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Editorial

Nebulization guidelines: The long wait is over!

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Editorial

Inhalation therapy has existed since ancient times and historically its evolution can be traced to India 4000 years ago when it was employed as a method to deliver medications directly to the lungs. Hippocrates (460-377 BC) also advocated the inhalation of vapours of herbs and resins boiled with vinegar and oil which were then drawn into the lungs through a tube. The benefits of delivering medication directly to the affected site, the lungs, have been understood for more than 200 years. The Egyptian *Ebers papyrus* (1554 BC); describes throwing of the weed onto hot bricks, causing the alkaloid contents of the plant to vapourise, so that the breathless patient could inhale. Inhaled route is the simplest and most natural route of drug delivery to the lungs. It was Greek, *Pedanus Discorides*, who during the first century, prescribed inhaled fumigation. Datura leaves fumigation was used as a treatment modality for asthmatics and was also commercially available in the form of cigarettes and pipes.[1] Sales-Girons, in 1858 in France, were the first to invent a portable nebulizer or pulverisateur to convert liquid medication into spray. It operated like a bicycle pump that draws liquid from the reservoir and forces it through an atomiser. In 1864, The first steam-powered nebulizer known as "Siegle's steam spray inhaler" was invented in Germany and it used the Venturi principle to atomise liquid medications. This was the beginning of nebulizer therapy. The first compressor-based electricity driven pneumatic nebulizer, 'Pneumostat', was invented in the early 1930s.

In the present era, the nebulizers use oxygen, compressed air, or ultrasonic power, to break up solutions and suspensions of drug into small aerosol droplets that are inhaled into the lungs. Nebulizer delivers a therapeutic dosage of a drug, generated from a drug solution or suspension, through the mouth, nose or artificial airway (including endotracheal and tracheostomy tubes) into the airways and lungs. There is no coordination required between the inhalation and actuation that makes nebulizer suitable delivery devices for unconscious patients, those with severely compromised lungs, elderly, and infants or small children. They are also helpful when one has trouble using an inhaler or needs a large dose of an inhaled medication. Nebulization therapy, thus, emerges as a good option in all these cases besides being useful in the home care, emergency room and critical care settings.

Today, nebulization therapy is expanding at a very rapid pace, and this is attributed to the rising number of cases of chronic respiratory diseases, increasing demand for home nebulization, and the growing geriatric population. This is also reflected in the fast-growing global market size of the nebulizer, which according to a recent report has been valued at US$ 1075.89 million in 2021 and is expected to grow at a compound annual growth rate (CAGR) of 7.8% from 2022 to 2030, becoming 2122.14 million at the end of this period.[2] This poses a great challenge to all of us since there has not been enough progress and efforts in providing proper guidance in the form of guidelines for the end user, may it be the physicians, para-medical professionals, caregivers, or patients themselves. Most of the nebulizer use in the present time may not be proper in absence of appropriate directions of its use. The European Respiratory Society (ERS) acknowledges that although nebulizers are used

in hospitals and at home, quite frequently, however, much of their use, they claim, may not be evidence-based. Some of these present practices may be ineffective or some even harmful.[3] A comprehensive guideline was released by ERS in 2001, more than two decades back, during a period when nebulization was not so much in practice, and there were no good quality evidences available in form of the randomized clinical trials, observational studies, reviews and analysis, to properly guide and support present clinical practice of nebulization. Unfortunately, these guidelines, after their formulation, have not been revised, even though so many advancements and developments have occurred in this field, which certainly calls for an update. Prior to these guidelines, the British Thoracic Society had also formulated an exhaustive guideline in 1997 which, too, was never revised. Subsequently, there have been some sporadic reports and recommendations which do not fill up the gap thus created. Under these circumstances the lead taken by The National College of Chest Physicians (India), appreciating these deficiencies and to bridge this gap, is quite appreciable and addresses this long-standing deficiency. These guidelines formulated by the task force of experts, have been evidence based; comprehensive yet exhaustive; incorporating all the aspects of nebulization practice. The guidelines target an audience from hospital and home, comprising of pulmonologists, internists, intensivist, paediatricians. The document will not only be useful to the doctors, but also to the nurses, pharmacists, paramedics, respiratory therapists, physiotherapists, and all others involved in the practice of nebulization.

While these guidelines were under preparation, the pandemic of COVID-19 suddenly struck, which had an adverse effect on the progress of this project. During this pandemic, a controversy had also cropped up regarding the fear of transmission of SARS-CoV-2, to the health care professionals and others, by getting exposed to the fugitive aerosol escaping into the surrounding environment during nebulization in these cases or their suspects. The scare was to the extent that it created a panic situation, world over, leading to a sudden fall in the nebulizer use, in all the cases, new and old, requiring inhaled drugs, who were switched over to the other devices, mostly the metered dose inhalers (generally albuterol), leading to a scarcity of this device in the market due to sudden increase in its demand. Considering these apprehensions and uncertainties, a separate section was created in these guidelines, which was not proposed originally, to address all the issues related to nebulization, not only to the SARS-CoV-2 but all other contagious infections, prevalent in the present time, and that may emerge in future, to provide suitable guidance in such situations as and when faced.

The pandemic of COVID-19 thus has given new insights into the use of nebulization therapy, however, relevant data either in favour or against its use in these cases is limited, and in view of insufficient evidence in the present time, various organizations like World Health Organization, and Centre for Disease Control, USA, have recommended for the continued use of nebulization in such cases. The fact that needs consideration, is that the 'medical aerosol' produced during nebulization is generated in the nebulizer chamber and unless it is contaminated with 'bio-aerosol' produced from the patient's airways, it does not carry a risk of infection. However, there always is the risk of patient coughing during nebulization which can contaminate the fugitive aerosol, creating the risk of infection to those exposed. Moreover, an indirect risk of infection is always there, by being in close contact with the patient, and for prolonged periods. Infection control measures and preventive steps have been recommended in the guidelines to take care of even the potential risk of transmission of the infection. The same recommendations are also applicable in the cases of contagious infections, other than SARS-CoV-2 and during their epidemics, that may be seen in the future.[4,5]

Indiscriminate use of nebulizers is also to be avoided since there is enough evidence to show that nebulizers are not more effective than MDIs with spacers.[6] However, these findings refer specifically to the asthma cases, and not for the other cases of obstructive lung disease (OAD) including cases of chronic obstructive pulmonary disease (COPD, especially those having severe disease.[7] For COPD cases, especially when managing their exacerbations, there is no evidence to indicate that MDI (with a spacer) delivered medicine is more or equally effective than administration of the same medicine with a nebulizer. Although there are studies that show that nebulizers deliver larger dosages at a faster rate, recent data suggests that the actual lung deposition rates are the same for the two modes.

It has also to be considered that the use of a nebulizer is not very simple, and truly it is cumbersome, compared to the other handheld devices which relatively are not so labyrinthine, yet quite toilsome to use, and in the real sense their correct technique is adopted only in a limited number of patients. The use of a nebulizer though looks simple, yet is complicated and burdensome, and is dependent on several variables, which affect the drug deposition in the lungs. These include the type of equipment, the flow rate, the fill volume, the interface used, the drug type and its form (solution or suspension), and many more. The impact of simply changing the type of nebulizer, for example, from a pneumatic jet to a vibrating mesh nebulizer, has a ten-fold more deposition of drug in the lungs, requiring adjustment in the dosage to avoid the adverse effects and drug wastage. Further, use of a nebulizer in situations, like, mechanically ventilated patients, its simultaneous use with non-invasive ventilators, and high flow nasal cannula (HFNC), complicate it further and enough literature is yet not available on these uses.

The use of nebulizer with medications beyond bronchodilators, corticosteroids, and mucolytics; is altogether a different facet of this therapy, which is quite wide and is still evolving with a plethora of drug formulations becoming available, widening the scope of its use, not only limited to pulmonary disease, but even beyond, in many systemic disorders. This includes use of antibiotics for pulmonary infections (bacterial, viral, and fungal); drugs for pain management; haemostatics in haemoptysis; diuretics in refractory dyspnoea for palliation; local anaesthetics during bronchoscopy; inhaled drugs for pulmonary arterial hypertension; and many more. There also are several drugs in the pipeline which have a potential for their use in the nebuliza-

tion therapy and are likely to be added to this list. Off-label use of drugs, often practised, needs to be avoided, since lungs are delicate organs, and they should not be offended and harmed with new drugs without going through the different stages of trials to assess their efficacy and safety. Several factors such as pH, physical and chemical characteristics, the preservatives, and excipients used, can all have influence on the lungs. Mixing of drugs for nebulization, often practised for convenience, is only to be done where their physico-chemical compatibility has been demonstrated and not to be done just indiscriminately without evidence.[6]

Cleaning, disinfection, storage, and maintenance are the other essential components of nebulization, especially in the home nebulization. Nebulizers that are not properly cleaned and disinfected, get contaminated with microorganisms, including bacteria and fungi, and can cause colonization of microorganisms and pulmonary infections in those using such equipment. It has been observed that most of the nebulizers used at home, are contaminated, often with potentially pathogenic bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, multidrug resistant *Serratia marcescens*, *Escherichia coli* and multi-resistant Klebsiella species, Enterobacteriaceae and fungus *Fusarium oxysporum*, which may often be responsible for exacerbations in COPD. Thus, regular cleaning and drying, periodic disinfection, and proper storage, though quite inconvenient, are imperative and of paramount importance to prevent their getting contaminated and becoming a source of nosocomial infection rather than being an aid to the management. Nebulization is like a coin that has two sides to it, separated by an expanse so small, but wide enough that one side can't see the other, where one side is salubrious but the other can be ominous, which has to be taken care of well and we need to know about both the sides well enough, and that these are connected.[8]

Nebulization therapy today, with the newer technologies evolving in the equipment, availability of a wide range of drugs for nebulization, diverse uses of this therapy in pulmonary and other conditions; concomitant use with other lifesaving equipment's; considering also the risk of contamination with microorganisms; and risk of transmission of infection from contagious infections during epidemics to the HCP; it is evolving more as an art rather than simply an aid to the management. Its use also is gradually becoming more common, especially the home nebulization, which in itself is emerging as an important segment in this therapy, however, enough guidance on its proper use is yet not available, highlighting the importance of educating the HCP, physicians, caregivers and to the patients and their households on all the aspects of this therapy. The current use of nebulization, mostly, is not quite authentic and needs to have a better scientific approach to make it still more useful, evading the flaws and trying to achieve perfection to have the optimal results.

The Indian Guidelines on Nebulization Therapy, published as a supplement in the Indian Journal of Tuberculosis, are quite elaborate, yet comprehensive, incorporating almost all the aspects of nebulization therapy that one needs to know about. It has six sections which deal with basic principles and technical aspects of nebulization; types of nebulizers, how to choose, and use; uses in OAD and other diseases; its applications in the intensive care unit; home nebulization; training of the health care professionals; and how to use the equipment during epidemics and pandemics of contagious pulmonary infections. It also provides instructions on proper cleaning, disinfection, and storage to keep it contamination free, without risk of nosocomial infection and directions for its proper maintenance, to have best performance out of it. It is high time to understand, educate, and implement a rational use of nebulization therapy in view of its wider application with improved and optimal results. These guidelines fill the gap which has been existing in the country, for such a long time, for a treatment modality whose use is increasing and getting diversified too at a very fast pace. The guideline is a great welcome and it is likely to change the entire outlook of this therapy making it more evidence based.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Viewpoint

Genomic revolution: Transforming tuberculosis diagnosis and treatment with the use of Whole Genome Sequencing - A consensus statement

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ABSTRACT

Tuberculosis (TB) is a preventable, treatable, and curable disease. However, in 2020, 9•9 million people were estimated to have developed tuberculosis, and 1.5 million people were estimated to have died from it. Whereas in India, 2.6 million were diagnosed with TB and 436,000 succumbed to TB in 2019. India (26%) is the major contributor to the global drop in TB cases. The COVID-19 pandemic has substantially reduced access to services for the diagnosis and treatment of TB, resulting in an increase in deaths and a reversal in global progress. [1]

Presently, TB incidence is falling at a rate of 2% per year, obstructed mainly by the rearing pandemic of drug-resistant tuberculosis (DRTB). Particularly concerning is multi-drug resistant TB (MDRTB), defined as resistance towards isoniazid (INH) and rifampicin (RIF). [2] The World Health Organization (WHO) targeted to reduce worldwide TB incidence by 90% until 2035. (1) Early initiation of effective treatment based on susceptibility patterns

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of the *Mycobacterium tuberculosis complex* (MTBC) is considered key to successful TB control in countries with high DRTB incidence. Worldwide MDRTB treatment outcomes are poor, with cure rates less than 60% (2) due to the lack of comprehensive Drug Susceptibility Testing (DST) in most high MDRTB burden countries. This is leading to the inadequate anti-TB activity of the provided regimens (3–5), unlike regimens advised for DST assure optimal results. (6) In addition to resistances to the established regimens, the resistance to the newer DRTB drugs is increasing.

