how far the tube needs to be inserted to reach the stomach. A syringe was attached to nasogastric tube and nasogastric tube was gently inserted through the nose, and advanced into the stomach. The position of the NG tube was checked by pushing some air (3–5 ml) from the syringe into the stomach and listening with a stethoscope over the stomach. 2–5 ml GA was withdrawn using the syringe attached to the nasogastric tube. If no fluid is aspirated, 5–10 ml of sterile normal saline was inserted and attempted to aspirate again and withdraw 5-10 ml gastric contents. GA was transferred from the syringe into a sterile container (sputum collection falcon tube).[13] After the procedure specimen container was wiped with alcohol/Chlorhexidine to prevent cross-infection and labeled, the laboratory requisition form was filled out.

Laboratory procedure

Each GA specimen was divided into 3 parts immediately after collection. Part one was used for Cartridge Based Nucleic Acid Amplification Test (CBNAAT)[14] and AFB microscopy (Auramine staining)[15] for *M.tuberculosis* (MTB) immediately after collection.[13] Second part was pH neutralized with equal volume of sterile 10% sodium bicarbonate, while the third part was not neutralized.[13] CBNAAT positive GA (n = 59) specimens were enrolled and further processed after decontamination with help of NALC-NaOH method. Both remaining (pH neutralized and non neutralized) parts were further divided in 3 parts; one part was processed immediately after CBNAAT result (within 2 hours) for culture, second part was stored at 4 °C and processed after 24 hours and third part was stored at room temperature and then processed after 24 hours (Figure- 1). Processing of all the parts of samples were done by *Mycobacterium tuberculosis* culture on MGIT.[15] Plan of study is shown inFig. 1.

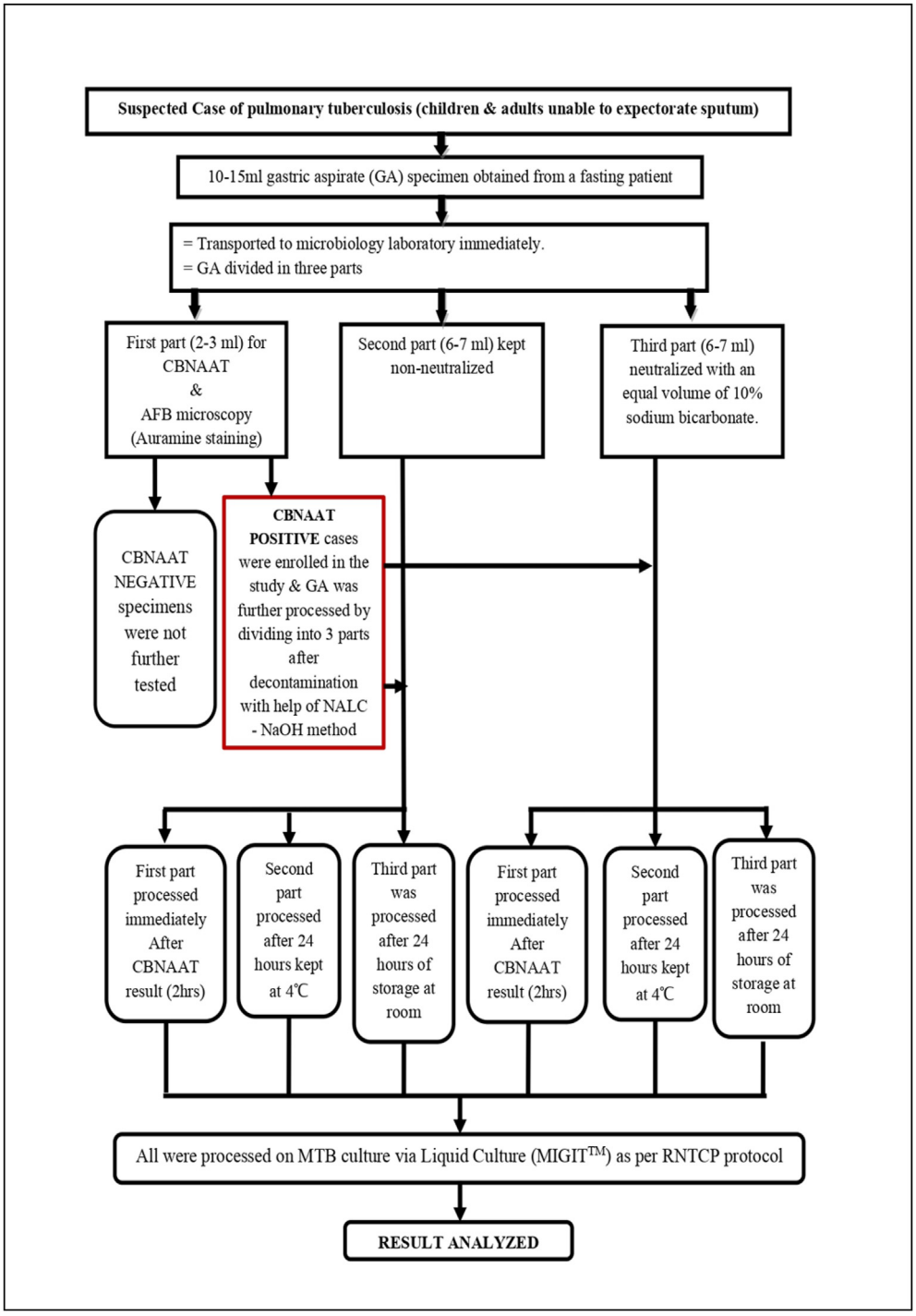


Fig. 1 – Study flow chart.

Sample decontamination[15]

All CBNAAT positive GA specimens were processed after decontamination with help of NALC-NaOH method. About 4–5ml of gastric aspirate was transferred to 50 ml falcon tubes. Equal volume of sterile 4% NaOH solution was added aseptically. Caps of the 50 ml falcon tubes were tightened and mixed well. Each falcon tube was inverted to ensure that NaOH contacts all the sides and inner portion of caps. The falcon tubes were kept at room temperature for 15 minutes. At the end of 15 minutes, 15 ml of sterile distilled water was added. Ingredients were mixed well and centrifuged at 3000 g for 15 minutes. Falcon tubes were carefully removed from the centrifuge without shaking. The supernatant fluid was carefully discarded into a container with 5% phenol solution. After that 40 ml, sterile distilled water was added to the sediment, and pellets were washed. The supernatant was discarded. Pellet was used for culture.

Data Analysis

All the results were entered into a Microsoft Excel sheet and correlation tables were made. Results were statistical analysis by using SPSS (Statistical Package for Social Sciences) Version 21.0 statistical Analysis Software. The sensitivity were calculated by 2*2 tables for *M. tuberculosis* culture on MGIT processed by various methods taking the CBNAAT as the gold standard and also by taking result of GA specimen processed immediately after CBNAAT (within 2 hours) on MGIT culture. P-value was calculated with help of Chi-square test. $P > 0.05$ was not significant and $p < 0.05$ was consider as significant.

3. Results

Of 865 samples, MTB was detected in 31 (3.6%, 31/865) GA specimens by AFB microscopic examination and 59 (6.8%, 59/865) GA specimens by CBNAAT. All the AFB smear positive samples were detected by CBNAAT as well. Adults had higher CBNAAT positivity (17.0%, 15/88) than children (5.7%, 44/777). All 59 samples were cultured on MGIT. Results are elaborated in Table 1. Culture positivity of pH neutralized GA (84.7%, 50/59) when processed within two hours of collection, was higher compared to paired non-neutralized GA [(67.8%, 40/59) (p value = 0.037]. pH neutralized GA samples kept at 4 °C for 24 hours had better culture positivity (45.8%, 27/59) than paired acid non-neutralized specimens (25.4%, 15/59); however, this difference was not statistically significant (p value-p = 0.068). Culture positivity was found to be low if kept at room temperature for 24 hours in both neutralized and non-neutralized samples as only 6.8% (4/59) of neutralized and 3.4% (2/59) non-neutralized GA specimens yielded MTB growth. Neutralized specimens had higher contaminated culture rate, than non-neutralized specimens (Table 1). Sensitivity of culture positivity by using each processing protocol was calculated using CBNAAT as gold standard. Details are shown in table- 1. Sensitivity was maximum (84.75%) when neutralized samples were cultured within 2 hours of collection. Sensitivity decreased as low as 3.39% when samples were stored at room temperature for 24 hours and were not neutralized for pH. Storage at 4 °C had a positive impact on culture positivity and so had neutralization of acid (Table- 1). All CBNAAT positive for MTB GA were not liquid culture positive, of 59 CBNAAT positive specimens, 55 GA (93.22%, 55/59) were positive on liquid culture processed by various methods. In reference of CBNAAT, sensitivity of liquid culture for GA specimen decreases when GA were not neutralized, or there was delay in processing by 24 hrs and not kept at lower temperature (4 °C).

4. Discussion

Tuberculosis (TB) is one of the top ten causes of death among children worldwide.[16,17] There is a significant challenge in the diagnosis of suspected pulmonary tuberculosis (TB) in children, therefore pediatrics pulmonary TB diagnosis is generally made based on the patient's clinical history, clinical examination, tuberculin skin test, chest radiograph, AFB

Table 1 – *Mycobacterium tuberculosis* culture positivity of gastric aspirate after storage at different time, temperature and pH.

	Gastric aspirate (GA); CBNAAT Positive for *M.tuberculosis* (n = 59)					
	NEUTRALIZED GA			NON-NEUTRALIZED GA		
	Immediately processed	Kept at 4°C for 24hr and then processed	Kept at room temp for 24hrs and then processed	Immediately processed n = 59	Kept at 4°C for 24hrs and then processed	Kept at room temp, for 24hrs and then processed
POSITIVE for *M tuberculosis* culture (MGIT™ Culture)	50 (84.7%) *(Reference)*	27 (45.8%) **p<0.001** $\chi^2 = 19.94$[a]	4 (6.8%) **p<0.001** $\chi^2 = 72.32$[a]	40 (67.8%) **p=0.037** $\chi^2 = 6.57$[a]	15 (25.4%) **p<0.001** $\chi^2 = 42.2$[a]	2 (3.4%) **p<0.001** $\chi^{2=}79.21$[a]
Sensitivity using CBNAAT positivity as reference	**Sensitivity 84.75%**	55.6% 45.75%	12.5% 6.78%	75.5% 67.8%	33.3% 25.42%	2.9% 3.39%
CONTAMINATED	3 (5%)	8 (13.55%)	22 (37.28%)	2 (3.38%)	10 (16.94%)	19 (32.20%)

Significant values are highlighted
[a] Chi square test.

microscopy, and when available, microbial culture.[5–7] Children and people with altered sensorium often are not able to expectorate and swallow sputum, as a result another means of obtaining specimens for diagnosis of pulmonary TB is required such as sputum induction (SI), bronchoalveolar lavage (BAL), gastric lavage (GL), and gastric aspirate (GA). However, the approach chosen is contingent upon the health center's facilities, cost, and the patient's compliance. GA is favored specimen for diagnosing pulmonary TB in youngsters and patients who swallow their sputum and are unable to expectorate.[8]

In the current study, adult were observed to have a considerably greater rate of GA specimen positivity on CBNAAT for pulmonary TB than children [17.0% (15/88) vs. 5.7% (44/777)].[18] Only 5.7 percent of children in our study tested positive for pulmonary tuberculosis, which is in line with the current tuberculosis prevalence in India, which is 5.67 percent.[19] Though females had a higher rate of CBNAAT positive than males in this study [8.0% (27/336) vs. 6.0% (32/529)], the difference was not statistically significant ($p = 0.259$).

Only 6.8 percent (59/865) of the study participants were found to be CBNAAT positive for *M.tuberculosis*, while 3.6 percent (31/865) had a positive microscopic result (Auramine staining method). All of the GA specimens that tested positive on AFB microscopy tested positive on CBNAAT as well. As expected, CBNAAT is 47.45% more sensitive than AFB microscopy, making it a better screening method for GA specimens than AFB microscopy. These results were concordant with previous research.[11,20,21] The WHO, now recommends using Xpert MTB/CBNAAT in gastric aspirate as the initial diagnostic test for tuberculosis in children <10yr age.[16]

All CBNAAT positive GA specimens ($n = 59$) were processed using various procedures on Liquid culture (MGIT™ culture). Of the 59 CBNAAT positive GA specimen, 55 (93.22%, 55/59) were culture positive. This finding was consistent with previous researches,[22] who concluded that polymerase chain reaction (PCR) testing (CBNAAT) is the most sensitive, whereas culture is specific. As a result, culture is still considered the gold standard for diagnosing tuberculosis due to various advantages like increased sensitivity, indication of viability and phenotypic antibiotic susceptibility testing for various drugs.[17,19]

Instantly neutralized GA specimens had significantly higher *M.tuberculosis* culture positive than non-neutralized GA specimens processed immediately ($p = 0.037$; sensitivity = 75.5%). Under comparable conditions, neutralized GA specimens held at 4 °C for 24 hours yielded a greater rate of *M.tuberculosis* culture positive than non-neutralized GA ($p < 0.001$). In GA specimens left at room temperature for 24 hours with or without neutralization, *M.tuberculosis* culture produced minimal output ($p < 0.001$; sensitivity = 12.5% & 2.9%, respectively). Other research articles have yielded similar results.[23–26] However, our result were not concordant with some studies[10] that strongly suggested that neutralization with sodium bicarbonate should be avoided. There is no evident explanation for how the observation disparity occurred, and a large study is needed to clarify and improve study observation.

Currently, 10% NaOH is recommended for neutralization; however, *M.tuberculosis* development is inhibited by either low or high pH, therefore we should investigate utilizing a different buffer solution. These findings were in line with other studies and believes that standardizing gastric lavage methods for the diagnosis of pulmonary tuberculosis in children is necessary.[27]

The percentage of culture contamination in non-neutralized GA specimens immediately processed was 3.4%, but neutralization of the GA samples resulted in a greater percentage of culture contamination (i.e. 5%) as in gastrointestinal samples, neutralization stimulated the proliferation of commensal bacteria, which are present in considerably greater numbers in a dormant state, resulting in a high contamination rate in liquid cultures. This is concordant with prior researches.[10]

The current study found that neutralization of GA specimens had no negative consequences. Neutralization, on the other hand, appears to be favorable for further culture processing of GA samples. Even if the HCl in the gastric aspirate is neutralized, other factors such as proteolytic enzymes, nutritional insufficiency, decreased Mg^{+2}, etc may impede bacterial development and are still unknown.[26–28] Furthermore, because pediatric tuberculosis is paucibacillary, stomach aspirate often has low bacilli counts, which may be eliminated after extended cleaning. As a result, if GA decontamination is required, an alternative technique should be considered if available.[27]

The small sample size and the single centric study was one of the limitations of this study. The author recommends multi-centric studies with a large sample size to increase the reliability and make the present observations more generalizable. Availability of GA is anyway difficult, and it becomes still more due to Covid-19 pandemic.

5. Conclusion

CBNAAT is more sensitive than AFB microscopy, making it a better GA specimen screening approach than AFB microscopy in non expectorating patients. Instantly acid neutralized GA specimens processed within two hours of collection had significantly higher *M.tuberculosis* culture positivity than acid non-neutralized GA specimens. If neutralization is not possible, process GA immediately for higher culture positivity. Hence, we conclude that, we should provide most favorable environment conditions by neutralizing acidic pH by sodium bicarbonate for earlier growth of *M. tuberculosis* on culture to prevent delay in the diagnosis by gastric aspirate specimen non expectorating patients.

Ethical approval & consent for participation

Approved by Institutional Ethical Committee (IEC), KGMU, Lucknow, UP, India. Relevant Ref. code is 101th ECM IIB-Thesis//P30. Written informed consent was obtained from study population or their parents/guardian in case study subject was a minor according to Ethical committee protocol.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article. However, due to privacy issues, any information that could identify the patient involved will not be shared.

Author's contributions

All authors reviewed and approved the final version. AJ helped in conceptualizing and planning. SS, US, & AJ consequently contributed to drafting and editing. RS contributed to testing of samples. SG helped in collection & procurement of clinical samples.

Conflicts of interest

The authors have none to declare.

REFERENCES

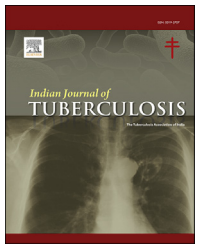
1. *Global Tuberculosis Report 2020*. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
2. Mirsaeidi MS, Tabarsi P, Farnia P, et al. Trends of drug resistant Mycobacterium tuberculosis in a tertiary tuberculosis center in Iran. *Saudi Med J*. 2007 Apr;28(4):544–550. PMID: 17457475.
3. Sputum-smear-negative pulmonary tuberculosis: controlled trial of 3-month and 2-month regimens of chemotherapy. *Lancet*. 1979 Jun 30;1(8131):1361–1363. PMID: 87829.
4. Newton SM, Brent AJ, Anderson S, Whittaker E, Kampmann B. Paediatric tuberculosis. *Lancet Infect Dis*. 2008 Aug;8(8):498–510. 10.1016/S1473-3099(08)70182-8. PMID: 18652996; PMCID: PMC2804291.
5. Stop TB, Partnership Childhood TB Subgroup World Health Organization. Guidance for National Tuberculosis Programmes on the management of tuberculosis in children. Chapter 1: introduction and diagnosis of tuberculosis in children. *Int J Tuberc Lung Dis*. 2006 Oct;10(10):1091–1097. PMID: 17044200.
6. Coulter JB. Diagnosis of pulmonary tuberculosis in young children. *Ann Trop Paediatr*. 2008 Mar;28(1):3–12. 10.1179/146532808X270626. PMID: 18318944.
7. Abadco DL, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of Mycobacterium tuberculosis in childhood pulmonary tuberculosis. *Pediatr Infect Dis J*. 1992 Sep;11(9):735–738. 10.1097/00006454-199209000-00013. PMID: 1448314.
8. Somu N, Swaminathan S, Paramasivan CN, et al. Value of bronchoalveolar lavage and gastric lavage in the diagnosis of pulmonary tuberculosis in children. *Tuber Lung Dis*. 1995 Aug;76(4):295–299. 10.1016/s0962-8479(05)80027-9. PMID: 7579310.
9. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. *Lancet Infect Dis*. 2003 Oct;3(10):624–632. 10.1016/s1473-3099(03)00771-0. Erratum in: Lancet Infect Dis. 2004 Apr;4(4):251. Dosage error in article text. PMID: 14522261.
10. Parashar D, Kabra SK, Lodha R, et al, Delhi Pediatric TB Study Group. Does neutralization of gastric aspirates from children with suspected intrathoracic tuberculosis affect mycobacterial yields on MGIT culture? *J Clin Microbiol*. 2013 Jun;51(6):1753–1756. 10.1128/JCM.00202-13. Epub 2013 Mar 27. PMID: 23536406; PMCID: PMC3716107.
11. Sharma S, Shulania A, Achra A, Jeram H, Kansra S, Duggal N. Diagnosis of pulmonary tuberculosis from gastric aspirate samples in nonexpectorating pediatric patients in a tertiary care hospital. *Indian J Pathol Microbiol*. 2020 Apr-Jun;63(2):210–213. 10.4103/IJPM.IJPM_694_19. PMID: 32317517.
12. *Draft Updated IAP RNTCP Pediatric TB Guidelines*; 2019. https://indianpediatrics.net/aug2019/692-693.pdf. accessed on 01.09.2020.
13. *Guidelines for PMDT in India*. New Delhi, India: REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME Central TB Division, Directorate General of Health Services, Ministry of Health & Family Welfare, Nirman Bhavan; 2019. https://www.google.com/search?client=firefoxbd&q=8.+Guidelines+for+PMDT+in+India+2019%2C+REVISED+NATIONAL+TUBERCULOSIS+CONTROL+PROGRAMME+Central+TB+Division%2C+Directorate+General+of+Health+Services%2C+Ministry+of+Health+%26+Family+Welfare%2C+Nirman+Bhavan%2C+New+Delhi%2C+India. accessed on 01.09.2020.
14. Xpert® MTB/RIF, GXMTB/RIF-US-10, Cepheid®, In Vitro Diagnostic Medical Device, 301-1404, Rev. F August 2019, Copyright © 2019 Cepheid. All rights reserved, https://www.cepheid.com/en/campaigns/India/CepheidIndia?gclid=Cj0KCQiAhf2MBhDNARIsAKXU5GR3TkudUvDnrj1A8wLo45IQglWTFWCClWgyBtHXOZIPQMcsoyqvtAaAo9nEALw_wcB, accessed on 01.01.2020.
15. *Mycobacteriology Laboratory Manual, Global Laboratory Initiative Advancing TB Diagnosis, A Publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership*. 1st ed.; April 2014. https://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf. accessed on 01.01.2020.
16. https://www.who.int/health-topics/tuberculosis#tab=tab2 accessed on 28.09.2021.
17. *Indian Academy of Pediatrics, NEWS Letter*; march 2021. Pediatrics TB in INDIA https://iapindia.org/pdf/child-india/2021/CHILD-INDIA-MARCH-2021.pdf. accessed on 23.09.2021.
18. Brown M, Varia H, Bassett P, Davidson RN, Wall R, Pasvol G. Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate. *Clin Infect Dis*. 2007 Jun 1;44(11):1415–1420. 10.1086/516782. Epub 2007 Apr 20. PMID: 17479935.
19. https://tbcindia.gov.in accessed on 28.09.2021.
20. Qureshi S, Sohaila A, Hannan S, Amir Sheikh MD, Qamar FN. Comparison of Xpert MTB/RIF with AFB smear and AFB culture in suspected cases of paediatric tuberculosis in a tertiary care hospital, Karachi. *J Pak Med Assoc*. 2019 Sep;69(9):1273–1278. PMID: 31511711.
21. Dunn JJ, Starke JR, Revell PA. Laboratory diagnosis of Mycobacterium tuberculosis infection and disease in children. *J Clin Microbiol*. 2016 Jun;54(6):1434–1441. 10.1128/JCM.03043-15. Epub 2016 Mar 16. PMID: 26984977; PMCID: PMC4879301.
22. Akkaya O, Kurtoglu MG. Comparison of conventional and molecular methods used for diagnosis of Mycobacterium tuberculosis in clinical samples. *Clin Lab*. 2019 Oct 1;65(10). 10.7754/Clin.Lab.2019.190145. PMID: 31625374.

23. Singh S, Singh A, Prajapati S, et al. Delhi Pediatric TB study group. Xpert MTB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. *BMC Microbiol*. 2015 Sep 29;15:191. 10.1186/s12866-015-0528-z. PMID: 26420261; PMCID: PMC4589030.
24. Chapman JS, Bernard JS. The tolerances of unclassified mycobacteria. I. Limits of pH tolerance. *Am Rev Respir Dis*. 1962 Oct;86:582–583. 10.1164/arrd.1962.86.4.582. PMID: 14020151.
25. Banda HT, Harries AD, Boeree MJ, Nyirenda TE, Banerjee A, Salaniponi FM. Viability of stored sputum specimens for smear microscopy and culture. *Int J Tuberc Lung Dis*. 2000 Mar;4(3):272–274. PMID: 10751076.
26. Piddington DL, Kashkouli A, Buchmeier NA. Growth of Mycobacterium tuberculosis in a defined medium is very restricted by acid pH and Mg(2+) levels. *Infect Immun*. 2000 Aug;68(8):4518–4522. 10.1128/IAI.68.8.4518-4522.2000. PMID: 10899850; PMCID: PMC98362.
27. Maciel EL, Brotto LD, Sales CM, Zandonade E, Sant'anna CC. Gastric lavage in the diagnosis of pulmonary tuberculosis in children: a systematic review. *Rev Saude Publica*. 2010 Aug;44(4):735–742. English, Portuguese 10.1590/s0034-89102010005000019. Epub 2010 Jun 25. PMID: 20585739.
28. Cook GM, Berney M, Gebhard S, et al. Physiology of mycobacteria. *Adv Microb Physiol*. 2009;55, 81-182, 318-319 10.1016/S0065-2911(09)05502-7. PMID: 19573696; PMCID: PMC3728839.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

A systematic review on correlates of risk of TB disease in children and adults

P.S. Siddhi [a,e,*], R. Raveendranath [b], P. Puligari [c], A. Chinnaswamy [d], R. Song [e,g], S.B. Welch [f,g]

[a] Walsall Manor Hospital, Paediatrics, Moat Road, Walsall WS2 9PS, UK
[b] Medical University of Lodz, Medical School, 90-647 Lodz, 1 Hallera Square, Poland
[c] Sandwell and West Birmingham Hospitals, Library Services, Hallam St, West Bromwich B71 4HJ, UK
[d] Coventry University, Priory Street, Coventry CV1 5FB, UK
[e] University of Oxford, Paediatric Infectious Diseases, Oxford, OX1 2JD, UK
[f] Department of Paediatrics, Birmingham Chest Clinic and Heartlands Hospital, University Hospitals Birmingham, Birmingham B9 5SS, UK

ARTICLE INFO

Article history:
Received 11 May 2022
Accepted 16 May 2022
Available online 21 May 2022

Keywords:
Correlates of risk (COR)
TB disease
Systematic review

ABSTRACT

Background: Tuberculosis (TB) remains one of the leading causes of death in the world. Targeted treatment to prevent progression from TB exposure and infection to disease is a key element of WHO End-TB strategy. A systematic review to identify and develop correlates of risk (COR) of TB disease is timely.

Method: EMBASE, MEDLINE, PUBMED were searched using relevant keywords and MeSH terms published between 2000 and 2020 on COR of TB disease in children and adults. Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) framework was used for structuring and reporting of outcomes. Risk of bias was assessed using Quality Assessment of Diagnostic Accuracy Studies tool-2 (QUADAS-2).

Results: 4105 studies were identified. Following eligibility screening, 27 studies were quality assessed. Risk of bias was high in all studies. Broad variations in COR type, study population, methodology and result reporting were observed. Tuberculin skin test (TST) and interferon gamma release essays (IGRA) are poor COR. Transcriptomic signatures although promising require validation studies to assess wider applicability. Performance consistency of other CORs-cell marker, cytokines and metabolites are much needed.

Conclusion: This review identifies the need for a standardized approach to identify a universally applicable COR signature to achieve the WHO END-TB targets.

* *Corresponding author*. Walsall Manor Hospital, Paediatrics, Moat Road, Walsall WS2 9PS, UK.
E-mail address: p.siddhi@nhs.net (P.S. Siddhi).
[g] These authors contributed equally to the work.

https://doi.org/10.1016/j.ijtb.2022.05.001

1. Background

Tuberculosis (TB) is one of the top 10 causes of death globally. In 2019, there were an estimated 10 million new TB cases including 1.2 million children worldwide.[1] Of the one-fourth of the world's population who are infected only 5–10% evolve into active disease (TB disease) during their lifetime.

The majority of TB disease in the world is caused by *Mycobacterium tuberculosis (M.tuberculosis)*. The susceptibility of the host (immune status), the infectiousness of the transmitter (number of tubercle bacilli that are expelled), the environmental factors and the duration of exposure all influence the probability of transmission.[2]

TB affects all age groups and once infected progression to disease is greater in the first two years after being infected.[3] Children (<5-years of age), individuals with HIV and those immune-suppressed are at increased risk of progression to TB disease.[1] Malnutrition and vitamin D deficiency have been associated with risk of TB disease progression (reference). Genetic deficiencies related to IFNγ and IL-12 are linked to an increased susceptibility to less virulent mycobacteria that causes severe disseminated and/or recurrent infections termed as mendelian susceptibility to mycobacterial diseases (MSMD).[4]

Current available TB screening tests, tuberculin skin test (TST) and interferon gamma release assays (IGRA) identify host immune responses suggestive of either an exposure to TB or evidence of a chronic non-progressive state. Uncertainty in screening is common, particularly when cross-reaction with other *atypical mycobacterium* occurs (TST) or an indeterminate IGRA result is encountered.

Bacteriological confirmation (culture or PCR) is the gold standard for TB disease diagnosis. Mycobacterial Growth Indicator Tube (MGIT-BACTEC) a liquid culture is a sensitive method to isolate mycobacterium in comparison to the solid culture method (Lowenstein–Jenson medium).[5] Xpert MTB/RIF (Nucleic Acid Amplification test) by Cepheid, was endorsed by WHO in 2011 as the next generation assay to diagnose TB disease. An advantage over culture is that it can detect *Mycobacterium TB complex* and resistance to rifampicin in less than two hours. Xpert MTB/RIF is reported to have a sensitivity of 64% in sputum samples and a specificity of 98% in smear positive samples.[6] The next-generation assay, Xpert MTB/RIF Ultra incorporates a PCR amplification technique and two additional molecular targets for *Mycobacterium tuberculosis*. In adults, Xpert MTB/RIF Ultra studies demonstrated an improved sensitivity both in smear positive and smear negative specimen.[7] Two accuracy studies in children conducted on cryo-preserved samples showed an increment in sensitivity by 11%, and a specificity of 97%.[8]

Considering a fourth of the world's population are latently infected, offering prophylactic treatment for such huge numbers is impractical. A consensus meeting report (2017) by WHO and the Foundation for Innovative New Diagnostics (FIND) on the development of a Target Product Profile (TPP) for a test predicting the progression to TB disease outlined operational and performance characteristics for predictive tests and recommendations for standardization of future studies. A minimum sensitivity and specificity for a TB predictive test of ≥75% and specificity of ≥90% was set.[9]

To date a few systematic reviews on biomarkers with TB disease diagnostic potential have been published.[10,11] One systematic review/metanalysis on patient level pooled transcriptomic signatures for diagnosis of incipient TB state was published by Gupta et al (2020). This evaluated only transcriptomic signatures as TB disease predictors on adult blood samples overlooking other potential serological markers.

In this systematic review, we aim to evaluate outcomes of studies on non-sputum-based COR of TB disease in children and adults, compare the results against the TPP targets for a TB prognostic test and identify the knowledge gaps for future exploratory studies.

2. Method

A systematic review on COR TB disease in children and adults published between January 1st, 2000 to May 25th, 2020 was conducted. EMBASE, MEDLINE, PUBMED were searched for relevant publications. In addition, key journals were searched. English language, year of publication and age groups [infants- (0–1 year), children (1–10 years), adolescents (10–19 years) and adults (19–65 years)] were used as filters. For each database, the search terms were transposed as appropriate.

2.1. *Identification of relevant studies*

Table- 1 illustrates the keywords that were transcribed appropriately in each database searched.

2.2. *Eligibility criteria*

To ensure the selection of relevant studies for this review, the study selection was guided by the eligibility criteria as specified under the inclusion/exclusion criteria.

2.3. *Inclusion criteria*

We included studies that met the following criteria:

1. Study question-studies that addressed the review question i.e. COR of TB disease progression were included.
2. Study type- Case-control, longitudinal cohort studies that utilized new study samples/subjects and studies that included prior recruited study samples/subjects were included.
3. Study subjects-studies with human subjects were included. And ages between 0 and 65 years were included.
4. COR-studies that included COR derived from host immune response to TB mycobacterium were included.

2.4. *Exclusion criteria*

Studies on correlates of treatment (COT) and correlates of diagnosis (COD) of latent and TB disease were excluded. Cross-sectional studies, conference papers and review articles were excluded. Studies on animal subjects were excluded.

2.5. *Study selection*

A structured preparation and reporting of systematic review in line with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines was performed.[12]

The data extraction criteria were developed electronically using google forms. The Table 2 shows the data extraction form that was used.

Titles of the studies were screened by one of the authors (PS) and exclusions were made based on-target condition (non-LTBI/TB infection and non-TB disease were excluded), and publication type (review articles, meta-analysis and conference abstracts were excluded). Abstracts and full-text studies were reviewed for eligibility by PS and RR independently. Inter-reviewer disagreements were resolved through consensus decision process overviewed by SW and RS. Data extraction was completed by PS utilizing google forms. For the purpose of standardization and consistency, one signalling question was selected in each domain of the QUADAS-2 framework and this process was completed by PS. In domain 1 (patient selection), signalling question on consecutive or convenience sampling was considered relevant to assess the sampling strategy. Under domain 2, signalling question on the conduct and interpretation of the index test (i.e. blinding) was chosen to be relevant. "Index test" indicates the biomarker test of prognostication. Under domain 3, signalling question on conduct and interpretation of the reference standard was considered relevant. "Reference standard" indicates either a culture proven or an agreed composite standard to classify latent TB and/or TB disease states was used. Under Domain 4, signalling question on patient flow was regarded relevant. The terms "Yes", "No" and "Unclear" were stated for each study for each domain.

If all responses in all four domains were "yes", then the risk of bias was considered "low" and "low applicability concerns", however if any of the responses were "no" or "unclear" then the risk of bias was considered "high" and hence "high applicability concerns".

3. Results

4105 studies were identified from the search. A further five studies were identified from references of review articles. Twenty-seven duplicates were eliminated. Titles of the resulting 4083 studies were screened, based on:

- Target condition (non-LTBI/TB studies) - 3699 were excluded
- Review/meta-analysis/conference abstracts - 73 were excluded

3772 studies were eliminated in the screening phase. The resulting 311 (abstract and full-text) studies were assessed for eligibility. 284 studies were excluded based on the eligibility criteria. Finally, twenty-seven studies qualified for quality assessment (Fig. 1).

3.1. Quality of studies (*Figure 2*)

1) Patient selection - (Signalling question- Was a consecutive or convenience sampling of patients used?) Fifteen studies used consecutive sampling.[13–26] Twelve studies used convenience sampling.[27–29]
2) Index test - (Signalling question-was the conduct and interpretation of the index test blinded?) Overall, eight studies clearly stated and implemented the process of blinding.[29–34] No clarity on the process of blinding in either the conduct or interpretation in five studies.[13,16,27,31] Fourteen studies had no documented process of blinding in their conduct and interpretation.
3) Reference standard: (Signalling question-the reference standard likely to correctly classify the target condition (i.e.

Table 1 – Database and keywords.

Date	Database	Keywords
25th May 2020	PubMed	(pulmonary tuberculosis [MeSH Terms]) OR (infection, latent tuberculosis [MeSH Terms])) OR (latent tuberculosis [MeSH Terms])) OR (mycobacterium tuberculosis [MeSH Terms])) OR ("latent tuberculosis")) OR ("active tuberculosis")) OR ("active infection")) OR ("latent infection")) OR ("active tuberculosis infection")) OR ("latent tuberculosis infection")) AND (biomarkers [MeSH Terms])) OR ("biological markers")) OR ("transcription biomarker")) OR ("bio signature")) OR (genetic transcription [MeSH Terms])) OR ("RNA transcription")) OR (proteome [MeSH Terms])) OR (proteomics [MeSH Terms])) AND (disease progression [MeSH Terms])) OR ("risk progression")) OR ("attributable risk")) OR ("correlates of risk")) OR ("correlates of TB risk")
25th May 2020	HDAS	("latent tuberculosis").ti,ab, ("active tuberculosis").ti,ab, ("active infection").ti,a, ("latent infection").ti,ab, ("latent tuberculosis infection").ti,ab, ("active tuberculosis infection").ti,ab, ("biological markers").ti,ab, ("transcription biomarker").ti,ab, ("bio signature").ti,ab, ("pulmonary tuberculosis").ti,ab, ("latent tuberculosis infection").ti,ab, ("latent tuberculosis").ti,ab, ("active tuberculosis").ti,ab, ("active tuberculosis infection").ti,ab, ("latent tuberculosis infection").ti,ab, ("biomarkers").ti,ab, ("transcription biomarker").ti,ab, ("bio signature").ti,ab, ("risk progression").ti,ab, ("attributable risk").ti,ab, ("correlates of risk" OR "correlates of TB risk").ti,ab.

Table 2 – Data extraction form.

DATA EXTRACTION form
First author
Year of publication
Study title
Study setting (country)
Study design
Sampling strategy
Study time period
Number of study subjects
Number followed-up
Age groups of subjects included
Characteristics of study group
Characteristics of control/comparison group
Identification of disease state- latent infection/TB disease
Number of subjects who developed TB disease
COR type
Type of sample used
Tests to measure COR
Outcomes reported in the study

LTBI/TB infection and TB disease). Twelve studies specified use of microbiological culture or a composite standard as a reference. [18,19,21,27,29–37] And three studies had no clear definition of reference standard. [15,17,30] Twelve studies lacked clarity in defining reference standard to classify target condition.

4) Patient flow and timing- (Signalling question-were all participants included in the analysis?) Eight studies had a clear account on all the participants included in the analysis of the study.[13,18,23,28,32,34,35,38] In five studies exclusion of participants was unclear.[17,24,25,31,33] Fourteen studies did not account for all participants and no explanations were provided.

Hence a "high risk" of bias was observed in the studies and hence a "high applicability" concerns (Fig. 3).

3.2. *Characteristics of studies*

3.2.1. *Year of publication*

All studies included were published between 2007 and 2020. Nineteen (70%) studies were published in the last five-years (2016–2020). Thirteen (48%) were conducted in sub-Saharan Africa, seven in Europe, six in Asia and one in South America. Based on the WHO classification of TB endemicity-twenty (74%) studies were conducted in high TB incidence countries (Incidence rate >40 per 100,000 population) and seven (26%) studies were conducted in low TB incidence countries (incidence rate <40 per 100,000 population).

3.2.2. *Study population*

Mean sample size in the twenty-seven studies was 745(n). Mean number of study subjects who developed TB disease during the study period was 42 (n). Nineteen (70%) studies included adult, children and adolescent groups. Five studies included the Adolescent Cohort Study (ACS) with 6363 healthy adolescents aged between 12 and 18years in South Africa.[29,32,34,35,37] Four studies included the Grand Challenges 6–74 (GC6-74) cohort with 4462 healthy household contacts of 1098 index TB cases in Africa.[33] Median duration of studies was two years (IQR-1.5–5 years). Twenty (74%) studies evaluated >1 COR for TB disease (Table 1).(see Table 3)

3.3. *Analysis based on COR type*

3.3.1. *i) Tuberculin skin test (TST) based*

Five cohort studies reported TST as a COR of TB disease.[19,20,22,26,30] The risk of bias was high in all studies. Four were conducted in low endemic countries and one in a high endemic country. Despite a large sample size, the proportion developing TB disease was low (range 0.9–2.5%). As a COR for TB disease, a pooled sensitivity of 94% and specificity of 44% was calculated.

3.3.2. *ii) IGRA based*

Ten cohort studies reported on IGRA.[16,18–20,22–24,28,30,39] The risk of bias was high in all studies. Seven were conducted in low endemic countries. Despite a large sample size, the proportion developing disease was low (Range-0 - 3.1%). As a COR for TB disease, a pooled sensitivity of 90% and specificity of 86% was calculated. PPV was reported to be <4% in four studies.