On World TB Day 2022, Academy of Advanced Medical Education, Thyrocare Technologies Limited and HyastackAnalytics - IITB along with expert pulmonologist and renowned physicians from India convened for an advisory board meeting in Delhi on 20th March 2022 to discuss the role of Whole Genome Sequencing (WGS) in the diagnosis and management of TB. Objectives and specific topics relating to WGS in MDRTB were discussed, each expert shared their views, which led to a group discussion with a commitment to putting the patient first, and increasing their collective efforts, the organizations recognized that it is possible to make this goal a reality. The organizations involved in the discussion have declared their commitment to engaging in collaborative efforts to tackle DRTB detection efficiently. They advocate for strengthening access to WGS TB services, controlling and preventing TB, improving surveillance and drug resistance management, and investing in research and development. This Round Table serves as a framework to build on and ensure that the goal of ending TB is achievable with WGS services wherever needed.

Post discussion, a uniform consensus was said to be arrived if more than 80% board members agreed to the statement. The present paper is the outcome of aspects presented and discussed in the advisory board meeting.

1. Consensus statements

In the past few decades, the emerging Genotypic DST (gDST) tests replaced the stream of TB diagnosis. Sequencing technologies of WGS and targeted next Genome sequencing (tNGS) have shown great prospective for rapid and reliable determination of TB drug resistance patterns [1,2] The availability of WGS as a tool for the diagnosis and clinical management of TB offers considerable promise in the fight against this stubborn epidemic. UDST for all TB patients has been an important target for the WHO and the NTEP of India. In recent years, PMDT guidelines issued by NTEP has already mandated the need for UDST based anti-TB therapy for all TB patients in India.[3–6] True UDST can only be achieved by a mix of molecular and microbiological tests or complete microbial testing. With an estimated incidence of >30 lakh TB cases in India annually, the current laboratory capacity (private & public) may be inadequate to perform UDST for any significant proportion of India's total annual TB burden. TB WGS can significantly and drastically change the coverage of UDST.[7]

Also Patients with MDR-TB and XDR-TB and relapse or re-infection will benefit from WGS. WGS of MTBC may be beneficial when results of other molecular tests are inconclusive or indeterminate. Besides this WGS may be beneficial when phenotypic DST is not available for newer drugs like Bedaquiline and Delamanid. WGS is beneficial to detect disease transmission (identify cluster and strain). Also it is an important part of epidemiological surveys. WGS provides additional information such as drug susceptibility profile and mutation information. Currently WGS has certain limitations (data, operational and technical related) that need to be addressed and taken care of as early as possible.

Critically, WGS has not been shown to be reproducible for direct-from-sample detection of drug resistance. Consequently, its application is limited to samples which shown positive growth when subjected to enrichment through liquid culture. While this enables the gDST of live bacilli, samples may not be subjected to it in case of no growth on liquid culture. Additionally, while >500 mutations across >35 genes are currently screened for gDST against 18 antibiotics, it still cannot provided conclusive prediction of sensitivity, and can only be used for definitive prediction of resistance. Though WGS is not the only epidemiological tool for TB surveillance, it is one of the most useful diagnostic tools with maximum advantages and uses. WGS has the potential to be an adjunct diagnostic tool to routinely used conventional diagnostic methods in TB patients.

WHO has indicated that; the uptake of these technologies for DRTB diagnosis is hindered by concerns regarding costs, integration into laboratory workflows, technical training and skill requirements of utilization, and the need for expert guidance regarding the management and clinical interpretation of sequencing data.[8]

Also discussed about key challenges to the routine rollout of TGS; Several programmatic and technical challenges can be addressed with a few interventions by reviewing recent publications. These challenges are programmatic costs, training, technical (inter-laboratory portability and comparability of WGS data) portability of raw data/FastQ files, portability and

mutual recognition of interpreted relatedness data, Relatedness analysis output & reproducibility.

Research needs to be encouraged to definitely observe the benefits of WGS in TB patients. Due to lack of Indian data WGS is not recommended for routine use. Further research and clinical outcome data in Indian setting should be promoted for gathering data and making recommendations in the future.

2. Current challenges in DST

There are three main challenges with a standardized, short regimen based on limited DST results and treatment history.[2] Firstly, there is a problem with detecting fluoroquinolone (FQ) resistance. Factors associated with FQ resistance does not correlate with actual resistance in many instances, which could exclude patients who would benefit from shortened treatment regimens and include patients who are unlikely to be successfully treated. Secondly, the standardized all-oral shorter regimen recommended by WHO includes multiple drugs that are unlikely to be effective for many patients with MDR/Rifampicin resistance (RR-TB) including isoniazid, pyrazinamide, ethambutol, and ethionamide, which significantly add to the pill burden and toxicity of treatment. Lastly, patients needing to receive a longer individualized regimen often require a more comprehensive DST profile to guide regimen construction. Limited DST in these circumstances could lead to the inclusion of ineffective drugs and exclusion of effective drugs making treatment less effective, more toxic, and challenging to complete. Less effective treatment can also lead to the generation of additional resistance mutations and the transmission of DRTB.

3. Whole Genome Sequencing

WGS, where all known resistance-conferring mutations can be identified simultaneously, is increasingly being used to provide comprehensive DST to individualize MDR/RR-TB treatment in well-resourced settings.[9,10] In support, the WHO has released technical guidance on the use of next-generation sequencing to infer MTB drug resistance.[8] Furthermore, the WHO recently released a catalog of mutations conferring MTB drug resistance, which can be updated regularly as more data emerges.[11] Advantages of WGS over Targeted Next Genome Sequencing (tNGS) include a) full genome sequenced, b) No pre-specified targets needed c) Obtaining more information d) Comprehensive solution.[12](Fig. 1).

Since tNGS does not offer any advantage over WGS in terms of sensitivity and specificity, cost per sample emerges as an important differentiator. In terms of sample processing, tNGS uses an additional kit post WGS to select specified genomic targets for sequencing, which adds to the total cost of sample processing. Thus WGS remains economically more viable with higher predictive value and greater access to data for better prediction of gDST.

The uses of WGS in TB diagnosis include a) Distinguishing relapse from reinfection in recurrent pulmonary TB, b) WGS to identify missed rifampicin and isoniazide resistance among TB isolates, c) Adoption of WGS as a high-throughput technology offering an unprecedented level of accuracy in identification, prediction of drug resistance, and molecular surveillance of MTB is significant in the context of disruption of TB services caused by COVID-19 pandemic and associated reductions in TB notification and drug resistance detection.[14]

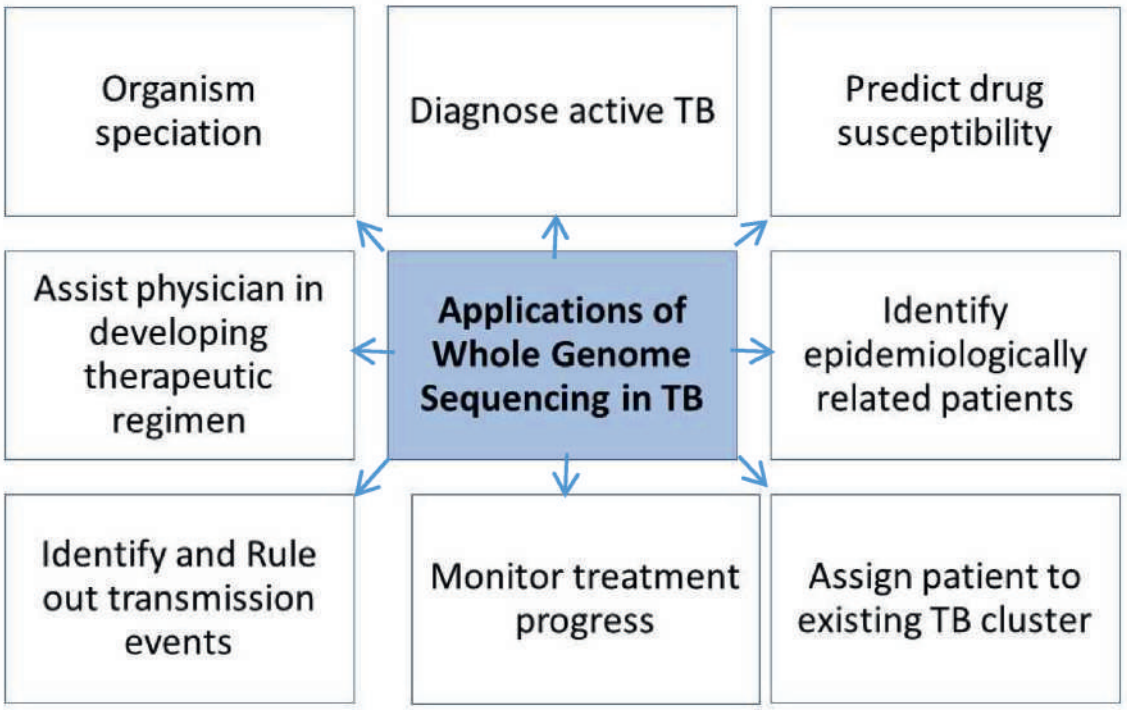

Fig. 1 – Application and added advantages of WGS in TB diagnosis.[13]

4. Place of WGS in current guidelines for the diagnosis & management of TB

CDC began performing retrospective WGS for isolates in select genotype-matched clusters in 2012. In 2018, the National TB Molecular Surveillance Center was established to perform WGS prospectively on all new MTB isolates.[15] In 2017, Public Health England (PHE) introduced routine WGS in the clinical setting of the National Health Service (NHS); and England is the first country in the world to pioneer its use at a population level for the diagnosis, detection of drug resistance, and typing of MTB.[16]

As mandated by the Programmatic Management of Drug Resistance TB(PMDT) guidelines; and based on several scientific studies,[17–22] the application of TGS for rapid and comprehensive Universal DST (UDST) has emerged as a significant breakthrough for reducing diagnostic delays, enabling early resolution of the disease, and stemming the transmission of drug-resistant forms of TB.[21]

The integration of gDST into the national TB diagnostic algorithm demands regular policy updates[9] because of the rapidly growing catalog of mutations associated with resistance to interpret genomic resistance profiles.[17,23] This requires a high level of autonomy and flexibility within the national board of experts accompanying and supervising gDST and continuous technical support from international partners.[17,24]

5. Discussion

Despite being a key part of the End-TB strategy, UDST remains largely un-implemented, with over 70% of DRTB cases remaining poorly diagnosed. There are several reasons for limited access to UDST, chief among which are unreliability of culture DST for several drugs, extremely stringent laboratory infrastructure needed for performing culture DST, and poor culture conversion of clinical isolates.

Several studies have demonstrated direct WGS from sputum. However, nearly all studies have been demonstrated the outcome in spiked or artificial samples. In real world, results are not reproducible. The primary reason for lack of reproducibility are sample quality, prior anti-TB therapy and inefficient DNA extraction. These are problems which can be resolved.

Molecular diagnostics overcome some of the limitations of culture DST. However, given the expansive and ever-growing list of mutations required to be screened for each of the drugs reduces their sensitivity unless kits and probes are updated frequently. WGS, is already available at a cost which is lesser than culture based universal DST, can replace all tests post CBNAAT with rapid increase in uptake of genomic diagnostics. Economies of scale will reduce the cost per test, which will enable further accessibility to the technology. One route to such efforts may include nationwide pilot for genomic based DRTB diagnostics, which can be done within six months, and the reviewed for roll out in the NTEP.

WGS of MTBC is essential irrespective of results of other molecular tests. Several factors like heteroresistance, low coverage of DRTB mutations and inconsistent LPA results. These result in poor concordance between treatment outcome and predicted DRTB profile by molecular tests. Nearly all these shortcomings are overcome by WGS, which can not only provide 18 drug DST on the basis of the most updated mutation catalogue, but also detect heteroresistance and detect NTMs.

For long phenotypic DST has been the proverbial gold standard not only for diagnosing TB, but also for all bacterial infections. However, the past 20 years' research has unequivocally demonstrated sever lacunae in phenotypic DST including non-representative nature of microbial culture. In case of TB; this shortcoming has a much greater impact wherein successive culturing of the isolate for testing on several drugs results in poor concordance with clinical breakpoints and low positive rate of culture testing.

The clear association of mutations with resistance and treatment outcome has already established. DNA as a better analyte than in-vitro microbial culture. While this is most pronounced in newer drugs since they are actively screened for hot spots of drug resistance, the unreliability of phenotypic DST when considered with the extreme risks of performing the test makes WGS relevant for DST of all drugs.

While new research has been able to determine association between mutations and MICs, this is yet to be validated for many drugs. Further, since WGS has always been compared to phenotypic DST for concordance, WGS can only be as sensitive as culture. WGS cannot be used currently for reporting MICs and that WGS does not specify mutations associated with clinical outcome are the two major shortcomings of WGS.

In terms of potential to scale, cost per sample, ease of use and future proofing, WGS will certainly replace molecular tests. To understand clearly, a molecular test examines a fraction of the molecule (the DNA) at nearly the same cost at which WGS examines the entire molecule. Obviously, WGS can never be less sensitive that a molecular test, but WGS will always be more specific than the combined outcome of all molecular tests (Table 1).

The recent PMDT guidelines recommends the use of universal DST for all patients.[25] In case of MDR/XDR cases, the subsequent tests include LPA's and then culture DST. Clearly, the need for universal DST if highest for MDR-TB and XDR-TB patients, whereas, in addition to the choosing the most effective therapy, a clinician also needs to tailor the least toxic therapy. This is even more critical, since in the absence of universal DST for newer drugs like Bedaquiline, Delamanid and Pretonamide, have been prescribed. Eventually the efficacy of these drugs, will result in the same cycle of amplified drug resistance.

Because of lengthy treatment TB patient *give up*. They give up due to *not getting better* soon enough & they give up due to the economic distressed and extremely high disability-adjusted life year (DALYs). WGS provides universal DST in a single test. This replaces the current cascading diagnostic algorithm, due to which a patient is often given less effective, more toxic and longer regimens. Defaulting anti-TB therapy by patients is primarily due to high toxicity, poor disease prognosis post therapy and extremely long therapies. By using WGS a patient can be administered least number of drugs, with the least amount of toxicity for the least duration of

Table 1 – Solutions to existing weaknesses of WGS.[11]

Weakness	Solution
Requires culture isolates	Agencies putting efforts to do it directly from samples or by enrichment.
Slower than targeted sequencing	Agencies putting efforts to do it directly from samples or by enrichment.
Complicated bioinformatics	Bioinformatics tools are in the pipeline.
Expensive	Cost can be reduced by batching up the samples. Cost is also getting reduced by knowing public health needs.

therapy, which will not only improve treatment outcome, but also enable faster return to regular life for a patient.

It is now well established that when considering universal DST, the cost of WGS is lesser than the combined cost of molecular and culture DST tests, thereby making WGS the ideal platform for scaling access to universal DST. Considering that universal DST functions in a hub and spoke model, WGS can be seamlessly operationalized for widespread access.

WGS requires no kit upgrade, can provide results for samples with scanty bacillary load and can be performed for 1000+ samples in each run. Thus, WGS is likely most economical, equitable and efficient platform for increasing access to universal DST.

6. Hurdles in implementing WGS in LMICs

There are few hurdles in implementing WGS in routine clinical use in LMICs. As currently in India, with developers, WGS been rolled out with several partners, the access to the test has expanded to nearly 500 pulmonologists.

1. No guideline for starting therapy on the basis of genome sequencing based diagnostics: While the PMDT and the WHO technical recommendations clearly outline the need for using genomics based universal DST in TB, the guidelines have not been updated to support the dispensing of therapy on the basis of genomic diagnostics. Since anti TB therapy is highly regulated and provided freely through the public health system, a patient's inability to access the therapy creates a significant financial burden, which is limiting the uptake of the technology.
2. Limited knowledge of personalised multidrug therapy: With significant rise in DRTB, the clinical fraternity widely educated to the need for personalised therapy in DRTB cases. Further recent reclassification of drugs mandates the use of multidrug combinations which can be a conflict with a universal DST report, thereby reducing the applicability of the test.
3. Patient delays: the well-known circuitous route that a TB patient takes due to several anthropological reasons means that by the time a patient is able to reach a pulmonologist with access to WGS, the patient has already undertaken several antibiotic regimens which reduce the effectiveness, thereby reducing the sensitivity of the test.

These experiences are likely to be similar across all LMICs as compared to developed countries and are going to be the key bottlenecks in the uptake of the technology.

There is no known case of *denovo* TB infection. Every case of TB is a case of TB transmission. When *Walker et al* in 2015 identified the source of TB outbreak on Oxfordshire from a common public house, it resolved one of the greatest enigma of public health. Prior to that study, the only way to conclusively determine transmission chains was through verbal autopsies wherein two patients were deduced to have been connected through detailed questionnaires. The use of well-tuned phylogenetic approaches of maximum parsimony and WGS, it is now possible to connect every new TB patient anywhere and create a global transmission chain, samples from all samples were to be subjected to WGS.

As described above, WGS for TB, when performed to cover maximum, possibly 100% of the denominator, cannot just determine transmission clusters, evolution of drug resistance, impact of strain types of disease progression, but can theoretically connect every sample with another to provide the highest resolution of epidemiology known to science today. In addition to UDST, WGS can detect heteroresitance, and can explain the *rise and fall* of TB. WGS can also detect NTMs at the species resolution. These are two critical aspects of treating a TB patient which is not addressed by any current diagnostic methods, and which a clinician can use to prescribe a highly effective therapy with very low treatment failures.

TB elimination is only possible if we can prevent transmission of TB. Currently there are two major causes of TB transmission which cannot be prevented. First is latent TB and second is ineffective therapy of DRTB. To address the second cause of active transmission of TB, every sample which is positive for TB should be subjected to WGS so that the most effective therapy is administered to every patient, which can reduce transmission by >90%. After launching WGS, nearly 100% of samples tested were from re-infection, re-lapse and non-responding patients. This clearly indicated the exacerbated need for the test in such cases. Whereas the patients remained culture positive despite months, and sometimes years of therapy. In case of re-infections, the trauma of previous episode and greater possibility of DRTB is considered for deciding on a wider DST testing strategy, making WGS a potent option.

Thus a treatable epidemic will be replaced by a difficult to treat and more expensive to treat epidemic. It is essential to implement universal TB for all TB patients in India to realistically target TB elimination. In the current state of science and evidence, WGS is the most likely tool to be able to scale fast enough to have any tangible impact. Crucially, since WGS not only enables better therapy, it will provide a granular spatiotemporal trend of drug resistance and drug requirements thereof, which in turn can reduce procurement of ineffective drugs. In some way WGS can enable NTEP to formulate public health policy with surgical precision at national scale.

7. Results of panel discussion

In the light of the above information, a panel of experts discussed on the consensus statements.

8. The need

Identifying the nature of the mutations requires an accurate diagnosis of resistance (e.g., mutations not associated with DR) and resistance level (e.g., mutations causing low-level R, combination of mutations). Occurrence of discrepancies between DST performed on MGIT system and molecular assays. Not every genotypic modification of a DR-associated gene affects phenotypic resistance equally (MIC values, silent mutations) to different drugs of the same class. Also, border line/low-level resistance is strongly associated with treatment failure. Superimposed on classic workflow (smear microscopy, culture, then DST), clinical laboratories have overlaid molecular testing over the past two decades, using various platforms and clinical strategies.

9. Diagnostic challenges in MDR-TB cases

Drug resistance TB is an obstacle to end TB. Early diagnosis of MDR-TB and DST is vital for better results. Improper or late diagnosis leads to less effective treatment and poor clinical outcomes. Thus WGS can help in precision diagnosis and identifying resistance/susceptibility to newer anti-TB drugs.

10. Results of WGS and treatment strategy

- Over the years, the diagnosis of TB has shifted from only clinical to adjunctive lab-based results.
- Furthermore, treatment has transitioned from standard treatment to targeted/precision therapy
- WGS will help in smoothening the transition by removing unnecessary drugs from the treatment regimen and thereby reducing drug toxicity.
- Tuberculosis is beyond diagnosis, treatment, and prevention. It is beyond us.
- Laboratory-based WGS results for individual TB patients is additional information that will ultimately help in making a bigger difference at the community level.
- In 10 years, regular follow-up and faster treatment attributed to early MDR-TB diagnosis can help the country in eradicating TB.

11. Conclusion

TGS is a viable and financially feasible tool for the timely and comprehensive diagnosis of DRTB in developed countries. With the increase in MDR-TB incidence, second-line anti-TB drugs are gaining importance. However, genetic resistance to second-line anti-TB drugs based on WGS is yet to be thoroughly studied. Drug resistance in TB is public health emergency that can derail several current efforts towards TB elimination. Moreover, incorporating WGS into the national diagnostic algorithms involves intensive training and programmatic planning, challenged by a lack of advanced molecular biological education. These sensed barriers need to be addressed in high-prevalence countries where the added benefits of genomic DST could make significant progress towards decreasing MDR-TB incidence.

Conflicts of interest

The authors have none to declare.

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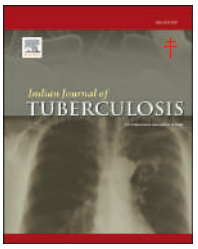

Original article

Deaths among tuberculosis patients of the western state of India: A secondary record based analytical study on its determinants

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ABSTRACT

Introduction: India has a significant TB burden, and ongoing attempts are being made to eradicate the disease. Globally, the number of TB deaths is declining, but not quickly enough to meet the End TB Goals. The National Strategic Plan (NSP) 2017–2025 in India set in motion an ambitious effort to expand the scope and efficacy of the National Tuberculosis Elimination Program (NTEP).

Methods: A descriptive retrospective study based on secondary data was conducted on information obtained from the electronic TB notification register for 2019, abstracted from Ni-kshay. Further, descriptive analysis was undertaken to identify the factors associated with deaths and successful treatment outcomes. The binomial logistic regression model estimates the crude relative risk and a 95% confidence interval to describe the association between predictor variables and TB treatment outcomes.

Results: After applying the eligibility criteria for the study population, a total of 1,44,643 (88%) TB patients were included in the study. 1,35,934 (94%) TB patients had completed the treatment and survived, while 8709 (6%) TB patients died. A significant association of treatment outcomes was observed in age, gender, key population, site of diseases, type of case, type of health facilities, HIV and Diabetes. When a logistic regression was applied, the model showed the association of the independent variables with the risk of death in TB patients.

Conclusion: The epidemiological factors associated with treatment outcomes among TB patients should be audited systematically. A structure of TB death surveillance and

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response system should be established with a mortality audit, including a community-based death review (CBDR) and a facility-based medical audit (FBMA) in case the patient is hospitalized or discharged from a hospital.

1. Introduction

Despite TB being a preventable illness, it is the 13th significant cause of mortality globally.[1] Until the COVID -19 pandemic, tuberculosis was the most prominent infectious cause of mortality, surpassing HIV/AIDS. World Health Organization (WHO) defines TB death as a number of TB patients dying during treatment, irrespective of the underlying cause.[2] According to the Global Tuberculosis Report 2021, tuberculosis fatalities climbed from 1.4 million in 2019 to 1.5 million in 2020. The African and Southeast Asian regions are responsible for 85 percent of deaths. While the case fatality ratio (estimated mortality/incidence) in high-income countries is 5%, it continues to be around 20% in high-burden countries.[1,2] WHO END TB strategy seeks to reduce tuberculosis-related mortality by 95% by 2035 compared to 2015.[3] Despite the fact that TB-related mortality has decreased globally, the slower rate of decline will preclude a 35% drop between 2015 and 2020.[4]

The ambitious target of reducing TB mortality is achievable only if the crucial contextual gaps are addressed. From the patient-centered care perspective, evidence indicates various factors mainly contributing to the mortality are predictable and preventable, too.[5] The epidemiological understanding of the distribution and determinants of TB mortality should inform and reflect in programmatic strategies and treatment guidelines. There are gaps in the health system preparedness leading to delays in diagnosis, difficulties in access, and a lack of comprehensive clinical care for patients with severe diseases.[6] Identifying the underlying causes of death and comorbidity will give policymakers and program managers critical information for developing strategies and early interventions to reduce TB morbidity and mortality.