3.3.3. *iii) Cytokine based*

Nine studies on cytokines were identified. [14–17,25,27,29,36,37] Five were case–control and four were cohort studies. Eight were conducted in high TB endemic regions. A high risk of bias was noted in these studies. Three studies had large sample sizes, however the percentage who progressed to have TB disease was <1%. Four studies were on IL-10 based on children and adults and demonstrated no consistent correlation to TB disease progression.[15,37,39,40] Complement factors were studied in two studies. Both were South African based case–control studies (validation sets) on adolescent and adult patient groups that demonstrated good correlation to TB disease progression. [28,37]

3.3.4. *iv) Cell marker based*

All five studies were conducted in high TB endemic regions.[26,27,36–38] Two case–control and three cohort studies were identified. The risk of bias was high in all studies. Large sample sizes were noted in these studies and the proportion who progressed to have TB disease at the end of the study period ranged between 0.9% and 35%. Monocyte levels were reported in four studies. An aHR of 6.25 (95% CI-1.63–23.95) and 1.17 (95%CI - 1.01-1.34) was reported in infants and children.[26] Identification of HLA DR + CD4+ cells in infants exposed to TB (part of the MVA85A vaccine trial) increased the risk of developing TB disease (OR -1.12 (95%CI-1.05–1.19).[27]

3.3.5. *v) Antibody based*

Two antibodies-based studies in adults were identified. Both studies had high risk of bias.[16,21] The proportion who developed TB disease at the end of the study period were negligible. Hence outcomes reported were not significant.

3.3.6. *vi) Transcriptional signature based*

Eight transcriptional signature studies were conducted in high TB endemic countries.[13,28,31,32,34–37] All were case–control and

Table 3 – Studies and outcomes.

	Study location	Study type	Age groups	Sample size	Sample type	COR	Index test	Reference standard	Negative population	Number developed TB disease	Outcomes
TST studies											
Rakotosamimanana N (2015)	Madagascar	cohort	children, adolescents, adults	482	whole blood	TST	TST >/ = 5mm, absolute cell counts	culture, clinical consensus	healthy controls	12	Monocytes >7.5% +TST >14mm - aHR −8.46 (95% CI-1.74–41.22, p < 0.01) at the onset of TB disease
Abubakar I (2018)	United Kingdom	cohort	Adolescents, adults	10,045	whole blood	TST, IFN	QuantiFERON and TST	culture, clinical consensus	migrants	97	IFN- gamma-threshold value >/ = 0.35IU/ml (PPV-3.3%, NPV-99.4%), T-Spot (PPV-4.2%, NPV-99.5%).
Altet N (2015)	Spain	cohort	infants, children, adolescents and adults	1335	plasma	IFN, TST	QuantiFERON and TST	TB case definition not specified	household contacts, LTBI	15	Sensitivity 5-9mm- 1.0, 10-14mm-0.93 (95%CI-0.79–1),>15mm-0.79 (95%CI-0.57–1), Specificity- 5-9mm- 0.26 (95%CI-0.22–.3), 10-14mm-0.5 (95%CI-0.45 –0.55), >15mm-0.78 (95%CI-0.68–0.8)
Bakir M (2008)	Turkey	cohort	infants, children, adolescents	908	whole blood	IFN, TST	ELISpot test-secretary peptides- ESAT-6, CFP-10	culture, clinical consensus	household contacts, LTBI	15	
Kik SV (2010)	Netherlands	cohort	Adolescents, adults	433	whole blood	TST, IFN	QuantiFERON, T-SPOT, TST	culture, clinical consensus	household contacts	9	PPV for TST>10mm-3.1%, TST>15mm-3.8%,
IFN gamma studies											
Scriba TJ (2017)	South Africa	Case-control + validation	Adolescents, adults	150	whole blood, plasma	IFN, T-cell/ monocytes, cytokines, 16-gene signature	RNA-sequencing, plasma proteomic analysis, SOMA scan, flow cytometry, transcriptomic analysis of sorted T cells and monocytes	culture	LTBI	44	Elevated expression of 16 gene TB signatures, expression of Interferon responses genes and complement activation up to 18 months before TB diagnosis in progressors. Changes in myeloid inflammation, lymphoid, monocyte and neutrophil gene modules occurred proximally to tuberculosis disease. Gene expression in purified T cells revealed suppression of IL-17 responses in progressors.
Kik SV (2010)	Netherlands	cohort	Adolescents, adults	433	whole blood	TST, IFN	QuantiFERON, T-SPOT, TST	culture, clinical consensus	household contacts	9	For an IFN-gamma threshold of >/ = 0.35IU/ml (sensitivity-63%, specificity– 46%, PPV-2.8% (95% CI-1.0–4.6%) and T-Spot test (sensitivity- 75%, T Spot-40%, PPV-3.3% (95% CI-1.3–5.3%).
Abubakar I (2018)	United Kingdom	cohort	Adolescents, adults	10,045	whole blood	TST, IFN	QuantiFERON and TST	culture, clinical consensus	migrants	97	IFN- gamma-threshold value >/ = 0.35IU/ml

											(PPV-3.3%, NPV-99.4%), T-Spot (PPV-4.2%, NPV-99.5%).
Diel (2008)	Germany	cohort	infants, children, adolescents and adults	601	whole blood	IFN, TST	QuantiFERON	TB case definition not specified	household contacts, LTBI	6	A 2-year predictive rate for QFT (>0.35IU/ml) to be 14.2%.
Bakir M (2008)	Turkey	cohort	infants, children, adolescents	908	whole blood	IFN, TST	ELISpot test-secretary peptides- ESAT-6, CFP-10	culture, clinical consensus	household contacts, LTBI	15	Adjusted Incident rate-for ELISpot (ESAT-6/CFP-10)- 3.86 (95% CI - 1.19-12.5) (p = 0.024).
Altet N (2015)	Spain	cohort	infants, children, adolescents and adults	1335	plasma	IFN, TST	QuantiFERON and TST	TB case definition not specified	household contacts, LTBI	15	Sensitivity- 0.35–5IU/ml- 1.0, 5–10IU/ml-1.0, >10IU/ml-1.0, Specificity-0.35–5 IU/ml- 0.8, 5–10IU/ml- 0.77, >10IU/ml- 0.84, PPV 0.35–5IU/ml- 3%, 5–10IU/ml- 3%, >10IU/ml- 6%
Delogu G (2016)	Italy	cohort	adolescents	41	whole blood	IFN to Heparin binding haemagglutinin antigen (HBHA)	ELISA	culture	HIV positive TB infected	2	A lack of HBHA responses indicated an increased risk to develop active TB in HIV-LTBI
Michelsen (2016)	Greenland	cohort	infants, children, adolescents and adults	978	whole blood	IFN against Rv1284, Rv2659, Rv266	ELISA	TB case definition not specified	healthy endemics	31	Hazard Ratio - Rv1284 −0.96 (95% CI-0.28–3.04) p value-0.89, RV2659 −1.05 (95% CI-0.51–2.13) p-value- 0.90, RV2660 −3.06 (0.70–13.37) pvalue-0.14
Talat N (2009)	Pakistan	cohort	adults	94	whole blood	IFN, IL-10, IL-6, IgG1 antibodies	ELISA	TB case definition not specified	healthy endemics	0	Reported no statistically significant correlation between IFN levels, IL-10, 6 and IgG1 antibodies with TB progression
Andrews JR (2017)	South Africa	cohort	Infants, children	2512	whole blood	IFN	QuantiFERON	culture, clinical consensus	Health infants wirh BCG vaccination and MVA85A vaccine	28	0.35IU/ml-Sensitivity-0.9, specificity- 0.9, PPV -8%, NPV-100% 0.4IU/ml-Sensitivity- 0.36, specificity- .99, PPV-16%, NPV- 99%.
Cytokine studies											
Talat N (2009)	Pakistan	cohort	adults	94	whole blood	IFN, IL-10, IL-6, IgG1 antibodies	ELISA	TB case definition not specified	healthy endemics	0	Reported no statistically significant correlation between IFN levels, IL-10, 6 and IgG1 antibodies with TB progression
Hussain R (2007)	Pakistan	cohort	Adolescents, adults	109	whole blood	IFN/IL-10 ratio	ELISA	culture, clinical consensus	household contacts	7	Linear trends for CF protein induced IFN-gamma/IL-10 ratio showed significant differences between contacts with TB disease- and disease-free contacts between 0 and 12 months (odds ratio = 0.45, 95% confidence interval = 0.295–0.685; p = 0.0002).
Penn- Nicholson A (2018)	South Africa, Gambia	Case-control + validation	Adolescents, adults	8311	plasma	TRM 5- CF-9,IGFBP-2, B-cell- ARCP, MRA-7, NrCAM 3PR	SOMA scan	culture	LTBI	80	ACS test set- Sensitivity-0.79 (95%CI - 0.54-0.94), Specificity-0.79 (95% CI-

						signature- C9, CK-MB, C1qTNF					0.54–0.94), AUC- 0.80 (95% CI-0.70–0.89), GC6-74 validation set- Sensitivity- 0.49 (95%CI - 0.33-0.65), specificity- 0.75 (95%CI-0.68–0.81), AUC-0.66 (95% CI-0.56 –0.75)
Bapat PR (2015)	India	cohort	Adolescents, adults	419	serum	Alpha-2 macroglobulin, Sero-transferrin, Haptoglobin	One dimensional SDS PAGE, MALDI-TOF analysis	culture, clinical consensus	malnourished healthy endemics	1	A 2-fold increase in the levels of the three proteins in the participant (n = 1) who developed TB,
Hussain R (2011)	Pakistan	cohort	Adolescents, adults	107	plasma	Il-4	cytometric bead array	culture, clinical consensus	LTBI, healthy endemics	10	A pair-wise comparison of IL-4 levels between disease free-healthy contacts (FHC) and contacts with TB disease (DHC) showed statistical significance (p = 0.0035) at 12 months from follow-up
Scriba TJ (2017)	South Africa	Case-control + validation	Adolescents, adults	150	whole blood, plasma	IFN, T-cell/ monocytes, cytokines, 16-gene signature	RNA-sequencing, plasma proteomic analysis, SOMA scan, flow cytometry, transcriptomic analysis of sorted T cells and monocytes	culture	LTBI	44	Elevated expression of 16 gene TB signatures, expression of Interferon responses genes and complement activation up to 18 months before TB diagnosis in progressors. Changes in myeloid inflammation, lymphoid, monocyte and neutrophil gene modules occurred proximally to tuberculosis disease. Gene expression in purified T cells revealed suppression of IL-17 responses in progressors.
Fletcher HA (2016)	South Africa	Case-control	Infants	284	PBMC	T cell surface receptor (HLADR + CD4 +T cells)	Flow cytometry	culture, Xpert MTB	Healthy infants with BCG vaccination and MVA85A	101	BCG specific T cells secreting IFN- gamma associate with reduced risk of TB (OR = 0.502, 95% CI = 0.29–0.86,P = 0.013, FDR = 0.14).
Chen DY (2016)	Taiwan	Case-control	Adults	238	plasma	Neopterin	ELISA magnetic bead arry	culture, clinical consensus	Non-rheumatoid Arthritis TB, healthy endemics	7	Non-stimulated and TB antigen stimulated neopterin levels were significantly higher in RA patients who developed TB (median -24.5 pg/ml and 23053 pg/ml respectively) and non-RA TB patients (12.2 pg/ml and 9633 pg/ml respectively) compared with QFT-Gold converters without TB (3pg/ml and 2720 pg/ml respectively both p < 0.01).
Cell marker studies											
Sutherland JS (2011)	Gambia	Case-control	adults	2348	whole blood, plasma		PBMC thawing, flow cytometry,	culture	household contacts, LTBI	22	A significantly higher level of CD4+CD25+cells

						Mononuclear cells, plasma cytokines, RNA	Multiplex cytokine analysis o plasma samples, Reverse transcription multiplex ligation-dependent probe amplification (RT-MLPA)				($p < 0.05$) at recruitment in comparison to non-progressors.
Fletcher HA (2016)	South Africa	Case-control	Infants	284	PBMC	T cell surface receptor (HLADR + CD4+T cells)	Flow cytometry	culture, Xpert MTB	Healthy infants with BCG vaccination and MVA85A	101	OR of TB disease - HLA DR + CD4+ cells at day 0, AUC- 0.619, OR = 1.12 (1.04–1.21) p = 0.002, FDR-0.047 and at day 28, AUC- 0.653, OR = 1.12 (95% CI-1.05–1.19) p = 0.001, FDR-0.013
Rakotosamimanana N (2015)	Madagascar	cohort	children, adolescents, adults	482	whole blood	blood monocyte levels	TST >/ = 5mm, absolute cell counts	culture, clinical consensus	healthy controls	12	Blood monocytes -aHR −6.25 (95% CI-1.63 −23.95) and increased ML ratio -aHR- 4.97, (95% CI 1.3–18.99); (p = 0.03).
Scriba TJ (2017)	South Africa	Case-control	Adolescents, adults	150	whole blood, plasma	IFN, T-cell/ monocytes, cytokines, 16-gene signature	RNA-sequencing, plasma proteomic analysis, SOMA scan, flow cytometry, transcriptomic analysis of sorted T cells and monocytes	culture	LTBI	44	Elevated expression of 16 gene TB signatures, expression of Interferon responses genes and complement activation up to 18 months before TB diagnosis in progressors. Changes in myeloid inflammation, lymphoid, monocyte and neutrophil gene modules occurred proximally to tuberculosis
Naranbhai V (2014)	South Africa	cohort	infants	1336	whole blood	Monocyte: lymphocye ratio	flow cytometry	Protocol defined criteria for categorization of Tuberculosis Disease and infection	HIV exposed but unaffected, randomised in IMPAACT trial	49	An increasing ML ratio was associated with TB disease/death within 2 years (aHR-1.17 per unit increase in ML ratio, 95% CI-1.01-1.34, p = 0.03). The association of baseline ML ratio to probable or definite TB disease was significant. (HR 1.50; 95% CI - 1.19-1.89; p = 0.006).
Antibody studies											
Talat N (2009)	Pakistan	cohort	adults	94	whole blood	IFN, IL-10, IL-6, IgG1 antibodies	ELISA	TB case definition not specified	healthy endemics	0	Reported no statistically significant correlation between IFNg levels, IL-10, 6 and IgG1 antibodies with TB progression
Maekura R (2019)	Japan	Case-control	adults	74	plasma	Antibodies to Mtb antigens- ESAT6, MVA85A, CFP10, Acr,	ELISA	TB case definition not-specified	healthy endemics, LTBI	1	Pre-clinical TB was identified in five individuals (among recent LTBI)- antibodies

											against ESAT-6 ($p = 0.03$), Ag85 A ($p = 0.048$) Acr ($p = 0.057$) and MDP1 ($p = 0.0001$) were significantly higher than those in the remote LTBI group.
Transcriptional signature studies											
Duffy FJ (2018)	South Africa, Uganda	Case-control + validation	children, adolescents, adults	1696	Serum	Serum circulating miRNA, RNA-COR signature	qRT-PCR	culture, clinical consensus	Household contacts, LTBI	54	Circulating -miRNA-AUC- 0.7 (95%CI-0.58 −0.82), PPV- 59%, NPV-81%, RNA COR, RNA-COR- AUC-0.77 (95% CI-0.68−0.87), combined miRNA + RNA-COR-AUC- 0.78 (95% CI-0.69 −0.88)
Scriba TJ (2017)	South Africa	Case-control + validation	Adolescents, adults	150	whole blood, plasma	IFN, T-cell/ monocytes, cytokines, 16-gene signature	RNA-sequencing, plasma proteomic analysis, SOMA scan, flow cytometry, transcriptomic analysis of sorted T cells and monocytes	culture	LTBI	44	Elevated expression of 16 gene TB signatures, expression of Interferon responses genes and complement activation up to 18 months before TB diagnosis in progressors. Changes in myeloid inflammation, lymphoid, monocyte and neutrophil gene modules occurred proximally to tuberculosis
Zak DE (2016)	South Africa, Gambia	Case-control + validation	adolescents, adults	9508	whole blood	RNA signature- 16 gene- ANKRD22, APOL1, BATF2, ETV7, FCGR1A, FCGR1B, GBP1, GBP2, GBP4, GBP5, SCARF1, SEPT4, SERPING1, STAT1, TAP1, TRAFD1,	RNA sequencing, qRT-PCR	culture	LTBI, ORD	119	ACS cohort- sensitivity-0.66 (95%CI-0.63−0.68), specificity-0.81 (95%CI-0.79−0.82), ACS + GC6-74 validation cohort (12 months prior to TB disease diagnosis), sensitivity-0.54 (95% CI-0.43−0.64), specificity 0.83 (95% CI-0.77−0.86)
Sutherland JS (2011)	Gambia	Case-control	adults	2348	whole blood, plasma	Mononuclear cells, plasma cytokines, RNA	PBMC thawing, flow cytometry, Multiplex cytokine analysis plasma samples, Reverse transcription multiplex ligation-dependent probe amplification (RT-MLPA)	culture	household contacts, LTBI	22	Significantly lower level of Bcl2 in progressors ($p = 0.011$). Progressors had significantly higher levels of chemokine receptor 7 (CCR7) compared to non-progressors.
Suliman S (2018)	South Africa, Gambia, Ethiopia, Uganda	Case-control + validation	Adolescents, adults	4466	plasma	RISK4 signature-Gas6/CD1C, SEPT4/BLK, SEPT4/CD1C, GAS6/BLK	PAXgene (PreAnaltiX), RNA sequencing-Gene	culture, clinical consensus diagnosis	household contacts, LTBI	120	AUC for RISK4 on all samples-AUC-0.67 [95% CI-0.57−0.77] $p = 0.0026$. RISK 4 signature-validated against time to

							Expression Omnibus database				TB within 1 year of diagnosis -AUC-0.66 [95% CI-0.55–0.78] p = 0.002 between 1 and 2 years of TB diagnosis-AUC-0.69 [95% CI-0.51 –0.86] (p = 0.02)
Leong S (2020)	Brazil	Case-control + validation	Adolescents, adults	37	Peripheral Blood Mononuclear cells	Predict –29 signature	RNA sequencing	clinical consensus based on Brazilian TB program guidelines	household contacts	55	Brazilian cohort-PREDICT 29 had a sensitivity of 0.74 (95% CI-0.70–0.78), specificity of 0.85 (95% CI-0.82 –0.88), AUC- 0.911 (95% CI - 0.894-0.928), PPV of 20.2% (95%CI - 13.1 –29.4%), NPV- 98.5%, (95%CI - 96.8–99.3%). GC6 cohort, sensitivity of 0.56 (95% CI - 0.53-0.59), specificity of 0.76 (95% CI-0.73–0.78), AUC-0.68 (95% CI-0.67–0.69) 4.1% (95% CI - 2.3–7.4%), NPV-98.7% (97.6–99.4%).
Fletcher H (2016)	South Africa	Case-control	Infants	5726	whole blood	Cytokines, RNA transcriptomes, monocyte/T cell ratios IFN	Gene expression analysis, flow cytometry, Human cytokine LINCO plex 29- bead array	culture, clinical consensus	household endemic controls	29	Comprehensive gene expression analysis failed to identify a correlate od risk.
Warsinke HC (2018)	South Africa, Brazil	Case-control + validation	Adolescents, adults	3866 (ACS-153)	plasma, whole blood	3-gene signature (GBP5, DUSP3, KLF2)	RNA sequencing, qRT-PCR	culture, clinical consensus	LTBI, healthy controls, ORD	43	<7 days of TB diagnosis-sensitivity-0.9, specificity-0.63, NPV-99% at 4% prevalence [AUC- 0.86 (95% CI - 0.77-0.96)]. Between 8 and 180 days- sensitivity of 0.86, specificity- 0.84, NPV-99% at 4% prevalence. [AUC- 0.86 (95% CI-0.70 –1.00)]
Metabolite studies											
Weiner J (2018)	sub-Saharan Africa	Case-control + validation	adolescents, adults	4462	whole blood, plasma	cysteine, histidine, mannose, taurocholenate sulfate, phenylalanine, tryptophan, glycocholenate sulfate, citrulline, citrate, creatine (TB-HEALTHY signature)	metabolite profiling, Mass spectrometry analysis	culture and XPERT MTB	healthy endemics	141	Sensitivity-0.5, specificity- 0.75, AUC-0.68 (95%CI-0.64–0.73)

1 Note- TST- Tuberculin skin prick test.

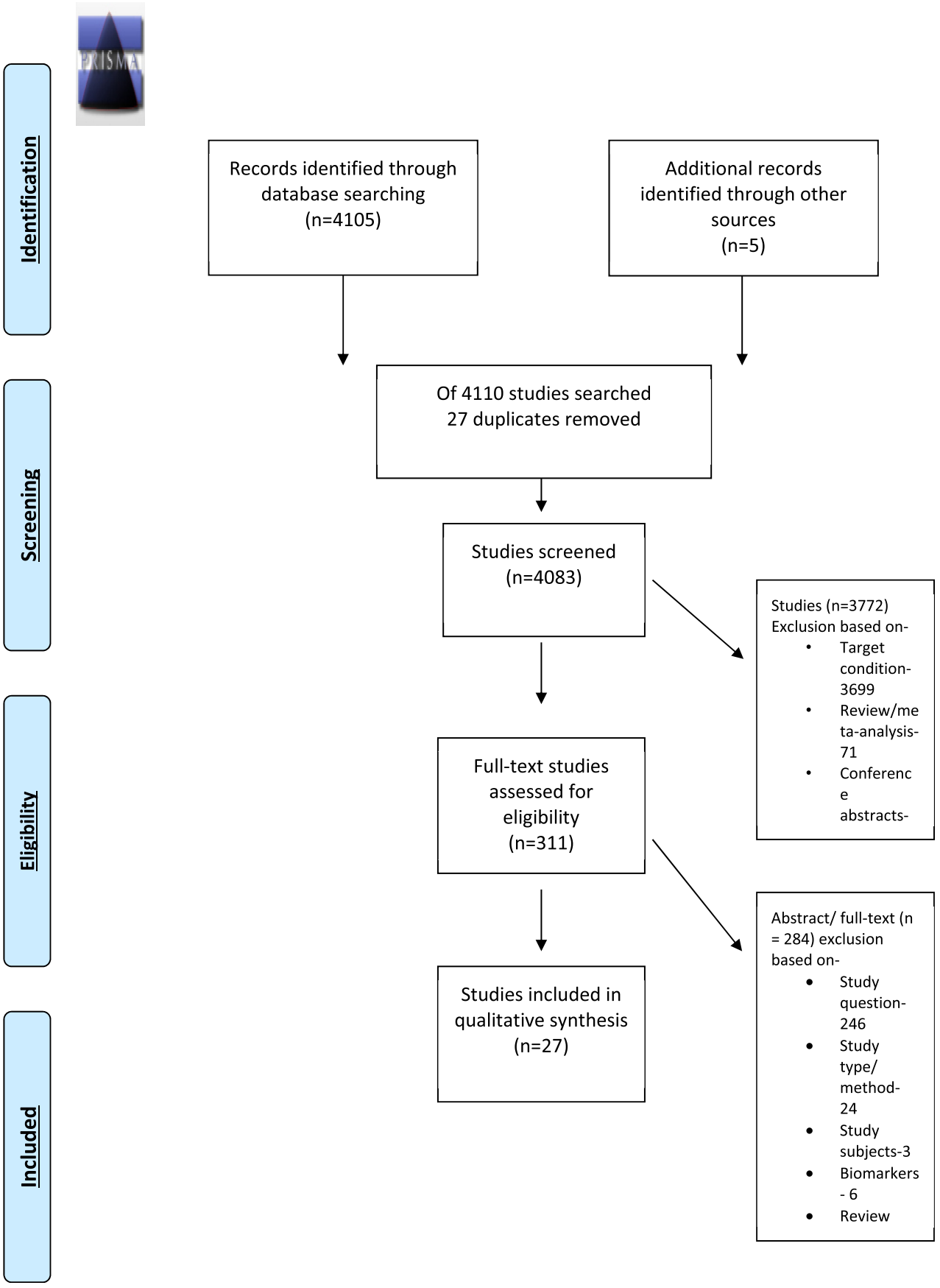

Identification
Screening
Eligibility
Included
Records identified through database searching (n=4105)
Additional records identified through other sources (n=5)
Of 4110 studies searched
27 duplicates removed
Studies screened (n=4083)
Studies (n=3772)
Exclusion based on-
Target condition-3699
Review/meta-analysis-71
Conference abstracts-
Full-text studies assessed for eligibility (n=311)
Abstract/ full-text (n = 284) exclusion based on-
Study question-246
Study type/ method-24
Study subjects-3
Biomarkers - 6
Review
Studies included in qualitative synthesis (n=27)

Fig. 1 – Prisma.

First Author (year)	Signalling question 1 (Patient selection)	Signalling question 2 (Index test)	Signalling question 3 (Reference Standard)	Signalling question 4 (Patient flow/timing)
1.Altet (2015)				
2.Maekura (2019)				
3.Chen (2016)				
4.Fletcher (2016)				
5.Fletcher, Filali-Mouhim (2016)				
6.Penn-Nicholson (2019)				
7.Duffy (2018)				
8.Rakotosamimanana (2015)				
9.Bapat (2015)				
10.Hussain (2011)				
11.Scriba 2017)				
12.Leong (2020)				
13.Zak 2016)				
14.Talat (2009)				

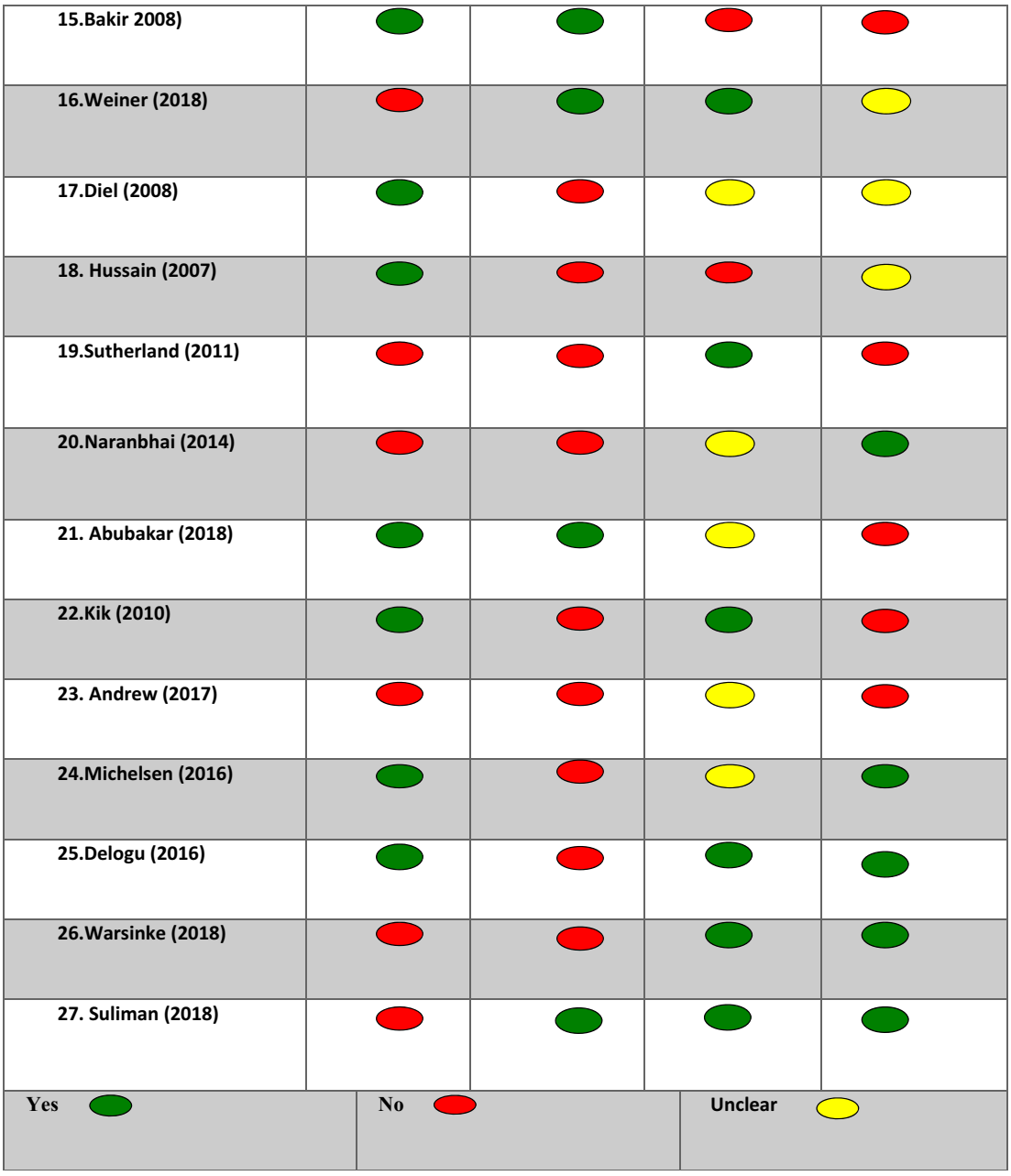


Fig. 2 – Assessment of Quality of studies (QUADASII).

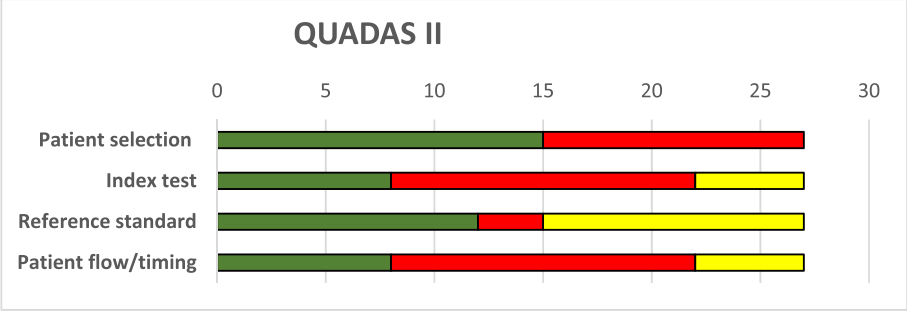


Fig. 3 – Overall depiction of risk of bias as per QUADAS II.

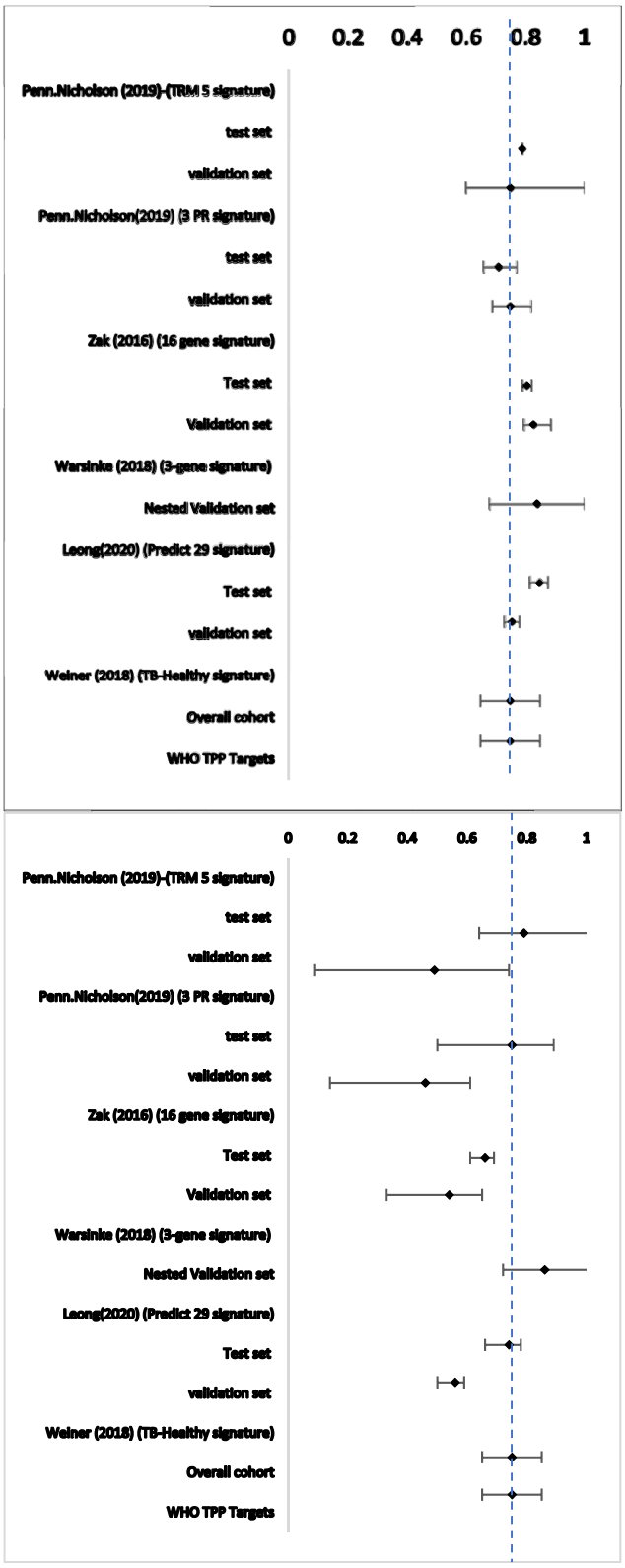


Fig. 4 – Sensitivity and Specificity for COR compared against WHO TPP Targets.

six had validation sets included. The studies included 16-gene, RISK-4, 3-gene, PREDICT-29 and c-miRNA signatures and predominantly included adolescent and adult cohort from South Africa. Two studies reported on the 16-gene signature.[32,35] A large sample size was observed in all studies, and the proportion who developed TB disease at the end of the study period ranged between 0.9% and 29%. 16 gene, 3-gene, PREDICT-29 reported sensitivities and specificities of 66%, 86%, 74% and 81%, 84%, 85% respectively in the test sets. However, in the validation sets, 16-gene and PREDICT-29 signatures reported sensitivities and specificities of 54%, 56% and 83%, 76% respectively. RISK 4 signature and the c-miRNA studies reported AUC of 0.7 (95% CI-0.58–0.82) and 0.67 (95CI-0.57–0.77) respectively.

3.3.7. *vii) Metabolite based*

A case–control study on 4462 adolescents and adults in the sub-Saharan African region, reported a sensitivity-50%, specificity- 75%, AUC- 0.68 (95%CI-0.64–0.73) on TB-Healthy signature.[33] The proportion of those who developed TB disease was 3%.

3.4. *Performance of COR in high-risk groups*

Twelve studies included high risk groups-ten studies on infants and children, one on HIV infected adults, one on HIV exposed and un-infected infants, one on malnourished adolescents and adults and one on Rheumatoid Arthritis (RA) adults. A lack of correlation (not statistically significant) of IFN against Heparin binding haemagglutinin antigen (HBHA) in HIV + ve adults was observed. A higher neopterin levels in RA patients who developed TB was observed (median –24.5pg/ml and 23053pg/ml respectively) and non-RA TB patients (12.2pg/ml and 9633pg/ml respectively) compared with QFT-Gold converters without TB (3pg/ml and 2720pg/ml respectively both $p < 0.01$). Alpha-2 macroglobulin, sero-transferrin, haptoglobin levels in malnourished endemic population demonstrated a two-fold increase in levels, however the number/s followed up were negligible.

3.5. *COR and WHO TPP targets*

WHO and FIND set an optimal TPP for a TB predictive test with a sensitivity and specificity of ≥90% or a minimum of ≥75% (within 2-years of exposure) and a high PPV with low NNT. Outcomes of five included studies could be compared against the WHO TPP targets (cytokine signature-3PR, TRM-5,[29] transcriptomic signatures- 16-gene,[32] 3-gene,[35] PREDICT-29 [14] and metabolomic signature-TB-healthy[33]) The performance of cytokine signatures as COR were comparable in the test sets but fell short in the validation sets. Similarly, the performance of 16-gene signature and PREDICT29 signature fell short of comparison in the validation sets. The 3-gene transcriptomic signature showed consistency in its performance in the nested cohort (validation set) and was comparable to the targets set for a TB predictive test (Fig. 4).

4. Discussion

We conducted a systematic review on blood-based, host-derived COR for TB disease in children and adults in accordance with PRISMA framework. We intended to analyze the

outcomes reported by studies on TB COR and compare their performance against the WHO/FIND TPP targets. TST and IGRA are poor COR of TB disease progression. A meta-analysis reported a PPV of 2.4% for TST and 6.8% for IGRA, implying the number needed to treat (NNT) to prevent one case of TB disease to be as high as 67.3 for TST and 37.3 for IGRA.[24] Among the cytokine studies, IL-10 (anti-inflammatory) was analyzed in five studies that demonstrated poor co-relation to TB disease progression.[16,17,28,36,37] Complement components[29,37] and M:L ratio [26,28,38] demonstrated a good correlation to TB progression, however larger prospective studies in diverse populations are required. Activated T-cell as a COR can be confounded by several factors that include environmental triggers and childhood immunization and hence this is a poor predictor in children.[27] Studies on transcriptomic signatures have demonstrated the most encouraging results to date.[13,31,32,34–37] However, their predictive performance when validated in geographically diverse cohorts reported inconsistencies. In addition, their predictive performance improved closer towards TB disease diagnosis which reduces the usefulness for early detection of incident TB for targeted preventive TB treatment. A meta-analysis on eight transcriptomic signatures reported comparable results (at pre-test probability of 2%), PPV between 6.8 and 9.4% over 24 months and 11.2–14.4% over 3-months before TB disease diagnosis.[40] The recent CORTIS trial, a randomised open label study that tested the prognostic accuracy of RISK-11 signature (derived from Zak-16 signature) and efficacy of targeted TB preventive treatment reported that the signature performed well as a COR, however, the targeted preventive TB treatment in comparison to signature positives and negatives showed poor efficacy in preventing TB disease during the 15-month follow-up.[41] Though this study incorporated diverse population in South Africa, it raises a few questions on the accuracy of the signature as a predictor of TB disease, the targeted preventive TB treatment approach and tackling the distinct complexities in high and low TB endemic regions.

This review has some potential limitations. The restricted search terminologies might have resulted in a narrow search. Only pulmonary TB studies were included in the search terminology hence that excluded studies on extra-pulmonary TB. All blood-based biomarkers were included in this review, making this review very broad. However, this review consolidates information on most potential COR studied till date. A narrative analysis was entailed due to the heterogenous nature of the studies which highlights the need for a standardized reporting for future studies in this field. A large number of studies incorporated high-risk groups, however the outcomes cannot be extrapolated into clinical practice due to the relatively small sample sizes and even smaller proportion of those evolving into TB disease. This highlights the practical challenges in conducting large longitudinal observational studies.

5. Conclusions

A worldwide accelerated effort to derive at a blood-based, host-derived COR for TB disease has been observed in the last two decades. TB disease progression is marked by a cascade of events and the studies on cell markers, metabolomic and transcriptomic signatures identified in this review have shown good potential as COR. As future prospects, validation studies on combination of these COR (as a signature) would be ideal.[1]

Author's contribution

P.S, S.W and R.S envisaged the idea for the review and its purpose. P.S and P.P conducted the systematic literature search. P.S and R.R screened the studies as per PRISMA framework. P.S assessed the quality of the studies based on the QUADAS II framework overviewed by S.W and R.S. P.S and A.C wrote the manuscript text and prepared all figures and tables. All authors reviewed the manuscript.

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval and consent to participate

Not applicable.

Consent for publication

All authors have read the final manuscript and provided their consent for publication.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available in the supplementary file titled Studies and outcomes.

Conflicts of interest

The authors have none to declare.

Acknowledgements

We would like to thank Professor Andrew Pollard for taking time and reviewing this paper.

REFERENCES

1. WHO. *Tuberculosis [Internet]. World Health Organization*; 2020 (WHO). Available from: https://www.who.int/news-room/fact-sheets/detail/tuberculosis.
2. Grover S, Hasnain SE, Ehtesham NZ. *Mycobacterium Tuberculosis: Molecular Infection Biology, Pathogenesis, Diagnostics*

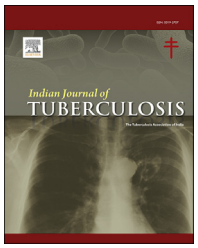
and New Interventions [Internet]. Singapore: Springer Singapore; 2019. Available from: http://link.springer.com/10.1007/978-981-32-9413-4.
3. Behr MA, Edelstein PH, Ramakrishnan L. *Revisiting the timetable of tuberculosis* 2018 Aug 23:362. k2738. Available from: https://doi.org/10.1136/bmj.k2738.
4. Bustamante J, Boisson-Dupuis S, Abel L, Casanova J-L. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-γ immunity. *Semin Immunol*. 2014 Dec;26(6):454–470. Available from: https://search.datacite.org/works/10.1016/j.smim.2014.09.008.
5. Brent AJ, Mugo D, Musyimi R, et al. Bacteriological diagnosis of childhood TB: a prospective observational study. *Sci Rep*. 2017 Sep 18;7(1):11808–11809. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28924198.
6. Detjen AK, DiNardo AR, Leyden J, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. *Lancet Respir Med*. 2015 Jun;3(6):451–461. Available from: https://search.datacite.org/works/10.1016/s2213-2600(15)00095-8.
7. Donovan J, Thu DDA, Phu NH, et al. Xpert MTB/RIF Ultra versus Xpert MTB/RIF for the diagnosis of tuberculous meningitis: a prospective, randomised, diagnostic accuracy study. *Lancet Infect Dis*. 2020 Mar;20(3):299–307. Available from: https://doi.org/10.1016/S1473-3099(19)30649-8.
8. Atherton RR, Cresswell FV, Ellis J, Kitaka SB, Boulware DR. *Xpert MTB/RIF Ultra for Tuberculosis Testing in Children: A Mini-Review and Commentary*. 2019 Feb, 1;7. Available from:: https://explore.openaire.eu/search/publication?articleId=od 267: d1122b750442b058954f4e8c7fc4d878.
9. [Internet]. *WHO. Consensus Meeting Report: Development of a Target Product Profile (TPP) and a Framework for Evaluation for a Test for Predicting Progression from Tuberculosis Infection to Active Disease*. Geneva: World Health Organization; 2017. Available from: https://apps.who.int/iris/handle/10665/259176.
10. MacLean E, Broger T, Yerlikaya S, Fernandez-Carballo BL, Pai M, Denkinger CM. A systematic review of biomarkers to detect active tuberculosis. *Nat Microbiol*. 2019 Feb 25;4(5):748–758. Available from: https://search.datacite.org/works/10.1038/s41564-019-0380-2.
11. Togun TO, MacLean E, Kampmann B, Pai M. Biomarkers for diagnosis of childhood tuberculosis: a systematic review. *PLoS One*. 2018 Sep 13;13(9), e0204029. Available from: https://search.datacite.org/works/10.1371/journal.pone.0204029.
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Reprint—preferred reporting Items for systematic reviews and meta-analyses. *The PRISMA Statement*. 2009 Sep 1;89(9):873–880. Available from: https://search.datacite.org/works/10.1093/ptj/89.9.873.
13. Leong S, Zhao Y, Ribeiro-Rodrigues R, et al. Cross-validation of existing signatures and derivation of a novel 29-gene transcriptomic signature predictive of progression to TB in a Brazilian cohort of household contacts of pulmonary TB. *Tuberculosis*. 2020 Jan;120:101898. Available from: https://doi.org/10.1016/j.tube.2020.101898.
14. Bapat PR, Satav AR, Husain AA, et al. Differential levels of alpha-2-macroglobulin, haptoglobin and sero-transferrin as adjunct markers for TB diagnosis and disease progression in the malnourished tribal population of melghat. India *PLoS One*. 2015 Aug 4;10(8), e0133928. Available from: https://search.datacite.org/works/10.1371/journal.pone.0133928.
15. Hussain R, Talat N, Ansari A, Shahid F, Hasan Z, Dawood G. Endogenously activated interleukin-4 differentiates disease progressors and non-progressors in tuberculosis susceptible families: a 2-year biomarkers follow-up study. *J Clin Immunol*. 2011 Oct;31(5):913–923. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21755390.
16. Talat N, Shahid F, Dawood G, Hussain R. Dynamic changes in biomarker profiles associated with clinical and subclinical tuberculosis in a high transmission setting. *A Four-Year Follow-Up Study*. 2009 Jun;69(6):537–546. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-3083.2009.02250.x.
17. Hussain R, Talat N, Shahid F, Dawood G. Longitudinal tracking of cytokines after acute exposure to tuberculosis: association of distinct cytokine patterns with protection and disease development. *Clin Vaccine Immunol*. 2007 Dec 1;14(12):1578–1586. Available from: http://cvi.asm.org/content/14/12/1578.abstract.
18. Delogu G, Vanini V, Cuzzi G, et al. Lack of response to HBHA in HIV-infected patients with latent tuberculosis infection. *Scand J Immunol*. 2016 Dec;84(6):344–352. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/sji.12493.
19. Kik SV, Franken WPJ, Mensen M, et al. Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. *Eur Respir J*. 2010;35(6):1346–1353. Available from: https://www.narcis.nl/publication/RecordID/oai:pure.amc.nl:publications%2F266bb5c8-4782-410c-b5f9-2e0dc98afedc.
20. Abubakar I, Drobniewski F, Southern J, et al. Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. *Lancet Infect Dis*. 2018 Jan 1;18(10):1077–1087. Available from: https://explore.openaire.eu/search/publication?articleId=od267, 76e61c599a1a3b8691077f65110ded02.
21. Maekura R, Kitada S, Osada-Oka M, et al. Serum antibody profiles in individuals with latent Mycobacterium tuberculosis infection. *Microbiol Immunol*. 2019 Mar;63(3–4):130–138. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/1348-0421.12674.
22. Altet N, Dominguez J, Souza-Galvão M-L de, et al. Predicting the development of tuberculosis with the tuberculin skin test and QuantiFERON testing. *Ann Am Thorac Soc*. 2015 May;12(5):680–688. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25699406.
23. Wilk M Sascha, Bolette S, Else Marie A, et al. Host immunity to Mycobacterium tuberculosis and risk of tuberculosis: a longitudinal study among Greenlanders. *Vaccine*. 2016;34(48):5975–5983. Available from: https://www.clinicalkey.es/playcontent/1-s2.0-S0264410X16308726.
24. Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive value of a whole blood IFN-γ assay for the development of active tuberculosis disease after recent infection with. *Mycobacterium Tuberculosis*. 2008 May 15;177(10):1164–1170. Available from: https://search.datacite.org/works/10.1164/rccm.200711-1613oc.
25. Chen D-Y, Li J-P, Chen Y-M, et al. Elevated neopterin levels are associated with increased tuberculosis risk in rheumatoid arthritis patients with QuantiFERON conversion during biologic therapy. *PLoS One*. 2016;11(11), e0166301. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27861525.
26. Rakotosamimanana N, Richard V, Raharimanga V, et al. Biomarkers for risk of developing active tuberculosis in contacts of TB patients: a prospective cohort study. *Eur Respir J*. 2015 Oct;46(4):1095–1103. Available from: https://search.datacite.org/works/10.1183/13993003.00263-2015.
27. Fletcher HA, Snowden MA, Landry B, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. *Nat Commun*. 2016 Apr 12;7(1):11290. Available from: https://search.datacite.org/works/10.1038/ncomms11290.
28. Fletcher HA, Filali-Mouhim A, Nemes E, et al. Human newborn bacille Calmette–Guérin vaccination and risk of tuberculosis disease: a case-control study. *BMC Med*. 2016 May 16;14(1):76. Available from: https://search.datacite.org/works/10.1186/s12916-016-0617-3.

29. Penn-Nicholson A, Hraha T, Thompson EG, et al. Discovery and validation of a prognostic proteomic signature for tuberculosis progression: a prospective cohort study. *PLoS One*. 2019 Apr 16;16(4), e1002781. Available from: https://search.datacite.org/works/10.1371/journal.pmed.1002781.
30. Bakir M, Millington KA, Soysal A, et al. Prognostic value of a T-cell-based interferon-gamma biomarker in child tuberculosis contacts. *Ann Intern Med*. 2008;149:777–787. Available from: https://explore.openaire.eu/search/publication?articleId=od.267::5726805d0c59792616a3d6bd9dcea8c0.
31. Duffy FJ, Thompson E, Downing K, et al. *A Serum Circulating miRNA Signature for Short-Term Risk of Progression to Active Tuberculosis Among Household Contacts*. vol. 9. 2018:661. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29706954.
32. Zak DE, Penn-Nicholson A, Scriba TJ, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet*. 2016 Jun;387(10035):2312–2322. Available from: https://search.datacite.org/works/10.1016/s0140-6736(15)01316-1.
33. Weiner J, Maertzdorf J, Sutherland JS, et al. Metabolite changes in blood predict the onset of tuberculosis. *Nat Commun*. 2018 Dec 6;9(1):5208–5212. Available from: https://search.datacite.org/works/10.1038/s41467-018-07635-7.
34. Suliman S, Thompson EG, Sutherland J, et al. Four-gene pan-african blood signature predicts progression to tuberculosis. *Am J Respir Crit Care Med*. 2018 May;197(9):1198–1208. Available from: https://search.datacite.org/works/10.1164/rccm.201711-2340oc.
35. Warsinske HC, Rao AM, Moreira FMF, et al. Assessment of validity of a blood-based 3-gene signature score for progression and diagnosis of tuberculosis, disease severity, and treatment response. *JAMA Netw Open*. 2018 Oct 5;1(6), e183779. Available from: https://doi.org/10.1001/jamanetworkopen.2018.3779.
36. Sutherland JS, Hill PC, Adetifa IM, et al. Identification of probable early-onset biomarkers for tuberculosis disease progression. *PLoS One*. 2011;6(9), e25230. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21966464.
37. Scriba TJ, Penn-Nicholson A, Shankar S, et al. Sequential inflammatory processes define human progression from M. tuberculosis infection to tuberculosis disease. *PLoS Pathog*. 2017 Nov 1;13(11). Available from: https://explore.openaire.eu/search/publication?articleId=od267::5837b114ee8f37fc9da2b9be9ac8d85e.
38. Naranbhai V, Kim S, Fletcher H, et al. The association between the ratio of monocytes:lymphocytes at age 3 months and risk of tuberculosis (TB) in the first two years of life. *BMC Med*. 2014 Jul 17;12(1):120. Available from: https://search.datacite.org/works/10.1186/s12916-014-0120-7.
39. Andrews JR, Nemes E, Tameris M, et al. *Serial QuantiFERON Testing and Tuberculosis Disease Risk Among Young Children: An Observational Cohort Study*. vol. 5. 2017:282–290. https://doi.org/10.1016/S2213-2600(17)30060-7.
40. Gupta RK, Turner CT, Venturini C, et al. *Concise Whole Blood Transcriptional Signatures for Incipient Tuberculosis: A Systematic Review and Patient-Level Pooled Meta-Analysis*. vol. 8. 2020:395–406. https://doi.org/10.1016/s2213-2600(19)30282-6.
41. Scriba TJ, Fiore-Gartland A, Borate B, et al. Biomarker-guided tuberculosis preventive therapy (CORTIS): a randomised controlled trial. *Lancet Infect Dis*. 2021 Mar;21(3):354–365. Available from: https://doi.org/10.1016/S1473-3099(20)30914-2.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Quality of life and associating factors in pulmonary tuberculosis patients

Richard Samuel [a], *Senthilkumar Natesan* [b], *Madhu Keshava Bangera* [c,*]

[a] *National Health Mission, Government of Tamil Nadu, Chennai 600006, Tamil Nadu, India*
[b] *Indian Institute Public Health Gandhinagar, Gandhinagar 382042, Gujarat, India*
[c] *Vivid Dental Care, Pandeshwar 575001, Mangalore, India*

ARTICLE INFO

Article history:
Received 21 April 2022
Received in revised form
25 April 2022
Accepted 17 May 2022
Available online 22 May 2022

Keywords:
Tuberculosis
Quality of life
WHOQOL-BREF
Vellore
India

ABSTRACT

Background: Quality of life is a significant issue among patients with tuberculosis and is used for evaluating treatment responses and therapeutic outcome. This study aimed to assess the quality of life in tuberculosis patients receiving anti-tuberculosis therapy for a short duration in the Vellore district of Tamil Nadu and its associated variables.

Methods: A cross-sectional study was designed to evaluate pulmonary tuberculosis patients receiving treatment under category –1 registered in the NIKSHAY portal at Vellore. A total of 165 pulmonary tuberculosis patients were recruited from March 2021 to the third week of June 2021. On obtaining informed consent, the data were collected through the telephone interview by administering WHOQOL- BREF structured questionnaire. The data were examined with descriptive and analytical statistics. Multiple regression analysis for independent quality of life variables was done.

Results: The lowest median scores, 31(25,38) & 38(25,44) was, related to psychological and environmental domains, respectively. In addition, the Man-Whitney & Kruskal Wallis showed a statistically significant variation in the mean quality of life for gender, employment status, duration of treatment, persistent symptoms, the location of residence of patients, and the stage of therapy. Age, gender, marital status, and persistent symptoms were the main associating factor.

Conclusion: Tuberculosis and its treatment influence psychological, physical functioning, and the environmental domain of patient quality of life. Attention is required in the follow-up and treatment of patients by monitoring their quality of life.

1. Introduction

Tuberculosis (TB) remains a significant health issue that contributes to one of the Top 10 major health problems that have dominated the history of human civilization for thousands of years.[1] Pulmonary tuberculosis, which accounts for 78% of TB types, is considered predominately high compared to other types of TB in India. The microbiologically confirmed

* *Corresponding author*. Vivid Dental Care, Pandeshwar 575001, Mangalore.
E-mail address: madhu.bangera@gmail.com (M.K. Bangera).

https://doi.org/10.1016/j.ijtb.2022.05.005

prevalence of pulmonary TB was 24.5–1518 per 100,000 population and the pooled one was 295.9 per 100,000 population. The prevalence of pulmonary tuberculosis was higher among rural male residents and increased with age.[2,3] The milestone for 2020, with a 20% and 35% reduction in TB incidence and death respectively from 2015, was not on track. The global target with the goal of 'End the TB epidemic and target TB strategy' is to achieve an 80% reduction in the TB incidence. Fewer than 80 cases for one lakh population per year by 2030 is a dubious question for developing countries like India as they cannot contain this disease.[4,5] Further with involvement of the COVID-19 pandemic threatens to deviate the reduction in the pulmonary TB cases in the country. Hence it is a big challenge for the government of India that has aimed to have TB-free India by 2025, five years ahead of the global target, 2030.[5,6]

NTEP (National Tuberculosis Elimination Program), previously known as RNTCP (Revised National Tuberculosis Control Programme) in India, provides a strategy to diagnose, treat and support TB patients. Although this program provides the best strategy in diagnosis and treatment, advanced information and communication technology-based tracking, treatment adherence, and direct benefit transfer for TB patients, only minimum attention is paid to other dimensions of health such as psychological support, social stigma, and environmental domain.[7,8] Evidence says that many psychological, social, and environmental domain problems are faced by TB patients.[7] It is a well-known fact that TB disease in developing countries like India is attached with a social stigma.[9] Therefore, all of these domains and the disease management determine the patient's quality of life (QOL). There occur differences in the QOL between different socio-demographic groups.[10,11,12] There were many factors associated with QOL, such as socio-economic, demographic, unhealthy behaviors, clinical history of the disease, etc.[13,14,15] The contribution of these factors can exceed the medical illnesses of this disease.[16] Therefore, it is essential to consider the QOL and the factor associated with QOL among PTB cases. Hence that will lead to better compliance, adherence, and completion of treatment, and thus the cure rate will increase.[8]

For a long time, the evaluation of the QOL among TB patients has been a neglected aspect in the Indian context. Many studies are confined only to medical outcomes with TB cases. A systematic review in 2004 reported that only 60 articles addressed the health-related QOL among TB cases.[17] Based on the literature survey, this would be the first study done in south India with the World Health Organization Quality of Life Instrument (WHOQOL-BREF) assessment tool to evaluate the QOL among Pulmonary TB patients receiving treatment in Vellore district of Tamil Nādu from South- India.

The present study aimed to quantify QOL and determine its associating factors among pulmonary tuberculosis (PTB) patients under category-1 receiving treatment in the Vellore district of Tamil Nādu, India. The study was designed to evaluate the QOL among the PTB patients receiving treatment in the Vellore district of Tamil Nadu and compare mean well-being scores between different socio-demographic groups of PTB patients under treatment. Additionally, the study determined the factors affecting the QOL among PTB patients under treatment.

2. Methods

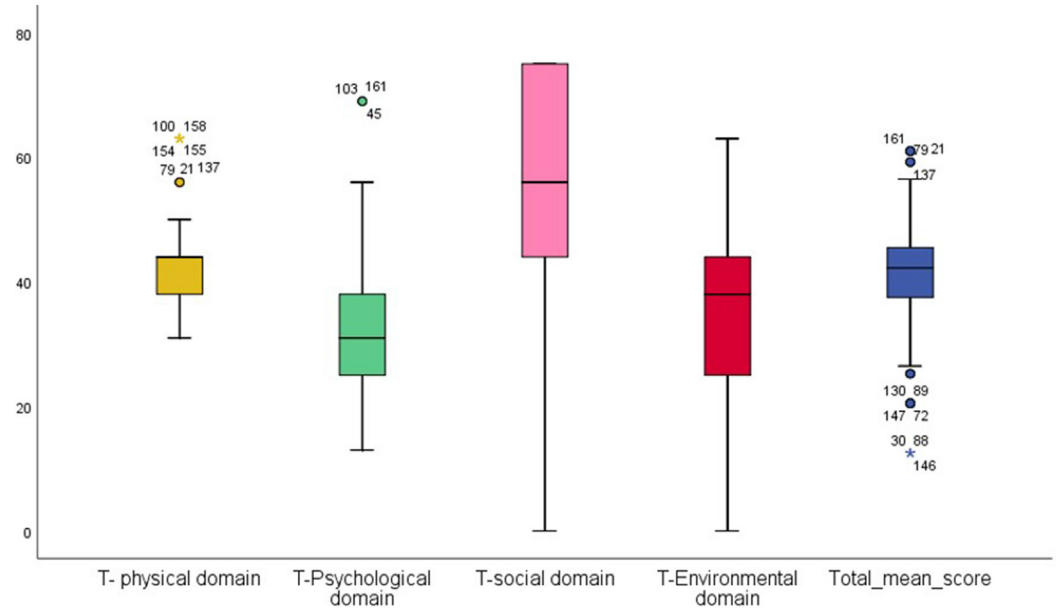
A cross-sectional study was designed in which the PTB patients receiving treatment in the Vellore district of Tamil Nādu served as study participants. Patients with PTB (microscopically or culture-confirmed or clinical confirmed with histopathological findings, x-ray findings, or miliary TB), new TB case (category 1), under DST-treatment, above 18 years of age who provided informed consent, who are fluent in Tamil and English language and under more than two months of treatment were included in the study. Patients with only extrapulmonary TB, all DR-TB cases (MDR-TB, XDR-TB, mono-resistant TB, poly-resistant), previously treated/recurrent TB and treatment change (category- 2 and 3), affected by COVID-19 within six weeks, HIV patients, children, pregnant women, defaulted, died, or refused treatment, and under intravenous venous anti-TB drug administration were excluded.

The sample size was calculated using the sample size equation in OpenEpi software, using a 95% confidence level, a design effect of 1.0 for random sampling, and set at $n = 164$ samples. The design involved two-stage sampling, convenience sampling at the initial stage and random sample at a later stage. A simple sampling was done using a computer-generated random number to select the PTB patient receiving treatment. Sampling frames include all the tuberculosis patients from March 2021 to the 3rd week of June 2021 in the Nikshay -portal maintained by the district tuberculosis center in Vellore district in Tamil-Nadu. Data was collected from the PTB patient receiving treatment from 1st March 2021-3rd week of June 2021 using Epi-collect 3.0 version software. On obtaining the patient's verbal consent, a telephonic interview was undertaken. The type of house, urban or rural setting (residence), education, employment, income, smoking, alcohol, comorbidity, and persistent symptoms were collected. An interviewer-administered semi-structured questionnaire using section A -socio-demographic profile and section B-WHOQOL-BREF (abbreviated version of the WHOQoL-100) questions, validated and piloted tested in Chennai and New Delhi, available both in English and Tamil language from the WHO portal was used. Demography factors, socio-economic factors, the median duration of treatment, comorbidity, persistent symptoms, smoking, and alcohol consumption served as independent variables. QOL (mean score for each domain and total mean score) served as the dependent variable. The statistical analysis was performed in SPSS (Version 19) software at a 5% significance level, and a P-value < 0.05 was considered statistically significant.

3. Results

3.1. *Characteristics of the study population*

All categorical variables in the study, like gender, religion, residence, etc., were summarised as frequency and



Box-plot graph displays the median score of Physical, psychological, social,environmental domain and total median score of pulmonary tuberculosis patient under category -1 receiving treatment in Vellore district of Tamil-Nadu, south India (WHOQOL-BREF scores)

Fig. 1 – Median score of physical, psychological, social, environmental domain and total median score of pulmonary tuberculosis patient under category –1 receiving treatment in Vellore district of Tamil-Nadu, south India (WHOQOL-BREF scores).

percentage. Age was summarized as mean and standard deviation. In contrast, the outcome variables of physical, psychological, social, and environmental domains were summarised as the median and interquartile range (IQR) as they were not normally distributed. The total mean score of all the domains of physical, psychological, social, and environmental factors was estimated by taking the average of all the scores. 78.2% were male and 21.8% were female participants. 95.8% were Hindus, 3.0% were Christians, and 1.2% were Muslim participants. 62.4% belonged to the rural set up and the remaining 37.6% to the urban household. 41.2% were housed in a kutcha and 58.8% in pucca household set up. 87.3% were married, 9.7% were single, whereas 3.0% were widowed. 27.3% were illiterate and 72.7% were literate, out of which 64.2% were unemployed. 57.6% were treated for three months, 10.9% for four months, 21.2% for five months, and 10.3% for six months. The socio-Economic status as per B.G Prasad classification 29.7% belonged to Class 1, 24.8% belonged to Class 2, 15.8% belonged to Class 3, 13.3% belonged to Class 4, and 16.4% to Class 5.66.1% were non-smokers and 49.7% non-alcoholic. 71.5% presented with comorbidities and 57% presented with persistent symptoms.

3.2. *Validity and reliability*

The Spearman correlation was used to analyze the strength measures between the variables, ranging from 0.711 to 1.286. Cronbach's alpha was used to measure the reliability or internal consistency, ranging from 0.724 to 0.916.

3.3. *Quality of life (QOL) score*

The median score for physical, psychological, social, and environmental domains and the total median score of PTB patients under category –1 receiving treatment are shown in Fig. 1. The psychological domain's median score was lowest compared to other domain median scores and the median score of the social domain was comparatively higher among other domain scores. Second-most affected domain was environmental.

3.4. *Comparison of QOL score between socio-demographic and associated factors*

The comparison of median scores of physical, psychological, social, environmental, and total mean scores domains between gender, type of house, residence, education, marital status, occupation, smoking status, alcohol status, comorbidities, and persistent symptoms was carried out using Mann Whitney *U* test as shown in Table 1. The comparison of median scores of physical, psychological, social, environmental, and total mean scores domains between duration of symptoms and socio-economic status (according to B.G Prasad classification 2019) was performed using the Kruskal Wallis

Table 1 – Comparison of domain score and total score between socio-demographic and socio-economic groups under category –1 treatment.

Outcome Variables	Categorical baseline variable- Age		P-Value
	18–46 median (Q1, Q3)	47–75 median (Q1, Q3)	
Physical	44 (38,44)	38 (38,44)	0.630
Psychological	31 (25,38)	31 (25,31)	0.381
Social	53 (34,75)	69 (44,75)	0.688
Environmental	38 (31,50)	31 (25,44)	0.051
Total Median score	42.25 (39,46.62)	42.25 (36,45.50)	0.879
Outcome Variables	**Categorical baseline variable- Gender**		**P-Value**
	Male median (Q1, Q3)	Female median (Q1, Q3)	
Physical	44 (38,44)	38 (31,44)	0.000[a]
Psychological	31 (25,38)	25 (25,31)	0.021
Social	69 (44,75)	50 (34,73)	0.245
Environmental	38 (31,47)	28 (25,42)	0.011
Total Median score	42.25 (37.50,47)	40.75 (31.5,42.43)	0.015
Outcome Variables	**Categorical baseline variable-Residence**		**P-Value**
	Rural median (Q1, Q3)	Urban median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.253
Psychological	25 (25,38)	31 (31,38)	0.027
Social	56 (44,75)	69 (31,75)	0.614
Environmental	31 (25,44)	44 (25,50)	0.149
Total Median score	42.25 (37.50,48.50)	42.37 (37.50,48.50)	0.190
Outcome Variables	**Categorical baseline variable-House type**		**P-Value**
	Kutcha median (Q1, Q3)	Pucca median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.639
Psychological	31 (26,38)	25 (25,31)	0.022
Social	50 (34,75)	69 (50,75)	0.247
Environmental	44 (38,50)	31 (25,44)	0.000[a]
Total Median score	42.37 (37.75,47)	42.25 (36.75,45.50)	0.264
Outcome Variables	**Categorical baseline variable-Marital status**		**P-Value**
	Married median (Q1, Q3)	Single/widowed median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.568
Psychological	31 (25,38)	31 (19,38)	0.876
Social	69 (50,75)	25 (0,28)	0.000[a]
Environmental	38 (25,44)	44 (31,56)	0.009[a]
Total Median score	42.25 (39,47)	36 (28.5,40.87)	0.000[a]
Outcome Variables	**Categorical baseline variable-Education**		**P-Value**
	Illiterate median (Q1, Q3)	Literate median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.695
Psychological	31 (25,38)	31 (25,38)	0.490
Social	50 (40,75)	69 (44,75)	0.698
Environmental	38 (25,44)	38 (25,50)	0.426
Total Median score	42.25 (36.75,44.62)	42.25 (37.56,47)	0.263
Outcome Variables	**Categorical baseline variable -Employment**		**P-Value**
	Unemployed median (Q1, Q3)	Employed median (Q1, Q3)	
Physical	44 (38,44)	44 (38,50)	0.000[a]
Psychological	31 (25,31)	31 (25,38)	0.032
Social	62 (44,75)	50 (25,75)	0.802
Environmental	31 (25,44)	31 (31,50)	0.000[a]
Total Median score	42.25 (37.50,45.25)	42.25 (36,53.5)	0.078
Outcome Variables	**Categorical baseline variable-Smoking**		**P-Value**
	Smoking median (Q1, Q3)	Not smoking median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.402
Psychological	31 (25,38)	31 (25,38)	0.712
Social	75 (50,75)	50 (37,75)	0.022
Environmental	31 (25,44)	38 (28,50)	0.057
Total Median score	42.25 (37.50,47)	43.75 (37.50,45.50)	0.396

Outcome Variables	Categorical baseline variable-Alcohol		P-Value
	Drink Alcohol median (Q1, Q3)	No alcohol median (Q1, Q2)	
Physical	37 (36,44)	44 (38,44)	0.034
Psychological	31 (25,38)	31 (25,32)	0.093
Social	69 (50,75)	50 (31,75)	0.010
Environmental	38 (31,50)	38 (25,44)	0.437
Total Median score	40.75 (36,42.50)	43.75 (37.75,48.50)	0.003[a]
Outcome Variables	**Categorical baseline variable- comorbidity**		**P-Value**
	Comorbidity present Median (Q1, Q3)	Comorbidity absent Median (Q1, Q3)	
Physical	44 (38,44)	44 (38,44)	0.216
Psychological	31 (25,38)	31 (25,38)	0.870
Social	56 (50,75)	69 (25,75)	0.294
Environmental	38 (25,44)	38 (31,44)	0.304
Total Median score	42.25 (31.50,47)	42.25 (37.50,45.50)	0.938
Outcome variables	**Categorical baseline variable-persistent symptoms**		**P-Value**
	Persistent symptoms median (Q1, Q3)	No persistent symptoms median (Q1, Q3)	
Physical	38 (38,44)	44 (44,50)	0.000[a]
Psychological	31 (25,38)	31 (25,38)	0.104
Social	50 (44,75)	69 (44,75)	0.054
Environmental	38 (25,44)	38 (25,50)	0.172
Total Median score	42.25 (37.75,48.50)	42.25 (36,45.50)	0.088
Outcome variables	**Categorical baseline variable- Duration of treatment**		**P-Value**
	3–4 months median (Q1, Q3)	5–6 months median (Q1, Q3)	
Physical	44 (38,44)	44 (44,50)	0.001[a]
Psychological	31 (25,38)	31 (25,38)	0.429
Social	50 (50,75)	69 (26,75)	0.317
Environmental	38 (25,44)	44 (31,56)	0.000[a]
Total Median score	42.25 (37.50,45.25)	44.50 (39,50)	0.16

[a] Significant association if $p < 0.05$.

test and no significant association was found between any categories based on the p-value.

3.5. *Factors associated with QOL score*

Multiple linear regression was performed for each outcome variable to assess the multifactorial measure of the relationship of the baseline characteristics to the outcome. The adjusted regression coefficient, 95% confidence interval of the regression coefficient, and the adjusted R square of each regression model were reported (Table 2).

4. Discussion

The validity and reliability of this study using the WHOQOL-BREF study tool are higher than the SF-36 item study tool.[18] The present study reports that QOL scores were very low compared to previous reports among PTB patients receiving treatment in India and elsewhere.[19,20] A study reported domain scores of 45.3 for physical, 49.3 for a psychological domain, 59 for social domain, and 50.5 for environmental domain among PTB patients receiving treatment.[20] A study conducted in China reported that the physical domain was the most affected one, while this study portrays the psychological domain as the most hampered in comparison to other domains with similar findings found in other QOL assessment studies in India.[21,22]

The physical domain was low among PTB patients receiving treatment, particularly in disease severity, persistent symptoms, duration of treatment, and employment status.[20] This study reports low scores among the female, unemployed, alcoholic patients, and those with persistent symptoms 3–4 months of treatment. It is likely that the decreased physical domain due to pathology and its associating factors, such as persistent symptoms and unemployment, irrespective of co-morbid condition. Evidence suggests that in developing countries like India, employment is directly linked to physical activity. A PTB individual with retained symptoms can affect the job status and unemployment, which will affect physical activity.[10,17] A review reported that PTB patients with symptoms that persist for a long time have refrained from the work, called TB induced unemployment; A culmination of disease, symptoms, weakness, fatigue, and loss of energy leads to unemployment followed by low socio-economic status. About twelve percent of the probability of relapse cases were seen among PTB cured patients with persistent symptoms, most of which occurred in the first six months after treatment.[18]

Psychological health was the most affected domain compared to other domains, and studies reported similar findings compared to our research.[10] We observed that the low score was particularly among women, villagers living in pucca house, and the unemployed. It has been seen that associating age, gender, house type, marital status, socio-economic class, and persistent symptoms played a role to a greater extent.

Table 2 – Multiple linear regression (multivariate analysis) shows factors associated with different domains and total scores among pulmonary tuberculosis patients under category –1 receiving treatment.

Domains	Variable	Regression coefficient (95% Confidence Interval)	t-statistic	P-value
Physical	**Age**	–0.043 (–0.145 -0.060)	–0.820	0.414
	Gender	1.843 (–1.974 – 5.659)	0.954	0.342
	Religion	1.520 (–2.689 -5.729)	0.714	0.476
	Residence	–0.573 (–3.003 – 1.857)	–0.466	0.642
	Duration of treatment	–0.057 (–1.380 – 1.266)	–0.085	0.932
	Type of house	0.815 (–1.616 – 3.246)	0.663	0.509
	Marital status	–2.216 (–6.001 – 1.569)	–1.157	0.249
	Employment status	5.761 (3.019–8.503)	4.151	0.000[a]
	Socioeconomic-B. G Prasad classification	–0.504 (–1.465 – 0.457)	–1.036	0.302
	Smoking	–0.438 (–3.445 – 2.569)	–0.288	0.774
	Alcohol	2.592 (–0.602 – 5.787)	1.603	0.111
	Comorbidity	–2.702 (–5.603 – 0.200)	–1.840	0.068
	Persistent symptoms	–3.126 (–5.876––0.376)	–2.246	0.026[a]
	Education	–0.473 (–3.306 – 2.360)	–0.330	0.742
Psychological	**Age**	–0.273 (–0.414––0.133)	–3.850	0.000[a]
	Gender	5.873 (0.663–11.084)	2.227	0.027[a]
	Religion	4.743 (–1.004 – 10.489)	1.631	0.105
	Residence	1.316 (–2.002 – 4.634)	0.784	0.434
	Duration of treatment	–1.448 (–3.255 – 0.358)	–1.584	0.115
	Type of house	–4.930 (–8.249––1.611)	–2.935	0.004[a]
	Marital status	–6.174 (–11.341––1.006)	–2.361	0.020[a]
	Employment status	1.004 (–2.740 – 4.747)	0.530	0.597
	Socioeconomic- B. G Prasad classification	–1.450 (–2.762––0.138)	–2.184	0.031[a]
	Smoking	–2.621 (–6.727 – 1.484)	–1.262	0.209
	Alcohol	1.691 (–2.671 – 6.052)	0.766	0.445
	Comorbidity	2.399 (–1.563 – 6.361)	1.197	0.233
	Persistent symptoms	–6.698 (–10.453––2.943)	–3.525	0.001[a]
	Education	2.304 (–1.564 – 6.172)	1.177	0.241
Social	**Age**	–0.415 (-0.673––0.158)	–3.190	0.002
	Gender	11.075 (1.521–20.628)	2.291	0.023
	Religion	–6.000 (–16.536-4.536)	–1.125	0.262
	Residence	–1.560 (–7.643 -4.524)	–0.507	0.613
	Duration of treatment	6.777 (3.465–10.089)	4.043	0.000
	Type of house	0.657 (–5.428 – 6.742)	0.213	0.831
	Marital status	–54.231 (–63.706––44.756)	–11.310	0.000
	Employment status	–6.107 (–12.971 - 0.757)	–1.758	0.081
	Socioeconomic- B.G Prasad classification	–1.987 (–4.392 – 0.419)	–1.632	0.105
	Smoking	–5.641 (–13.168 – 1.886)	–1.481	0.141
	Alcohol	6.566 (–1.431 – 14.563)	1.622	0.107
	Comorbidity	4.890 (–2.374 – 12.154)	1.330	0.186
	Persistent symptoms	–4.604 (–11.488-2.281)	–1.321	0.188
	Education	–1.530 (–8.621-5.562)	–0.426	0.671
Environmental	**Age**	–0.365 (-0.516––0.213)	–4.761	0.000[a]
	Gender	7.414 (1.792–13.036)	2.606	0.010[a]
	Religion	13.261 (7.061–19.461)	4.226	0.000[a]
	Residence	0.984 (–2.596 – 4.564)	0.543	0.588
	Duration of treatment	1.822 (–0.127 – 3.771)	1.847	0.067
	Type of house	–5.608 (–9.189––2.027)	–3.095	0.002[a]
	Marital status	–5.327 (–10.903 – 0.249)	–1.888	0.061
	Employment status	4.831 (0.792–8.870)	2.363	0.019[a]
	Socioeconomic- B.G prasad classification	–0.489 (–1.905 – 0.926)	–0.683	0.496
	Smoking	–7.201 (–11.630––2.771)	–3.212	0.002[a]
	Alcohol	1.805 (–2.900 – 6.511)	0.758	0.450
	Comorbidity	5.890 (1.615–10.164)	2.722	0.007[a]
	Persistent symptoms	–2.437 (–6.488 – 1.615)	–1.188	0.237
	Education	**–0.625 (-4.799–3.548)**	**–0.296**	0.768

[a] Significant association if $p < 0.05$.