As about 34% of global TB fatalities are attributed to India[7]; the Government of India has set forth an ambitious National Strategic Plan 2017–2025 to decrease tuberculosis fatalities in India by 3 per 1 lakh people by 2025, in accordance with WHO's END TB strategy and with a goal of a TB-free India.[8] The estimated death rate in India has decreased significantly, from 60 per 100,000 in 2000 to 32 per 100,000 in 2018.[4] The major gaps remain in understanding the slow decline and strengthening of the program. With essential evidence documentation to understand the contextual factors related to the death of TB patients, this research was carried out to identify socio-demographic, epidemiological and programmatic factors contributing to TB patients' death and suggest potential interventions that could be incorporated into the existing program in one of the Western Indian State, Gujarat.

2. Material and methods

2.1. Study design

A descriptive retrospective study was done through a record review of the routinely collected programmatic data from the Ni-kshay online portal. NI-KSHAY- (Ni = End, Kshay = TB) is the web-enabled patient management system for TB control under the National Tuberculosis Elimination Program (NTEP). It is developed and maintained by the Central TB Division (CTD), Ministry of Health and Family Welfare, Government of India.[9]

2.2. Study settings

This study was conducted in the western state of India, Gujarat, with a 60.4 million population scattered across 33 districts. NTEP has been implemented in all districts of the state. To implement NTEP, each district has a district TB center, which monitors the program for the entire district. The district is further divided into sub-district, i.e., Tuberculosis unit (TU) at each block. These TUs situated at the sub-district level have one medical officer - tuberculosis control (MO-TC) and two full-time supervisory staff - senior treatment supervisor (STS) and senior treatment lab supervisor (STLS) for implementing the TB control activities. Each TU has a network of designated microscopy centres (DMCs) and peripheral health institutions (PHIs) for carrying out diagnostic, treatment, and preventive activities under NTEP.[10]

2.3. Study population

2.3.1. Study definition

2.3.1.1. Ni-kshay online portal. The health facilities diagnosed patients with tuberculosis, clinically or microbiologically, are enrolled on the Ni-kshay. The enrolled TB patients are mentioned as notified TB patients for the program management to track their treatment parameters through several register reports. One of the reports is a notification register report containing variables like; date of diagnosis, treatment and treatment outcomes, type of TB case, demographic variables, comorbidity variables, and types of facilities providing program services.

2.3.1.2. Type of TB case. The study followed the case definitions as advised in the NTEP guideline. The key population is a socially vulnerable and clinically high risk, as per the NTEP guidelines. There are three types of TB cases, i.e., the new cases (drug-sensitive), retreatment cases (drug-sensitive TB patient with a history of the previous episode of TB) and

programmatic management of drug-resistant TB (PMDT) cases (drug-resistant TB cases who are on drug-resistant treatment regimen).[10]

2.3.2. Study sample and sampling

This study samples a full-year cohort of TB patients irrespective of the type in which the outcomes have already been assigned. Therefore, the secondary data of notified TB patients of Gujarat enrolled in the notification register of the Ni-kshay 2019 cohort was retrieved for the study. The reason for considering the cohort for the 2019 year was to include all cases where the outcome was already declared at the time of onset of this study, i.e., early 2022. E.g., PMDT patients on an oral longer or conventional regimen require 24 months-long treatment, where the outcome is only expected by the end of the year 2021. The notification registers of 2019 that contained 1,64,425 TB patients were extracted from Ni-kshay. Eligible TB patients for analysis were identified based on the inclusion and exclusion criteria mentioned below.

1) *Inclusion criteria:*
   a) TB patients enrolled in Ni-kshay remained mapped with health facilities belonging to Gujarat state until the outcome was declared.
   b) TB patients with the treatment outcomes date within the two years of the date of TB notification and outcome reported category assigned was 'successfully completed or cured' or 'died'.
2) *Exclusion criteria:*
   a) The TB patients who migrated out of the state or outcome declared as 'not traceable'; 'lost to follow-up' who interrupted the treatment continuously for more than one month; 'Failed' patients with no response to the treatment (treatment failure); 'regimen changed' patients with changes in the regimen for extension, duplicate records; and pending outcome reporting.

After applying the eligibility criteria for the study population, a total of 1,44,643 (88%) TB patients were included in the study. 1,35,934 (94%) TB patients had completed the treatment and survived, while 8709 (6%) TB patients died (Fig. 1).

2.4. Data variables, sources of data and data collection

The Notification report was obtained from the Ni-kshay Portal. The secondary data of the Ni-kshay under NTEP were enumerated to define variables for the study design. The following information was obtained: - TB registration number, age, gender, a patient from the vulnerable population who is more prone to get infected with TB (key population), type of TB (Pulmonary TB - PTB and extrapulmonary TB - EPTB), type of TB case and comorbidity status. The line list of the TB patients using non-identified numbers for each patient allotted.

2.5. Analysis and statistics

Data were entered into an Excel file (MS Excel 2021) and imported into the statistical software - SPSS (version 23.0 IBM, NY, USA) for analysis. The patient data on various variables have been summarized using numbers and proportions. The chi-squared test was used to compare groups, while the chi-square for trend examined linear trends. Risk measures were determined using odds ratios (OR) and 95% confidence intervals (CI), with the level of significance set at p-value <0.05. The duration (in days) between TB diagnosis and treatment initiation in TB patients has been described using median and interquartile ranges.

The "null hypothesis" of no association between death and successful outcome variables was tested. We quantified two key parameters: (a) TB patient reported as "Died" and (b) TB patient reported with successful treatment outcome. The primary outcome variable was descriptive; the dichotomous variables, death or successful outcome, were compared with the input variables. The input variables were socio-demographic variables, TB (key population), type of TB (Pulmonary TB - PTB and extrapulmonary TB - EPTB), type of TB case and comorbidity status. The study did not exclude TB patients with missing data, as excluding patients with missing data on variables will exclude patients who may have experienced/received sub-optimal care. Hence, created a separate category for missing data (unknown) within each variable and used that variable for analysis.

Logistic regression was used to identify variables independently associated with the risk of death. Binary logistic regression was done by the 'enter' method, considering the death of patients as the dependent variable and entering the following variables as the independent variables (predictors predicting death) in Step 1: age in years, gender, key population, site of diseases, type of case, microbiological confirmation of the presence of M. TB, comorbid conditions; HIV and diabetes. Bivariate analysis and logistic regression were reported as chi-square test, crude and adjusted odds ratio (OR), respectively, with 95% confidence intervals (CI).

2.6. Ethics and consent

The ethics for carrying out the study were obtained from the Institutional Ethical Committee of the Indian Institute of Public Health, Gandhinagar, Gujarat. The administrative permission for carrying out the study was obtained from the state TB office, Government of Gujarat.

2.7. Public and patient involvement (PPI)

Patients and the public were not involved in developing the research question, design, or implementation of this analysis. The study design was secondary data analysis, and we did not have funding to support such involvement. The non-identifier data was used to undertake the data analysis where the objective of the study to generate and present evidence on determinants of deaths among TB patients at program level.

3. Results

3.1. Characteristics of the study population

The study observed around 6% of deaths among TB patients who were diagnosed and treated in the health facilities of Gujarat. Out of all the patients in the selected cohort (N = 1,44,643), almost half of the patients belong to 26–50 years. Only 1.3% were of more

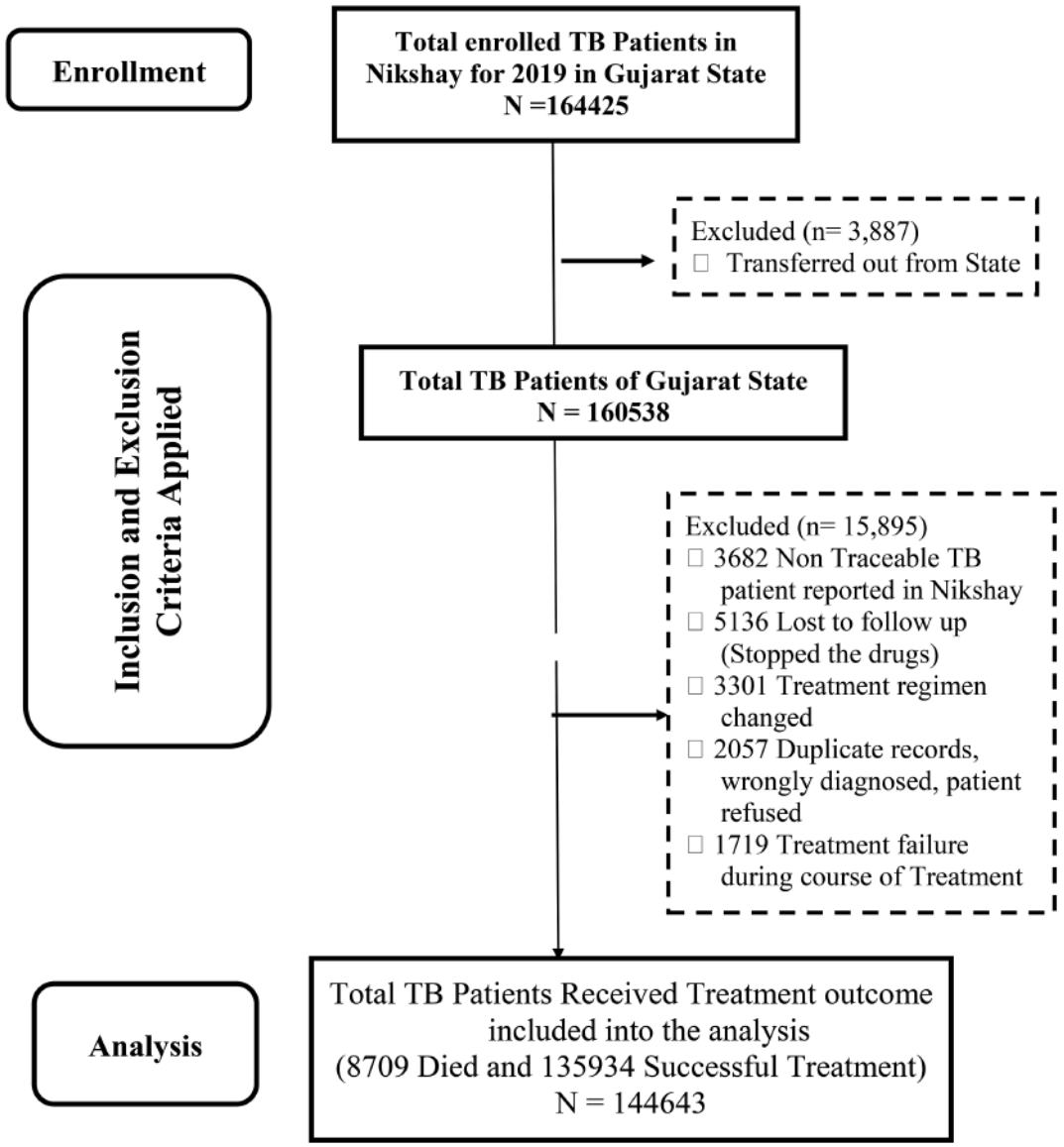

Fig. 1 – Flow diagram of the TB patients enrolled in the retrospective study of the Western State of India, Gujarat, for the Year 2019 (N = 164,425).

than 75 years and above. The distribution of deaths (n = 8709) among the age group of 26–50 years was 42.9%, followed by 39.8%, 12.8% and 4.4% in respective 50–75 years, less than 25 and more than 75 years. The association of categories with outcome was found statistically significant. (Chi-square 3239.1, $p < 0.001$). Similarly, more deaths were observed in male patients (74.1%). The key population accounted for 42.7% of the study population, with 6.5% of cases died during treatment. According to the study, those from key population demographics were 1.15 times (p 0.000, OR 95% CI 1.1063 to 1.2068) more likely to die than patients from non-key populations (Table 1).

When looking at the treatment outcomes, it was discovered that 78.7% of TB patients reported pulmonary TB and 6.5% of pulmonary TB patients died during therapy. There was a significant association between treatment outcome and illness location (site of TB) ($p < 0.005$, OR 95% CI 1.5533 to 1.7556). Individuals with pulmonary tuberculosis were 1.65 times more likely to have an unfavourable outcome (death) than those with extrapulmonary tuberculosis. It was observed that the proportion of deaths among the microbiologically confirmed TB patients (63.1%) was two times ($p < 0.001$, OR 95% CI 1.9195 to 2.0995) more than the clinically diagnosed TB patients (36.9%) without having microbiological confirmation (Table 1).