While the study had similar findings compared to ours, the evidence suggests that the mental trauma incurred by female patients on account of their illness persists for a prolonged time.[18,20] Persistent symptoms, marital status affected the physiological domain, leading to depression and anxiety.[17,18] The present study's social domain scores were not as low as

the previous study among PTB patients receiving treatment. A study reported domain scores of 59.0 among drug sensitivity-PTB patients.[20] Overall, social domain scores appeared to be higher than other domains. In contrast to other domains, this was not affected by gender and persistent symptoms. With the similar findings from other studies, our study reports that single/widowed have a lower score as compared to married individuals, and the essential associating factor was marital status; as the evidence suggests that there were two different types of stigmas associated with TB are public discrimination, and the internal stigmatization that patients feel after contracting TB, especially in developing countries like India TB associated with stigma both from family and public which in turn leads to isolation of people with TB but the support provided by a spouse, partners, family, friends, health-care professionals had deviated from the negative influence on the social domain among PTB patients.[18,20] Furthermore in this present study, age, gender, duration of treatment in a month have been associating with social domain while an Indian study reports the strong association found on similar independent factors with the addition of residence and socio-economic status.[8,20]

The environmental domain was the second primarily affected domain with the lowest score, comparatively lower than other Indian studies.[12,20] While a study reported lower score was observed among the female individual who lives in a rural pucca house, are married, have Hindu religion and have low economic status. Similar findings account for this present study among PTB patients receiving treatment in the Vellore district.[20] The factors that influence this domain include age, gender, religion, house type, employment status, smoking, and comorbidity, similar to the environmental domain score in the Indian study.[12,20] Evidence suggests that external stigmatization along with low resources environment affect the environmental domain, especially in individuals living in pucca house and with lower socio-economic status.[20] Employment plays a vital role in determining the workplace environment and living environment; thus, it impacts the QOL.[21] And other factors of contribution were not apparent. A study from Baltimore showed that the root cause of low socio-economic status is the out-of-pocket expenditure incurred by TB patients. At the same time, our study also strongly supports this issue. Most of the respondents in our study have quit jobs due to their health status or social stigma, which were similar to the findings of the other studies.[23]

Though diabetes occupies a major proportion of the comorbidity of PTB, comorbidity is only associated with the environment domain, which is similar to a study.[15] With similar findings from Asian and African studies, this research also reports on the significant prevalence of PTB cases among the male gender.[11] In contrast to the survey in African studies, females had low scores in all the domains, similar to Muniyandi et al study, which evaluated QOL using the SF-36 questionnaire.[18] Evidence suggests the prevalence of patriarchal society and very few coping skills of the feminine gender towards the peer pressure and challenges that incurred every day would determine the social and psychological aspects of quality of health.[11]

There occurs a difference in the duration of treatment in a month; during the initial phase (3–4 months) of treatment, the domain score was lower when compared to 5–6 months of treatment; a similar finding was observed earlier.[20] This also leads to subside symptoms throughout treatment, with similar results found in another study.[24] WHOQOL-BREF study tool consists of high validity and reliability compared to the SF-36 study tool used by Rajeswari et al, 1999. In contrast to other studies, there were no significant differences in this study; about more than half were from rural incurred PTB, while Rajeswari et al, 1999 reported 75% of urban patients.[24] This study strongly supports that TB induces unemployment, while studies from Asia and Africa reported the same.

However, the need of the hour would be the psychometric assessment tool designed explicitly for TB patients.[18,20] Hence it is necessary to bring psychometric assessment throughout treatment for PTB patients. A comprehensive approach is much needed in the context of the TB arena. The main strengths of our study lie in using the WHOQOL-BREF study tool, which is validated and reliable compared to other QOL evaluation tools.[20] This study reports its finding based on community. This study was conducted in the Vellore district; hence it is a generalization of the TB diseased population of South India. As this research deals with the dimensions of pulmonary TB patients and QOL who are receiving treatment in Vellore, this research does not interfere with another research done in this field.

This study reports certain limitations, such as the exclusion of certain groups of TB patients (all drug-resistant and extrapulmonary TB) and restricted only to pulmonary TB patients. Language barrier in this study refrain generalization of the population of India. This study is only confined to pulmonary TB patients undergoing treatment in Vellore and the method of this study was the telephonic interview; hence rapport building was not there. This study also had reporting bias, since this doesn't involve any direct observation of the study participant. Recall bias may be present because the present study used the WHOQOL-BREF questionnaire to assess the study participant's feelings and experience for the last two weeks.

5. Conclusions

Quality of life is significantly impaired among PTB patients under category-1 receiving treatment in the Vellore district of Tamil Nadu. In this study, the most affected aspect of QOL is the psychological domain of the patients, and the main associating factors with low QOL that predominately prevail over all the domains are marital status, persistent symptoms, and unemployment, with a significant difference, was also observed in these variables. The research fraternity would have a new novel exposure to the various dimensions and issues related to pulmonary TB patients and their QOL in India with specific reference to Vellore through this study. Health policymaker/program manager would have practical knowledge and mindset of pulmonary TB patients towards QOL and associating factors. Researchers can develop

suitable strategies to effectively manage active pulmonary TB patients, systems, and practices in India to have an optimal outcome.

Funding

The study did not receive any financial or material support for the conduct of the research and/or preparation of the article.

Conflict of interest

The authors have none to declare.

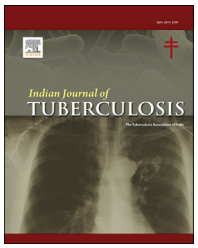
REFERENCES

1. Ali DK, Mohammad RM. Factors associated with health-related quality of life in tuberculosis patients referred to the national research institute of tuberculosis and lung disease in Tehran. *Tuberc Respir Dis*. 2015;78:309–314.
2. Vikas GR, Jyothi B, Rajiv Y, et al. Prevalence of pulmonary tuberculosis - a baseline survey in central India. *PLoS One*. 2012;7:e43225.
3. Ramadass S, Mani K, Praveen A, Sanjeev KG. Prevalence of pulmonary tuberculosis in India: a systematic review and meta-analysis. *Lung India*. 2020;37:45–52.
4. Abhijit GH, Annsmol PM, Lalzikpuii C, et al. Unmasking the human face of TB- the impact of tuberculosis on the families of patients. *J Fam Med Prim Care*. 2020;9:5345–5350.
5. *Global Tuberculosis Report 2020*. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
6. India Tb report. *Annual Reports: Central TB Division*. Ministry of Health and Family Welfare-Government of India; 2021.
7. Meera D, Nandini S, Ingle GK. Impact of tuberculosis on the quality of life. *Indian J Community Med*. 2008;33:58–59.
8. Rameshchandra MT, Gunjan PU. Psychosocial reaction of diagnosing tuberculosis – an experience of tertiary care center of rural Gujarat. *Int J Med Sci Publ Health*. 2014;3:1498–1500.
9. Dhingra VK, Shadab K. A sociological study on stigma among TB patients in Delhi. *Indian J Tubercul*. 2010;57:12–18.
10. Rajeswari R, Muniyandi M, Balasubramanian R, Narayanan PR. Perceptions of tuberculosis patients about their physical, mental and social well-being: a field report from south India. *Soc Sci Med*. 2005;60:1845–1853.
11. Olufunke OA, Olayinka OO, Ayodele C, et al. Factors influencing quality of life and predictors of low quality of life scores in patients on treatment for pulmonary tuberculosis: a cross-sectional study. *J Publ Health Afr*. 2014;5:366.
12. Laxmeshwar C, Stewart AG, Dalal A, et al. Beyond 'cure' and 'treatment success': quality of life of patients with multidrug-resistant tuberculosis. *Int J Tubercul Lung Dis*. 2019;23:73–81.
13. Carlo AM, Fawziah M, Victoria CC, Anita P, Mark FM. Factors influencing quality of life in patients with active tuberculosis. *Health Qual Life Outcome*. 2004;2:58.
14. Shoichi M, Taro Y, Akihiro O, Shoji Y, Aurora GQ, Yasuhiko K. Factors associated with health-related quality of life among pulmonary tuberculosis patients in Manila, the Philippines. *Qual Life Res*. 2014;23:1523–1533.
15. Alemayehu D, Tsega H, Mezgebu Y, Getasew A, Andualem YA. Quality of life and associated factors among patients with tuberculosis at the University of Gondar comprehensive specialized hospital, Ethiopia. *Qual Life Res*. 2021;30:1173–1181.
16. Cassileth BR, Lusk EJ, Strouse TB, et al. Psychosocial status in chronic illness. A comparative analysis of six diagnostic groups. *N Engl J Med*. 1984;311:506–511.
17. Betty C, Albert WW, Nadia NH, Gregory BD. Quality of life in tuberculosis: a review of the English language literature. *Qual Life Res*. 2004;13:1633–1642.
18. Muniyandi M, Rajeswari R, Balasubramanian R, et al. Evaluation of post-treatment health-related quality of life (HRQoL) among tuberculosis patients. *Int J Tubercul Lung Dis*. 2007;11:887–892.
19. Carlo AM, Fawziah M, Lindsey C, et al. Health-related quality of life trajectories among adults with tuberculosis: differences between latent and active infection. *Chest*. 2008;133:396–403.
20. Aggarwal AN, Gupta D, Janmeja AJ, Jindal SK. Assessment of health-related quality of life in patients with pulmonary tuberculosis under programme conditions. *Int J Tubercul Lung Dis*. 2013;17:947–953.
21. Chamla D. The assessment of patients' health-related quality of life during tuberculosis treatment in Wuhan, China. *Int J Tubercul Lung Dis*. 2004;8:1100–1106.
22. Raman S, Ravinder Y, Meenakshi S, Varinder S, Vipin K. Quality of life of multi-drug resistant tuberculosis patients: a study of north India. *Acta Med Iran*. 2014;52:448–453.
23. Khan A, Walley J, Newell J, Imdad N. Tuberculosis in Pakistan: socio-cultural constraints and opportunities in treatment. *Soc Sci Med*. 2000;50:247–254.
24. Rajeswari R, Balasubramanian R, Muniyandi M, Geetharamani S, Thresa X, Venkatesan P. Socio-economic impact of tuberculosis on patients and family in India. *Int J Tubercul Lung Dis*. 1999;3:869–877.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Recovery rates of mycobacterium from suspected extra-pulmonary tuberculosis patients using liquid culture at a tertiary referral centre of India

Gavish Kumar [a], *Manpreet Bhalla* [a], *Niti Singh* [a], *Ajoy Kumar Verma* [a,*], *Ravindra Kumar Dewan* [b]

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

[b] *Department of Thoracic Surgery and Surgical Anatomy, National Institute of Tuberculosis and Respiratory Diseases, Sri Aurobindo Marg, New Delhi 110030, India*

ARTICLE INFO

Article history:
Received 29 November 2021
Received in revised form
29 April 2022
Accepted 17 May 2022
Available online 23 May 2022

Keywords:
N-Acetyl-L-cysteine
Extra-pulmonary tuberculosis
MGIT 960

ABSTRACT

Tuberculosis still remains a serious public health problem in developing countries. Rapid isolation of mycobacteria is critical for accurate diagnosis and management of tuberculosis. In the present study BACTEC MGIT 960 system was evaluated against Lowenstein Jensen (LJ) medium for isolation of mycobacteria from different extra-pulmonary specimens (N = 371). The samples were processed using NaOH-NALC method and inoculated in BACTEC MGIT and on LJ medium. The BACTEC MGIT 960 system detected 93 (25.06%) samples positive for acid fast bacilli and by LJ only 38 samples (10.24%) was positive. Furthermore, total 99 (26.68%) samples were detected positive by both the culture methods. The mean turnaround time to detection of mycobacteria by MGIT 960 were significantly less (12.4 days) as compared with LJ (22.76 days). In conclusion, BACTEC MGIT 960 system is more sensitive and rapid culture system for isolation of mycobacteria. However LJ culture method also suggested to further increase the detection rate of EPTB cases.

1. Introduction

Extra-pulmonary tuberculosis (EPTB) is a diagnostic dilemma as the clinical symptoms may be non-specific and microscopic demonstration of the acid fast bacilli in these cases yields mostly negative results. Although, EPTB cases are not contagious, yet high rate of mortality and morbidity has been noted all over the world.[1] Rapid and accurate diagnosis of EPTB is also very important to facilitate proper treatment. Although molecular probe based assays are valuable for rapid screening of smear positive cases of tuberculosis, sensitivity of these assays for smear negative cases is very low.[2,3] Furthermore, molecular method like line probe assay is invalid for mycobacterium other than tuberculosis (MOTT) and also not capable to follow up progress of treatment as it can not differentiate viable and dead bacilli. Early isolation of mycobacteria is necessary for drug susceptibility testing and also to

* *Corresponding author*. Department of Microbiology, National Institute of tuberculosis and respiratory diseases, New Delhi 110030, India. Tel: 9818255342 (mobile).
E-mail address: akv_580@yahoo.co.in (A.K. Verma).

https://doi.org/10.1016/j.ijtb.2022.05.006
0019-5707/

follow up the success of treatment. Culture on Lowenstein Jensen (LJ) is the conventional technique to isolate viable mycobacteria from specimen of patient. LJ cultures require 3–8 weeks of incubation time to interpret the culture result and additional 3–4 weeks for drug susceptibility testing.[4] Broth media based culture systems are known for rapid isolation of mycobacteria with reduced time and increased sensitivity. Among broth based culture systems BACTEC MGIT 960 is a fully automated and non-radiometric method for the isolation of mycobacteria using a fluorescent oxygen-quenched sensor embedded in silicone at the bottom of the tubes. World health organization (WHO) recommended liquid culture for timely diagnosis and treatment of the disease. There are some reports showing the value of MGIT 960 instrument for isolation of mycobacteria.[5–7]

In the present study, turnaround time for isolation of mycobacteria from EPTB specimens was evaluated using MGIT 960 instrument and compared with LJ culture method at a tertiary care centre in India.

2. Materials and methods

2.1. Study subject

This study was carried out at National Institute of Tuberculosis and Respiratory Diseases formerly known as Lala Ram Swarup Institute of Tuberculosis and Respiratory Diseases, New Delhi, India. A total of 371 clinically suspected cases of extra pulmonary tuberculosis attending the OPD and Indoor were collected. The clinical samples included FNAC (166), Pus (96), Pleural fluid (41), Pleural pus (26), Cold abscess (6), Chest/breast abscess (5), Ascitic fluid (4), CSF (2), Skin biopsy (2), Endometrial fluid (2), Tissue (2), Synovial fluid (1), Para-spinal fluid (1), Pericardial fluid (1), Knee abscess (1), Liver abscess (1), back abscess (1), urine sample (1), and unknown (12).

2.2. Samples processing

Samples were processed using sodium hydroxide and N-acetyl-L-cysteine (4% NaOH/0.5% NALC in 2.9% sodium citrate solution) method.[8] In brief, the equal volume of specimen and NaOH/NALC solution were mixed and vortex. Phosphate buffer (0.067 M, pH 6.8) was added to make final volume 50 ml After 15 min of incubation at RT. It was centrifuged at 3000×*g* and 4 °C for 20 min. The supernatant was discarded and sediment was resuspended in phosphate buffer to a final volume of 2 ml. This suspension was used for inoculation in MGIT 960 and LJ culture medium. Specimens were usually processed on the day of collection and otherwise stored at 4 °C (i.e., received during the weekend or holiday). Smear of suspension was prepared to examine the presence of acid fast bacilli.

2.3. Inoculation in MGIT and LJ medium

The suspension (0.5ml) was inoculated in MGIT (Becton, Dickinson, and company) culture tube containing PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) antibiotics. The tubes were entered into the MGIT 960 instrument. The tubes were incubated at 37 °C and were monitored automatically after every 60 min for generation of fluorescence upto 6 weeks. Sample flagged as positive, was taken out from the MGIT 960 instrument. 0.1 ml of the suspension was used for inoculation on LJ media. LJ media were incubated at 37 °C and reading was taken after interval of one week upto a duration of 8 weeks.

2.4. Sterility and smear examination of positive flagged MGIT tube

Smear of positive tube was prepared and examined for AFB after ZN staining. Sterility of MGIT culture was checked by streaking on blood Agar plate.

2.5. Identification of M. tuberculosis complex

Immunochromatographic assay using TbcID kit (BD diagnostics) was performed for MGIT cultures positive for acid fast bacilli as described by the manufacturer. In brief, 0.1 ml of liquid culture was applied onto the TbcID strip. Strips were incubated for 15–30 min at RT. Pink colour band in the control (C) region confirmed test validity and band in the test (T) region was interpreted as positive for MPT64 Ag.

2.6. Statistical analysis

The following formulas were used for statistical calculations-Sensitivity was true positives/(true positives + false negatives) ×100, specificity was true negatives/(true negatives + false positives) x 100. A predictive value (PV) to define the probability of a disease is very important as it provides the significance of a disease to characterize a patient for the particular disease from the patient's population (+PV), and a high -PV is also needed to exclude the disease. PPV was calculated as true positives/(true positives + false positives) x 100 and NPV was true negatives/(true negatives + false negatives) x 100.

3. Results

All the extra-pulmonary patients included in the present study were over 18 and under 52 years of age. The mean age of patients suffering from extra-pulmonary tuberculosis was 27.8 days. The male and female ratio of tuberculosis patients was 1:1.3. In the present study, 317 cases belong to Delhi State and 54 cases were outside of Delhi State.

The focus of the present study was to compare the MGIT 960 and LJ culture methods for diagnosis of extra pulmonary tuberculosis. The culture of extra pulmonary tuberculosis patients was inoculated on both medium. The clinically suspected 371 EPTB patients (123 New EPTB, 147 Failure, 85 relapsed TB, 8 defaulters and 8 chronic execrators) cases were included (Fig. 1). The different sites of all extrapulmonary tuberculosis (EPTB) specimens are summarized in Table 1.

The comparative positivity rate for EPTB subjects is summarized in Table 2. Out of 371 EPTB cases, 25.06% of clinical samples were positive for MTB complex by MGIT 960 system whereas positivity for LJ method was 10.8%. The result showed that isolation of MTBC in MGIT 960 was higher by 15% when compared with the LJ culture. The

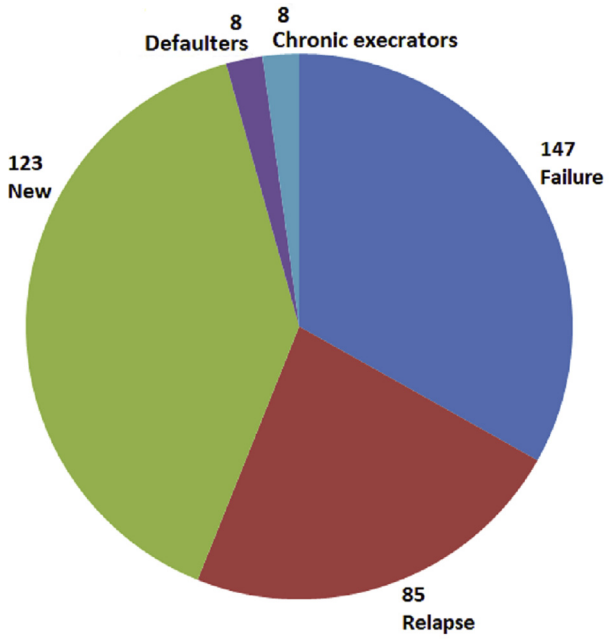


Fig. 1 – Different categories of extra-pulmonary tuberculosis cases (N = 371)

combined results of both LJ & MGIT 960 showed 26.68% positivity for MTBC. However, 1.06% of MTBC were isolated in LJ culture media had not grown in MGIT 960 culture system (Table 2). The contamination rate was found to be 5.12% on MGIT 960 media as uniform turbidity in the entire tube was noted and culture was negative to ZN staining. In MGIT media 8 nontuberculous mycobacteria were recovered and only 2 of these were positive for LJ media (Table 3). In the present study it was observed that among 38 EPTB, 32 specimens (84.21%) those were positive by LJ detected positive by MGIT also. However, 61 (16.44%) EPTB

Table 1 – Different sites of extra pulmonary specimens

Specimen site	No
FNAC	166
Pus	96
Pleural fluid	41
Pleural pus	26
Cold abscess	6
Chest/breast abscess	5 (4 + 1)
Ascitic fluid	4
Cerebro spinal fluid	2
Skin biopsy	2
Endometrial fluid	2
Synobial fluid	1
Para-spinal fluid	1
Tissue	2
Peri cardial fluid	1
Urine	1
Knee abscess	1
Liver abscess	1
Back abscess	1
Unknown	12
	371

Table 2 – Culture positivity rate for extra-pulmonary tuberculosis subjects

Smear	Culture positive			Contaminated	
	LJ	MGIT	LJ + MGIT	LJ	MGIT
Negative EPTB subjects (N = 371)	38 (10.24%)	93 (25.06%)	99 (26.68%)	8 (2.15%)	19 (5.12%)

cases detected as negative for mycobacteria by LJ media were recovered in MGIT media (Table 4). Among specimens the turnaround time to positivity was shorter with MGIT 12.4 days as compared to detection time on LJ media (22.76 days) (Table 5). All the culture negative tubes were further inspected visually for any growth and growth noticed in one MGIT 960 tube and it was reported as positive for MTB by ZN staining and Immunochromatographic test.

4. Discussion

The World Health Organization recommends liquid culture system as standard method for rapid isolation of mycobacteria. MGIT 960 method is known to higher culture positivity rate among smear negative specimens.[9] MGIT 960 system has been extensively evaluated for recovery of mycobacteria from tuberculosis specimens.[5–7] The present study was done to evaluate the utility of MGIT 960 culture method against widely used conventional LJ media for diagnosis of extrapulmonary tuberculosis.

Table 3 – Organisms identified as MOTT from positive mycobacterial cultures

MGITculture positive	LJ culture positive
8/371 (2.15%)	2/371 (0.5%)

Table 4 – Correlation of positivity rate on LJ and MGIT culture

MGIT	LJ		Total
	Positive	Negative	
Positive	32 (84.21%)	61 (18.31%)	93 (25.06%)
Negative	6 (25.79%)	272 (81.68%)	278 (74.93%)
Total	38	333	371
PPV; 34.40%, NPV; 97.8%, Accuracy; 81.94%.			

Table 5 – Turnaround time for culture using LJ and MGIT 960

Days	Culture positive	
	LJ	MGIT 960
<7 days	0	4
7–14 days	6	17
>14 days	32	72

In our study MGIT positivity is higher for MTBC and NTM as compared to solid media underlines the importance of liquid media for identification in combination with immunochromatographic assay. Various studies showed limitation of MGIT 960 due to relatively high proportion of cultures contamination.[6,10,11] In our study the contamination rate was within the range 2.1% and 5.1 for LJ and MGIT 960 media respectively. Study suggests that MGIT 960 requires careful processing and timely handling of specimens. Our data showed increase in number of positive cultures by MGIT 960 system compared to conventional LJ method. In addition, our study also showed increased rate of positivity taking both the culture methods in combination for diagnosis of tuberculosis. It was noticed that culture contaminated on MGIT 960 were recovered on LJ media. Therefore, increased yield of positivity reported in our study was due to less contaminated rate of LJ in comparison to MGIT culture. The average culture positive detection time for specimens was reported less for MGIT 960 system as compared to LJ. In contrast only 6 cultures were detected on LJ in less than 14 days and in MGIT 960 detected 17 culture positive. In the present study only limited number of samples were included and drug susceptibility data is not available. In our study visual appearance of growth was noticed in a MGIT 960 tube declared negative by instrument after 42 days of incubation and it was positive to MTB complex by ZN smear and Immunochromatographic test. Therefore, further incubation of MGIT 960 tubes containing EPTB samples may increase the yield of culture. In the present study default setting of incubation period i. e 42 days was used.

Our study highlighted the value of MGIT 960 system as a highly efficient, semi-automated system for isolation of mycobacteria from extrapulmonary specimens. Shifting from LJ to MGIT culture in existing laboratory and by strengthening the peripheral laboratory with MGIT 960 system will help to find out more EPTB cases in short span of time. This in turn will help the clinicians to manage and treat the EPTB patients in shortest/earliest possible time. In conclusion, although MGIT 960 cultivation system has higher sensitivity and rapid isolation rate, solid media also suggestive for isolation of mycobacteria from extrapulmonary specimens to further increase the yield.

Conflicts of interest

All authors have none to declare.

Acknowledgements

The authors thank the Foundation for Innovative Newer Diagnostics for the MGIT 960 7 ML media and kits. The authors are grateful to Ms Sana Tabassum for their technical support during the experiment. We declare no conflicts of interest.

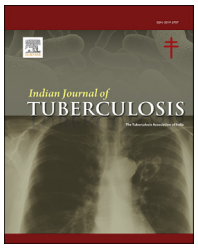
REFERENCES

1. Yang Z, Kong Y, Wilson F, et al. Identification of risk factors for extrapulmonary tuberculosis. *Clin Infect Dis*. 2004;38:199–205.
2. Cheng VC, Yew WW, Yuen KY. Molecular diagnostics in tuberculosis. *Eur J Clin Microbiol Infect Dis*. 2005;24:711–720.
3. Telenti A, Marchesi F, Balz M, Bally F, Boittger EC, Bodmer T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol*. 1993;31:175–178.
4. Raviglione MC, O'Brien RJ. Tuberculosis. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, eds. *Harrison's Principles of Internal Medicine*. 16th ed.. USA: McGraw-Hill; 2005:953–966; 1.
5. Chihota VN, Grant AD, Fielding K, et al. Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *Int J Tubercul Lung Dis*. 2010;14(8):1024–1031.
6. Mehedi H, Munshi SK, Sabiha BMM, et al. Evaluation of the effectiveness of BACTEC MGIT 960 for the detection of mycobacteria in Bangladesh. *Int J Mycobacteriology*. December 2013;2(4):214–219.
7. Kalpana T, Imola J, Kalaiarasan E, et al. Comparison of MGIT 960 with Lowenstein jensen media for recovery of mycobacteria from extrapulmonary specimens in southern India. *J Clin Diagn Res*. 2021;15(3):DC01–DC04.
8. Kent PT, Kubica GP. *Public Health Mycobacteriology, A Guide for the Level III Laboratory*. Atlanta, Ga: Centers for Disease Control; 1985.
9. Shinu P, Nair A, Singh V, et al. Evaluation of rapid techniques for the detection of mycobacteria in sputum with scanty bacilli or clinically evident, smear negative cases of pulmonary and extra-pulmonary tuberculosis. *Mem Inst Oswaldo Cruz*. 2011;106(5):620–624. Rio de Janeiro.
10. Lee JJ, Suo J, Lin CB, et al. Comparative evaluation of the Bactec MGIT 960 system with solid medium for isolation of mycobacteria. *Int J Tubercul Lung Dis*. 2003;7:569–574.
11. Hanna BA, Ebrahimzadeh A, Elliott LB, et al. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. *J Clin Microbiol*. 1999;37:748–752.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Stakeholders' perspective on the daily regimen of tuberculosis treatment- A qualitative approach

Nayanabai Shabadi [a,*], *N.K. Prashanth Kumar* [b], *B. Prakash* [c], *Praveen Kulkarni* [a], *M.R. Narayana Murthy* [a]

[a] *Department of Community Medicine, JSS Medical College, JSS Academy of Higher Education and Research (JSSAHER), Mysuru, Karnataka, India*

[b] *School of Public Health, JSS Academy of Higher Education and Research (JSSAHER), Mysuru, Karnataka, India*

[c] *Department of Community Medicine, Panimalar Medical College Hospital & Research Institute, Varadharajapuram, Poonamalle, Chennai, Tamilnadu, India*

ARTICLE INFO

Article history:
Received 2 May 2022
Received in revised form
15 May 2022
Accepted 20 May 2022
Available online 28 May 2022

Keywords:
Tuberculosis
DOTS
Daily regimen
Stakeholder perspectives
Qualitative research

ABSTRACT

Introduction: In the context of changing over from an intermittent treatment regimen to a daily regimen, it becomes crucial to understand the impact of a daily regimen on the treatment process and outcome. It enables health professionals to strengthen strategies, to enhance the quality of treatment as well as the quality of life of TB patients. The perspective of each stakeholder involved in the process is important in assessing the impact of the daily regimen.
Objectives: To understand patients' and providers' perspectives on the daily regimen of Tuberculosis treatment.
Methodology: A qualitative study was conducted between March 2020 to June 2020, including in-depth interviews with TB patients on treatment and DOT providers, and Key Informant Interview(KII) with TB Health Visitors(TBHV) and family members of TB patients. A thematic-network analysis approach was utilized to get the results.
Results: Two sub-themes emerged: (i) Acceptance of the daily regimen of treatment; (ii) operational difficulties of the daily regimen. No injections in the regimen, fewer side effects of drugs as dose depends on weight band, family members can be treatment supporter, awareness about disease and treatment, the drugs are as same as private drugs available, adherence has improved, monthly DBT were found to some of the enablers in the study. The Barriers found in the study were traveling daily to get drugs, loss of daily wages, accompanying patients daily, tracing private patients, pyridoxine is not given free in this regimen, increased workload for treatment providers, etc.
Conclusion: The study points out that acceptance of the patient to the daily regimen is better as they have lesser side effects. The operational difficulties in the implementation of the daily regimen can be addressed by providing family members as treatment supporters.

* *Corresponding author*. Department of Community Medicine, JSS Medical College, Mysuru, 570015, Karnataka, India.
E-mail address: nayanabaishabadi@jssuni.edu.in (N. Shabadi).

https://doi.org/10.1016/j.ijtb.2022.05.008

1. Introduction

Tuberculosis (TB) remains a major global health problem. About a third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*. India has one-fourth (27%) of the global TB burden.[1] According to the Global TB report 2019 India has an incidence of 199 cases per lakh population per year. TB now ranks alongside HIV as a leading cause of death worldwide.[1] TB kills more adults in India than any other infectious disease. In India, every day more than 6000 develop TB disease; more than 600 people die of TB (i.e. 2 deaths every 5 minutes). India has the highest burden of MDR TB, it has around 3% of new TB cases and 12%–17% of previously treated TB cases have MDR-TB.[2]

The revised National TB Control Program (RNTCP), now called as National TB Eradication Program(NTEP), is an ongoing Centrally Sponsored Scheme, being implemented under the umbrella of the National Health Mission. India adopted the internationally recommended Directly Observed Treatment Short-Course (DOTS) in the management of TB patients.

Following the recommendations of WHO, the RNTCP on 30th October 2017 made a major shift in regimen from intermittent (thrice-weekly) to daily regimen of TB treatment throughout the country. The daily strategy is known to improve program coverage along with compliance. The principle of treatment for drug-sensitive TB with a daily regimen is to administer a daily fixed-dose combination of first-line anti-TB drugs with inappropriate weight bands for pulmonary and extra-pulmonary TB in all age groups. Such strategic shifts will have both management and operational implications.[3]

Understanding the impact of daily regimens on treatment outcomes is crucial to enable health professionals to strengthen strategies to enhance the quality of treatment as well as the quality of life of TB patients. Through this study it was attempted to explore the personal experience of TB patients, family members of patients, DOTS providers as well as TB Health visitors regarding and daily regimen. This study can provide a holistic picture of the functioning of DOTS in daily regimen can be understood and further initiatives to be taken to address unmet needs.

2. Objectives

1. To explore TB patients' and providers' perceptions concerning the daily regimen of TB treatment by DOTS.
2. To explore the perception of Family members of TB patients regarding the daily regimen of TB treatment through DOTS.
3. To identify the barriers, challenges, and supportive needs of both TB patients and DOT providers.

3. Methodology

The study was conducted in the DOTS center of JSS hospital to explore the experiences and perceptions of TB patients on associates of treatment adherence, phenomenological approach was employed to carry out qualitative data collection. Accordingly, Telephonic in-depth interviews with TB patients, TB Health Visitors, DOTS Providers, and family members of TB patients were conducted from 9th to 20th of May 2020.

3.1. *Study participants and sampling*

Age (18 years old and above) and duration of treatment (3 weeks or more) were considered as criteria for selection. Selection continued until conceptual saturation was reached (to the point no further new information was obtained anymore).

- **Recruitment of participants**

Eligible participants were contacted by the investigators within the selected facilities either in person or through a telephone call. These clients were informed of the study and given the chance to decide to willingly participate. And Patients who were above the 18 years of age group were included in the study and the TB patients who did not give consent for the study, patients who were seriously ill were excluded from the study.

- **Data collection techniques and tools**

The interview guide was prepared before the interview, which consists of Semi-structured interview questions. Some of the probing questions were formed to use if the information received was incomplete or the participant faced problems in understanding the question and in answering.

The questions included awareness of the diseases, patients' experience with the daily regimen, family members' experience, TBHVs, and DOTS providers' experience with the daily regimen.

All the in-depth interviews were conducted in the Kannada language and were audio-recorded. Each In-depth interview lasted for an average of 30mins. The Interviews guide was used to initiate discussions and to gear the whole session of the discussions toward the topic.

The data analysis was done by following the steps.

i. Transcription-the raw data will be transformed into written text format.
ii. Each of the audio records will be repeatedly listened to and transcribed verbatim and translated into English by the principal investigator.
iii. Deciding the unit of analysis-either sentences or paragraphs
iv. Coding the text
v. Drawing conclusions from coded data

ATLAS-ti software version 2.0 was used to organize and save the codes and themes.

4. Results

A total of 15 participants took part in the study giving in-depth interviews. Of the 15 members, 6 were TB Patients, 5 were family members of TB Patients, 2 were TB Health Visitors and 2 were DOTS providers/Treatment supervisors (Fig. 1).

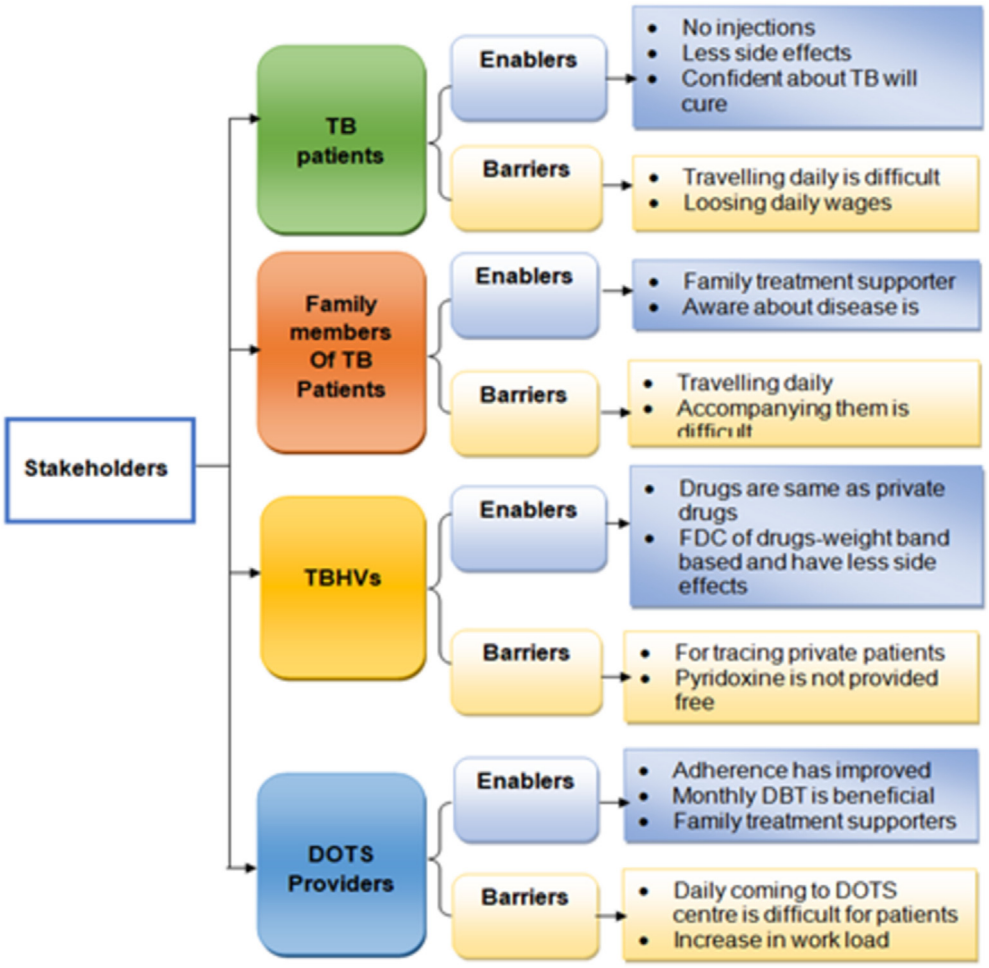


Fig. 1 – Flowchart of the enablers and barriers of daily regimen from Stakeholders' perspective.

4.1. *Theme 1. Awareness about the disease*

Most TB patients knew about the cause and spread of the disease. They said it's through the respiratory route. One of the patients said he doesn't know about the cause of the disease, he just has to take treatment so he is taking it.

One of the DOTS providers said, "They(patients) usually know how they get the disease, but one who is addicted to alcohol they are not bothered to know it. Once they come for treatment we give complete awareness about the disease".

4.2. *Theme 2. Patient's perspective on a daily regimen of DOTS*

Most of the patients opined that they are satisfied with the treatment given to them. The patient knows that for a complete cure of tuberculosis they have to complete the treatment without interruption. They said the health workers are very cooperative and supportive to them in completing the treatment.

A 52-year-old male patient said, "I don't have any problem in taking the tablets daily, disease has come to me so I have to take the treatment and get cured as early as possible and the government is doing it for our good only".

One of the college-going girls said she doesn't have any problem with the daily regimen and it's convenient for her to take the drug regularly as the DOTS center is near to her house. She is getting full support from her family as well as the health system.

4.3. *Theme 3. Family members' perspective on a daily regimen of DOTS*

The daily regimen and the longer duration of treatment are challenging for both patients and their family members. As most of the family members are aware of the disease and treatment course, they are very much supportive of the patients; they have complete confidence in DOTS in curing the disease. According to this newer regimen, the family members have a provision of being family DOTS providers/supporters, who can monitor the treatment and be the support throughout the completion of the course.

One of the family members of a patient expressed that being a daily wager sometimes it becomes difficult to collect the tablets daily, especially for the first two months.

4.4. *Theme 4. Patient-related enabling factors of a daily regimen of DOTS in TB patients' care and support*

One of the patients who had received intermittent regimen 2 years back and then on a daily regimen as well, shared his experience of both the experiences. In the initial stage first, he took six months of treatment, and then he took eight months of treatment along with a streptomycin injection. He had phased problem with the injections. He says "I don't have any problem in taking tablets but can't tolerate the pain of the injections". Now in this recent regimen, the streptomycin injections are not given.

He said he is a self-motivated person and he wants to get cured and he doesn't want any of his family members to suffer. Also, the health personnel including DOTS providers, TBHVs, and health workers were very much supportive in completing the treatment.

4.5. *Theme 5. Program-related enabling factors of the daily regimen of DOTS in TB patients' care and support*

4.5.1. *According to the TBHVs*

The number of tablets has reduced in the daily regimen compared to the 7 tablets in a day in the intermittent regimen. This factor has improved drug adherence among the patients. The FDC and daily regimen have given the feeling of improved quality of treatment among patients because the regimen is the same as that of the private regimen available.

They said even the adverse events/reactions are less with the daily regimen because the FDC and tablets are given according to weight band unlike in intermittent regimens where the same doses were given to everyone irrespective of their weight.

Having the option of family treatment supporter has improved the TB patient care and support for treatment completion.

4.5.2. *According to the DOTS providers and TBHVs*

Even though the treatment completion rate is almost the same with the older regimen compared to the newer regimen, with the effort of the program the awareness level regarding the prevention, treatment of the TB disease has increased among the patients, family members, and the community as a whole. This has helped in treatment completion.

They also said that a Direct benefit transfer of rs 500 to all the TB patients to support their nutrition has helped and encouraged many patients to complete the treatment. The DOTS provider said with a laugh "the patients ask for money even after completing the treatment".

4.6. *Theme 6: Health system barriers to TB patients' care and support*

According to the TB health visitor (TBHV), increased workload on the health workers because of the daily regimen, initially only patients coming to the public sector were taken care of by the health workers but now because of the newer regulations even the private sector patients also have to be monitored and followed up. In the recent regulation, the patient is TB free is declared after two years, till then every six months the patient has to be tested for sputum AFB.

Tracing some of the private patients is a difficult task, as they will not be willing to share their details and most of the time they will not be comfortable with health workers' visits to their houses, therefore health workers have to be dependent on private doctors data on follow up the status of the patients.

One of the TBHV expressed that, as the fixed-dose combination drug regimen will not be providing the pyridoxine, the patients have to bear the cost of pyridoxine it is needed.

4.7. *Theme 7. Patient-related barriers to TB patients' care and support*

Some of the patients expressed that they face difficulty in getting the tablets daily by coming to DOTS centers.

Daily Travelling to the DOTS center is difficult for them in terms of money spent on the transportation and sometimes for female patients coming alone will be difficult. Somebody has to accompany them. One of the patients said he has stopped going to his job since the diagnosis of the disease, only his wife is earning now and his sons are also supporting some time.

5. Discussion

A total of 15 participants took part in the study giving in-depth interviews. Of the 15 members, 6 were TB Patients, 5 were family members of TB Patients, 2 were TB Health Visitors and 2 were DOTS providers/Treatment supervisors.

5.1. *Awareness about the disease*

Most TB patients knew about the cause and spread of the disease. They said it's through the respiratory route. One of the patients said he doesn't know about the cause of the disease, he just has to take treatment so he is taking it. Similarly, a study conducted by **Negandhi et al, Kasa et al and Kigozi et al,** reported that the majority of patients knew about the route of transmission of disease.[4,5,6] In a study conducted by **Negandhi H** the patients described TB, its mode, and the spread of infection which shows an adequate level of knowledge.[4] The good knowledge among patients in our study is in contrast to the study conducted by **O.Boyle** et al. where they found that majority of patients in the study stated that the health workers should give information about TB and DOTS.[7]

5.2. *Patients' perspectives on a daily regimen of DOTS*

Most of the patients opined that they are satisfied with the treatment given to them. The patient knows that for a complete cure of tuberculosis they have to complete the treatment without interruption. They said the health workers are very cooperative and supportive to them in completing the treatment. Similarly, In a study conducted by **Aibana O et al,** the patients opined that missing doses of TB medications may decrease treatment effectiveness.[8]

Similarly, in a study conducted by **Aibana O**, many participants said that they have a desire to recover or stay healthy/alive.[8]

In our study, some of the participants opined that they don't have any problem with their daily regimen, and it's convenient for them to take the drug regularly as the DOTS center is near to their house. Many of them received full support from their family as well as the health system. A study conducted by **Daniel Fhisea et al**. found a similar view of patients that the advice they got from the health care providers(DOT providers) helped them to continue their treatment.[9]

In a study conducted by **Sahil,** patients said that they were having good relationships with health care service providers and they obtained information and support from the DOTS providers. In a study conducted by **Daniel Fiseha et al,** the majority of TB patients stated they got support and encouragement from family members during treatment.[9]

5.3. *Family members' perspective on a daily regimen of DOTS*

The daily regimen and the longer duration of treatment are challenging for both patients and their family members. As most of the family members are aware of the disease and treatment course, they are very much supportive of the patients; they have complete confidence in DOTS in curing the disease. According to this newer regimen, the family members have a provision of being family DOTS providers/supporters, who can monitor the treatment and be the support throughout the completion of the course. One of the family members of a patient expressed the difficulties in collecting the tablets daily, especially for the first two months. In a study conducted by **Marahatta SB**, the participants said that economic problem is one of the main barriers to attending facilities to get TB drugs.[10]

5.4. *Patient-related enabling factors of the daily regimen of DOTS in TB patients' care and support*

One of the patients who had received intermittent regimen 2 years back, and then on a daily regimen as well, shared his experience that he won't have any problem in taking tablets but can't tolerate the pain of the injections. He was a self-motivated person and he wants to get cured and he doesn't want any of his family members to suffer. And he opined that the health personnel including DOTS providers, TBHVs, and health workers were very much supportive in completing the treatment. In a study by **Lee et al.**, it was found that patient education by health workers regarding TB disease and treatment showed an increased adherence to treatment in Bangladesh.[11]

5.5. *Program-related enabling factors of a daily regimen of DOTS in TB patients' care and support*

5.5.1. *According to the TBHVs*

Reduction in the number of tablets compared to the intermittent regimen. This factor has improved drug adherence among the patients. The FDC and daily regimen have given the feeling of improved quality of treatment among patients because the regimen is the same as that of the private regimen available. The adverse events/reactions are less with the daily regimen because the FDC and tablets are given according to weight band unlike in the intermittent regimen where the same doses were given to everyone irrespective of their weight. In a study conducted by **Sanneh AFNS**, the participants expressed that daily medication with its fixed-dose combination was more convenient. In the present study they have stated that having the option of family treatment supporter has improved the TB patient care and support for treatment completion. In another study, conducted by Yellappa et al. participants stated that the family was the main source of support during the patient's recovery.[12]

5.5.2. *According to the DOTS providers and TBHVs*

With the effort of the program, the awareness level regarding the prevention, and treatment of the TB disease has increased among the patients, family members, and the community as a whole which helped in treatment completion. A direct benefit transfer of Rs 500 to all the TB patients has helped and encouraged many patients to complete the treatment. A study conducted by **Daniel Fiseha et al** found that the support from the family and community was very important for patients to comply with their treatment.-cite daily regimen.[9]

In the other study conducted by Tadesse T et al., it was found that family support is identified as the most important factor for influencing treatment compliance.[13]

5.6. *Health system barriers to TB patients' care and support*

According to the TB health visitor (TBHV), increased workload on the health workers because of the daily regimen, Tracing some of the private patients is a difficult task, as they will not be willing to share their details and most of the time they will not be comfortable by health worker's visit to their houses, therefore health workers has to be dependent on private doctors data on follow up the status of the patients. A study conducted by **Negandhi H et al** described that complete notification of all patients attending private hospitals has still not been attained. And also they described that increased supervision in daily regimen increases health worker workload which may undermine the implementation of DOTS.[4]

5.7. *Patient-related barriers to TB patients' care and support*

Some of the patients expressed that they face difficulty in getting the tablets daily by coming to DOTS centers. Daily Travelling to the DOTS center is difficult for them in terms of money spent on the transportation and sometimes for female patients coming alone will be difficult. Somebody has to accompany them. In the present study, it was observed that long traveling distances to DOTS centers and inconvenient timings were making it difficult to receive regular treatment. Similar observations were done in other studies.[11]

Another study conducted by **Daniel Fiseha et al** observed that the barrier was money, where patients were going to the clinic barefoot every day due to lack of money and the high

cost of transportation.[10] Similar factors were found in other studies conducted by **I Tanimura T et al., and S. J. O'Boyle. et al.**[14,7] In a study conducted by **Sahil Z** it was observed that the barrier was distance and money, where two participants said that the health facilities were far from their homes and relatively costly for transportation services.[15]

A study conducted by Mette sagabekken et al found some similar other barriers like daily physical demands, food insecurity, and hunger. Rigid routines work of the day, and poor support from some of the health workers.[16]

5.8. *Conclusion*

The study was carried out to explore the stakeholder perspectives on the Daily regimen of DOTS mainly to identify the barriers, challenges, and supportive needs of both TB patients and DOT providers. We found that the barriers and challenges among patients were Daily Travelling to the DOTS center, time inconvenience of patients due to their work, economic problems, side effects of Anti-TB drugs, etc. Among DOTS providers the barriers and challenges increased in workload, monitoring of private patients, etc., and enabling factors among patients were the support of DOTS providers and family members. Supportive needs for patients are recognized as economic support, motivation among defaulters, etc. among DOTS providers, extra manpower resources, and logistical support. The limitations of this study are the study was conducted only in one DOTS center of JSS hospital in Mysore. Further studies should concentrate more on defaulters rather than adherent patients.

5.9. *Limitation*

As it is a qualitative research method, the results obtained cannot be generalised to the larger group.

Funding

None.

Conflict of interest

The authors have none to declare.

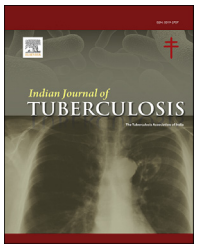
REFERENCES

1. *Global Tuberculosis Report 2021*. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO https://www.who.int/publications/digital/global-tuberculosis-report-2021.
2. Revised National Tuberculosis Control Programme. *National Health Portal India*; April 6-2015. Available on https://www.nhp.gov.in/revised-national-tuberculosis-control-programme_pg.
3. Technical and operational guidelines for Tuberculosis control in India 2016, Revised National TB Control Programme, Central TB Division, Director General of Health Services, Ministry of Health and Family Welfare, New Delhi, India. https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4573&lid=3177.
4. Negandhi H, Tiwari R, Sharma A, et al. Rapid assessment of facilitators and barriers related to the acceptance, challenges and community perception of daily regimen for treating tuberculosis in India. *Glob Health Action*. 2017 Jan 1;10(1):1290315. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5496091/.
5. Kasa AS, Minibel A, Bantie GM. Knowledge, attitude and preventive practice towards tuberculosis among clients visiting public health facilities. *BMC Res Notes*. 2019;12:276. https://doi.org/10.1186/s13104-019-4292-2.
6. Kigozi NG, Heunis JC, Engelbrecht MC, et al. Tuberculosis knowledge, attitudes and practices of patients at primary health care facilities in a South African metropolitan: research towards improved health education. *BMC Publ Health*. 2017;17:795. https://doi.org/10.1186/s12889-017-4825-3.
7. OBoyle SJ, Power JJ, Ibrahim MY, Watson JP. Factors affecting patient compliance with anti-tuberculosis chemotherapy using the directly observed treatment, short-course strategy (DOTS). *Int J Tubercul Lung Dis*. 2002 Apr 1;6(4):307–312.
8. Aibana O, Dauria E, Kiriazova T, et al. Patients' perspectives of tuberculosis treatment challenges and barriers to treatment adherence in Ukraine: a qualitative study. *BMJ Open*. 2020;10, e032027. https://doi.org/10.1136/BMJopen-2019-032027.
9. Fiseha D, Demissie M. Assessment of Directly Observed Therapy (DOT) following tuberculosis regimen change in Addis Ababa, Ethiopia: a qualitative study. *BMC Infect Dis*. 30 Sep. 2015;15:405. https://doi.org/10.1186/s12879-015-11422. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4590704/.
10. Marahatta SB, Yadav RK, Giri D, et al. Barriers in the access, diagnosis and treatment completion for tuberculosis patients in central and western Nepal: A qualitative study among patients, community members and health care workers. *PLoS One*. 2020;15(1), e0227293. https://doi.org/10.1371/journal.pone.0227293.
11. Lee S, Khan OF, Seo JH, et al. Impact of physician's education on adherence to tuberculosis treatment for patients of low socioeconomic status in Bangladesh. *Chonnam Med J*. 2013;49:27–30.
12. Yellappa V, Lefèvre P, Battaglioli T, Narayanan D, Van der Stuyft P. Coping with tuberculosis and directly observed treatment: a qualitative study among patients from South India. *BMC Health Serv Res*. 19 Jul. 2016;283. https://doi.org/10.1186/s12913-016-1545-9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4950693/.
13. Tadesse T, Demissie M, Berhane Y, Kebede Y, Abebe M. Long distance travelling and financial burdens discourage tuberculosis DOTs treatment initiation and compliance in Ethiopia: a qualitative study. *BMC Publ Health*. 2013 Dec;13(1):1–7.
14. Tanimura T, Jaramillo E, Weil D, Raviglione M, Lonnroth K. Financial burden for tuberculosis patients in low- and middle-income countries: a systematic review. *Eur Respir J*. 2014;43:1763–1775.
15. Sahile Z, Yared A, Kaba M. Patients' experiences and perceptions on associates of TB treatment adherence: a qualitative study on DOTS service in public health centers in Addis Ababa, Ethiopia. *BMC Publ Health*. 2018;18:462. https://doi.org/10.1186/s12889-018-5404-y.
16. Sagbakken M, Frich JC, Bjune G. Barriers and enablers in the management of tuberculosis treatment in Addis Ababa, Ethiopia: a qualitative study. *BMC Publ Health*. 2008 Dec;8(1), 1-1.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Costing of services under National Tuberculosis Elimination Program at public health facilities of northern India

Dinesh Kumar [a,*], *Shankar Prinja* [b]

[a] *Dr. Rajendra Prasad Government Medical College, Kangra at Tanda, Himachal Pradesh, 176001, India*
[b] *School of Public Health and Community Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India*

ARTICLE INFO

Article history:
Received 10 November 2021
Received in revised form
17 May 2022
Accepted 20 May 2022
Available online 27 May 2022

Keywords:
Costing
Tuberculosis
Services

ABSTRACT

Background: Costing of resources helps to measure financial implications and effective utilization of resources of national programs. As there is limited evidence about cost per service, current study was done to assess the cost of services under National Tuberculosis Elimination Program (NTEP) at Community Health Centres (CHCs) and Primary Health Centres (PHCs) of northern state of India.

Material and methods: Cross-sectional study carried out in two districts and from each district eight CHCs and PHCs were randomly selected.

Results: Mean annual cost of providing NTEP services at CHCs and PHCs were US$5243.1 (95%CI: 3008.0–7225.4) and US$1031.9 (95%CI: 669.1–1447.1) respectively. Across both centres human resource contributes to the most (CHC: 72.9%; PHC: 85.9%). One way sensitivity analysis was carried out for all health facilities and observed that human resource cost influences most cost per treated case by providing services under NTEP. Although relatively very less but cost of drugs also influences cost per treatment.

Conclusion: Cost of delivering services was high for CHCs as compared to PHCs. At both types of health facilities, human resource contributes the most to cost of delivering services under the program.

1. Background

India has an estimated population of about 1.2 billion living across its 29 states and 8 Union Territories.[1] India provides organized health services for Tuberculosis (TB) through Revised National TB Control Program (RNTCP) which has now renamed as National TB Elimination Program (NTEP). NTEP structure at state level is headed by state TB officer (STO) that heads state TB cell (STC). Services are provided at sub-district level like TB Unit (TU)- a program management unit-covering about 200,000 population in rural and urban population and about 100,000 in tribal area/hilly terrains. Each TU has designated microscopy centre (DMC) covering about 100,000 and

* Corresponding author.
E-mail addresses: dinesh9809@gmail.com (D. Kumar), shankarprinja@gmail.com (S. Prinja).
https://doi.org/10.1016/j.ijtb.2022.05.009

50,000 population in urban/rural and tribal/hilly area respectively. DMC provides diagnostic services whereas TU provided management services for TB treatment, supervision, and monitoring. The TU and DMC are integrated in health care delivery system of India like at community health centre (CHC) for about 120,000 people in urban/rural and 80,000 in tribal/hilly area. CHC manages primary health centres (PHCs) which further covers about 30,000 and 20,000 people in urban/rural and tribal/hilly area respectively.[2,3] At CHC and PHC level patient-centred services like routine laboratory tests, microscopic sputum examination, chest roentgenogram, cartridge based nucleic acid amplification test (CBNAAT), and dispensation of medicines are provided. In addition, under the NTEP, activities like advocacy, communication and social mobilization (ACSM) are given to the population. Referrals are generally required for change in treatment regimen due to treatment failure to TB and adverse effects due to medicines.[2]

Treatment initiation after diagnosis and success are major indicators to monitor progress of NTEP. Across public health facilities in country, treatment initiation was done in 94.0% of notified people with TB with 83.0% success rate. NTEP is one of the largest public funded health care programs of India that has provided diagnostic and treatment services free of cost to about 25 million patients since its inception.[2] Free services to people with TB are financed by government of India and international donors, so sustained finances are required to manage standard of services for TB in India. Central TB Division (CTD) carry out financial management (disbursement to state treasuries) and State TB cells (STC) in states receive and manage funds at state and district levels. There has been gap in financing of NTEP and from 2014 to 2020 about US$1.8 billion were requested and US$1.5 billon (836.3%) budget was approved. Of approved budget about 86.6% (US$1.3 billion) expenditure was incurred by the states to provide NTEP services.[2,4] Evidences do mention about financial facets of NTEP but data on costing of TB services are limited. However, studies assessed costing for diagnostic services and from perspective of patient.[5–9]

Financing of NTEP gives an underestimate for quantification of services as additional resources like time of human resources, capital investments, other laboratory equipment for assessment of presumptive patient of TB etc. are also used. It becomes important to estimate the cost of providing NTEP services which will serve as foundation of economic evaluations.[10] Costing of resources helps to measure financial implications and effective utilization of resources of NTEP. Information about cost levied for delivery of type of service is useful for planners and policy makers to improve allocative efficiency. Evidence of cost per unit exist where it was observed that cost to diagnose and treat per patient with drug susceptible TB was US$51.2 and US$180.7 respectively in Viet Nam and US$135 for 6 month therapy in Kenya.[11,12] Systematic review observed average cost of treatment for drug sensitive TB from S$14,6559 in high income countries to US$258 in lower income countries (LIC).[13] There is limited evidence mentioning cost spent per service delivery at the level CHCs and PHCs. We have adopted an organizational perspective to estimate annual cost for all type of services delivered at CHCs and PHCs in public sector under NTEP. The study was planned with an objective to assess the cost of services under NTEP at CHCs and PHCs of northern state of India.

2. Material and Methods

The study was a cross-sectional study and was undertaken in two districts of state of Himachal Pradesh, a northern state of India with about 6.8 million population. Considering primary health care facilities of Himachal Pradesh as study universe, first, two districts were selected randomly, followed by selection of Community Health Centres (CHCs) and Primary Health Centres (PHCs). No sample size estimation was done for selection of number of health facilities. The selected districts are western districts of the state and have about 2 million (29.6%) population. In financial year 2019–20, with base year 2011–12, state has gross domestics product per capita of US$3097 at current price US$2401 at constant price. and In State, rural area has high average monthly per capita consumer expenditure of US$26 (Food: US$11; Non-food: US$15) as compare to US%17.9 (Food: US$8.7; Non-food: US$9.2) of country. Study was carried out in randomly selected CHCs and PHCs of two selected districts. State has total 4132 health facilities in public sector across state; Hospitals: 127; CHCs: 94; PHCs: 586; Sub-Centres (SCs): 2096; and Dispensaries: 1229 with about an average of 60 health facilities per 100,000 population and about 74 facilities per 100 square kilometres (Km^2). In state on an average of 1.37 CHCs and 8.54 PHCs are available per 100,000 population which are accessible as about average number of 1.69 CHCs and 10.53 PHCs per 1000 Km^2. In selected districts, total 26 CHCs and 87 PHCs are in first and 111 PHCs provides health care services.[14] State has case notification rate of person with TB as 235/100,000 population with 89.0% treatment success rate and 67.0% cure rate. State was given award for its best performance for NTEP.[2] Districts were selected based on significant share of population and requirement of costing of NTEP by the state health services. Simple random sampling was adopted for selection of health facilities for each district. Firstly, four CHCs were selected and then from each selected CHC one PHC was again selected.

At CHC, medical officer assesses and examines a person with presumptive TB. Then person was sent to laboratory for microscopic sputum examination for Acid Fast Bacilli (AFB) and chest roentgenogram. The said tests were performed by LT and radiographer at health facility. If person shows signs of TB then medical officer inform the TU for registration and initiate the treatment for TB. If required, person was also assessed with basic investigations like hemogram and biochemistry. Person with signs of TB was registered for treatment and anti-tubercular drugs (ATT) were dispensed by the pharmacist. Person on ATT was followed-up at the health facility for treatment response with microscopic sputum examination and adverse effects of drugs. In case of non-response to treatment person with TB was referred to next health facility for further management. Under the programmatic settings, home-based visits were also done in case if person failed to report at facility on scheduled date for treatment. Senior treatment supervisor (STS) also visits home of

person with TB to monitor the progress of treatment and any other problem. Supervision and monitoring of laboratory services at CHC was done by the senior TB laboratory supervisor (STLS) regularly to ensure quality of activities. At PHC, there were no laboratory and radiography services as person were referred to CHC for assessment. Whereas, medical officer carried out assessment and examination at PHC and person with TB receives ATT at PHC.

In current study, bottom-up costing methods were used to assess economic costs of NTEP at CHCs and PHCs. Data was collected from health facilities from April 2019 to January 2020 by field investigators who were trained to collect data on costing. Data on capital and recurrent resources were collected for providing delivery of services at health facilities during last year i.e., from April 2018 to March 2019. Capital resources were comprised of items which lasted for a period of more than one year. It included building, equipment, and other non-consumables. For study purpose, costing of equipment and furniture are presented separately, whereas cost of land and building were considered as capital resource. Trainings which did not repeat and less likely to be repeated within one year were also kept as capital resource. Recurrent cost included staff salaries, drugs, consumables, and overheads such as water, electricity bills etc.

With NETP perspective, data were collected retrospectively from health facilities through review of registers, reports, individual interview of health staff, and direct observation of facility. Record review like outpatient registers, laboratory register, pharmacist register, and their respective monthly reports was done to quantify the diagnostic and treatment services provided to persons seeking care at health facilities. Stock registers were reviewed for enlisting and quantifying medicines and consumables used to provide services. Utilization of equipment and non-consumable items was also assessed form stock registers.

In addition, facility survey was carried out to assess capital and physical infrastructure like land, building, space, furniture, and equipment for their presence and utilization. Physical infrastructure was assessed by direct observation and measurement for space along with listing for type of delivered services. Interviewer administered semi-structured questionnaire was used for facility staff to collect information on monthly income, trainings received for NTEP, allowances/honorariums received in a month, and time allocation for different type of services in last week. Information to quantify frequency of activities like daily, weekly, monthly, quarterly, semi-annual, and annual were also gathered using questionnaire. Activity like review meetings and trainings were less frequent and allocated for entire day. Time allocation was also done for staff members on administrative work related to the NTEP. Administrative approval was sought from health authorities and informed consent was also taken form participants. All cost were in Indian National Rupees (INR) and later transformed in to United State Dollars (US$) of year 2019 (INR 1 = US$0.014).

Cost of utilized resources like equipment and furniture were annualized with annuity factor based on price and life of item. Equal discounting approach was considered and a discount rate of 5.0% was considered and applied.[15] Capital costs for resources like building and space were calculated based on the measurement of floor area (feet^2). Prevalent market rental prices were then obtained from key informants of local area of located health facilities. Standard literature on the life of furniture and equipment were reviewed.[16,17] In addition, local staff was also asked about their perceptions for the life of capital resources and cost of equipment were also obtained from local distributors. State government rate contract was use to estimate cost of recurrent resources like drugs, consumables etc. and then costs were applied to amount of resources consumed.

Complete cost was allocated to resource if it was used exclusively to deliver particular service. If resources like room space, furniture, and equipment were used to provide more than one service then cost was apportioned according to time utilized to provide particular service. The apportionment criteria are inclusive of the effect of number of beneficiaries for services and time utilized to offer that particular service to per beneficiary. The overall NTEP cost at CHCs and PHCs were presented as human resource, consumables, medicines, stationary, equipment, furniture, capital, and miscellaneous. Then the costing of various types of offered services were also presented like outpatient, diagnostic, treatment, monitoring, meetings and trainings, recoding, IEC (information, education, and communication), and auxiliary.

In addition to the costing for head and services, costs per capita unit per year were also calculated like cost per outpatient consultation, per person screened for TB, and per treated. Cost to provide NTEP services was also calculated for per population. It was calculated by calculating the total annual cost to provide service and then division by number of beneficiaries in a year. Sample was bootstrapped 1000 times and mean estimates were calculated for unit costs along with 95% confidence interval using Microsoft excel 2003–7. In addition, effect of type of cost on cost per treated patients was calculated using probabilistic one-way sensitivity analysis (OSA). First Monte-Carlo simulation of observed costs was done and mean estimate of simulated values was calculated. Thereafter, OSA was performed wherein effect of changing mean cost more and less than 20.0% was observed on cost per treatment.

3. Results

A total of 18 health centres (CHC: 8; PHC: 8) were sampled from two districts of the state. Total population covered by CHCs and PHCs were 363,785 and 151,448 respectively. Total persons with presumptive TB were high in PHCs (10,309) as compare to CHCs (7,703). Likewise, number of persons with TB receiving treatment from health facility were high in PHCs (342) than in CHC (232). Doctor to population ratio was similar across both types of facilities (CHC: 13,991.7; PHC: 13,768.0), whereas nurse to population ratio was observed to be low in CHC (24,252.3) compared to PHC (75,724.0). Overall health professional to population ratio high for PHC (6,884.0) than for CHC (5,196.9) (Table: 1).

Mean annual cost of providing NTEP services at CHCs and PHCs were US$5243.1 (95%CI: 3008.0–7225.4) and US$1031.9 (95%CI: 669.1–1447.1) respectively (Table: 2). Across both centres human resource contributes to the most (CHC: 72.9%;

PHC: 85.9%). Cost of consumables and medicines was high at CHCs (16.3%) as compared to PHC (6.4%). Description of nature of services showed that at CHCs outpatient services, diagnostic, and treatment services cost about US$1753.5 (95%CI: 1010.1–2543.9), US$1279.2 (95%CI:20.9-6-2682.0), and US$1216.2 (95%CI: 512.0–2178.6) respectively. Outpatient services cost about US$513.5 (95%CI: 381.2–727.4) at PHC (Table: 3). At CHC outpatient, diagnostic, and treatment cost the most (81.0%) and at PHC activities like outpatient, monitoring, and meetings and trainings had major share in cost (90.0%).

Cost per service deliver was calculated where cost per population, per outpatient consultations, per person diagnosed for TB, and per person treated for TB covered under NTEP were US$0.4 (95%CI: 0.3–0.6), US$0.3 (95%CI:0.2–0.5), US$6.1 (95%CI: 3.6–8.9), and US$182.5 (95%CI: 118.0–257.9) respectively at CHCs. For respective services, cost at PHCs were about US$0.2 (95%CI: 0.2–0.4), US$0.2 (95%CI: 0.1–0.3), US$4.0 (95%CI: 3.1–4.8), and US$101.4 (95%CI: 19.1–48.6) (Table: 4). OSA was carried out for all health facilities and observed that human resource cost influences most cost per treated case by providing services under NTEP. Although relatively very less but cost of drugs also influences cost per treatment (Figure: 1).

4. Discussion

Costing of various health services delivered at health facilities of public health sector had been done in India. Extensive analysis of all these services at CHCs and PHCs is available.[18–20] Evidence is available for costing of diagnostic services for TB infection, however, information does not exist for cost of providing programmatic services for TB control at public health facilities.[5,6] Resources are being made available at public health facilities of primary care under NTEP to improve case detection and cure rate. Current study was planned to estimate the annual cost of delivering services under NTEP at sixteen health centres (CHCs and PHCs) of northern state of India.

With best of our knowledge, current study is a maiden effort where costing for diagnostic, therapeutic, and information services was done. Bottom-up standard costing methodology was adopted and data was observed for complete one year to remove bias due to seasonal variation. TB is a chronic insidious disease, so seasonal variation exists due to indifferent pattern in symptomology, occupational constraints, and health care seeking behaviour and utilization. Current study estimated annual cost of US$0.4 and US$0.2 per capita at CHC and PHC respectively. Mean annual cost was about US$0.3 at CHC and US$0.2 at PHC per outpatient consultations. At CHC, annual cost for per diagnosed and treated was observed to be US$6.1 and US$182.5 respectively, however same at PHC respective mean annual cost was US%4.0 and US$101.4. Human resource-salaries-constituted large part in delivering NTEP services at health facilities. More than four-fifth of annual cost at CHCs was utilized for outpatient, diagnostic, and treatment services. Whereas, in addition to outpatient services activities like monitoring and meetings shared about three-fourth of annual cost.

Cost of service provision varies according to country settings due to differential financing of health care services. In Viet Nam, cost to diagnose and treat per patient with drug susceptible TB was US$51.2 and US$180.7 respectively.[11] In Kenya, at national level, per patient cost first line treatment of 6 month was US$135 for drug sensitive TB.[12] In a systematic review of 71 research articles, average treatment cost of drug sensitive TB was US$14,6559 in high income countries (HIC), US$840 in upper-middle income countries (UMIC), US$273 in lower middle-income countries (LMIC), and US$258 in lower income countries (LIC). In LMIC countries, total 10 studied were available and per patient cost was highest for hospitalization (US$215) followed by for outpatient visits (US$75), diagnostics and monitoring (US$48), drugs (US$39), and other (US$25).[13]

Although, costing of NTEP services have not been done but evidence observed share of salaries dominated the annual cost. Study estimated that share of human resource to total cost was about 65.0% for chest roentgenogram, 87.0% for Ziehl-Neelsen microscopy, and 95.0% digital roentgenogram.[5] Evidence observed that salaries of staff constitute about 50.0–60.0% to deliver primary health care services in public sector.[18–20]

Cost share of resources depends upon range of reasons like population covered, nature of disease, deployment of laboratory support, and medicines. In current study, radiological facility, laboratory support, and additional staff like treatment supervisor and educator were available at CHC. Relatively additional manpower at CHC, increased the annual cost for diagnostic and treatment cost to US$1279.2 and US$1216.2 respectively. However, despite being a higher facility, persons with TB receiving treatment at CHC was less as compare (232) to PHC (342). It is due to the mechanism under NTEP where person with features of presumptive TB can be referred for diagnostic care to CHC but receives treatment form health facility in his/her residential facility like PHC. More patients receiving medicines from PHCs require more supervision and monitoring, which is explained by its share in annual cost of services. Another important reason which influences cost estimates is population covered by health facility. In current study CHCs covered a way less population (average: 45.473.1) than the norm of 80,000 population per CHC. It is due to upgradation of PHCs to CHCs with provision of additional

Table 1 – Health facility details assessed for costing of National Tuberculosis Elimination Program (NTEP), 2020, Himachal Pradesh India.

Characteristics	CHC (8)	PHC (8)
Doctors	26	11
Nurses	15	2
Laboratory Technicians	7	1
Pharmacists	14	8
Radiographers	4	0
Senior Treatment Supervisor	2	0
Educator	2	0
Population covered	363,785	151,448
Presumptive TB Patients	7703	10,309
TB patients on treatment	232	342
Doctor to population ratio	13,991.7	13,768.0
Nurse to population ratio	24,252.3	75,724.0
Health professionals to population ratio	5196.9	6884.0

Table 2 – Cost centres of Community Health Centre (CHC) and Primary Health Centre (PHC) delivering services under National Tuberculosis Elimination Program (NTEP), 2020, Himachal Pradesh India.

Centres	CHC (8)			PHC (8)		
	Mean	LCI	UCI	Mean	LCI	UCI
Human Resource	3822.2	2112.0	5386.6	886.8	542.5	1236.8
Consumables	176.5	53.1	310.5	7.9	2.1	14.3
Medicines	676.6	368.1	1044.7	58.5	19.2	104.4
Stationary	11.4	7.8	15.1	2.6	1.7	3.9
Equipment	84.5	23.6	169.3	2.3	1.2	4.3
Furniture	126.0	41.7	236.3	25.9	18.2	36.3
Capital	18.3	6.9	32.7	7.2	5.4	9.1
Miscellaneous	32.6	10.4	64.7	7.8	6.9	8.7
Total	5243.1	3008.0	7255.4	1031.9	669.1	1447.1

Table 3 – Costing at Community Health Centre (CHC) and Primary Health Centre (PHC) for services and activities under National Tuberculosis Elimination Program (NTEP), 2020, Himachal Pradesh India.

Services/Activities	CHC (8)			PHC (8)		
	Mean	LCI	UCI	Mean	LCI	UCI
Outpatient	1753.5	1010.1	2543.9	513.5	381.2	727.4
Diagnostic	1279.2	209.6	2682.0	84.6	4.8	239.5
Treatment	1216.2	512.0	2178.6	86.1	26.3	178.3
Monitoring	301.3	47.8	759.6	139.0	34.4	306.9
Meetings and Trainings	294.3	119.2	516.7	105.7	52.8	161.2
Recording	71.1	15.2	161.7	63.6	11.8	172.9
IEC	36.5	20.3	49.8	16.8	5.4	28.5
Auxiliary	258.4	42.8	544.3	16.0	3.9	31.1

Table 4 – Cost effectiveness at Community Health Centre (CHC) and Primary Health Centre (PHC) for services and activities under National Tuberculosis Elimination Program (NTEP), 2020, Himachal Pradesh India.

Services/Activities	CHC (8)			PHC (8)		
	Mean	LCI	UCI	Mean	LCI	UCI
Cost per capita	0.4	0.3	0.6	0.2	0.2	0.4
Cost per out patient consultations	0.3	0.2	0.5	0.2	0.1	0.3
Cost per diagnosed	6.1	3.6	8.9	4.0	3.1	4.8
Cost per treated	182.5	118.0	257.9	101.4	19.1	48.6

capital, human, and diagnostic resources. Whereas, on an average 18,931 population is covered by each PHCs which is within norm of 20,000 population per PHC for hilly area.

Globally, since 2006, requirement of resources for TB control have doubled that are largely attributed to diagnostic and treatment services. Domestic funding was advocated to be increased to meet the about 68.0% money gap for TB control. India has invested in its TB control efforts with increase in its domestic funding up to 92.0% from year 2016–2019 and about US$2.3 billion in year 2017–20, was estimated for diagnostic, treatment administrative, patient support services.[21,22] Economic costing assists comprehensibility of various strategic heads of program and allocative efficiency. Current results of costing information for various cost centres will be useful for reframing policy and programmatic strategies. It will be found useful to set up and upgradation of peripheral health facilities for delivering TB control services under NTEP. It will also be potentially useful to monitor money flow to health sector activities with national and state health accounts.

Study findings should be read with certain limitations of the study. First, about 6 PHCs were upgraded to CHCs by the government with positing of additional resources at reduced population norms. Large investment was done for the upgraded CHCs in terms of capital, human resources, equipment, and furniture. It effects the unit cost per service delivered but current study still captures the average cost of delivering NTEP services at CHC level. Secondly, time motion study was not carried out to monitor the time consumed to deliver various forms of multiple activities by health professionals. While such study gives a robust estimate for time allocation patterns.[23] However, different types of services at CHCs and PHCs under NTEP limited application of time-motion study, and methods used in current study considered to be useful for estimation.[20,24,25] Thirdly, current study was carried out from health system perspective and does not account for out-of-pocket expenditure incurred by patients. Fourthly, every state invests in health facilities depending upon available resources, so findings of current study cannot be generalized to other states. A larger sample, stratified according to geography and population norm, across states will be nationally representative. It adjusts the cost estimates for difference in number of beneficiaries availing health services at different

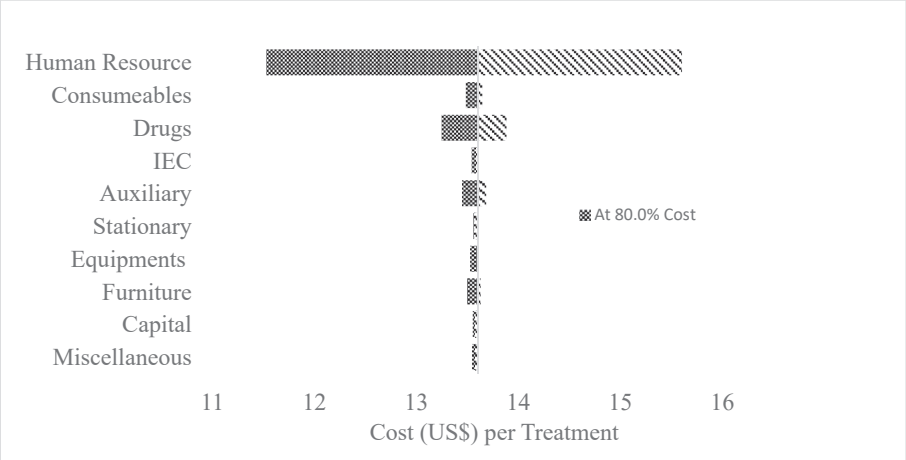


Fig. 1 – Tornado plot for influence of various type of costs on cost per treatment at both types of health facilities for provision of services and activities under National Tuberculosis Elimination Program (NTEP), 2020, Himachal Pradesh India.

types of health facilities. Current study, apart from facility level cost estimates, bootstrapping was done to calculate population level average of unit cost and its dispersion. It was applied to draw mean cost along with confidence interval as original sample was considered to be sub-optimal to draw estimates.

It is to conclude that human resource share most of the cost and influence outcome in terms of cost per treated. Moreover, costing studies have been done at CHCs and PHCs to estimate cost of delivering services, but evidence in grey literature about cost to deliver NTEP services at primary care health facilities is not available to our best knowledge. This study gives an estimate for cost of delivery of programmatic services at CHCs and PHCs as peripheral health institutions (PHIs). The findings can be utilized to plan and set up PHIs in hilly area under NTEP. Since under the program the services are given free of cost to the people, so the findings can be helpful to government to assess incremental cost to deliver additional services. Consistent studies under the program at sentinel health facilities will guide NTEP to capture pattern of public health expenditure.

Conflicts of interest

The authors have none to declare.

Acknowledgement

We thank State Task Force (STF) for involvement of medical colleges under NTEP to provide financial assistance.