During the study, about 80.6% TB patients were diagnosed with TB as a new case. While only 3% of patients were drug-resistant (PMDT) and others (16.4%) were receiving treatment for the second or more episodes. As per the NTEP guidelines, the type of regimen will be based on the type of case. The new and retreatment cases are assigned drug-sensitive regimens, whereas PMDT cases are assigned as drug-resistant regimens. The study identified that out of all TB deaths reported as a treatment outcome, the number of deaths was 67.8% of new patients, 8.7% of PMDT patients, and 23.5% of retreatment patients. Among the type of case categories, the deaths were observed among the multidrug-resistant TB - PMDT cases (17.2%), followed by retreatment cases with a history of failure

Table 1 – Determinants of TB death: A logistic regression analysis of Socio-demographic and clinical profile of TB patients (cohort 2019) in Gujarat, India (N = 144,643).

Variables	Treatment Outcome			aOR (95%CI)
	Total N (%)	Death n (%)	Successful Treatment outcome n (%)	
Age				1.037 (1.036–1.039)
≤25 years	44,788 (31.0)	1117 (2.5)	43,671 (97.5)	
26–50 years	66,877 (46.2)	3739 (5.6)	63,138 (94.4)	
51–75 years	31,253 (21.6)	3468 (11.1)	27,785 (88.9)	
>75 years	1725 (1.2)	385 (22.3)	1340 (77.7)	
Gender				
Female	51,408 (35.5)	2254 (4.4)	49,154 (95.6)	1.578 (0.490–5.080)
Male	93,155 (64.4)	6452 (6.9)	86,703 (93.1)	1.969 (0.612–6.3360
Transgender	80 (0.1)	3 (3.7)	77 (96.3)	Ref
Site of TB				
Pulmonary	113,861 (78.7)	7456 (6.5)	106,405 (93.5)	0.995 (0.928–1.067)
Extrapulmonary	30,782 (21.3	1253 (4.1)	29,529 (95.9)	Ref
Type of case				
New	116,648 (80.6)	5906 (5.1)	110,742 (94.9)	Ref
PMDT	4398 (3.0)	755 (17.2)	3643 (82.8)	2.873 (2.626–3.144)
Retreatment: Recurrent	8387 (5.8)	691 (8.2)	7696 (91.8)	1.317 (1.246–1.392)[a]
Retreatment: Treatment after failure	643 (0.4)	71 (11.0)	572 (89.0)	
Retreatment: Treatment after lost to follow up	1979 (1.4)	197 (10.0)	1782 (90.0)	
Retreatment: Others	12,588 (8.7)	1089 (8.7)	11,499 (91.3)	
Current health facility				
Government	95,304 (65.89)	6974 (7.3)	88,330 (92.7)	Ref
Private	49,339 (34.04)	1735 (3.5)	47,604 (96.5)	0.506 (0.472–0.542)
HIV status				
Reactive	3186 (2.2)	629 (19.7)	2557 (80.3)	Ref
Non-reactive	127,996 (88.5)	7116 (5.6)	120,880 (94.4)	3.976 (3.613–4.375)
Unknown	13,461 (9.3)	964 (7.2)	12,497 (92.8)	1.255 (1.143–1.379)
Diabetic status				
Diabetic	6718 (5)	612 (9.1)	6106 (90.9)	1.131 (1.034–1.237)
Non-diabetic	119,980 (83)	6595 (5.5)	113,385 (94.5)	Ref
Unknown	17,945 (12)	1502 (8.4)	16,443 (91.6)	2.299 (2.120–2.494)
Key population	61,788 (42.7)	4012 (6.5)	57,776 (93.5)	0.948 (0.904–0.993)
Microbiologically confirmed	68,088 (47.1)	5499 (8.1)	62,589 (91.9)	1.555 (1.471–1.644)

Footnote: Number in () indicates the row% across the group and column % in total.
All variables shown here significantly differ across the group with p-vale <0.001.
Cox & Snell R Square 0.044, Nagelkerke R2 value was 0.121, classification accuracy of 94% in prediction.
[a] All retreatment sub-categories are categorized as retreatment.

of treatment (11%). The association of cases was found to be statistically significant with the outcome. (chi-square 15432.1, $p < 0.001$). Looking further into the type of facility from which the patients availed the services, 65.9% of the notified patients had received the services from the government health facilities, from which 7.3% of patients died compared to 3.5% deaths among private-sector TB patients (34.1%) (Table 1). There was a significant difference in the number of death and service provided by the facility. (chi-square 897.4, $p < 0.001$).

Majority patients (88.5%) were non-reactive, and only 2.2% were Human Immunodeficiency Virus-infected (HIV) reactive positive TB patients. However, the HIV status of about 9% of patients was unknown. Among the HIV status categories, almost 20% of patients with HIV-positive status died during the treatment, while the proportion was less than HIV-positive in the other two categories. (5.6% deaths in non–reactive and 7.2% in the unknown status of HIV). This difference among the category of patients was significant (chi-square 1139.2, $p < 0.001$). The study found that 83% were non-diabetic, 5% were diabetic and the status of 12% of patients was unknown. Among the diabetes comorbidity category, 9% of diabetic patients (5%) died during the treatment. In contrast, in the other two categories, no. of patients who died was less than diabetic patients (8.4% in unknown diabetes status (12%) and 5.5% in non-diabetes patients (83%)), which shows a significant difference among the categories of patients. (chi-square 346.5, $p < 0.001$).

3.2. *Regression analysis of the predictors of the deaths of TB patients*

The study used a binomial logistic regression model to estimate the crude relative risk and a 95% confidence interval to describe the association between predictor variables and TB treatment outcomes. The study used all variables in our dataset to develop the predictive model. Only patients with complete data on outcome variables were retained for model building. The model showed that apart from gender, key population and site of diseases, the rest of the variables were statistically significant ($P < 0.05$). The regression model

showed that the Nagelkerke R2 value was 0.121 with a classification accuracy of 94% (Table 1).

4. Discussion

The infection with *Mycobacterium tuberculosis* is not the only factor that causes and contributes to death during TB treatment; there are host, disease, and healthcare-system-related variables. The determinants of TB-related death may vary according to the epidemiological setting concerning the burden of TB, HIV, undernutrition, and resources within the health systems. The present study could not identify the nutritional status and gaps in the program interventions. Globally, 95% of TB deaths occur in low and middle-income countries (LMIC).[4] Age, gender, tuberculosis type, and drug susceptibility profile influence the chance of mortality from tuberculosis.

There is a higher TB and mortality burden in younger age groups in LMICs. In this study, a higher number of deaths (43%) were observed in the young population aged 25–50 years, where the higher mortality proportion among the age category was observed age more than 75 years. The importance of age as a predictor of death varies by nation.[11] There is a higher burden of TB and TB mortality in younger age groups in LMICs. In the Global Burden of Disease (GBD) study; the largest proportion of TB deaths worldwide (37.4%) occurred in the age group under 49 years, of which the 15–49 age group contributed 84%.[11] In a population-based survey from Bangladesh, TB was the second major cause of death in 15–49 years.[12] The present study found a higher mortality proportion among males than in the other two categories. The reason for this is unknown, but it could be due to differences in risk factor exposure, the effects of sex-specific factors on immunity, or genetic factors. However, in rural cohorts from India, the age at death in women was much lower (mean age at death of 32 years)[13] The women are more likely to adhere to treatment following a TB diagnosis[14,15] and have better treatment outcome.[14,16] Similar to the study, TB mortality disproportionately affects those living in poverty, urban slums and tribal populations, marginalized groups, and manual labour work.[17–20]

Majority deaths were found in pulmonary and drug-sensitive TB patients in the study as they have larger proportions among all study populations. Deaths are more common in pulmonary tuberculosis (PTB); nevertheless, some forms of extrapulmonary tuberculosis (EPTB), such as meningeal TB, have a significant death rate (27–60%), according to findings from Africa,[21] India,[11,16,22] and Denmark.[23] During the analysis, the deaths among MDR TB (PMDT) patients were around 17.2%, followed by patients with failure of treatment (11%), higher than other categories. Despite the fact that multidrug resistance (MDR) is a risk factor, drug-susceptible tuberculosis is the most common cause of death in TB patients (DSTB), 68% in the current study. According to the GBD study 2017 results, of the estimated 1.18 million TB deaths, 1.04 million (88%) occurred in patients with DSTB.[11] The proportion of deaths was higher in the government health facilities due to inadequate reporting from the private sector into the Ni-kshay.[24,25]

The mortality ratio among HIV-positive TB patients was high (19.7%). The study included that the missing could add more numbers to deaths. HIV co-infection raises the risk of mortality by 3–8 fold; hence HIV status is routinely assessed in all newly diagnosed patients with TB.[20,26–28] Several studies now advise the early commencement of antiretroviral medication among HIV patients and preventive therapy of anti-TB medications[29,30] Like HIV infection, patients with diabetes mellitus (DM) are more prone to TB than non-DM patients; DM patients had a 3.59-fold increased risk of active TB.[31,32] The present study provides the prediction model of determinants associated with unfavourable outcomes. The logistic regression model predicted an accuracy of 94% with nine studied variables.

4.1. *Limitation*

The present study included all causes of death, irrespective of the mode and cause of death, so the causes do not influence the study population. If there are gaps in the recording or updating of information on Ni-kshay, our findings are likely to be biased. The data was secondary from the Ni-kshay reporting portal, so the study could not address the status of nutrition, severity of the current diseases, program gaps or delays encountered during the therapy, inadequate reporting from the private sector, and treatment compliance of the medications.

5. Conclusion

The study identified the socio-demographic and clinical parameters as determinants associated with mortality among TB patients. Higher mortality rates were observed among the TB patients over 75 years, males, key population, pulmonary TB, multidrug resistant TB, patients receiving treatment in government health facilities and patients with comorbidities of HIV and diabetes mellitus. When logistic regression was applied, most variables were independently associated with treatment outcomes. Patients' health-seeking behaviour, compounded by health-system deficits and poor-quality care, all play a role in the chain of causation that leads to the mortality of TB patients. The study recommends that in high-TB-burden areas, the health-care system and program interventions must be customized per local morbidity and mortality pattern to reduce TB mortality. TB deaths should be documented with the same urgency and structure as maternity death audits. They should be accompanied by a mortality audit that addresses delays in seeking medical help, gaps in diagnosis, treatment, and quality of care. A structure of TB death surveillance and response system should be established with a mortality audit, including a community-based death review (CBDR) and a facility-based medical audit (FBMA) in case the patient is hospitalized or discharged from a hospital.

Author contributions

All authors contributed equally to the development of this study. All authors contributed to data analysis, drafting, or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. All authors approved the final draft.

HD Shah, S. Yasobant, Deepak Saxena, PD Nimavat: Conceptualization, Methodology, Software.

S. Yasobant, HD Shah, Priya Bhavsar: Data curation.

HD Shah, S. Yasobant, Patel Yogesh, Modi Bhavesh, PD Nimavat: Original draft preparation.

HD Shah, S. Yasobant, Somen Saha, AK Sinha, Jay Patel, Deepak Saxena, Patel Yogesh, Modi Bhavesh: Visualization, Investigation.

Deepak Saxena, Patel Yogesh, Modi Bhavesh: Supervision.

HD Shah, S. Yasobant, Jay Patel, KM Narkhede: Software, Validation.

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Conflicts of interest

The authors have none to declare.

What is already known about this topic?

Tuberculosis is the world's second-leading infectious killer and the 13th leading cause of death. As about 34% of global TB fatalities are attributed to India; the Government of India has set forth an ambitious National Strategic Plan 2017–2025 to decrease tuberculosis fatalities in India to 3 per 1 lakh people by 2025, as per WHO's END TB strategy and with a goal of a TB-free India. The epidemiological understanding of the distribution and determinants of TB mortality should inform and reflect in programmatic strategies and treatment guidelines.

What are the new findings?

The study identifies the determinants associated with deaths among patients with tuberculosis and the related risk of unfavourable treatment outcomes. TB deaths should be documented with the same urgency and structure as maternity death audits. They should be accompanied by a mortality audit that addresses delays in seeking medical help, gaps in diagnosis, treatment, and quality of care.

What do the new findings imply?

A surveillance and response system for reviewing TB deaths at the community and facility levels should be established. Creating such a system can give us a glimpse of the bottlenecks to address through program interventions that must be addressed to meet the TB elimination targets by 2030.