REFERENCES

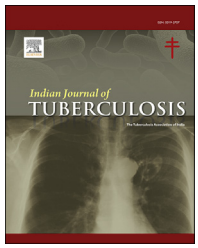
1. Office of registrar general of India. Government of India. Available at: http://www.dataforall.org/dashboard/censusinfoindia_pca/(Accessed on: March 5, 2021).
2. Central TB Division, Ministry of Health and Family Welfare (MoHFW), Government of India. India TB Report 2016 to 2020. National TB Elimination Programme Annual Report. Central TB Division, MoHFW, Government of India; 2016 - 2020. Available at: https://tbcindia.gov.in/showfile.php?lid=3538 (Accessed on: March 5, 2021).
3. Chokshi M, Patil B, Khanna R, et al. Health systems in India. *J Perinatol*. 2016;36(s3):S9–S12.
4. Babu R, Sagili K, Jacob A, Chadha AA. Resource optimisation for tuberculosis elimination in India. *Econ Polit Wkly*. 2016;L1:26–27.
5. Muniyandi M, Lavanya J, Karikalan N, et al. Estimating TB diagnostic costs incurred under the national tuberculosis elimination programme: a costing study from Tamil nadu, south India. *Int Health*. 2021 Feb 11;13(6):536–544. ihaa105.
6. Pallas SW, Courey M, Hy C, Killam WP, Warren D, Moore B. Cost analysis of tuberculosis diagnosis in Cambodia with and without Xpert® MTB/RIF for people living with HIV/AIDS and people with presumptive multidrug-resistant tuberculosis. *Appl Health Econ Health Pol*. 2018 Aug;16(4):537–548.
7. Bogdanova EN, Mariandyshev AO, Balantcev GA, et al. Cost minimization analysis of line probe assay for detection of multidrug-resistant tuberculosis in Arkhangelsk region of Russian Federation. *PLoS One*. 2019 Jan 29;14(1), e0211203.
8. Chandra A, Kumar R, Kant S, Parthasarathy R, Krishnan A. Direct and indirect patient costs of tuberculosis care in India. *Trop Med Int Health*. 2020 Jul;25(7):803–812.
9. de Siqueira Filha NT, de Fatima Pessoa Militao de Albuquerque M, Legood R, Rodrigues L, Santos AC. The economic burden of tuberculosis and latent tuberculosis in people living with HIV in Brazil: a cost study from the patient perspective. *Publ Health*. 2018 May;158:31–36.
10. Raftery J. Costing in economic evaluation. *BMJ*. 2000 Jun 10;320(7249):1597.
11. Minh HV, Mai VQ, Nhung NV, et al. Costs of providing tuberculosis diagnosis and treatment services in Viet Nam. *Int J Tubercul Lung Dis*. 2017;21(9):1035–1040.
12. Kairu A, Orangi S, Oyando R, et al. Cost of TB services in healthcare facilities in Kenya (No 3). *Int J Tubercul Lung Dis*. 2021;25(12):1028–1034.
13. Laurence YV, Griffiths UK, Vassall A. Costs to health services and the patient of treating tuberculosis: a systematic literature review. *Pharmacoeconomics*. 2015;33(9):939–955.
14. Department of Economic and Statistics. Government of Himachal Pradesh. Statistical abstract of Himachal Pradesh

2018-19. Available at: https://himachalservices.nic.in/economics/pdf/StatisticalAbstract_2018_19.pdf (Accessed on: March 5, 2021).
15. Attema AE, Brouwer WBF, Claxton K. Discounting in economic evaluations. *Pharmacoeconomics*. 2018 Jul;36(7):745–758.
16. Johns B, Baltussen R, Hutubessy R. Programme costs in the economic evaluation of health interventions. *Cost Eff Resour Allocation*. 2003;1(1):1.
17. World Health Organization. *Making Choices in Health: WHO Guide to Cost-Effectiveness Analysis* [Internet]. Geneva, Switzerland; 2003. Available from: http://www.who.int/choice/publications/p_2003_generalised_cea.pdf. Accessed March 9, 2021.
18. Prinja S, Gupta A, Verma R, et al. Cost of delivering health care services in public sector primary and community health centres in north India. *PLoS One*. 2016 Aug 18;11(8), e0160986.
19. Anand K, Kapoor SK, Pandav CS. Cost analysis of a primary health centre in northern India. *Natl Med J India*. 1993 Jul-Aug;6(4):160–163.
20. Prinja S, Jeet G, Verma R, et al. Economic analysis of delivering primary health care services through community health workers in 3 North Indian states. *PLoS One*. 2014 Mar 13;9(3), e91781.
21. World Health Organisation. *Global Tuberculosis Report 2019*. Geneva, Switzerland: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO. WHO/CDS/TB/2019.15.
22. Central TB Division, Ministry of Health and Family Welfare (MoHFW), Government of India. *National Strategic Plan for TB Elimination 2017-2025*. Central TB Division, MoHFW, Government of India; 2017. Available at https://tbcindia.gov.in/WriteReadData/NSP%20Draft%2020.02.2017%201.pdf. Accessed March 17, 2021.
23. Fox-Rushby J, Cairns J. *Economic Evaluation*. London: Oxford University Press; 2006.
24. Prinja S, Manchanda N, Mohan P, et al. Cost of neonatal intensive care delivered through district level public hospitals in India. *Indian Pediatr*. 2013;50(9):839–846. Epub 2013/03/19.
25. Prinja S, Mazumder S, Taneja S, et al. Cost of delivering child health care through community level health workers: how much extra does IMNCI program cost? *J Trop Pediatr*. 2013;59(6):489–495.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Vitamin D receptor genetic polymorphisms in severe and recurrent tuberculosis in children

Ira Shah [a,*], *Naman S. Shetty* [a], *Prameeta Chigari* [a], *Vandana Pradhan* [b], *Durga Chougule* [b], *Vishrutha S. Poojari* [a], *Akanksha Jaiswal* [a], *Manisha Madkaikar* [b]

[a] *Pediatric TB Clinic, Department of Pediatric Infectious Diseases, B. J. Wadia Hospital for Children, India*
[b] *ICMR-National Institute of Immunohaematology, K. E. M. Hospital, Parel, Mumbai, India*

ARTICLE INFO

Article history:
Received 16 May 2021
Received in revised form
28 September 2021
Accepted 20 May 2022
Available online 29 May 2022

Keywords:
Children
Polymorphism
Tuberculosis
Vitamin D receptor

ABSTRACT

Aim: To analyze the genetic polymorphisms of vitamin D receptor FokI, TaqI, ApaI and BsmI gene polymorphisms in children with severe and recurrent tuberculosis (TB).
Methods: A prospective, observational study was conducted in 35 children with severe and recurrent TB referred to our Pediatric TB clinic at a tertiary referral center for children. The blood samples were analysed for genetic polymorphisms of Vitamin D receptor with respect to FokI, TaqI, ApaI and BsmI genotypes and their individual alleles and association of various clinical and laboratory parameters were analysed.
Result: Ten (28.6%) children had recurrent TB and 26 (74.3%) had severe TB. The severity of TB was not associated with Ff and ff polymorphism of FokI (Odd's ratio 7.88) as compared to no FokI polymorphism. Absence of FokI polymorphism was associated with recurrent lymph node TB (Odds ratio 3.429). Presence of Tt polymorphism of TaqI ($p = 0.04$) and Fok1 Polymorphism [Odds ratio 7.88] were not associated with recurrent TB.
Conclusion: Recurrent TB was absent in presence of Tt polymorphism of TaqI. Severe TB was not associated polymorphism of Vitamin D receptor polymorphisms.

1. Introduction

Approximately 90% of tuberculosis (TB) infected individuals will remain asymptomatic with latent infection and only 10% will develop active disease suggesting that host genetic factors play an important role to regulate the progression of TB infection.[1] In fact, the mechanisms involved in restriction of disease development in latent infection or leading to severe active disease are still largely unknown. Studies such as case–control studies, twin, candidate gene approaches, family-based and genome-wide association studies (GWAS) revealed the association of genetic factors with susceptibility or resistance to TB.[1] Differential rates of TB infection and clinical outcomes among races, ethnicities, and families suggest a plausible genetic contribution toward tuberculosis susceptibility.[2]

* Corresponding author.
E-mail address: irashah@pediatriconcall.com (I. Shah).
https://doi.org/10.1016/j.ijtb.2022.05.011

In TB infection, vitamin D activates macrophage to restrict *Mycobacterium tuberculosis* (MTB) intracellular growth. This effect is achieved through binding to vitamin D receptor (VDR) in macrophages, and this activates cathelicidin synthesis,[3] and consequently eliminate MTB in phagolysosomes.[4] These processes might be affected by polymorphisms in the VDR gene. The VDR gene which is located on chromosome 12q13.11, includes various single nucleotide polymorphisms (SNPs), including ApaI (A/a), BsmI (B/b), FokI (F/f), and TaqI (T/t). These polymorphisms might affect the activity of VDR and subsequently downstream effects of vitamin D. Though there are several studies evaluating the VDR gene polymorphisms and their effects on susceptibility to or resistance against TB in different ethnicities or populations, the exact effect is still unknown and inconsistent.[5]

The different results of TB infection might be due to diverse ethnic background. Meta-analysis by Gao[6] (2010) found that in Asians, subjects with ff genotype were more susceptible to TB and subjects with bb genotype had decreased risk for TB. Several studies showed an association between vitamin D deficiency and certain diseases such as systemic lupus erythematosus,[7] diabetes mellitus,[8] TB,[9] etc.

Evidence suggests that the effects of vitamin D may vary based on vitamin D receptor (VDR) genotypes, implying that supplementation may only be of clinical benefit in subpopulations with a particular VDR genotype.[10] Our study aims to determine the association of VDR polymorphism with recurrent and severe TB in the Indian population.

2. Materials and methods

2.1. Patient population

This observational, non-interventional, prospective study was conducted over a period of 12 months from Dec 2018–Dec 2019 in 35 children in the age group of 0–18 years with recurrent and severe TB who were referred to the Pediatric TB clinic at a tertiary children's hospital. The study was conducted after approval of the institutional ethics committee. The sample size has been calculated based on the previous year's data or recurrent and severe TB available in the hospital. Children were diagnosed as TB based on bacteriological confirmation on GeneXpert or TB MGIT culture or were clinically diagnosed based on World Health Organization (WHO) criteria for diagnosis of TB.[11] Patients were determined to be drug-resistant (DR) TB based on WHO criteria.[11] Patients with HIV, on chemotherapy agents and non-tuberculous mycobacterial infections other than BCGosis were excluded from the study.

Severe TB was defined as disseminated TB, neuro TB, fibrocavitatory TB, bone TB, miliary TB or mediastinal lymph node TB with lung parenchymal involvement causing respiratory distress. Recurrent tuberculosis was defined as when a patient developed more than one infection of TB. A detailed history and physical examination of the patient were conducted. After obtaining parent/guardian consent, blood sample was collected in plain and EDTA (2 ml and 3 ml each) vacutainers for estimation of serum 25(OH) vitamin D levels and Vitamin D receptor (VDR) gene polymorphism respectively.[12] Duration of current TB treatment was determined and paradoxical reaction was defined as unusual expansion or formation of a new tuberculous lesion despite appropriate anti-tubercular treatment.[13]

2.2. Estimation of serum vitamin D levels

Serum 25(OH) vitamin D levels were detected using sandwich ELISA (Calbiotech) as per manufacturer instructions. Patients were considered Vitamin D sufficient if levels were >30 ng/ml, Vitamin D insufficient if levels were between 20 and 30 ng/ml and Vitamin D deficient if levels were <20 ng/ml. Patients with Vitamin D levels >100 ng/ml were considered to have toxicity.

2.3. Vitamin D receptor gene polymorphism analysis

Genomic DNA extraction from whole blood was done using QIAamp DNA Mini Kit (QIAGEN, Germany) as per manufacturer instructions. Purified DNA was eluted from the QIAamp Mini column in a concentrated form using tris edta (TE) buffer or water[14] PCR reaction was set up in volume of 25 μl containing genomic DNA (100 ng), 2 pM of each primer, 200 μM of each dNTPs, 2.5 μl of 10× PCR Buffer and 1.25 units of Taq DNA polymerase (Bioron GmbH) separately in each tube for FokI, TaqI, ApaI and BsmI polymorphic sites of VDR in a gradient thermal Master cycler (Eppendorf, USA) using primers given by Harris et al and Morrison et al. The PCR cycling conditions for FokI, TaqI, ApaI and BsmI were as follows: Initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 45 s, annealing as tabulated in Table 1 for 45 s, extension at 72 °C for 45 s, which was followed by final extension at 72 °C for 7 min. Post amplification PCR products were subjected to restriction digestion by FokI (FastDigest Thermo Fisher Scientific) at 37 °C for 1 h, TaqI (Thermo Fisher Scientific) at 65 °C overnight, ApaI (Thermo Fisher Scientific) at 37 °C overnight, and BsmI (CutSmart New England BioLabs) at 65 °C for 1 h respectively in separate tubes. After digestion the cut products were separated on 2.5% (w/v) agarose gel (Thermo Fisher Scientific).

2.3.1. Statistical analysis

Polymorphisms of FokI, BsmI, ApaI and TaqI genes were analysed with respect to the individual alleles. Association of various clinical parameters and laboratory parameters were analysed with the different polymorphisms by Fischer Exact test and Anova-1 test respectively. P value <0.05 was considered as significant.

3. Results

The mean age of patients was 6.56 ± 4.1 years. Male: Female ratio was 2:3. The mean duration of treatment was 1.23 ± 0.3 months with a range of 0.8–2 months at the time of doing the study. Demographic details of patients are depicted in Table 2. Five (14.3%) patients had paradoxical reaction in the form of LN TB in 2 (5.8%), pulmonary TB in 1 (2.9%), recurrent ascites in 1 (2.9%) and retropharyngeal abscess in 1 (2.9%) while on TB treatment.

The four different polymorphisms were analysed with respect to the individual alleles (homozygous dominant,

Table 1 – PCR-RFLP details for FokI, TaqI, ApaI and BsmI VDR gene.

rs ID	Polymorphism	Primer sequence	Annealing Temperature (°C)	PCR-RFLP products (bp)
rs2228570	Exon 2 (T/C) FokI	F 5′ -AGCTGGCCCTGGCACTGACTCTGCTC-3′ R 5′- ATGGAAACACCTTGCTTCTTCTCCCTC-3′	69	FF (196,69) Ff (265, 196, 69) ff (265)
rs731236	Exon 9 (C/T) TaqI	F 5′- CAGAGCATGGACAGGGAGCAA-3′ R 5′- GCAACTCCTCATGGCTGAGGTCTC-3′	64	TT (495, 245) Tt (495, 290, 245, 205) tt (290, 245, 205)
rs7975232	Intron 8 (A/C) ApaI	F 5′- CAGAGCATGGACAGGGAGCAA-3′ R 5′- GCAACTCCTCATGGCTGAGGTCTC-3′	64	AA (740) Aa (740, 520, 220) Aa (520, 220)
rs1544410	Exon 7/Intron 8 (A/G) BsmI	F 5′- CAACCAAGACTACAAGTACCGCGTCAGTGA-3′ R 5′- AACCAGCGGGAAGAGGTCAAGGG-3′	63	BB (650, 175) Bb (825, 650, 175) bb (825)

Table 2 – Demographic details of patients.

Variable	N (%)
Age	
0–3 years	8 (22.8%)
3–6 years	12 (34.2%)
6–12 years	13 (37.3%)
>12 years	2 (5.7%)
Type of TB	
lymph node (LN) TB	13 (37.1%)
neuro TB	9 (25.7%)
fibro-cavitatory TB	5 (14.3%)
disseminated TB	4 (11.4%)
bone TB	2 (5.7%)
miliary TB	2 (5.7%)
Recurrent TB	10 (28.6%)
Severe TB	26 (74.3%)
BCG vaccination	31 (88.6%)
BCG scar present	27 (77.1%)
TB contact	9 (25.7%)
Type of drug resistance	
MDR-TB	5 (14.3%)
Contact with MDR-TB	1 (2.9%)
pre-XDR TB	5 (14.3%)
XDR TB	1 (2.9%)
Type of previous TB	
pulmonary TB	1 (2.9%)
LN TB	9 (25.7%)
Positive test	
GeneXpert	25 (71.4%)
Mantoux	23 (66.7%)
MGIT	16 (45.7%)
Treatment status	
Completed	10 (28.6%)
On Treatment	25 (71.5%)
Vitamin D levels	
Deficient	7 (20%)
Insufficient	7 (20%)
Normal	10 (28.6%)
Unchecked	11 (31.4%)

homozygous recessive and heterozygous). Testing of FokI gene showed that 10 (28.6%) patients had Ff variant, 9 (25.7%) had ff variant while 1 (2.9%) had FF genovariant.

Testing of TaqI gene showed that 14 patients (40%) had TT genotype, 9 patients (25.7%) had Tt genotype and one patient (2.9%) had tt genotype. Recurrent TB was less common in the Tt TaqI polymorphism ($p = 0.036$).

Testing of ApaI gene showed that 11 patients (31.4%) had aa polymorphism, 6 patients (17.1%) had AA genotype and four patients (11.4%) had Aa genotype. The recurrence and severity of TB was not associated with ApaI polymorphism.

Testing of BsmI gene showed that 11 patients (31.4%) had bb genotype, 9 patients (25.7%) had Bb genotype and seven patients (20%) had BB genotype. Children with BB polymorphism were less likely to get drug resistant TB (0.048).

The various clinical and laboratory factors and their association with the 4 VDR gene polymorphisms are depicted in Table 3.

4. Discussion

$1,25(OH)_2D3$ plays immunomodulatory role in the activation of monocytes and suppression of human innate immunity to

Table 3

Clinical variable	FF (n = 1)	Fokl (%) Ff(n = 10)	ff (n = 9)	Fisher's Test p value	TT (n = 14)	Taq (%) Tt (n = 9)	tt (n = 1)	Fisher's Test p value	AA (n = 6)	ApaI (%) Aa (n = 4)	aa (n = 11)	Fisher's Test p value	BB (n = 7)	BsmI (%) Bb(n = 9)	bb (n = 11)	Fisher's Test p value
Sex				0.52				0.622				0.350				0.927
Male	1 (100)	4 (40)	4 (44.4)		7 (50)	4 (44.4)	0 (0)		4 (66.7)	1 (25)	4 (36.4)		3 (42.9)	4 (44.4)	4 (36.4)	
Female	0 (0)	6 (60)	5 (55.6)		7 (50)	5 (55.6)	1 (100)		2 (33.3)	3 (75)	7 (63.6)		4 (57.1)	5 (55.6)	7 (63.6)	
Diagnosis				0.35				–				0.773				0.442
Miliary TB	0 (0)	1 (10)	1 (11.1)		0 (0)	0 (0)	0 (0)		1 (16.7)	0 (0)	0 (0)		0 (0)	2 (22.2)	1 (9.1)	
Bone TB	1 (100)	1 (10)	0 (0)		2 (14.3)	1 (11.1)	0 (0)		1 (16.7)	0 (0)	1 (9.1)		0 (0)	1 (11.1)	1 (9.1)	
Disseminated TB	0 (0)	2 (20)	1 (11.1)		1 (7.1)	3 (33.3)	0 (0)		1 (16.7)	1 (25)	1 (9.1)		1 (14.3)	1 (11.1)	3 (27.3)	
Fibrocavitatory TB	0 (0)	2 (20)	1 (11.1)		2 (14.3)	2 (22.2)	0 (0)		2 (33.4)	1 (25)	2 (18.2)		0 (0)	1 (11.1)	0 (0)	
LN TB	0 (0)	2 (20)	3 (33.3)		3 (21.4)	2 (22.2)	1 (100)		1 (16.7)	1 (25)	3 (27.3)		5 (71.4)	1 (11.1)	4 (36.4)	
Neuro TB	0 (0)	2 (20)	3 (33.3)		6 (42.9)	1 (11.1)	0 (0)		0 (0)	1 (25)	4 (36.4)		1 (14.3)	3 (33.3)	2 (18.2)	
Recurrent				0.78				**0.036**				0.305				0.090
Yes	0 (0)	3 (30)	2 (22.2)		4 (28.6)	0 (0)	1 (100)		1 (16.7)	0 (0)	4 (36.4)		3 (42.9)	0 (0)	4 (36.4)	
No	1 (100)	7 (70)	7 (77.8)		10 (71.4)	9 (100)	0 (0)		5 (83.3)	4 (100)	7 (63.6)		4 (57.1)	9 (100)	7 (63.6)	
Severe				0.94				0.072				0.942				0.290
Yes	1 (100)	9 (90)	8 (88.9)		12 (85.7)	8 (88.9)	0 (0)		5 (83.3)	3 (75)	9 (81.8)		4 (57.1)	8 (88.9)	9 (81.8)	
No	0 (0)	1 (10)	1 (11.1)		2 (14.3)	1 (11.1)	1 (100)		1 (16.7)	1 (25)	2 (18.2)		3 (42.9)	1 (11.1)	2 (18.2)	
Vitamin D				0.43				0.116				0.623				0.487
Not Done	0 (0)	3 (30)	1 (11.1)		2 (14.3)	3 (33.3)	0 (0)		1 (16.7)	1 (25)	2 (18.2)		3 (42.9)	3 (33.3)	2 (18.2)	
Deficient	1 (100)	3 (30)	1 (11.1)		5 (35.7)	0 (0)	0 (0)		1 (16.7)	0 (0)	4 (36.4)		0 (0)	1 (11.1)	4 (36.4)	
Insufficient	0 (0)	2 (20)	3 (33.3)		1 (7.1)	3 (33.3)	1 (100)		2 (33.3)	2 (50)	1 (9.1)		2 (28.6)	2 (22.2)	1 (9.1)	
Normal	0 (0)	2 (20)	4 (44.4)		6 (42.9)	3 (33.3)	0 (0)		2 (33.3)	1 (25)	4 (36.4)		2 (28.6)	3 (33.3)	4 (36.4)	
Gene Expert				0.61				0.312				0.711				0.686
Negative	0 (0)	3 (30)	4 (44.4)		4 (28.6)	5 (55.6)	0 (0)		2 (33.3)	2 (50)	3 (27.3)		2 (28.6)	4 (44.4)	3 (27.3)	
Positive	1 (100)	7 (70)	5 (55.6)		10 (71.4)	4 (44.4)	1 (100)		4 (66.7)	2 (50)	8 (72.7)		5 (71.4)	5 (55.6)	8 (72.7)	
Resistance				–				0.390				–				**0.048**
No	1 (100)	7 (70)	8 (88.9)		10 (71.4)	7 (77.8)	1 (100)		6 (100)	3 (75)	7 (63.6)		7 (100)	6 (66.7)	7 (63.6)	
MDR	0 (0)	2 (20)	1 (11.1)		4 (28.6)	0 (0)	0 (0)		0 (0)	0 (0)	4 (36.4)		0 (0)	0 (0)	4 (36.4)	
Pre XDR	0 (0)	1 (10)	0 (0)		0 (0)	1 (11.1)	0 (0)		0 (0)	1 (25)	0 (0)		0 (0)	1 (11.1)	0 (0)	
XDR	0 (0)	0 (0)	0 (0)		0 (0)	1 (11.1)	0 (0)		0 (0)	0 (0)	0 (0)		0 (0)	2 (22.2)	0 (0)	
Outcome				0.43				0.821				0.277				0.536
On Treatment	0 (0)	6 (60)	5 (55.6)		8 (57.1)	5 (55.6)	1 (100)		2 (33.3)	3 (75)	7 (63.6)		4 (57.1)	6 (66.7)	7 (63.6)	
Treatment Completed	1 (100)	3 (30)	4 (44.4)		1(7.1) 5 (35.7)	0(0) 4(44.4)	0(0) 0(0)		4 (66.7)	1 (25)	3 (27.3)		3 (42.9)	3 (33.3)	3 (27.3)	
Paradoxical Reaction				0.59				0.690				0.7621				0.536
No	1 (100)	9 (90)	9 (100)		13 (92.9)	9 (100)	1 (100)		6 (100)	4 (100)	10 (90.9)		6 (85.7)	9 (100)	10 (90.9)	
Yes	0 (0)	1 (10)	0 (0)		1 (7.1)	0 (0)	0 (0)		0 (0)	0 (0)	1 (9.1)		1 (14.3)	0 (0)	1 (9.1)	

Laboratory Variable	Mean (SD)			P-value	Mean (SD)			P-value	Mean (SD)		P-value	Mean (SD)		P-value
Age (years)	11 (–)	6.86 (4.8)	4.36 (3.39)	0.230	6.67 (4.35)	4.03 (3.6)	5 (–)	0.333	3.38 (4.07)	6.75 (3.59)	0.298	5.51 (4.42)	6.07 (4.13)	6.5 (4.27)	0.891
Hb (gm%)	11.6 (–)	10.4 (1.94)	10.15 (0.93)	0.673	9.85 (1.15)	10.7 (1.41)	10.6 (–)	0.291	10.28 (0.77)	11.17 (1.82)	0.129	10.68 (0.98)	11.15 (1.76)	9.76 (1.15)	0.084
WBC (cells /cumm)	10,600 (–)	11,545 (3976)	11,450 (6116.5)	0.985	11028.57 (4011)	17083.3 (17,293)	9600 (–)	0.435	13,891 (6315)	7575 (450)	0.137	9814 (3613)	16,433 (17,430)	11,590 (4344)	0.429
Polymorphs (%)	40 (–)	46.9 (12.16)	38 (8.322)	0.208	40.86 (11.5)	42 (9.5)	40 (–)	0.963	42 (8.2)	41.5 (11.5)	0.296	40.17 (8.6)	46.56 (12.59)	39.91 (11.76)	0.383
Lymphocytes (%)	60 (–)	51.2 (14.4)	53.56 (9.15)	0.762	54.71 (11.8)	54.44 (8.8)	60 (–)	0.886	57.33 (8.26)	51.5 (8.69)	0.710	56.5 (7.8)	49.33 (14.3)	55.09 (12.47)	0.446
Platelets ($x10^9$ /cumm)	2.7 (–)	2.85 (0.8)	3.59 (1.59)	0.415	3.77 (1.43)	2.8 (0.744)	3.1 (–)	0.194	3.12 (0.5)	2.5 (0.86)	0.236	3.02 (0.92)	2.8 (0.85)	3.7 (1.56)	0.243
MPV	9.90 (–)	10.51 (1.259)	10.68 (1.148)	0.819	10.39 (1.26)	10.22 (0.746)	10 (–)	0.898	9.93 (0.747)	10.58 (0.506)	0.356	9.69 (1.05)	10.26 (1.2)	10.68 (1.23)	**0.240**
25 (OH) Vitamin D (ng/dl)	13.14 (–)	26.43 (16.2)	32.73 (15.8)	0.442	28.69 (15.2)	39.01 (18.9)	28.5 (–)	0.359	39.24 (22.4)	26.51 (6.61)	0.462	41.84 (15.38)	34.28 (19.39)	25.98 (13.4)	0.141

certain infectious agents which may be important in the body's defense against TB, in which the attack of macrophages is a key step in pathogenesis. Vitamin D exerts its actions through binding its receptor, VDR. Activation of Toll-like receptors (TLRs) due to TB infection leads to the upregulation of VDRs and 1-alpha hydroxylase. This facilitates the production of 1,25 dihydroxy vitamin D, which acts on the VDR leading to induction of cathelicidin. Cathelicidin has antimycobacterial action and promotes autophagy. VDR gene polymorphisms plays a role in the regulation of gene expression and VDR activity. As VDR polymorphisms determine the activity of the receptor; they have been suggested as potential markers of host susceptibility to TB.[6,15]

The pleiotropism of vitamin D and its highly conserved action in organisms may also explain the genetic polymorphism for the vitamin D receptor (VDR) in humans. The evolutionary pathway of the biology of vitamin D in humans generated a genetic polymorphism in the VDR, with 4 major single nucleotide polymorphisms (SNPs) and many additional minor ones.[16] Studies regarding prevalence of Fok1, Bsm1, Apa1 and Taq1 polymorphisms in VDR are being carried out in various cohorts. The VDR polymorphism sites most frequently studied are start codon Fok1 (rs 2228570), intron 8 Bsm1 (rs1544410), Apa1 (rs7975232).[17] Polymorphism BsmI is located on intron 8 and results of the adenine–guanine substitution (A–G).

In vitro studies have shown that the short protein appears to have higher transcriptional activity than the long protein. This could lead to increased functionality of the VDR, which would modify the effect determined by vitamin D in different cells and tissues. The effect of polymorphism FokI in the transcriptional activity of immune specific transcription factors in lymphocyte proliferation and protein synthesis by immune cells indicates the involvement of this polymorphism in immune regulation. The importance of VDR polymorphism study in population arises from the differences between genotypes and alleles according to the ethnicity. This requires comparison of genotypes and allele frequency between healthy individuals and patients in each population and then comparing the genotype and allele frequency with other different populations.[18]

Two large meta-analayses by Gao et al[7] (2010) and Chen C et al[19] (2013) which found the ff genotype of the FokI was associated with significantly increased risk among Asians but no evident association was observed among African or South American. *ApaI* polymorphism had no significant associations for African populations and a tentative negative association was observed for Asians. Cao et al[15] (2015) conducted a meta-analysis including 38 studies which showed a statistically significant correlations were observed between VDR TaqI gene polymorphism and TB risk in South and West Asians. Magee et al[10] (2017) conducted a prospective cohort study of adult MDR TB patients receiving second-line TB treatment in KwaZulu-Natal province. Each additional risk allele on SNP rs74085240 delayed culture conversion significantly. Djaharuddin et al[20] (2016) found significant correlation between VDR gene *FokI* and nutritional status with increase of BMI ($p = 0.019$) but not significant for clinical response during intensive phase of treatment. Panda et al[21] (2019) showed a significant association between Fok1 polymorphism and susceptibility to TB ($P < 0.0005$).

VDR mRNA, VDR protein and vitamin D levels were significantly lower in active TB group while cathelicidin levels were

higher in active TB patients compared to other groups. 'f' allele was associated with increased susceptibility to TB. Nnoaham et al[9] (2008) from UK found that low serum vitamin D levels were associated with higher risk of active tuberculosis. Presence of Tt polymorphism of TaqI ($p = 0.036$) was associated with a lower risk of recurrent TB. The severity of TB was not associated with TaqI polymorphism which was discordant to the study done by Wilkinson et al[22] but their definition of severe TB was involvement of >4 lung zones and presence of cavitation.

Our study shows that recurrent TB was less common in Tt polymorphism of Taq gene. The T allele of the Taq1 gene has been associated with decreased production of Tissue Inhibitor of Metalloproteinase-1 (TIMP-1), which inhibits matrix metalloproteinase-9 (MMP-9). MMP-9, a regulator of chronic inflammation, is upregulated by monocytes in response to TB. The T allele allows for increased production of MMP-9 and is associated with a higher susceptibility to TB infection.[23] The severity of TB was not associated with vitamin D receptor polymorphism.

The differential rates of TB infection amongst different countries and ethnicities can be attributed to a combination of genetic and environmental factors. The differential rates of susceptibility to TB and the risk of developing active TB may be attributed to the VDR polymorphism present in the individual. The VDR polymorphism status of a patient can also help determine the risk of developing active severe TB or recurrent TB. Vitamin D supplementation may improve clinical outcomes in particular VDR polymorphisms. Knowing the VDR polymorphism status can enable clinician to predict the course of TB.

4.1. *Limitations of the study*

We did not have a control group in the study. Only four polymorphisms of Vitamin D receptor were studied in this study.

Author contributions

IS designed the study. IS, PC, VP, DG, VSP, AJ, MM and NSS drafted and revised the article and approved the final version to be submitted for publication. IS, PC, VSP, AJ and NSS participated in the data collection. IS, PC, VP, DG, VSP, AJ, MM and NSS participated in the analysis and interpretation of the data. VP, DG and MM performed the investigations.

Conflicts of interest

The authors have none to declare.

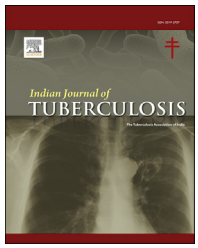
REFERENCES

1. Harishankar M, Selvaraj P, Bethunaickan R. Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. *Front Med*. 2018;5, 1–1.
2. Azad AK, Sadee W, Schlesinger LS. Innate immune gene polymorphisms in tuberculosis. *Infect Immun*. 2012;80:3343–3359.
3. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol*. 2007;179:2060–2063.
4. Chun RF, Adams JS, Hewison M. Back to the future: a new look at "old" vitamin D. *J Endocrinol*. 2008;198:261–269.
5. Mohammadi A, Jafari M, Khanbabaei H, Nasiri-Kalmarzi R, Khademi F, Tajik N. Vitamin D receptor ApaI (rs7975232), BsmI (rs1544410), Fok1 (rs2228570), and TaqI (rs731236) gene polymorphisms and susceptibility to pulmonary tuberculosis in an Iranian population: a systematic review and meta-analysis. *J Microbiol Immunol Infect*. 2020;53:827–835.
6. Gao L, Tao Y, Zhang L, Jin Q. Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. *Int J Tubercul Lung Dis*. 2010;14:15–23.
7. Handono K, Marisa D, Kalim H. Association between the low levels of vitamin D and Treg function in systemic lupus erythematosus patients. *Acta Med Indones*. 2013;45:26–31.
8. Afzal S, Bojesen SE, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and metaanalysis. *Clin Chem*. 2013;59:381–391.
9. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol*. 2008;37:113–119.
10. Magee MJ, Sun YV, Brust JCM, et al. Polymorphisms in the Vitamin D receptor gene are associated with reduced rate of sputum culture conversion in multidrug-resistant tuberculosis patients in South Africa. *PLoS One*. 2017;12:1–11.
11. World Health Organizaton. *GLOBAL TUBERCULOSIS REPORT*. 2019.
12. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Drug and therapeutics committee of the Lawson Wilkins pediatric endocrine society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics*. 2008;122:398–417.
13. Smith H. Paradoxical responses during the chemotherapy of tuberculosis. *J Infect*. 1987;15:1–3. https://doi.org/10.1016/s0163-4453(87)91276-x. PMID: 3668262.
14. QIAamp DNA. *Mini and Blood MiniHandbook*. 05/2016:11–15.
15. Cao Y, Wang X, Cao Z, Cheng X. Association of vitamin D receptor gene taqi polymorphisms with tuberculosis susceptibility: a meta-analysis. *Int J Clin Exp Med*. 2015;8:10187–10203.
16. Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. *Clin Therapeut*. 2017;39:894–916. Excerpta Medica Inc.
17. Emerah AA, El-Shal AS. Role of vitamin D receptor gene polymorphisms and serum 25- hydroxyvitamin D level in Egyptian female patients with systemic lupus erythematosus. *Mol Biol Rep*. 2013;40:6151–6162.
18. Haddad S. Vitamin-D receptor (VDR) gene polymorphisms (Taq-I & Apa-I) in Syrian healthy population. *Meta Gene*. 2014;2:646–650.
19. Chen C, Liu Q, Zhu L, Yang H, Lu W. Vitamin D receptor gene polymorphisms on the risk of tuberculosis, a meta-analysis of 29 case-control studies. *PLoS One*. 2013;8:1–11.
20. Djaharuddin I. Correlation between vitamin D receptor polymorphism with clinical and nutritional status in intensive phase treatment of pulmonary tuberculosis. *Eur Respir J*. 2016;48:PA2117.
21. Panda S, Tiwari A, Luthra K, Sharma SK, Singh A. Association of Fok1 VDR polymorphism with Vitamin D and its associated molecules in pulmonary tuberculosis patients and their household contacts. *Sci Rep*. 2019;9:1–10.
22. Wilkinson RJ, Llewelyn M, Toossi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet*. 2000;355:618–621.
23. Roth DE, Soto G, Arenas F, et al. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *JID (J Infect Dis)*. 2004;190:920–927.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case report

Acral involvement in lichen scrofulosorum: A diagnostic oddity with distinctive features on dermoscopy

Tejas Vishwanath [a,*,c], *Hershada S Mithari* [a,d], *Sunanda Mahajan* [a], *Siddhi Chikhalkar* [a], *Prachi Gole* [a], *Vidya Kharkar* [a], *Pooja Golwad* [a], *Harish Balaji* [b]

[a] *Department of Dermatology, K.E.M. Hospital, Mumbai, 400012, India*
[b] *Department of Dermatology, Sion Hospital, Mumbai, 400022, India*

ARTICLE INFO

Article history:
Received 8 November 2021
Received in revised form
1 April 2022
Accepted 17 May 2022
Available online 21 May 2022

Keywords:
Dermoscopy
Acral
Tuberculid
Delayed hypersensitivity
Lichen scrofulosorum

ABSTRACT

A commonly underdiagnosed harbinger of visceral tuberculosis, lichen scrofulosorum classically manifests as centripetally located erythematous to violaceous cutaneous papules. Perifollicular and perieccrine tuberculoid granulomas constitute the histologic hallmark. We report a case of lichen scrofulosorum with involvement of the acral areas which is unusual. Also, dermoscopy, which has not yet been widely utilized in this condition gave a novel insights into histopathology in this case.