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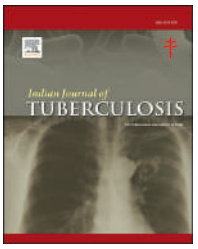

Original article

A study of analysis on prevalence, serological marker and prognosis of tuberculosis in tertiary care hospital

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ABSTRACT

Background: Tuberculosis is an infectious disease responsible for a significant cause of ill health. According to the WHO global tuberculosis report 2021. 9.9 million cases fell sick with TB in 2020. Significantly, the prevalence of tuberculosis in India is 25%.

Objective: To analyze the prevalence of tuberculosis in the suburban areas of the metropolitan city in South India. To analyze the serological marker and prognosis of tuberculosis among males and females. To determine the importance of molecular testing - PCR confirmation on TB after AFB smear.

Methods: A retrospective study to analyze 462 patients enrolled by the respiratory medicine department on suspecting pulmonary- 356 (M–264 & F-92) and extra-pulmonary-106 (M-73&F-33) patients and diagnosed Zhiel-Neelsen staining, Mantoux test, Chip-based RT-PCR test, Erythrocyte sedimentation rate, and analyzed serological test such as C-Reactive Protein, Chemiluminescence immune assay.

Results: 23 patients were positive in Ziehl-Neelsen staining, 65 were positive in molecular True-Nat PCR test, Mantoux skin test induration in 10 patients, 98 TB Positive patients examined in the serological analysis, 1 & 3 patients reacted in HIV/HBsAg, and HBsAg test respectively, by chemiluminescence immunoassay, 8 PTB and 4 EPTB and 47 non-TB patients were positive in C-reactive protein, 46 TB and 94 non-TB patients detected abnormal values out of these 160 patients in ESR test.

Conclusion: The Prevalence of tuberculosis is significantly rising, especially in the middle-aged population. The rapid molecular diagnostics to detect TB are highly sensitive and specific. Serological markers are essential for the analysis of disease prognosis and need to focus on the guidance of DOTS and RNTCP to End TB.

Abbreviations: WHO, World health organization; PTB, Pulmonary Tuberculosis; EPTB, Extra-pulmonary Tuberculosis; AFB, Acid-Fast Bacilli; RT-PCR, Real-time polymerase chain reaction; NAAT, Nucleic Acid Amplification Technique; M.tb, *Mycobacterium tuberculosis*; CLIA, Chemiluminescence immunoassay; CRP, C-reactive protein; LTB, Latent tuberculosis infection; HI, Human immune deficiency virus; HC, Hepatitis C Virus; HBsA, Hepatitis B Surface Antigen; DOTS, Directly Observed Therapy; RNTCP, Revised National Tuberculosis Control Programme.

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1. Introduction

Tuberculosis (TB) is an infectious disease responsible for a significant cause of ill health and one of the top ten causes of death from a single infectious agent. *Mycobacterium tuberculosis* (Mtb) is the etiological agent of tuberculosis. According to the WHO global tuberculosis report of 2021, 9.9 million (range 8.9–11.0 million) cases fell ill with TB in 2020. 1.3 million (range 1.2–1.4 million) tuberculosis deaths among HIV-negative patients in 2020, and additional 214,000 deaths among HIV-positive patients. Men accounted for 56% of the people who developed TB in 2020; women accounted for 33%, and children (aged< 15 years) for 11%. Geographically most people who developed TB in 2019, eight countries accounted for two-thirds of the global total; out of this, the number in India is 25%. The global drops in TB are significantly fair; India has 41% of drops in TB.[1] Drug-resistant TB is a global public health threat worldwide in 2020, and the Bacilli Calmette Guerin (BCG) vaccination is mandatory for all childbirth in India.[2] The Prevalence of TB in India is high because, most predictably, people live in poverty and have a poor quality of life. Hence have a higher risk of tuberculosis due to the household with muds floor, walls, asbestos roofs and higher sharing toilets in household contacts (HHC).[3] The decreased trend in TB prevalence was observed in the saharia tribal community of the central Indian state of Madhya Pradesh; based on the data analyzed, the burden of TB and disease effectiveness was reduced because of the programmatic effectiveness of DOTS and RNTCP.[4] The above factor is persisted, and this study could analyze the current scenario of prevalence as well as serological and molecular diagnostics to detect TB and help to understand the specificity of diagnostic markers and the correlation between viral disease and tuberculosis.

2. Materials & methods

A retrospective study to analyze 462 suspected pulmonary and extra-pulmonary patients were enrolled. We have included the patients who satisfied various parameters such as age, gender, specimen type, disease persistence, and symptoms of TB. Symptoms include persistent cough and fever, blood in sputum, chest pain, breathlessness, loss of appetite, loss of weight, and other co-morbidity conditions. The patients were advised to diagnose by Mycobacteriological, serological, and molecular diagnostics. Samples were collected directly from the hospital's respiratory and other clinical department wards and analyzed at SRM hospital's central laboratory from May 2020 to May 2021.

2.1. Ziehl-Neelsen staining

Ziehl-Neelsen staining (AFB-smear microscopy) processed for all suspected pulmonary and extra-pulmonary samples such as sputum, tracheal aspirate, bronchial wash, pus, tissue, synovial fluid, peritoneal fluid, pleural fluid, cerebrospinal fluid (CSF), and ascitic fluid. The samples were spread evenly (size-2 × 3) over the centre area of the slide. The slides were placed on the dryer to heat-fix the surface of the smear and covered with primary stain carbol-fuchsin. It was heat-fixed until the vapour began to rise for 5 minutes and washed with distilled water, and then flooded with a decolourizer (20% sulfuric acid) for 2–5 minutes, followed by washing with distilled water. The smear was counterstained with methylene blue for 1–2 minutes and washed with distilled water. The slides were allowed to air-dry and examined microscopically using the 100x oil immersion objective.

2.2. Tuberculin skin test (Mantoux test)

We have analyzed the tuberculin skin test (Mantoux test) as per the standard procedure. Briefly, we injected intradermal purified protein derivative (PPD) between the dermis layer on the flexor surface of the left forearm, midway between the elbow and wrist. The administration of a dosage of PPD was according to standard doses; 1 TU for paediatrics and 5 TU for adults, respectively, using a tuberculin syringe. Finally, we observed the indurations after 48–72 hours.

2.3. Modified-Petroff method

Based on the SOP for the mycobacteriology laboratory assay, we initially processed the modified-Petroff method for 5 sputum samples. The samples were mixed thoroughly with an equal amount of 4% NaOH using a vortex before being shaken for 15 minutes and centrifuged at 3000 rpm for 15 minutes. After carefully discarding the supernatant, sterile distilled water was added to the pellets in the centrifuge tube's rim to serve as a neutralizer. Centrifugation at 3000 rpm for 15 minutes followed, and the pellets for pre-treated with liquefaction buffer and lysis buffer for nucleic acid extraction to NAAT assay.

2.4. Chip-based RT-PCR technique

The suspected TB samples, including sputum, tracheal aspirate, bronchial wash, CSF, synovial fluid, peritoneal fluid, ascitic fluid, pleural fluid, and tissues, were processed by molecular analysis. We performed a Mol-Bio Chip-based RT-PCR assay according to the manufacturer's instructions. The samples of the pulmonary and extra-pulmonary (tissues were grained using sterilized scissors and mechanical blenders) were liquefied using 2–4 drops of liquefaction buffer. After 10 minutes, add 0.5 ml of the liquefied sample to 2.5 ml of lysis buffer and wait for 20 minutes, and 3 ml of the lysed sample was allowed to extract the mtbDNA by a universal cartridge. 6 μl of the elute was added to the PCR mixture and followed by the MTB chip for amplification.

2.5. C-reactive protein test (CRP)

We performed the C-Reactive protein test according to the Standard CRP test protocol. Patient blood samples were

Table 1 – TB prevalence among EP-TB &PTB.

N = 462	extra-pulmonary TB	pulmonary TB	P-Value
Total no of patients	106 (23%)	356 (77%)	
AFB POSITIVE	7 (6%)	16 (4%)	
True-Nat positive	22 (20%)	43 (12%)	p-0.33

There is no significant association between pulmonary and Extra-Pulmonary TB and TB in gender in both tests due to the table value being greater than the calculated value.

Table 2 – TB Prevalence among age groups.

Age	Male	Female	P-Value
0–10	0	0	
11–20	3 (1%)	1 (1%)	
21–30	11 (3%)	5 (4%)	
31–40	13 (4%)	10 (8%)	
41–50	13 (4%)	7 (6%)	
51–60	8 (2%)	1 (1%)	
61–70	13 (4%)	5 (4%)	
71–80	7 (2%)	1 (1%)	
81–90	0	0	p-0.04

There is a significant difference between the age group of the two populations male and female.

collected and centrifuged at 3500 rpm for 15 minutes. 50 μl of serum was added to test slides, followed by one drop of CRP reagent added and mixed with an applicator stick before being placed on a shaker for 2 minutes. The formation of clumps indicated positive results. Based on the positive CRP reaction of the qualitative analyses, we performed further quantitative analyses with dilution ratios of 1:12; 1:24; 1:48; 1:96 and 1:192, respectively.

2.6. *Chemiluminescence immunoassay (CLIA)*

The chemiluminescence immunoassay (CLIA) was processed for TB-positive patients to detect other viral infections. Based on the manufacturer's instruction, the sample was collected and centrifuged at 3500 rpm for 15 minutes, then air bubble-free serum-containing tubes placed in the tray and observed the results after 55 minutes for HCV, HIV, and HBsAg diagnosis.

2.7. *Erythrocyte sedimentation rate (ESR) test*

The blood samples were collected and placed on the sensor-based vesmatic cube 30-ESR, according to the manufacturer's guidelines, and observed the test results after 35 minutes.

2.8. *Statistical analysis*

The data were analyzed statistically by t-test and chi-square to test the study's significance.

3. Results

Out of 462 patients, we tested 356 (77%; M–264 & F-92) pulmonary and 106 (23%; M-73&F-33) extra-pulmonary patient samples. We found 23 (5%; M–20 & F-3) positive patients in AFB staining (Ziehl-Neelsen method), pulmonary TB 16 (4%; M–14 & F-2) and extra-pulmonary TB 7 (7%; M–6 & F-1) and 65 (14%; M–47 & F-18) patients detected MTB in TRU-NAAT chip-based RT-PCR test, PTB 43 (12%; M–32 & F-11) and EPTB 22 (20%; M–19 & F-8) respectively, 10 (2%; M–4 & F-6) patients induration seen in Mantoux skin test (p-0.33; There is no significant difference between the association of pulmonary and Extra-Pulmonary TB, and with gender; Table 1 & Fig. 1), analyzed patients among age groups, 43 (M–27 & F-16) positive between the age of 11–40 and 47 (M–34 & F-13) positive between the age of 41–70 (p-0.04, There is a

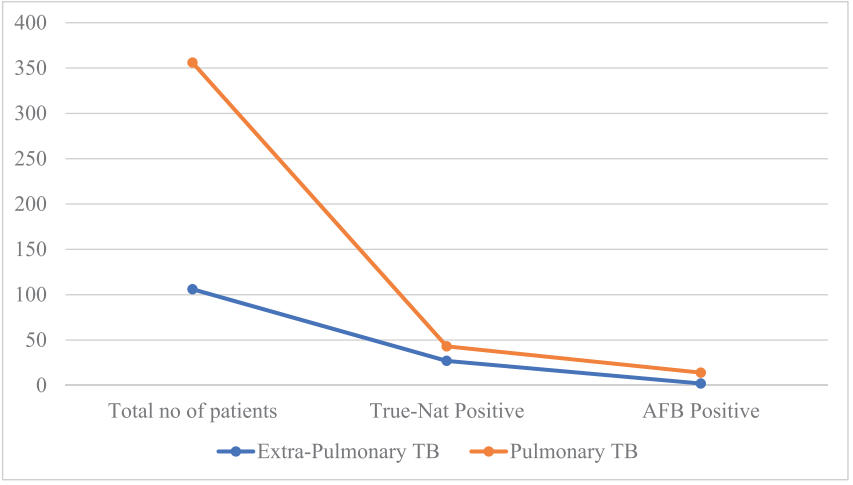


Fig. 1 – Diagnostics results on TB-positive patients.

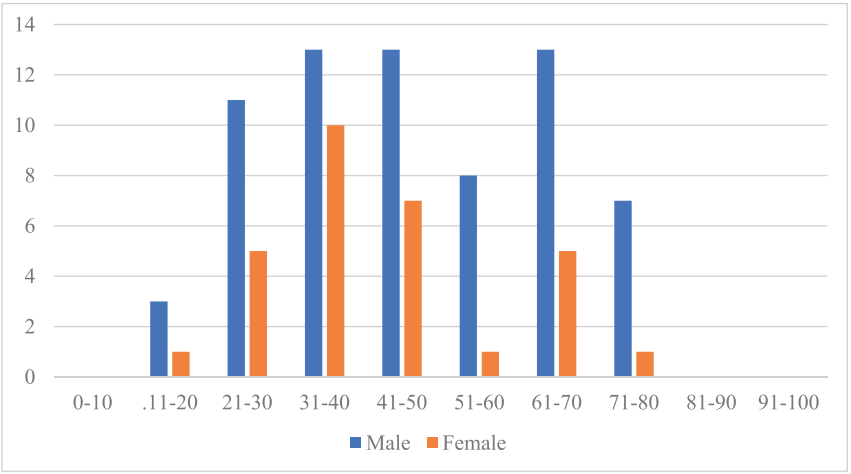


Fig. 2 – TB Prevalence among different age groups.

Table 3 – Serological analyzes of TB-positive and TB-symptomatic patients.

	CRP	HIV/HBsAg Reacted	HBsAg reacted	HCV/HIV/HBsAg non-reacted	P-Value
Pulmonary TB	8 (15%)	1 (1%)	3 (3%)	47 (36%)	
Extra-pulmonary TB	4 (19%)	0	0	4 (4%)	
non-TB	47 (13%)	0	1 (0.2%)	37 (10%)	p-0.02

There is a significant difference between the above test for HIV-positive TB and HIV-negative TB with the CRP test.

significant difference between the age group of two population male and female; Table 2 &Fig. 2), ESR abnormal sedimentation seen in 46 (47%; male-39 & female-10) TB patients and 94 (26%; male- 51 & female- 43) non-TB patients (p-0.03; There is a significant difference between ESR test value in TB and Non-TB patients, so ESR test is non-specific for TB). 12 TB patients positive, PTB 8 (14%), EPTB 4 (19%) and non-TB patients 47 (13%) in CRP test, 3 (4%) patients were reacted in HBsAg and 1 patient in HIV/HBsAg, 51 (15%) patients non-reacted in all HCV/HIV/HBsAg in TB positive and 37 (10%) non-reacted in TB negative patients in chemiluminescence immunoassay (CLIA) respectively (p-0.02; There is a significant difference between the above test to HIV-positive TB than HIV-negative TB with CRP test; Table 3 & Fig. 3).

4. Schematic diagram of TB diagnostics

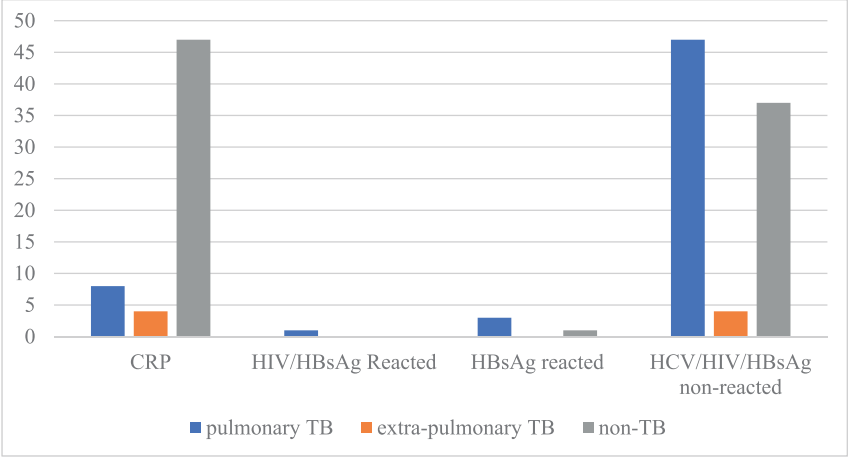
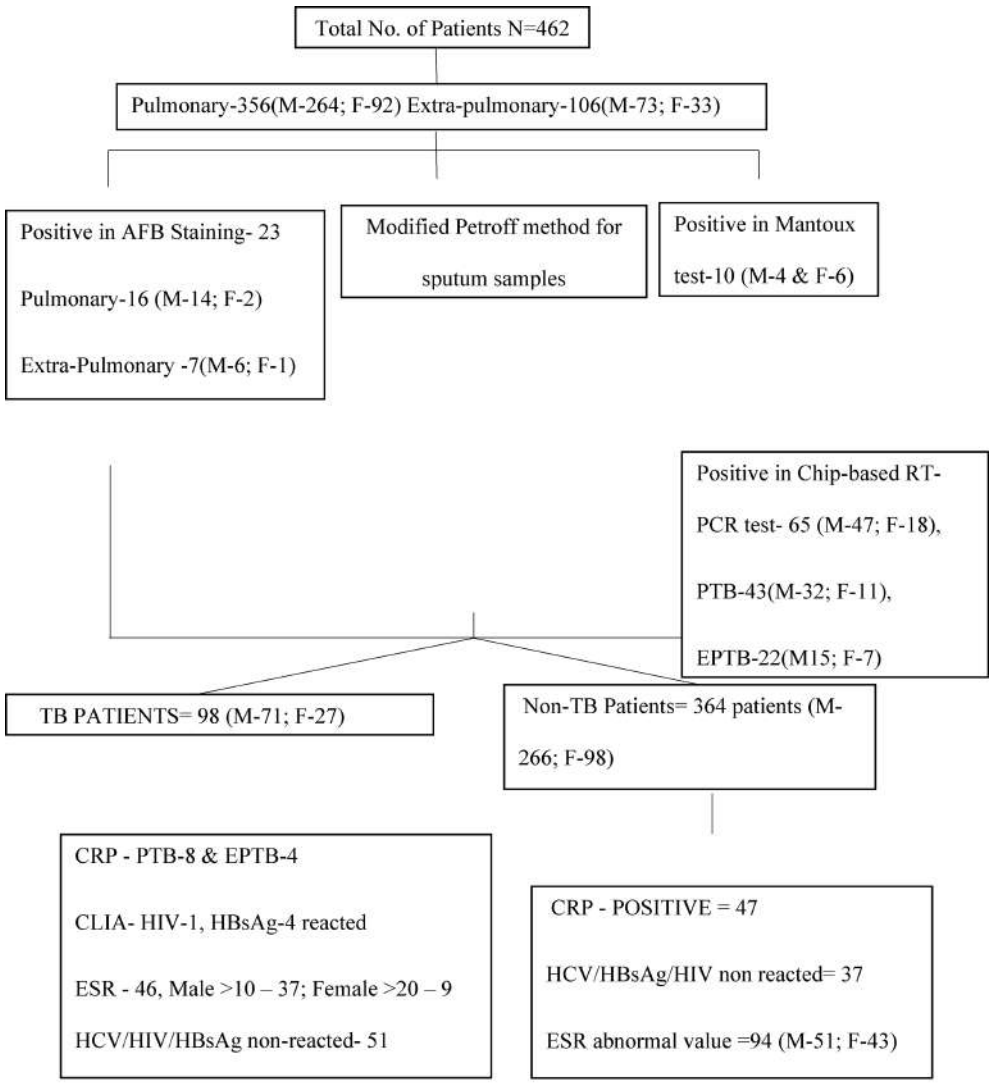


Fig. 3 – CLIA assay among TB positive & Negative patients.



5. Discussion

The study shows that the prevalence of tuberculosis in the Covid-19 pandemic between the first and second waves attacked India, especially in the suburban area of Chennai. We analyzed the modified Petroff method, included with chip-based RT-PCR detection for MTB based on Gene Xpert diagnostic procedure first time. Thus, the results were the same as a routine procedure of NAAT assay. Consequently, the technique could be beneficial to remove other normal microbes in the direct sample, which was then analyzed by the nucleic acid extraction cartridge-based pure Mtb DNA. In addition, in the comparison between TB and NON-TB patients in the ESR test, the patient's sedimentation rates showed abnormal so it could be a severe infection in TB and other illnesses. The ESR test may feasibly support analyzing the worsening of the disease but is not specific to TB. A similar study found that ESR is insignificant in evaluating TB-HIV co-infection. Routine determination of ESR tests has little value for patient care other than incurring additional costs.[5] When new patients are diagnosed with TB, analyzing disease progression using different diagnostics markers in the pandemic situation is advisable. A similar study states that the incidence of Hepatitis B Virus (HBV) was relatively high in tuberculosis patients, thus the need for routine screening of viral hepatitis markers for all TB patients before anti-TB treatment for better management of patients.[6] Tuberculosis management during the covid-19 is an important concern because it's a communicable infection and can easily spread and increase the patient's disease progression due to viral pneumonia in close contact with households, a similar study showed in Spain that Covid-19 caused substantial changes in TB care and 24% of active TB due to isolating in the household for suspect Covid-19.[7] The occurrence of TB in HHC is high in younger age and females in LTBI due to close contact, and who are self-isolating in the household, a coughing adult can spread the infection to others, and it could have TB that is easily passed on to other family members.[8,4] The mid-year survey in Chennai city between 2010 and 2012 showed that the total population of people living with TB is 4.7 million and

the proportion of the population living in urban areas are 100 in 2001 (RNTCP).[9] Connected with this, a study shows TB increased rapidly, and gender, age, and habits are all associated with each other. They need to focus on regular TB surveys, reflecting the same in a similar study that noted the incidence of latent tuberculosis infection (LTBI) among household contacts of PTB patients in India is very high and varies by test type, age, and exposure gradient. The prevalence of pulmonary TB is still a major problem in the Wardha district and is needed to strengthen TB control efforts.[10,11] C-Reactive protein test is advisable to monitor inflammation for covid-19 and other co-morbidity conditions because of lung inflammation. Still, TB patients had less positivity than non-TB patients in the CRP test. Comparatively, a study found that the CRP test is moderately specific for HIV in active TB.[12] Prevalence of TB in gender and age groups was analyzed, and patients aged between 40 and 70 are high exposure. Notably, a similar study showed male patients and the younger population were more affected, especially the middle-aged group in the metropolitan city in south India, and the male ratio is higher than females. The study analyzed the more suspected patients by sputum specimen, examined culture-positive and smear-positive pulmonary tuberculosis (PTB) and the data estimated 228, culture-positive PTB 259 and bacteriologically positive PTB 349 per 1,00,000 populations in south India.[13,7] Accelerating access to quality of life in the paediatric TB prevalence at major cities in India, where the rifampin-resistant population is high.[14] Since the TB culture remains the gold standard laboratory test for MTB with high sensitivity and specificity, it is particularly time-consuming to diagnose. Moreover, AFB smear microscopy is less sensitive for diagnosing extra-pulmonary tuberculosis. In this context, the rapid molecular diagnostics RT-PCR analysis to detect MTB and MTB-RIF is crucial for the timely monitoring of the disease progression. Moreover, the rapid molecular diagnostic is accurate, time-constrained, highly sensitive and timely treatment with anti-tubercular drugs might reduce mortality rates.[15] A recent study also found that molecular diagnostics provide accuracy and reduce the turnaround time and reliable results on smear-positive. Still, it cannot replace AFB smear and culture tests but is tested additionally with the standard method. Moreover, the patient had TB and Covid-19 simultaneously and based on the rapid application of NAAT to the diagnosis of MTB complex.[16–18] The newly diagnosed TB patients are advised to adhere to the anti-TB treatment, which promptly reduces the TB illness. It can emphasize appropriate treatment-seeking because of the similar symptoms of TB and Covid-19; patients added a protective measure should implement the Covid-19 out-breaks while engaging TB services.[19,20] CLIA assay for HCV, HIV and HBsAg reported the other viral infection in active TB patients. It could worsen the condition of TB patients. A recent study found a high frequency of HBV markers and a low frequency of HCV markers in HIV and TB, and the prevalence of TB, HIV, and HCV target population-based survey needed to reduce the infectious diseases, and premature mortality.[21–24] The incidence and factors involved in MDR-TB, most probably the female gender and HIV negative with HIV seropositivity suggests the immune system and sex hormone associated with the etiopathogenesis of MDR-TB, and co-infection HBsAg in TB can induce hepatotoxicity so careful monitoring is most needed. Moreover, the diagnosis of HBsAg is crucial in HIV-TB patients.[25] Statistical analysis shows that TB prevalence is especially higher in men than women because of poor quality of life, workplace atmosphere, smoking habits, alcohol consumption, and the need to diagnose timely. Thus, rapid molecular diagnostics is high accuracy in detecting MDR-TB along with standard diagnostics. A similar study correlates the high Prevalence of MDR-TB in western India revealed that rapid molecular diagnostic like line probe assay enhances the patients get prompt anti-TB treatment in all people living with HIV.[26]

6. Conclusion

According to the current study's findings, the prevalence of tuberculosis is rapidly increasing in South India, particularly in large suburban cities. Furthermore, men are more likely than women to develop tuberculosis, and middle-aged people are particularly vulnerable. A TB prevalence survey regularly is required to close this gap in the literature. Furthermore, rapid molecular assays for tuberculosis (TB) diagnosis improve sensitivity, specificity, and timeliness. In addition, C-reactive protein, ESR, CLIA- HIV, HCV, and HBsAg tests to predict the prognosis of other viral diseases in TB patients.