1. Introduction

Tuberculosis (caused by *Mycobacterium tuberculosis*) remains endemic in multiple countries across the globe. An important form of extrapulmonary tuberculosis, cutaneous tuberculosis constitutes 0.9% of patients attending the outpatient department.[1] Broadly, cutaneous tuberculosis can be classified as true cutaneous tuberculosis and tuberculids caused due to immune response mounted to disseminated antigens of the pathogen.[1]

Lichen scrofulosorum (LS) is the most commonly encountered cutaneous tuberculid in pediatric patients with high cell mediated immunity.[1] An under diagnosed condition, it usually manifests as innocuous centripetally located discrete or agminate follicular and perifollicular violaceous and reddish brown papules.[2] Although diverse morphological variants are known, LS on acral areas is distinctly unusual with only a

* *Corresponding author*. Department of Dermatology, K.E.M. Hospital, Mumbai, 400012, India. Tel.: +919920402705.
E-mail address: tejasvishwanath.igs@gmail.com (T. Vishwanath).
[c] Present Address: Disha skin and laser institute, Thane-400605, India.
[d] Consultant dermatologist, Mumbai.

https://doi.org/10.1016/j.ijtb.2022.05.003

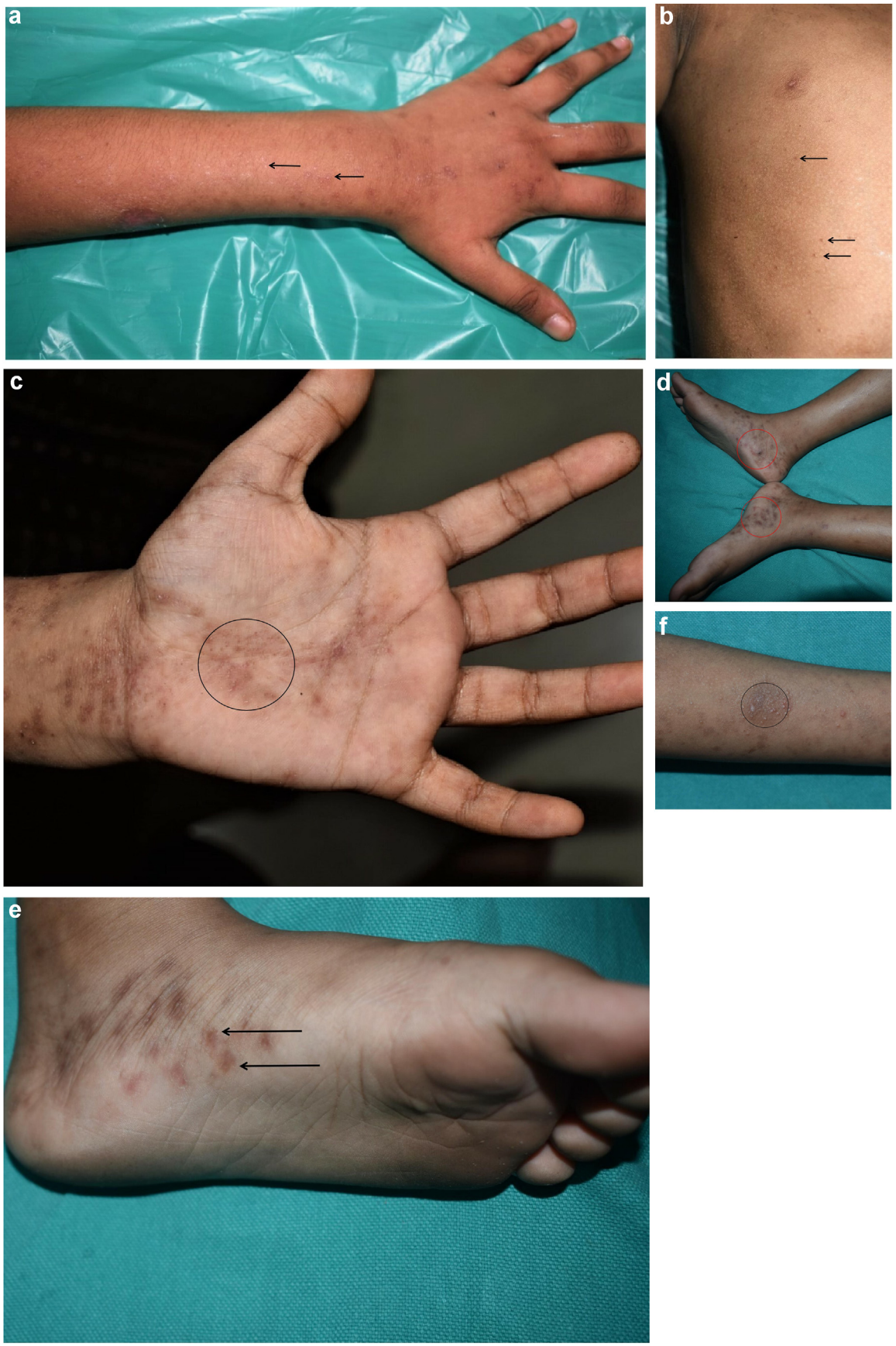


Fig. 1 – a- Skin colored and violaceous papules on the forearm (marked by arrows). b- Subtle dull skin colored papules on the chest and abdomen (marked by arrows). c- Dusky erythematous papules on the palms (encircled). d- Dusky erythematous papules on the feet extending onto the soles (encircled). e- Close up view of papules on the foot and sole (marked with arrows). f- Strongly positive tuberculin skin test with lichenoid papules (encircled).

single case reported previously. Dermoscopy, a useful non invasive diagnostic tool, has been used successfully to diagnose some forms of cutaneous tuberculosis such as lupus vulgaris, however, reports on the use of this modality in LS are surprisingly sparse. We report an unusual case of LS involving the extremities with dermoscopic features and histopathologic correlation.

2. Case

A nine year old Indian girl presented with mildly pruritic violaceous papules on the forearms, thighs, abdomen and genitals. She also had dull erythematous papules on the palms and feet extending onto the soles. There was associated with low grade evening fever, weight loss as well as ataxia and altered sensorium since two months. There were no pulmonary manifestations. Examination revealed subtle violaceous and skin colored discrete follicular and non-follicular papules on the trunk, forearms and thighs (Fig. 1a and b). Some papules had fine scales. Non-tender minimally elevated dusky erythematous papules were noted on the palms and soles (Fig. 1c,d,e). Mantoux test was strongly positive (2cm) with lichenoid papules (Fig. 1f). Multiple cerebral parenchymal and sulcal tubercular granulomas were noted on magnetic resonance imaging. A provisional diagnosis of LS along with acral papulonecrotic tuberculid (PNT) secondary to central nervous system (CNS) tuberculoma was made. Erythema multiforme and cutaneous small vessel vasculitis were also entertained as a differential diagnosis for acral lesions.

Dermoscopy of forearm lesions revealed central pale areas (some of which were folliculocentric) with surrounding yellow-orange background and ring of vessels (Fig. 2a). On the sole, irregular ill-defined white streaks with surrounding linear irregular vessels on a subtle orange background were noted (Fig. 2b).

Histopathologic examination of a papule on the forearm revealed perifollicular and periacrosyringeal tuberculoid granulomas with edema (Fig. 3a) whereas that of a papule on the foot showed tuberculoid granulomas in a periacrosyringeal distribution (Fig. 3b and c). Stains for acid fast bacilli were negative. Thus, a final diagnosis of lichen scrofulosorum secondary to tuberculoma of CNS was made and the patient was started on antituberculosis treatment. Improvement of cutaneous and systemic features was ascertained telephonically four weeks after initiation of the treatment since the patient refused to come to the hospital. Subsequently, she was lost to follow up.

3. Discussion

Lichen scrofulosorum is caused by delayed type hypersensitivity response to mycobacteria or their circulating fragments which disseminate hematogenously from a tuberculous focus.[3] Strong Mantoux test positivity and rapid response to anti-tuberculous therapy are important diagnostic features.

A high degree of suspicion is warranted especially in tuberculosis endemic locations. This is especially so since this tuberculid is known to manifest atypically: atypical morphologies reported include psoriasiform, annular and granuloma annulare like.[2] Atypical sites of involvement include the acral areas and genitals.[4,5] It may thus be missed by the unwary observer leading to delay in initiation of anti tuberculous therapy.

The present case is particularly unusual because of acral localization of lesions with unusual morphology clinically simulating a vascular pathology. A single case with involvement of the extremities has been described in the literature; this case also had a tuberculoma in the brain with good response to anti tuberculosis therapy akin to the present case.[4] However, the paper lacks dermoscopic and histologic characterization of the patient. A recent series has documented LS occurring exclusively on the flexor aspect of the forearms and legs, however this did not involve the acral areas.[6] This makes the present case unusual and noteworthy.

Important differential diagnoses with respect to the palmoplantar lesions in this include PNT, small vessel vasculitis and erythema multiforme. All these were conclusively ruled out on histopathologic analysis which did not show vascular

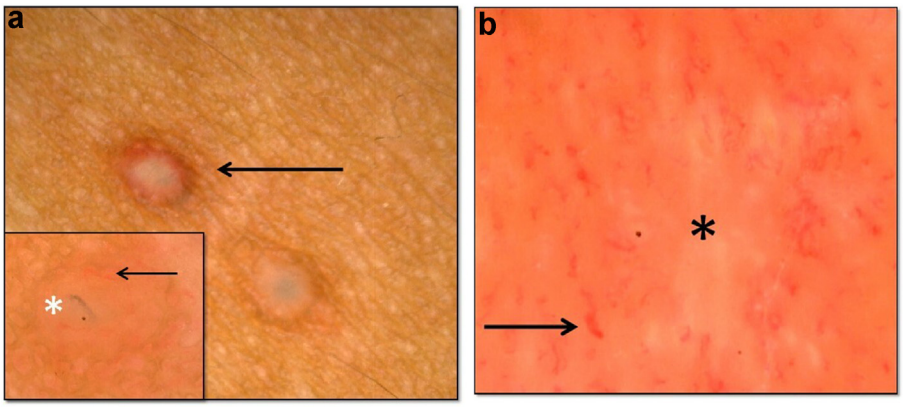


Fig. 2 – a- Dermoscopy of forearm lesions (arrow) displayed central pale areas (inset, white *) around hair follicles with surrounding yellow-orange background corresponding to granuloma on histopathology and ring of vessels (black arrow). b- Dermoscopy of lesions on the sole showed white streaks (black *) with surrounding linear irregular vessels (black arrow) on an orange background.

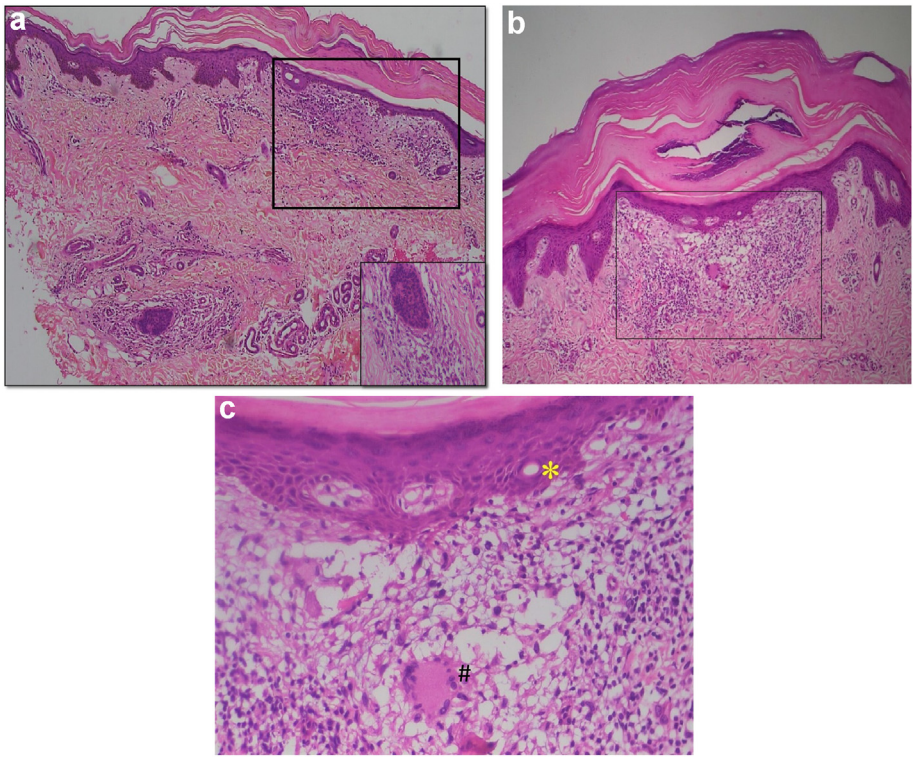


Fig. 3 – a- Histopathologic examination of a papule on the forearm (Hematoxilin and Eosin stain; 10x) revealed tuberculoid granulomas around the opening of a hair follicle and sweat duct (in black square) with edema. Inset (Hematoxilin and Eosin stain; 20x)- Perifollicular granulomas are seen. These features are characteristic of lichen scrofulosorum. b- Biopsy of a papule on the sole shows granuloma (black box) concentrated around the opening of a sweat duct (acrosyringeum; Hematoxilin and Eosin stain; 10x). c- Higher power view of same field as figure 3b showing the acrosyringeum (yellow *) and a Langhans giant cell with abundant central pink cytoplasm and peripherally arranged nuclei (#).

pathology, vacuolar interface dermatitis or epidermal cell necrosis which characterize these conditions.

Dermoscopy may be a useful tool in this regard in future. In this case it gave a vivid insight into the pathology. Central pale area and white streaks correspond to central edema in granuloma while the surrounding orange hue presumably corresponds to the granuloma proper. Dilated linear vessels at the periphery signify active inflammation. These features have not been described previously. Previous reports detail pale agminate folliculocentric dots and halos with brown follicular plug along with marginal hyperpigmentation, milia like cysts, altered pigment network and scaling.[7,8] Further case series may be informative since dermoscopy may aid in diagnosis of this underdiagnosed entity especially atypical variants.

4. Conclusion

Lichen scrofulosorum must be a diagnostic consideration in tuberculosis endemic areas especially since it may be an early indicator of tuberculosis, a curable condition. Awareness of rare variants is paramount to achieve early diagnosis. Histopathology occupies a place of prime importance in resolving diagnostic conundrums. Dermoscopy may be a useful adjunct to diagnosis since it acts as a bridge between clinical diagnosis and histopathology.

Conflicts of interest

The authors have none to declare.

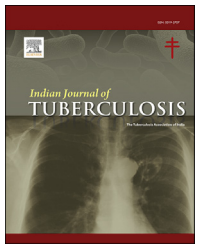
REFERENCES

1. Kumar B, Kumar S. Pediatric cutaneous tuberculosis: Indian scenario. *Indian J Paediatr Dermatol*. 2018;19:202–211.
2. Singhal P, Patel PH, Marfatia YS. Lichen scrofulosorum: a diagnosis overlooked. *Indian Dermatol Online J*. 2012;3(3):190–192.
3. Molpariya A, Ramesh V. Lichen scrofulosorum: importance of early recognition. *Clin Exp Dermatol*. 2017 Jun;42(4):369–373.
4. Beena KR, Ramesh V, Mukherjee A. Lichen scrofulosorum - a series of eight cases. *Dermatology*. 2000;201(3):272–274.
5. Gandhi V, Vij A, Bhattacharya SN. Lichen scrofulosorum on the genitalia–an unusual presentation. *Int J Dermatol*. 2007 May;46(5):548–549.
6. Kolalapudi SA, Konala S, Kotha S, Arumilli PC, Kalagarla S. Unusual presentations of cutaneous tuberculosis. *Indian J Tuberc*. 2020 Jul;67:433–437.
7. Jassi R, Yadav A, Chander R. Dermoscopy of lichen scrofulosorum. *Indian Dermatol Online J*. 2020;11:876–877.
8. Khopkar U, Bharti A. *Dermoscopy, Trichoscopy and Onychoscopy in the Diseases of Pigmented Skin: An Atlas and Short Text*. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 2020.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case report

A silent march-Post covid fibrosis in asymptomatics – A cause for concern?

Thomas Antony [a,b,*], *K. Vishak Acharya* [a,b], *B. Unnikrishnan* [c,b], *N.S. Keerthi* [a,b]

[a] *Dept. of Pulmonary Medicine, Kasturba Medical College, Mangalore, India*
[b] *Manipal Academy of Higher Education, Manipal, Karnataka, India*
[c] *Dept. of Community Medicine, Kasturba Medical College, Mangalore, India*

ARTICLE INFO

Article history:
Received 6 May 2022
Received in revised form
10 May 2022
Accepted 17 May 2022
Available online 25 May 2022

Keywords:
COVID-19
UIP
Post COVID fibrosis

ABSTRACT

We report a case series of patients presenting with undiagnosed pulmonary fibrosis as a primary manifestation. On evaluation, after excluding other causes, the fibrosis was attributed to asymptomatic or mild COVID illness in the past. This case series serves to highlight the difficulties posed to clinicians while evaluating pulmonary fibrosis in the post-COVID era, more so in mild to asymptomatic COVID-19. The intriguing possibility of fibrosis setting even in mild to asymptomatic COVID is discussed.

1. Introduction

COVID-19 pandemic caused by SARS-CoV-2 had a devastating effect on health, economic and social sectors globally. The presentation of COVID-19 infection varies from asymptomatic to hypoxemic respiratory failure and respiratory distress syndrome. Long-standing sequelae involving the lungs such as fibrosis are seen in patients who have recovered from COVID-19 infection.[1] Other pulmonary complications like cavitary lung disease, bronchiectasis are also seen in addition to persistent symptoms such as dyspnea and fatigue.[2,3] The pulmonary sequelae are mostly seen in hospitalized patients with severe COVID-19 infection.[4] Asymptomatic COVID-19 patients developing lung fibrosis is an unheard-of entity. It is alarming that, unlike in idiopathic pulmonary fibrosis where fibrosis usually occurs in the elderly, post-COVID fibrosis is seen irrespective of age and other co-morbidities. Here we report a series of four cases presenting with pulmonary fibrosis as initial manifestation and lacking a definitive association with microbiologically proven COVID. Pulmonary fibrosis in these patients was largely attributable to post COVID complications amongst asymptomatic COVIDs. Pulmonary fibrosis is generally seen in patients with moderate to severe disease and is very rarely described in asymptomatic and mild cases of COVID-19. The radiological similarity between usual interstitial pneumonia (UIP) and post COVID

* *Corresponding author*. Department of Pulmonary Medicine, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, 575001, India. Tel.: +91 8547437931.
E-mail address: t.antony5@gmail.com (T. Antony).

https://doi.org/10.1016/j.ijtb.2022.05.004
0019-5707/

fibrosis, symptomatology overlap, and difficulties posed by clinicians in differentiating these two entities in the post-pandemic era has been discussed in this case series.

2. Case 1

A 43-year-old female, homemaker, known diabetic, who was not vaccinated against COVID-19 infection, presented with a 1-month history of progressive exertional breathlessness. She reported no history of any joint pain or any other extra-pulmonary symptom. Her baseline vital parameters were within normal limits. She gave no prior history of respiratory infection, fever, or any hospital admission in the recent past. Chest X-ray showed bilateral reticular opacities. HRCT of the thorax showed fibrosis with traction bronchiectasis, primarily involving the lower lobes with predominant sub-pleural involvement (Fig. 1A). COVID testing using cartridge-based RT-PCR did not detect SARS-COV2 RNA. Connective tissue work-up was done and showed a negative rheumatoid factor, anti-cyclic citrullinated peptide (CCP), antineutrophilic cytoplasmic antibody (ANCA), and antinuclear antibody (ANA). Echocardiography did not show any abnormality. However anti-SARS-COV2 IgG quantitative test antibodies for COVID-19 came positive (>200 BAU/ml). Pulmonary function test showed restrictive pattern (FEV1: 0.80 L, 31% predicted; FVC: 0.92, 30% predicted), reduced DLCO (8.7 ml/min/mmHg, 34% predicted). She was unable to perform a six-minute walk test. She was started on anti-fibrotic therapy, injectable steroids, and pulmonary rehabilitation and is currently on follow-up.

3. Case 2

A 51-year-old male patient, ex-smoker, farmer, with no co-morbid illness, recently vaccinated with the first dose against COVID-19, presented to the respiratory medicine clinic with two weeks history of progressive exertional breathlessness and dry cough. He had a history of fever lasting for 2 days that preceded symptoms of dry cough. He had not undergone testing for COVID then. There was no history of any joint pain or fever. General examination was unremarkable, except for low peripheral oxygen saturation on presentation (92% on room air). Systemic examination showed bilateral basal crepitations on auscultation. Chest X-ray showed bilateral reticular opacities. Sputum aerobic culture and CBNAAT for tuberculosis did not reveal any organisms. COVID testing using cartridge-based RT-PCR did not detect SARS-COV2 RNA. HRCT thorax showed diffuse, predominantly subpleural areas of fibrosis and bronchiectasis (Fig. 1B). Echocardiography showed normal biventricular function and no evidence of pulmonary artery hypertension. Spirometry showed restrictive pattern (FEV1: 0.82 L, 28% predicted; FVC: 1.01, 28% predicted), reduced DLCO (6.6 ml/min/mmHg, 24% predicted). His six-minute walk test showed a reduction in total distance covered (120 meters at six-minute) and the lowest recorded

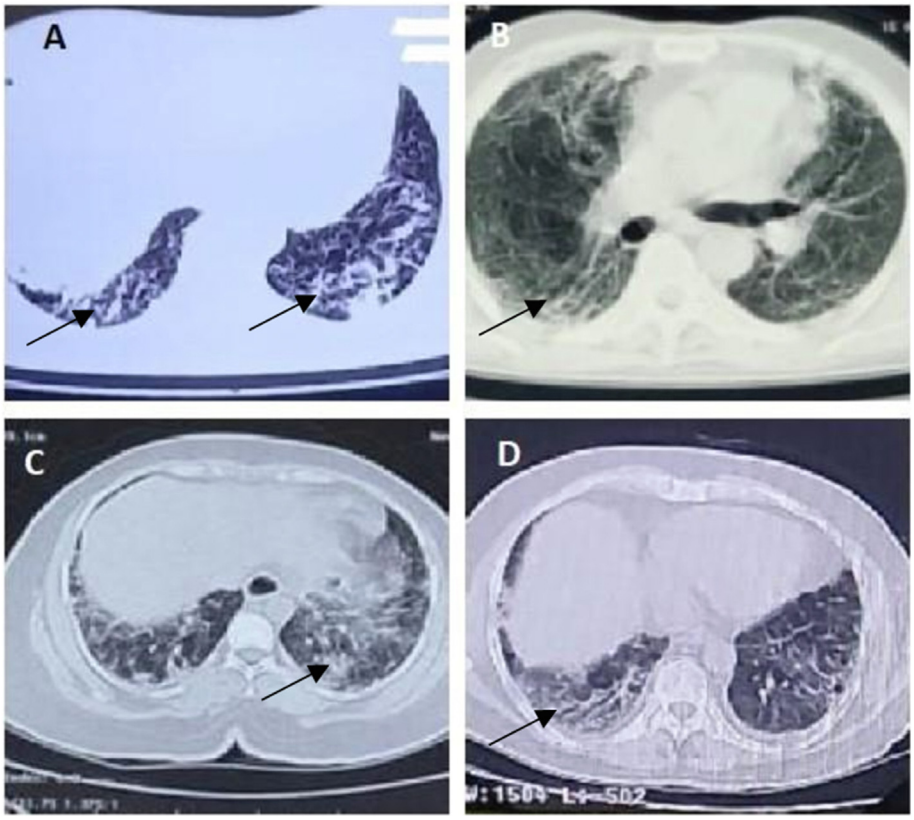


Fig. 1 – A: Case 1, B: Case 2, C: Case 3, D: Case 4. (A–D) Images of HRCT thorax showing areas of fibrosis, involving the lower lobes with dominant subpleural pattern of distribution (marked with black arrow).

oxygen saturation of 88% on room air. The patient was started on anti-fibrotic agent, injectable steroids. The patient partly responded to treatment and is currently on follow-up.

4. Case 3

A 28-year-old female patient, homemaker, with no comorbid illness, not vaccinated against COVID-19, presented with 20 days history of exertional breathlessness and dry cough. General examination was unremarkable and peripheral oxygen saturation on presentation was 95% on room air. Bilateral basal crepitations were present on auscultation. Chest X-ray showed reticular opacities bilaterally. HRCT thorax showed diffuse, predominantly sub-pleural areas of fibrosis involving lower lobes (Fig. 1C). COVID testing using cartridge-based RT-PCR did not detect SARS-COV2 RNA. The patient was tested for COVID-19 antibody using anti-SARS-COV2 IgG quantitative test and was found to be significantly elevated (>200 BAU/ml). Echocardiography did not reveal any significant abnormality. Pulmonary function assessment showed restrictive pattern (FEV1: 0.96 L, 38% predicted; FVC: 1.10, 38% predicted), reduced DLCO (7.8 ml/min/mmHg, 27% predicted). She covered a total distance covered (360 meters at six-minute) during a six-minute walk test with the lowest recorded oxygen saturation of 88% on room air. The patient was managed with supportive care, anti-fibrotic agent, and injectable steroids. The patient is currently on follow-up.

5. Case 4

A 75 -year-old male, ex-smoker, with diabetes mellitus, fully vaccinated against COVID-19, tested positive for COVID testing using cartridge-based RT-PCR. He was largely asymptomatic then and opted for home quarantine. He developed progressive breathlessness after a month of testing positive. The patient was tachycardic and with a respiratory rate of 28 breaths per minute. His peripheral oxygen saturation was 89% on room air at presentation. Chest X-ray showed bilateral reticular opacities. HRCT of the thorax showed bilateral basal predominant areas of fibrosis (Fig. 1D). Echocardiography did not show any significant abnormality. His spirometry showed restriction abnormality and DLCO was reduced. The patient was managed with supportive care, started on anti-fibrotic agent, injectable steroids. The patient was stable on discharge is on follow-up.

6. Discussion

There has been an unprecedented spurt in the cases of pulmonary fibrosis following the COVID-19 pandemic. Although it is a known complication in severe COVID-19 pneumonia, the possibility of lung fibrosis secondary to an asymptomatic or mild COVID-19 infection is also probable as reported in this case series.

Two of the patients had no history of COVID-19 infections, were unvaccinated, and had an elevated anti IgG titer for COVID-19 confirming an asymptomatic COVID-19 infection in the recent past. The third patient was partially vaccinated with a history suggestive of a recent mild COVID-19 infection. The fourth patient was fully vaccinated with a confirmed mild COVID-19 infection. All four cases were diagnosed with pulmonary fibrosis, which was unlike typical of post COVID fibrosis making the etiology of fibrosis a diagnostic dilemma. In all our cases, a comprehensive workup for baseline connective tissue disorders and detailed occupational history was inconclusive ruling out other possible etiologies for pulmonary fibrosis. Thus, it should be kept in mind that fibrosis could be sequelae in spite of the vaccination status of the patients. Raised IgG antibodies against COVID-19, who is not vaccinated and in those with no prior history of any past or current COVID-19 infection gives indirect evidence of asymptomatic COVID infection in the recent past.[5]

Although fibrosis of lungs in asymptomatic COVID patients is not yet studied, it could be assumed that the pathogenesis could simulate that in severe infection. The correlation between the antibody levels and the extent of lung fibrosis needs to be studied further. Possible mechanisms of injury could be direct injury related to viral infection or due to immune-mediated injury following the release of pro-inflammatory and pro-fibrotic cytokines/factors such as TGF-β, TNF-α, MMPs, TIMPs, IL-1, IL-4, IL-5, IL-6, IL-13, and IL-17.[6] Other possible causes such as cardiac function and an active infection should be ruled out in such patients.

A significant proportion of patients with asymptomatic to mild COVID-19 cases have demonstrated ground-glass opacities and pneumonia on HRCT.[7] However, the impact on lung function over medium to long term and silent progression to fibrosis remains unclear and has not been studied in great detail. Some studies have amply demonstrated pulmonary function impairment after 4 months of acute COVID-19 even in asymptomatic COVID-19 cases as characterized by diffusion impairment, reduced total lung capacity, and forced vital capacity. Surprisingly 20% of patients with the mild disease showed a decrease in paO2 on exercise indicating a subtle gas exchange abnormality.[8] Reduction in diffusion capacity persisting after 4 months suggests ongoing interstitial process even amongst those with mild disease.

There is also evidence that viral infections including COVID-19 may predispose, trigger or exacerbate respiratory pathologies like pulmonary fibrosis.[9,10] This has been explained by the "two-hit" hypothesis wherein an initiating insult causes changes in the lung microenvironment increasing its susceptibility to a fibrotic process following a secondary insult. Viral infections can upregulate expression, signaling pathway, and production of growth and pro-fibrotic factors triggering progressive fibrotic process.

In the post-COVID era, early and accurate diagnosis of post-COVID fibrosis from usual interstitial pneumonia (UIP) becomes a nightmare as both these entities have a near similar HRCT presentation with bilateral sub-pleural honeycombing with reticulation and traction bronchiectasis. In such clinical contexts, confirmation of past active COVID illness with RT-PCR or elevated anti-COVID antibody titers in the unvaccinated (as evidenced in two of our cases) becomes critical in clinching the diagnosis. Diagnosing post-COVID fibrosis in the asymptomatic untested but vaccinated, is often a probable diagnosis and hinges primarily on history

suggestive of COVID-like illness as related in the third patient from our series. It is probable that many elderly asymptomatic populations might have moderate–severe pneumonia but the symptoms might not be overt in their manifestation considering the age, immunity, and activity levels. Often this section of the population might present with pulmonary fibrosis as the initial and sole manifestation of COVID-19 sequel.

Established post- COVID pulmonary fibrosis has an unpredictable course and continues to confound clinicians' world over with its myriad presentations, variable response to therapy, and long-term prognosis. In some extensive fibrosis is known to resolve spontaneously over time, while in others anti-fibrotic therapy might slow down the fibrosis while in some the disease marches, all novel interventions notwithstanding. Though there exists a slew of data there is little consensus on factors modifying the onset and progression of fibrosis in individual patients.

Antifibrotics are often used commonly in patients with post-COVID fibrosis and UIP, it is extremely important to differentiate the two processes in terms of long-term prognostication and outcome. Clinicians are often faced with this dilemma as seen in our case series when patients present with pulmonary fibrosis as initial presentation and a history of microbiological confirmation of COVID positivity is lacking. This case series seeks to highlight this vexing clinical issue posed to physicians in the post-COVID era.

There is no definitive treatment for post-COVID fibrosis of the lungs. Studies have shown the potential benefit of antifibrotic agents in preventing the worsening of lung function. The use of anti-inflammatory agents like steroids has a possible role in reducing immune-mediated lung injury and fibrosis.[7,8] Other supportive such as pulmonary rehabilitation and regular physical exercise could have some role in management.

Conflicts of interest

The authors have none to declare.

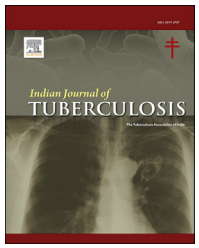
REFERENCES

1. Martin Rumende C, Susanto EC, Sitorus TP. The management of pulmonary fibrosis in COVID-19. *Acta Med Indones Indones J Intern Med*. 2021;53.
2. Ahmed OF, kakamad FH, Hama Amin BJ, et al. Post COVID-19 pulmonary complications; a single center experience. *Ann Med Surg*. 2021 Dec 1:72.
3. Esendağli D, Yilmaz A, Akçay Ş, Özlü T. Post-COVID Syndrome: Pulmonary Complications. *Turk J Med Sci*. 2021;51:3359–3371.
4. Crisan-Dabija R, Pavel CA, Popa IV, Tarus A, Burlacu A. "A chain only as strong as its weakest link": an up-to-date literature review on the bidirectional interaction of pulmonary fibrosis and COVID-19. *J Proteome Res*. 2020;19(11):4327–4338.
5. Ali H, Alahmad B, Al-Shammari AA, et al. Previous COVID-19 infection and antibody levels after vaccination. *Front Public Health*. 2021 Dec 1:9.
6. Huang WJ, Tang XX. Virus infection induced pulmonary fibrosis. *J Transl Med*. 2021;19.
7. Ali RMM, Ghonimy MBI. Radiological findings spectrum of asymptomatic coronavirus (COVID-19) patients. *Egypt J Radiol Nucl Med*. 2020 Dec 1;(1):51.
8. Munker D, Veit T, Barton J, et al. Pulmonary function impairment of asymptomatic and persistently symptomatic patients 4 months after COVID-19 according to disease severity. *Infection*. 2022 Feb 1;50(1):157–168.
9. Naik PK, Moore BB. Viral infection and aging as cofactors for the development of pulmonary fibrosis. *Expet Rev Respir Med*. 2010;4:759–771.
10. Qiao J, Zhang M, Bi J, et al. Pulmonary fibrosis induced by H5N1 viral infection in mice. *Respir Res*. 2009 Nov 12:10.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case report

Anti-tubercular therapy (ATT) induced exfoliative dermatitis—A case series

Anjaly Mary Varghese [a], *Nagavishnu Kandra* [a], *Praveen Kumar Uppala* [b,*], *Yakaiah Vangoori* [a], *Lavanya Butti* [c], *Swapna Masapogu* [d], *Upendrarao Uttaravalli* [e], *Venkata Saibaba Somarouthu* [f], *Murali Krishna Balijepalli* [g]

[a] *Dept. of Pharmacology, Santhiram Medical College & General Hospital, Nandyal, Kurnool, Andhra Pradesh, India*
[b] *(Employee ID - IPC – 291), NCC-PvPI, Indian Pharmacopeia Commission, Ghaziabad, India*
[c] *Dept. of Clinical Pharmacy, Santhiram Medical College & General Hospital, Nandyal, Kurnool, Andhra Pradesh, India*
[d] *Assistant Professor, Dept. of Pharmacy Practice, Santhiram College of Pharmacy, Nandyal, Kurnool, Andhra Pradesh, India*
[e] *Dept. of Pharmaceutical Analysis, Sri Sivani College of Pharmacy, Srikakulam, Andhra Pradesh, India*
[f] *Dept. of Pharmaceutical Analysis, K V K College of Pharmacy, Surmaiguda, Hyderabad, India*
[g] *Research Scholar, Centurion University of Technology and Management, Odisha, India*

ARTICLE INFO

Article history:
Received 6 July 2022
Accepted 12 August 2022
Available online 23 August 2022

Keywords:
Tuberculosis
Antitubercular therapy (ATT)
Isoniazid (INH)
Pyrazinamide exfoliative dermatitis
Erythroderma

ABSTRACT

Tuberculosis is a serious contagious disease mainly affecting the lungs and is common in the developing countries. The essential component of all antitubercular regimens include Isoniazid, pyrazinamide as first-line drugs. A serious cutaneous adverse drug reaction namely exfoliative dermatitis (erythroderma) is associated with isoniazid use though uncommon but is common among pyrazinamide users. Here we report 3 cases of tuberculosis patients on antitubercular therapy (ATT) for 8 weeks, came to hospital OP (outpatient) with severe generalized redness and scaling with itching distributed all over the body and trunk. Immediately ATT was discontinued and all the three patients were administered antihistaminic and corticosteroid. The patients recovered within 3 weeks. To confirm ATT induced erythroderma and narrow down the offending agents, sequential rechallenge with ATT was done and again these patients had similar lesions erupt all over the body only with isoniazid and pyrazinamide. Antihistamine, steroids were started and the symptoms resolved and completely recovered within 3 weeks. Prompt withdrawal of the culprit drug along with appropriate medications and supportive measures is necessary for good prognosis. Physicians must be judicious while prescribing ATT especially, isoniazid and pyrazinamide as these can precipitate fatal cutaneous adverse reactions. Strict vigilance may help in early detection of this type ADR and timely management.

* *Corresponding author.*
E-mail address: praveen.chintu32@gmail.com (P.K. Uppala).
https://doi.org/10.1016/j.ijtb.2022.08.001

1. Introduction

Erythroderma or generalized exfoliative dermatitis (GED), is a serious life-threatening cutaneous inflammatory condition characterized by diffuse erythema and scaling of greater than 90% of the body's surface that begins in patches, spreads and may slough off.[1] Exfoliative dermatitis (ED) may occurs as exacerbation of pre-existing skin condition like psoriasis or atopic dermatitis, eczema, or due to a reaction to certain medicines and malignancies like lymphoma. Drug induced ED is a cluster of severe, infrequent, dermal drug hypersensitivity reactions (DHR). Erythema multiforme (EM), Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are examples of drug induced ED occurring mostly within few days to weeks after exposure to the offending drug. The classical clinical signs and symptoms of ED include erythema or intense skin redness, glossy and thin skin, scaling (white or yellow), scabby lesions, skin thickening, itching, fever, dehydration (due to loss of body fluids through the marred skin) and protein deficiencies. Progressive lichenification and edema make cause tightening of the skin. Cutaneous adverse drug reaction (CADR) is defined as any secondary dermal reactions occurring after the systemic drug administration. The incidence of ED was reported as 0.035% (35 per 100,000 patients) in dermatology OP by an Indian study group. Extensive dermal inflammation and epidermal proliferation due to increased adhesion molecules expression in epithelial cells is the underlying etiology of ED. Markedly excessive exfoliation seen in erythrodermic patients. T cells play a major role in immune-mediated drug induced ED. Previously reports state that ED has a mortality range 4%–64%. Cardiac failure, septicemia, and pneumonia are the most common cause of death in exfoliative dermatitis patients.[2]

It is essential to diagnose properly, identify the underlying etiology, and appropriately manage erythroderma as this condition may be a potentially fatal condition and may require hospitalization. Drug induced ED usually rapidly resolves after the offending drug is stopped and precise treatment and maintaining metabolic, hemodynamic balance. Symptomatic supportive treatment with confinement to bed, oral antihistamines, topical corticosteroids and emollients for proper wound care must be asserted to improve quality of care and quicker recovery.[3]

Tuberculosis (TB) is a respiratory disease caused by *Mycobacterium tuberculosis*. TB spreads via droplet infection and can be treated successfully with antitubercular drug therapy. Fist line anti TB drugs include INH, rifampicin, pyrazinamide and ethambutol; ATT drugs may be associated with the development of ED within six to eight weeks of ATT initiation. Other drugs such as cimetidine, diltiazem, phenylbutazone, allopurinol, hydantoin derivatives (phenytoin), carbamazepine, penicillins, sulfonamides, dapsone can also cause ED. Early detection of the cutaneous reaction and discontinuation of the maleficent agent promptly and steroid administration are mainstay of ED management.[4]

2. Case report 1

A female patient, 40-year-old, diagnosed of TB and was on category I (CAT-I) ATT for ten weeks. She came to the dermatology OP with complaints of generalized itching and redness of skin on both upper limbs. On physical examination, generalized scaling eruption all over the body was seen. She was diagnosed to have exfoliative dermatitis due to ATT because she was not on any other medications. ATT was immediately withheld and she was put on tablet hydroxyzine 10 mg (antihistamine) once daily for 7 days, tablet prednisolone 5 mg three times daily for 21 days and topical emollients. She recovered in 3 weeks (dechallenge) after which individual ATT drugs were reintroduced in a sequential manner (rechallenge). Ethambutol was reintroduced first, then rifampicin, pyrazinamide and INH at the last, with 1 week interval between each drug. The patient did not develop any signs of CADR for the first three drugs, but after INH reintroduction, within 48 hours, she developed erythematous lesions and intense itching. INH was withdrawn and doctor confirmed this case as 'isoniazid induced erythroderma'. The patient was given symptomatic treatment. The lesions resolved in a weeks' time, and alternative regimen of ATT was prescribed to her which replaced INH with levofloxacin. No further cutaneous ADR was reported. This ADR was reported to our pharmacovigilance center. On WHO-UMC causality assessment scale, this case falls under 'certain' category (Fig. 1).

3. Case report 2

A 54-year-old woman presented with fever and productive cough, diagnosed as pulmonary TB was started on ATT under directly observed treatment short course (DOTS) regimen. In the 8th week, this patient came to dermatology OPD with complaints of redness, swelling and severe itching on her lower limbs for the last 7–10 days. Dermatological examination showed non uniform darkened erythematous lesion and scaling involving both legs and soles. Pruritic plaques on the body and legs showed increased in size day after day. The patient's history revealed no preexisting skin disorders or any other medical problem. ATT was abruptly halted. Symptomatic treatment including emollients, steroids and antihistamines were given after which lesions resolved in 21 days. To confirm ATT induced ED, rechallenge was done wherein individual drugs were reintroduced in a sequential manner with an interval of 1 week between each drug. INH was reintroduced at the last and within 48 hours, pruritic erythematous rashes was developed by the patient all over body. Offending drug was withdrawn and the case was diagnosed as "INH induced erythroderma". The lesions subsided in 1 week after appropriate treatment; INH was excluded and levofloxacin, a second line ATT drug was introduced. The patient did tolerate the alternate drug. Based on WHO-UMC causality assessment scale, the case was considered as "certain" and reported to PVPI office (pharmacovigilance program of India) from our pharmacovigilance center (Fig. 2).

4. Case report 3

A lady aged 45-year with a history of low-grade fever in evening, progressive loss of body weight and sputum positive TB was on standard ATT for the last 3 months. In the 4th month,

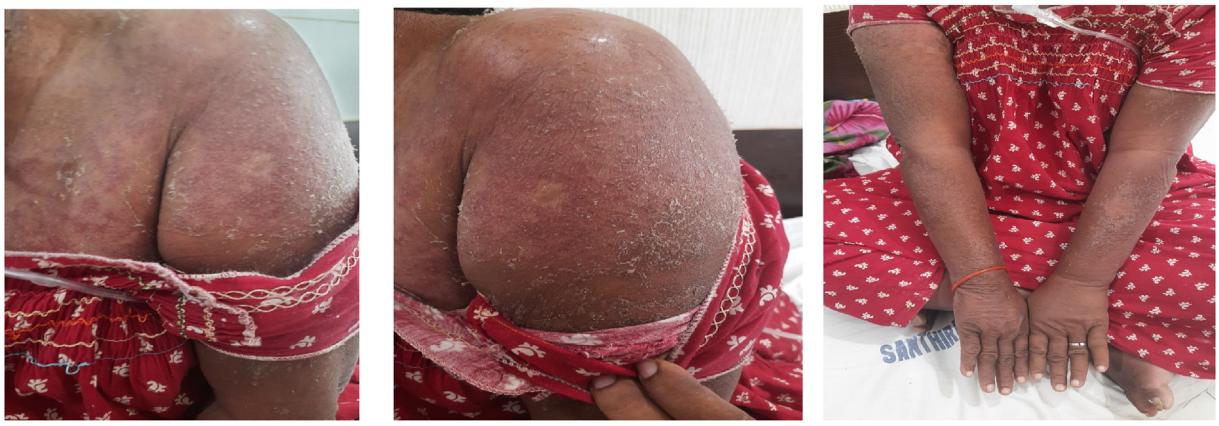


Fig. 1 – Case report 1. INH induced Exfoliative dermatitis.

she visited Dermatology OPD, complaining of red patches and itching over abdomen which lasted for more than 10 days. The erythematous rashes spread to the entire body gradually. Immediately ATT was withdrawn and antihistamine hydroxyzine 10 mg for 10 days, deflazacort (6 mg once daily) for 5 days and topical mometasone ointment were given. Conformation of ATT induced ED required rechallenge with ATT drugs sequentially at one week interval. Rechallenge with rifampicin in week 1 and then isoniazid in week 2, ethambutol in week 3 were tolerated without any significant symptoms. On reintroduction of pyrazinamide caused, within 48 hours the patient developed pruritis, erythema and fever. Offending drug was withdrawn and the dermatologist confirmed the diagnosis as "pyrazinamide induced erythroderma". The symptoms resolved on antihistamine, steroids therapy for 10 days and patient was discharged (Fig. 3).

5. Discussion

Erythroderma is a generalized cutaneous inflammatory disorder characterized by extreme peeling of skin, diffuse skin redness and scaling classically found on >90% of the body surface. ED has a high morbidity, mortality risk significantly due to its complications arising from severe metabolic dysfunction. Exfoliative dermatitis is a severe form of CADR that has been associated to all four first-line antitubercular medications namely INH, rifampicin, pyrazinamide and ethambutol. Cutaneous ADRs interfere with TB drug therapy and enhance treatment failure risk, development of ATT resistance, recurrence of TB and higher complication risks.[5] A study on CADR with ATT in a tertiary care hospital, the most common malicious offender was pyrazinamide (2.38%), next streptomycin (1.45%), followed by ethambutol (1.44%), rifampicin (1.23%) and least with isoniazid (0.98%).[6] Most common adverse effects of INH are peripheral neuritis and hepatitis, while that of pyrazinamide are bleeding gums, joint pains which are more common in alcoholics and older patients, but CADR are rare with incidence of <0.001%. From the findings of our study, rifampicin and ethambutol were found to be tolerated well but CADRs developed to INH and pyrazinamide. Case reports on exfoliative dermatitis with antitubercular drugs are available in plenty; but only 4 cases of erythroderma induced by isoniazid alone is reported so far. So, to the best of our knowledge, this is the fifth and sixth cases of INH induced ED reported globally.

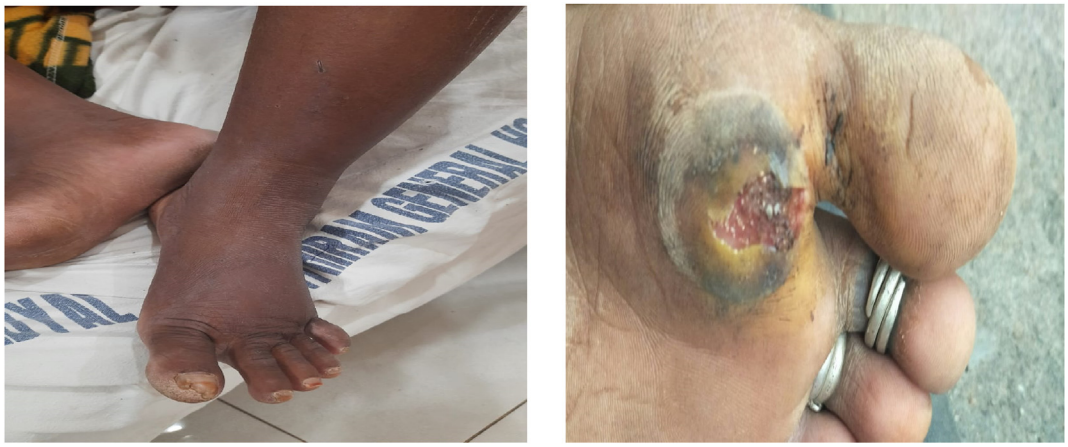


Fig. 2 – Case report 2. INH induced Exfoliative dermatitis.

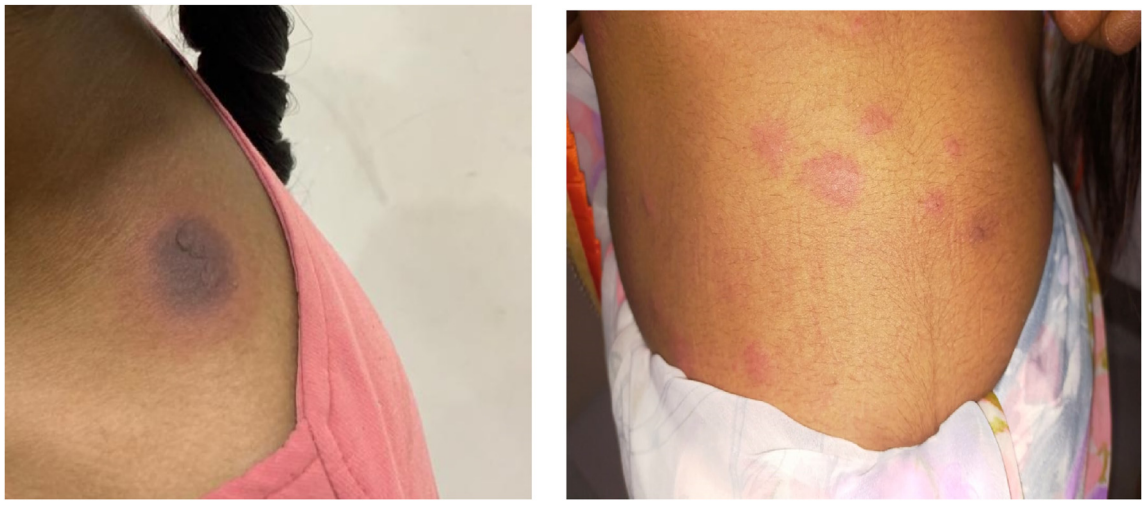


Fig. 3 – Case report 3. Pyrazinamide induced Exfoliative dermatitis.

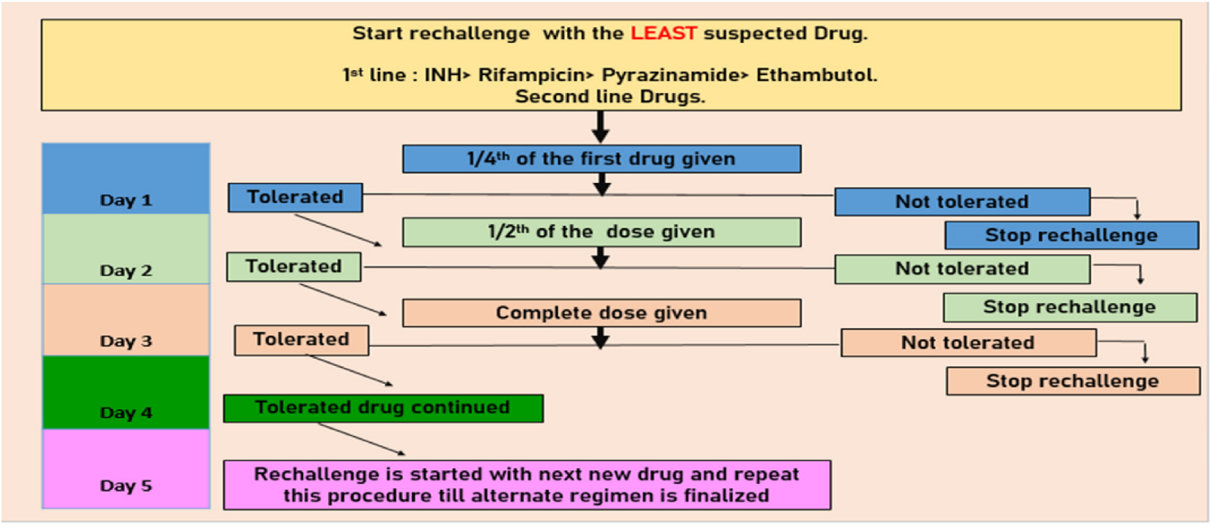


Fig. 4 – Algorithm for Rechallenge with ATT drugs.[8]

In India, the ATT regimen includes fixed drug combination (FDC) of drugs and this poses difficulty in pin pointing the single probable agent responsible for the ADR. ATT induced CADR confirmation is a lengthy process. Re-introduction of the drugs is the only way to detect the causative agent. Alteration of regimen results in use of a less effective regimen, increases the duration of treatment and may decreases patient compliance.[7] Though exfoliative dermatitis can be caused by multiple drugs, the temporal relationship between the introduction of INH, pyrazinamide and the onset of CADR, resolution following withdrawal and recurrence of symptoms when the drugs were reintroduced confirmed that INH, pyrazinamide as the causative agents (Fig. 4).

Among all the causes of erythroderma, drug-induced ED has the best prognosis and it usually resolves within 2–6 weeks. Factors predisposing for ATT induced hypersensitivity reactions include geriatric age group, polypharmacy, HIV infection, autoimmune disease, and liver or kidney dysfunction. The etiological mechanism of ED is postulated that the chemically active drug metabolite act as a hapten initiating an immunological response, cause apoptosis acceleration, or may directly result in cellular necrosis; an alternative mechanism proposed is that ED could be related to immunosuppression due to drug-allergy either immediate or delayed hypersensitivity reaction, due to drug accumulation or due to tuberculoproteins release.

Prompt resolution of the lesions after withdrawal of the ATT and treatment with oral antihistaminics, corticosteroids and emollients, reinforce the diagnosis as ATT induced erythroderma. Patients must be advised to avoid scratching, avoid precipitating factors and must be treated for the underlying cause and complications. In INH induced ED, ATT prescription was changed to rifampicin 150 mg, pyrazinamide 400 mg, ethambutol 275 mg, and levofloxacin 750 mg for a period of 2

months in intensive phase followed by levofloxacin 750 mg, rifampicin 450 mg for a period of 4 months in continuation phase. In pyrazinamide induced ED, pyrazinamide was excluded and remaining ATT drugs were continued.[9]

6. Conclusion

Exfoliative dermatitis are potentially fatal CADRs requiring a minimum of 2 weeks to several months of exposure to the trigger drug for the emergence of CADRs and can be avoided by discouraging drug over-prescription and use of combination drugs that interfere with the metabolism of the offending drugs. It is necessary to frequently monitor liver and kidney functions particularly in comorbid patients and those on polydrug therapy to prevent drug toxicity leading to increased incidence of CADR'S due to impaired metabolism or drug elimination. Several genes found to be involved in the genesis of exfoliative dermatitis include ABCB6, ALOX12B, C3, C4BPA, CARD14, CD4, CD8A, IL2, SPINK5, and TNF.[10] It may be recommended in specific high-risk ethnic groups (particularly Asiatic) to perform genotyping for ED associated genes before starting therapies with possible triggers.

After the diagnosis of ATT induced ED, multidisciplinary approach is initiated for management of the disease. At the earliest possible, identify and avoid the trigger and further complications. Antihistamines and steroids, topical emollients (to maintain skin moisture) are the cornerstone of treatment in any CADRs. New strategies like immunomodulatory therapies need to be looked into as CADRs are finally due to malfunctional immune system. To conclude, ED caused by isoniazid and pyrazinamide is a serious but potentially life threatening CADR. Abrupt withholding of the ADR triggering drug along with symptomatic management will help tide over this critical situation. Hence, physicians may be advised to follow caution while prescribing ATT regimen which includes INH and pyrazinamide. Early detection and judicious approach may be life-saving in patients with this type of ADR.

Conflicts of interest

The authors have none to declare.

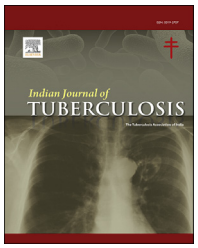
REFERENCES

1. Okoduwa C, Lambert WC, Schwartz RA, et al. Erythroderma: review of a potentially life-threatening dermatosis. *Indian J Dermatol*. 2009;54(1):1–6. https://doi.org/10.4103/0019-5154.48976.
2. Dua R, Sindwani G, Rawat J. Exfoliative dermatitis to all four rst line anti-tubercular therapy. *Indian J Tubercul*. 2010;57:53–56.
3. Sharma Dr Himanshu, Kishan Dr Jai, Singh Dr Sukhjinder Pal. Isoniazid induced exfoliative dermatitis in a patient with left renal agenesis. *Int J Sci Res*. 2019;8(2):8–9.
4. Yacoub, Mona-Rita, Berti Alvise, Campochiaro Corrado, et al. Drug induced exfoliative dermatitis: state of the art. *Clin Mol Allergy*. 2016;14(9):1–12. https://doi.org/10.1186/s12948-016-0045-0.
5. Jaisuresh Krishnaswamy. Pyrazinamide-induced exfoliative dermatitis in a patient on hemodialysis: a rare complication. *Case Rep Nephrol*. 2013;2013:1–3. https://doi.org/10.1155/2013/387293. Article ID 387293.
6. Roy Rajat, Bhattarai Anil, Shrestha Prativa, Paudel Upama, Parajuli Sudip. Dapsone induced exfoliative dermatitis: a case report. *J Coll Med Sci - Nepal*. 2010;6. https://doi.org/10.3126/jcmsn.v6i2.3621.
7. Hidayah Risa Miliawati Nurul, Anjani Andini Dwikenia, Ramali Lies Marlysa, Suwarsa Oki, Gunawan Hendra. Exfoliative dermatitis due to dermatophytosis. *J Infect Dev Ctries*. 2021;15(2):306–309. https://doi.org/10.3855/jidc.12218.
8. Modi Bina, Modha Jay. Spectrum of anti-tubercular therapy induced cutaneous adverse drug reactions and its management through rechallenge: a prospective study at a Tertiary Care Centre. *Indian J Tubercul*. 2021. https://doi.org/10.1016/j.ijtb.2021.07.018.
9. Garg Y, Gore R, Jain S, Kumar A. A rare case of isoniazid-induced erythroderma. *Indian J Pharmacol*. 2015;47(6):682–684. https://doi.org/10.4103/0253-7613.169575.
10. https://www.bosterbio.com/diseases/exfoliative-dermatitis.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Case report

Lest we forget spinal tuberculosis (Potts's spine): Case series with unusual presentation

Sameena Khan, Chanda Vyawahare, Shahzad Mirza, Nageswari R. Gandham, Sahjid Mukhida *

Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra 411018, India

ARTICLE INFO

Article history:
Received 31 July 2022
Accepted 20 August 2022
Available online 27 August 2022

Keywords:
Pott's disease
Tuberculosis
Bone
Culture
Growth

ABSTRACT

Pott's disease, also known as TB spondylitis, is a very uncommon extrapulmonary infection caused by *Mycobacterium tuberculosis*. As its prevalence is not high it can easily be underdiagnosed. Magnetic resonance imaging (MRI), computed tomographic (CT) guided needle aspiration, or biopsy are known to be the best techniques for early histopathological diagnosis along with confirmation by microbiological results. Ziehl Neelson stain (ZN) can detect Mycobacterium infections when clinically suspected samples are adequate and optimally stained. No single method or simple guideline can diagnose spinal tuberculosis. Early diagnosis and prompt treatment are necessary to prevent permanent neurological disability and to minimize spinal deformity. We are reporting three cases of Potts disease which could have been easily missed if we would have relied on one single investigation.

1. Introduction

Tuberculosis (TB) is an infectious disease that poses major health and socio-economic burden at a global level, especially in developing countries.[1] Tuberculosis can either be pulmonary or extra-pulmonary (EPTB) affecting any anatomic site of the body. One of them though uncommon is spinal tuberculosis or Pott's TB which affects the vertebral bones.[2] EPTB accounts for 15–20% of total tuberculosis cases with skeletal tuberculosis occurring in about 1–3% of the total TB cases only, it can lead to a destructive form of spinal tuberculosis. False-negative results in the diagnosis of EPTB is are not uncommon even with most advanced molecular tests like Cartridge Based Nucleic Acid Amplification Technology (CBNAAT)/Xpert and Line probe assay (LPA). It is due to low bacillary load and therefore needs to be correlated with the patient's clinical presentation.[3–5]

2. Case report 1

A 43-year-old woman presented to the orthopedic department with a complaint of severe back pain. She had similar complaints in the past four months for which she had visited a medical practitioner. She had been taking analgesics for pain

* *Corresponding author*. Tel.: +91 9974770469.
E-mail address: drssmukhida@rediffmail.com (S. Mukhida).
https://doi.org/10.1016/j.ijtb.2022.08.003

relief but her condition did not improve. The patient described the pain as progressively worsening and had no history of trauma.

On clinical examination, she had a temperature of 37.3 °C, a pulse rate of 100/min, and blood pressure of 120/75 mmHg. On physical examination, local tenderness on the lumbar area of the spine was observed. Laboratory blood examination showed results in the normal range. Magnetic resonance imaging (MRI) of the lumbosacral spine revealed vertebral changes and spondylodiscitis with Para -vertebral masses at D7-D8 (Fig. 1).

A CT-guided needle biopsy was performed and tissue was sent for histopathology and mycobacteriology investigations. The biopsy specimen was mechanically homogenized and resuspended in saline and smears were stained using the Ziehl-Neelsen method for detection of Acid-fast bacilli (AFB).

CBNAAT and LPA were also performed. Histopathology reported granulomatous inflammatory pathology. CBNAAT and LPA were found to be negative. The solid culture was done on in-house prepared Lowenstein-Jensen (LJ) media and liquid culture on MB Bact (BACT/ALERT MP Culture Bottle, BIOMERIEUX, USA) bottle after decontamination of specimen.[6,7] Rough, tough, and buff colonies were seen on the LJ medium (Fig. 2). Liquid culture media from Bact/Alert gave a positive signal after 4 weeks of incubation but no AFB was seen. At the end of the 6 weeks, again the positive signal was received but this time AFB was seen in the Z-N stain.

The MPT64 test was performed using the kit (SD MPT64TB Ag kit, Standard Diagnostics) with the solid–liquid culture which yielded a positive result (Fig. 3). LPA has performed once again from culture and gave a positive result. Hence the Patient was diagnosed with Pott's disease and was put on this using four drug regimen (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol). After six months of treatment, the patient is improving.

3. Case report 2

A 36- year-old female patient presented to the surgery outpatient department with complaints of back pain and swelling from the last 6 months. The pain was intense and gave the patient sleepless nights for the past 1 month. On clinical examination, she had a temperature of 38.1 °C, a pulse rate of 82/min, and blood pressure of 120/80 mmHg.

On physical examination, there was local tenderness and swelling which was painful during palpation. MRI lumbosacral spine revealed subtle extension into lateral recess at D5 vertebral level abutting spinal cord at this level and hypodense collection in the subcutaneous plane of the posterior

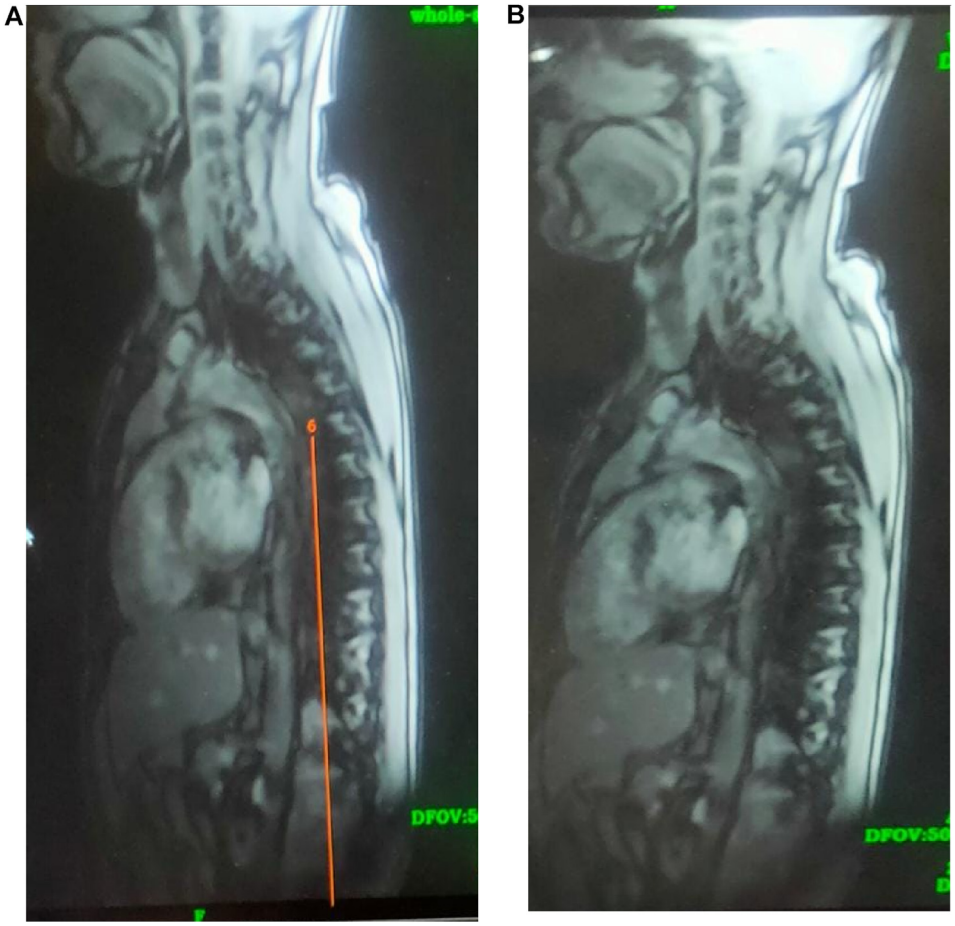


Fig. 1 – (A and B) MRI lumbosacral spine revealed involvement and collection at psoas muscle with edema at T11-L2.

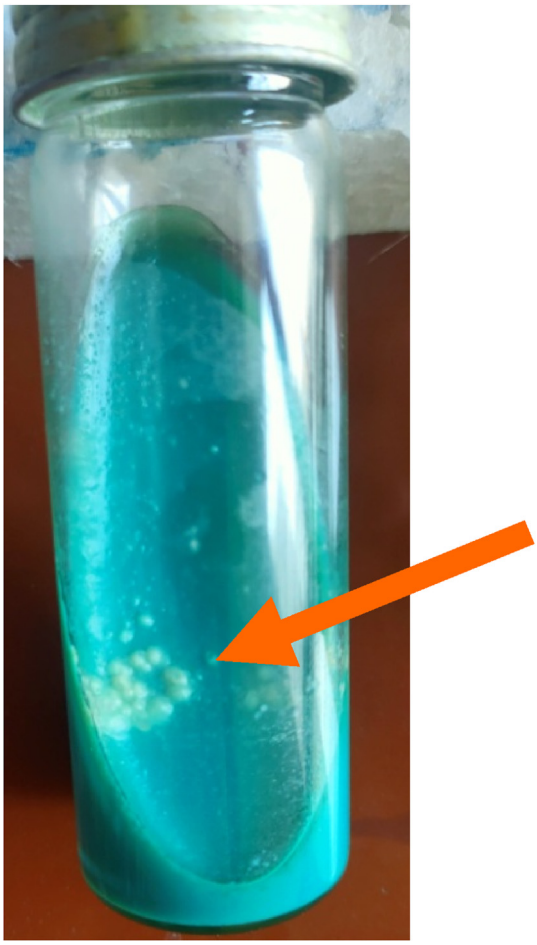


Fig. 2 – Growth of MTB on LJ media.

chest wall at the level of the upper border of right 3rd to lower border of 11th rib.

The abscess was drained under the USG guidance and sent for malignant cytology, bacterial culture, CBNAAT, and AFB culture. MTB was detected (low) with sensitive to rifampicin in CBNAAT. Based on CBNAAT, Anti-Tubercular treatment was started immediately and the remaining sample was processed for AFB solid and liquid culture as described in case 1. After 4 weeks of incubation, a positive signal was received from BacT/Alert 3D system for liquid culture, and acid-fast bacilli were seen in the Z-N stain smear. After 6 weeks of incubation, MTB colonies were grown on the L-J medium. MPT-64 was performed from L-J culture-condensation fluid which was also found positive. Post-treatment patient is improving.

4. Case report 3

A 27- year-old male patient presented to Orthopaedic OPD with the complaint of back pain which are radiating to the lower limb for the last 6 months. He had a tingling sensation in the lower back region. The patient was not able to sit or sleep for the past 15 days. The patient did not have any history of trauma or weight loss. On clinical examination, he had a temperature of 37.7 °C, a pulse rate of 93/min, and blood pressure of 110/75 mmHg. On physical examination, there was local tenderness on the lumbar area of the spine. Laboratory blood examination showed results in the normal range. On physical examination, there was swelling which was painful during the palpation. MRI lumbosacral spine revealed involvement and collection at psoas muscle with edema at T11-L2 (Figure- 1 A and B).

Para spinal abscess aspiration was performed under USG guidance and the fluid drained was sent for malignant cytology, bacterial culture, CBNAAT, and AFB culture. MTB was detected with low rifampicin sensitivity in CBNAAT. Malignant cytology reported necrotic material and

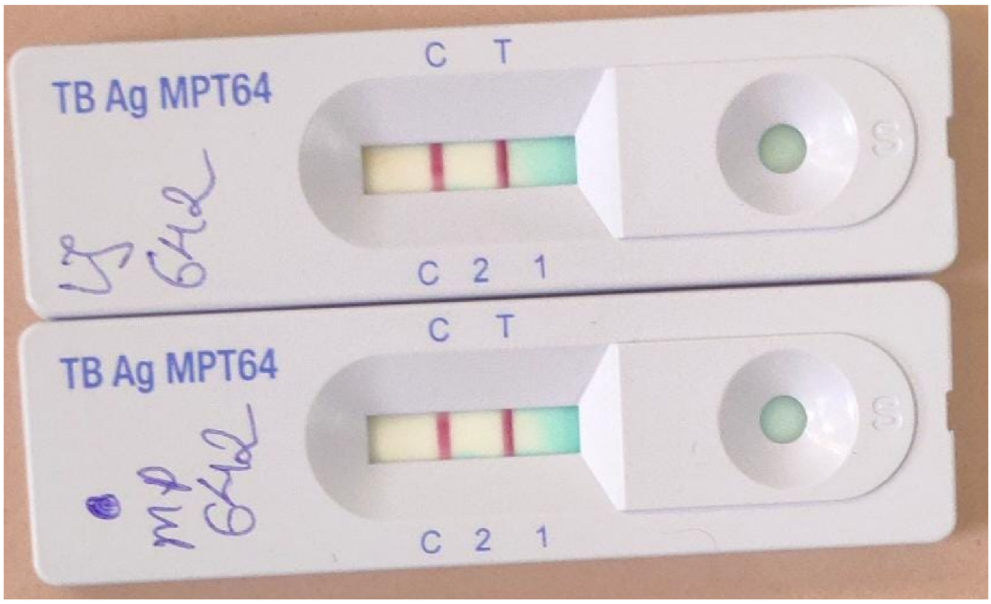


Fig. 3 – MPT 64 showing positive band for MTB.

Table 1 – Case details of all patients.

Details	Case 1	Case 2	Case 3
Age	43 years	36 years	27 years
Gender	Female	Female	Male
Affected area of spinal cord	D7-D8	3rd to lower border of 11th rib.	T11-L2
Hb (gm/dL)	8.20	6.60	15.2
TLC (per μL)	7200	6100	8700
Platelet (per μL)	275×10^3	564×10^3	391×10^3
ESR (mm/hr)	32	69	29
CRP (mg/L)	15.2	16.2	11.30
X-Ray	Not Detected	Not detected	Not detected
MRI	lumbosacral spine revealed vertebral changes and spondylodiscitis with Para -vertebral masses at D7-D8	hypodense collection in the subcutaneous plane of the posterior chest wall at the level of the upper border of right 3rd to lower border of 11th rib	MRI lumbosacral spine revealed involvement and collection at psoas muscle with edema at T11-L2
Type of Specimen	Vertebral Tissue	Aspirated Pus	Aspirated Pus
CBNAAT	MTB not detected	MTB Detected-Low; Rifampicin Sensitive	MTB Detected-Low; Rifampicin Sensitive
LPA	MTB not detected	MTB detected	MTB detected
Z-N Stain	AFB not seen	AFB not seen	AFB not seen
L-J culture	MTB- Growth	MTB- Growth	MTB- Growth
Liquid Culture	MTB Growth	MTB Growth	MTB Growth
MPT 64	MTB Detected	MTB Detected	MTB Detected
Treatment	Patients started ATT	Patients started ATT	Patients started ATT
Post Treatment outcome	Patient improved	Patient improved	Patient improved

degenerated changes. AFB was not seen in the Z-N stain smear. Based on CBNAAT, treatment was started and the remaining sample was processed for AFB solid and liquid culture as described in case 1. After 5 weeks of incubation, a positive signal was received from Bact/Alert 3D system for liquid culture, and acid-fast bacilli were seen in the Z-N stain smear. After 4 weeks of incubation, MTB colonies were grown on the L-J medium (Figure- 2) MPT-64 was performed from growth on L-J culture-condensation fluid which was found to be positive (Figure- 3). Post-treatment patient is improving. All 3 patients' details are mentioned in the Table 1.

5. Discussion

Pott's disease is not very common but can cause significant morbidity with serious consequences especially in developing economies. Prompt diagnosis of spinal tuberculosis remains challenging as compared to pulmonary tuberculosis. Suspicion of spinal tuberculosis needs to be correlated with clinical and microbiological investigations to confirm the diagnosis.[8]

Spinal Tuberculosis produces nonspecific symptoms with a slow clinical course, resulting in considerable delays in diagnosis and subsequent bone or joint damage. Although any section of the spine might be affected, the lower thoracic and upper lumbar vertebrae are most commonly affected. The symptoms of spinal TB are usually gradual, and the disease progresses slowly. Low-grade fever, agitation, weight loss, back pain, kyphosis, incorrect gait alignment, and paraplegia are common symptoms. Atypical manifestations of spinal TB have been recorded, including malignancy and fracture.[9,10]

We present three different cases of spinal tuberculosis with their clinical presentation and challenges in diagnosis. The most common symptom in all the three cases was backache similar to the presentation shown in a various studies[8,11] Back pain is the earliest and most common symptom in Pott's spine which can worsen with activity.

Due to the paucibacillary character of the specimen and the difficulty in acquiring a deep-seated specimen it is impossible to obtain multiple specimens for the diagnosis of Pott's spine. This inability and delay to diagnose and treat infected people leads to disease spread and impair the prognosis. With few accessible tests for MTB diagnosis, each with its onset of limitation on diagnosing the disease still remains tough.

Z-N stain, the feasible and highly efficient microbiological investigation of today often requires a large amount of sample and hence the chances of missing out on the bacilli remain high. Smear microscopy has low sensitivity with a range of 0%–40%, negative results cannot exclude the presence of TB.[11] In the current case series, we were not able to detect acid-fast bacilli on direct microscopy of specimen by Z-N stain.

CBNAAT and LPA are rapid molecular tests with good sensitivity and specificity.[12–14] They not only provide rapid results for MTB detection but also drug susceptibility testing results for rifampicin alone and in combination with isoniazid respectively. Both these tests have limitations related to bacilli load in the specimen exist here as well.

In Case-1, both the rapid molecular tests missed to detect MTB indicating that LPA is not a complete replacement for conventional methods of diagnosis as stated by World Health.[15] A positive MTB culture is a gold standard for detecting active tuberculosis, however it is not widely used in daily practice due to its time-consuming and laborious nature. MTB grew on solid culture in all our three cases whereas one case was undetected using the molecular technique.[16,17]

Radiological findings cannot reveal the exact etiology and may mimic malignancy or fungal nature unless accompanied by microbiological diagnosis.[18]

6. Conclusion

Potts disease is an extrapulmonary manifestation of tuberculosis that is difficult to diagnose. Rapid, expensive, and sophisticated molecular techniques, can often give false-negative results so the role of conventional solid culture cannot be ruled out. Although taking a long time can be helpful to start the treatment. Correlation between clinical presentation and diagnosing method can give an early sign of spine TB and also helpful to start treatment. Early treatment starting stop the growth of the disease and prevent rare complications in the patients. It can be considered in the differential diagnosis for patients presenting with back pain especially when clinical presentation and imaging findings are suspicious.

Source of support

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

Ethical committee approval

Applied.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. World Health organization. Fact Sheets. https://www.who.int/news-room/fact-sheets/detail/tuberculosis#:~:text=Key%20facts,with%20tuberculosis%20(TB)%20worldwide (Last accessed on 20th April 2022).
2. Bennett John E, Dolin Raphael, Martin J. Blaser in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. In: *Mycobacterium tuberculosis*. 9th Ed. 2020:2985–3021.
3. GenoType MTBDR Plus. *VER 2.0, Instructions for Use, IFU-304A-02*. Germany: Hain Lifesciences GmbH; 2012.
4. Somoskovi A, Deggim V, Ciardo D, Bloemberg GV. Diagnostic implications of inconsistent results obtained with the Xpert MTB/RIF assay in detection of Mycobacterium tuberculosis isolates with a rpoB mutation associated with low-level rifampicin resistance. *J Clin Microbiol*. 2013;51:3127–3129. https://doi.org/10.1128/JCM.01377-13.
5. Polley P, Dunn R. Noncontiguous spinal tuberculosis: incidence and management. *Eur Spine J*. 2009;118:1096–1101.
6. Bhirange S, Misra RN, Hatolkar S, Gupta N, Jadhav S. Comparative study between benzalkonium chloride tri-sodium phosphate and NALC-NaOH decontamination methods for recovery of mycobacterium tuberculosis from pulmonary sample. *Int J Microbiol Res*. 2019;11(3):1518–1520.
7. Balani K, Sahasrabudhe TR, Mehta k, Mirza S. TB patients: is sputum disinfection important? *Indian J Tubercul*. 2022. https://doi.org/10.1016/j.ijtb.2022.03.027 [Epub ahead of print] [cited 2022 Jul 29]. Available from:.
8. Cormican L, Hammal R, Messenger J, Milburn HJ. Current difficulties in the diagnosis and management of spinal tuberculosis. *Postgrad Med*. 2006;182:46–51.
9. Milburn H. Key issues in the diagnosis and management of tuberculosis. *J R Soc Med*. 2007 Mar;100(3):134–141. https://doi.org/10.1177/014107680710000312.
10. Beiner JM, Grauer J, Kwon BK, Vaccaro AR. Postoperative wound infections of the spine. *Neurosurg Focus*. 2003;115(3):14.
11. Canadian Thoracic Society and The Public Health Agency of Canada and Licensors. *Canadian Tuberculosis Standards*. 7th ed. Ottawa: Public Health Agency of Canada; 2013.
12. Lawn S, Zumla A. Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay. *Expert Rev Anti Infect Ther*. 2012;10(6):631–635.
13. Aricha SA, Kingwara L, Mwirigi NW, et al. Comparison of GeneXpert and line probe assay for detection of Mycobacterium tuberculosis and rifampicin-mono resistance at the National Tuberculosis Reference Laboratory, Kenya. *BMC Infect Dis*. 2019;19:852. https://doi.org/10.1186/s12879-019-4470-9.
14. Kannuri S, Mirza S, Misra RN, et al. Role of cartridge-based nucleic acid amplification test in diagnosing extrapulmonary tuberculosis. Med J DY Patil Vidyapeeth [Epub ahead of print] [cited 2022 Jul 9]. Available from: https://www.mjdrdypv.org/preprintarticle.asp?id=339947.
15. World Health Organization. *Molecular Line Probe Assay for Rapid Screening of Patients at Risk of Multidrug Resistant Tuberculosis (MDR-TB): Policy Statement*. Geneva: WHO; 2008.
16. Theron G, Venter R, Calligaro G, et al. Xpert MTB/RIF results in patients with previous tuberculosis: can we distinguish true from false positive results? *Clin Infect Dis*. 2016;62:995–1001.
17. Metcalfe JZ, Makumbirofa S, Makamure B, et al. Suboptimal specificity of Xpert MTB/RIF among treatment-experienced patients. *Eur Respir J*. 2015;45:1504–1506.
18. Jadhav S, Vyawahare C, Chaudhari N, Gupta N, Gandham N, Misra RN. Primary splenic tubercular abscess in an immunocompromised patient–rapid diagnosis by line probe assay. *J Clin Diagn Res*. 2013;7(9):1996–1998.