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Ethical statement

We have obtained an Ethical certificate and got approval from the head of the institution for analyzing this study. Hence, it's a retrospective analysis, so not applicable for informed consent from patients.

Conflicts of interest

The authors have none to declare.

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Original article

Prevalance of tuberculosis amongst healthcare workers, working in DOTS/sputum microscopy centre in two different districts of state of Uttarakhand & Uttar Pradesh of India

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ABSTRACT

Background & Objective: The Healthcare workers (HCWs) who work in DOTS/Sputum microscopy centre are exposed to higher risk of contacting tuberculosis (TB) comparatively to other health workers who are serving the other health sectors. The HCWs in DOTS are more exposed due to direct contact with patients suffering from TB or through sharing the infected air space with the infectious patients. The aim of the study is to know the prevalance of TB disease amongst the HCWs who are working in DOTS cum Sputum Microscopy Centre's under RNTCP in two different districts of state of Uttar Pradesh (UP) and Uttarakhand (UK) of india.

Methods: The prospective cross-sectional study is conducted in two districts of different states having high burden of TB disease in UP and low burden of TB disease in UK state. All 100% (130) staff i.e. Medical officers, Sputum microscopy technicians, DOTS providers of DOTS cum Sputum Microscopy centre's of both selected Ghaziabad (UP) and Dehradun (UK) districts are covered in the study.

Results: The 4.6% (6) healthcare workers of both the districts were taking ATT at the time of interview and 13.8% (18) HCWs had taken the ATT in past. The 62.5% (15) HCWs i.e 55.5% (5) from Dehradun district and 66.6% (10) from Ghaziabad district preferred to have a ATT from the private medical store inspite of taking DOTS with assumption of low efficacy of drugs and high toxicity. The 58.33% (14) HCWs ie 55.5% (5) staff members of DOTS/sputum microscopy centre in Dehradun & 60.0% (9) staff members of DOTS/sputum microscopy centre in Ghaziabad district had not notified about the status of their disease to the health care authority due the assumption that they may be asked to leave the job or to go on a long unpaid leave.

Conclusion: The 18.4% (24) HCWs of both the district got TB disease during their working in DOTS/Sputum microscopy centre and 4.6% (6) HCWs of both the districts were taking the ATT at the time of interview.

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by bacteria called *Mycobacterium tuberculosis* that most often affects the human lungs. Transmission of TB and multidrug-resistant TB (MDR-TB) or Extensively drug-resistant TB (XDR-TB) typically is more intense in congregates settings like in health facilities, laboratories, immunocomprised persons, prisons etc.[1]

Quarter of world TB cases are in India and 36% of deaths are occurring in India despite the disease being curable and free treatment (DOTS) is available. Another disturbing figure is that 9% of the cases are MDR - TB. MDR TB is a dreadful state of TB which requires longer treatment and having worse outcome.

The recent TB prevalence survey reveals that Uttar Pradesh is in top three states of the country in prevalence of TB with 481 cases per one lakh population while on another side Uttarakhand is having low prevalence of TB with 275 cases per one lakh population comparatively state of Uttar Pradesh. It is important to prevent occurrence and transmission of TB/MDR TB which are a threat to the community. Healthcare workers (HCWs) working in DOTS/Sputum microscopy centre are at an increased risk of acquiring tuberculosis (TB) compared to the other healthcare workers working in other healthcare settings and general population because of the frequent face to face contact of patients suffering with TB or potential exposure to TB through shared air or space with infectious patient(s).[2,3] However, data on TB among HCW working in DOTS cum sputum microscopy centre is limited. Therefore, this study is aimed to determine the prevalence of TB disease among healthcare workers who are working in DOTS cum sputum microscopy centre of two different states of two districts in India.

2. Methodology

A prospective cross-sectional study is conducted to know the prevalence of TB disease amongst HCWs working in DOTS cum Sputum Microscopy Centre's under Revised National Tuberculosis Control Programme (RNTCP) in two districts of different states having high burden of TB disease in UP and low burden of TB disease in UK state. The reason for the selection of these centre was because specialised TB hospitals admit and treat TB patients on a regular basis whereas tertiary clinics allow presumptive TB patients to be diagnosed and then send confirmed TB patients to either DOTS clinics or TB hospitals. All 18 centres from District Dehradun (UK) and 28 from District of Ghaziabad (UP) was covered under the study. A total of 130 HCWs i.e, 54 healthcare workers in dehradun district and 76 healthcare workers in Ghaziabad district i.e. Medical Officers, DOTS providers and Sputum microscopy technicians working in DOTS cum sputum microscopy centre of both the districts were included in the study. (Table 1)

3. Results

Nine (16.66%) staff members of DOTS/sputum microscopy Centre in Dehradun & 15 (19.73%) staff members of DOTS/sputum microscopy Centre in Ghaziabad district, they have/

Table 1 – Data of healthcare workers, working in DOTS/Sputum Microscopy Centre in two different districts of state of Uttarakhand & Uttar Pradesh of India.

Sr No	Question	Dehradun n 54		Ghaziabad n 76		Total n 130	
		Yes	No	Yes	No	Yes	No
1.	Do you have/had symptoms of TB	9 (16.66%)	45 (83.33%)	15 (19.73%)	61 (80.26%)	24 (18.46%)	106 (81.53%)
2.	Are you suffering from TB at present ?	2 (3.70%)	52 (96.29%)	4 (5.26%)	72 (94.73%)	6 (4.61%)	124 (95.38%)
3.	Do you had a TB in past while working here ?	7 (12.96%)	47 (87.03%)	11 (14.47%)	65 (85.52%)	18 (13.84%)	112 (86.15%)
4.a	Have you taken ATT in past while working here ?	7 (12.96%)	47 (87.03%)	11 (14.47%)	65 (85.52%)	18 (13.84%)	112 (86.15%)
4.b	If yes, then taken ATT under DOTS Centre/Private (Medical Store)	3 (42.85%) (DOTS)		4 (36.36%) (DOTS)		7 (38.88%) (DOTS)	
		4 (57.14%) (Private)		7 (63.63%) (Private)		11 (61.11%) (Private)	
4.c	Are you taking ATT at present ?	2 (3.70%)	52 (96.29%)	4 (5.26%)	72 (94.73%)	6 (4.61%)	124 (95.38%)
4.d	If Yes, then taking ATT under DOTS Centre/Private (Medical Store)	1 (50.00%) (DOTS)		1 (25.00%) (DOTS)		2 (33.33%)	
		1 (50.00%) (Private)		3 (75.00%) (Private)		4 (66.66%)	
6.	Have you notified the status of your TB to health care authority ?	4 (44.44%)	5 (55.55%)	6 (40.00%)	9 (60.00%)	10 (41.66%)	14 (58.33%)

had symptoms of TB recently. Two (3.70%) staff members of DOTS/sputum microscopy centre in Dehradun & four (5.26%) staff members of DOTS/sputum microscopy centre in Ghaziabad district, at the time of interview they are suffering from TB and they are taking ATT. Seven (12.96%) staff members of DOTS/sputum microscopy centre in Dehradun & 11 (14.47%) staff members of DOTS/sputum microscopy centre in Ghaziabad district, they had TB in past and they had taken ATT in past and completed the full course.

Seven (12.96%) staff members of DOTS/sputum microscopy centre in Dehradun were taking ATT in past, three (42.85%) are from DOTS Centre and four (57.14%) are from private medical store. 11 (14.47%) staff members of DOTS/sputum microscopy centre in Ghaziabad district, they were taking ATT in past, four (36.36%) are from DOTS Centre and seven (63.63%) are from private medical store having impression about the low quality and efficacy of the DOTS and hiding their illness considering that their status will be known to their colleagues and others.[4]

Two (2.70%) staff members of DOTS/sputum microscopy centre in Dehradun are taking ATT at present, one (50.00%) is from DOTS Centre and another one (50.00%) is from private medical store. Four (5.26%) staff members of DOTS/sputum microscopy centre in Ghaziabad district, they are taking ATT at present, one (33.33%) is from DOTS Centre and three (66.665) are from private medical store. Four (44.44%) staff members of DOTS/sputum microscopy centre in Dehradun & six (40%) staff members of DOTS/sputum microscopy centre in Ghaziabad district, they notified the status of their TB to health care authority to keep the status of their disease confidential and not disclosing to working colleagues or superiors including others.

4. Discussion

Healthcare workers who are working in DOTS cum Sputum microscopy centre are at risk to get TB infection due to their high exposure and direct contact with the patients.[5] 18.4% (24) HCWs of both the district got TB disease during their working tenure in DOTS/Sputum microscopy centre and 4.6% (6) HCWs were taking the ATT at the time of interview. Baussano et al (2011) discovered in the study that the risk for TB among HCWs is consistently higher than the risk among the general population worldwide.[6] G.de Vries et al (2006) mentioned in their study that 42% HCWs got the TB infection at work.[7] The risk for HCWs of TB attributable to occupational exposure as the conditions and practices are contributing to this risk.[8]

Tuberculosis disease is still a stigma amongst the community as well as amongst the few HCWs. TB is a notifiable disease and hence it should be notified to the nodal public health authority on diagnosis or initiation of ATT of a TB case diagnosed through microbiologically or clinically at least on monthly basis.[9] Majority of HCWs (58.33%) have not notified about the status of their disease to the health care authority and majority of HCWs (63.88%) prefer to have ATT from private medical stores inspite of taking DOTS considering that their status will be known to their colleagues, family and others. A few of the HCWs also have the impression about the low quality and efficacy of the DOTS. Banga RK, Singh J et al (2018) mentioned in their study that the patients on both i.e. daily ATT and DOTS therapies had good response to treatment and they had no relapse during the follow up.[10]

18 (13.84%) staff members of DOTS/sputum microscopy centres of both the districts had TB in past and they had taken ATT in past knowing that disease is preventable and treatable with compliance of treatment and completed the full course of ATT. The overall treatment success rate of TB patients is 90.7% as per *Genet C et al (2019) and* WHO recommends that a good performing tuberculosis program should achieve at least **90%** treatment success rate and 85% cure rate.[8,11,12]

It has been observed that inadequate supply of PPE to the DOTS/Sputum microscopy centre and lack of training and awareness on infection prevention and control practices are also a contributory factor for transmission of disease.[12,13] Preventive health checkup or screening of HCWs is recommended on regular interval by the health authorities/RNTCP and surveillance activities of TB amongst HCWs may be performed by the authorities considering the risk to get infected by TB. It has been also observed that many centres do not have a sufficient and well ventilated waiting area for the patients as they wait in a common and crowded waiting area along with their attendants. DOTS/Sputum microscopy centres do not have a designated sputum collection area as patients were asked to give the sputum sample in waiting area or in washrooms.[14] All these factors contribute to risk of getting infection amongst the HCWs.

5. Conclusion

The 18.4% (24) HCWs of both the district got TB disease during their working in DOTS/Sputum microscopy centre and 4.6%[6] HCWs of both the districts were taking the ATT at the time of interview. A multi-pronged approach is required to eliminate TB from the India by 2025 which is planned 5 years earlier than the global target of 2030.

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Conflicts of interest

The authors have none to declare.

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Original article

Pharmacovigilance monitoring and treatment adherence in patients on anti-tubercular drugs

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ABSTRACT

Background: Tuberculosis (TB) is a chronic infectious disease that involves 6–9 months regimen with different combinations of drugs. Poor adherence can lead to prolonged treatment, higher cost, spurt in new cases and the development of resistance. Directed Observed Therapy (DOTS) are recommended to reinforce adherence for the treatment of TB. Thorough literature reviews suggest limited studies on pharmacovigilance monitoring and treatment adherence in tuberculosis.

Methodology: This prospective study was conducted on patients suffering from tuberculosis after obtaining written informed consent. The patients underwent a thorough history taking and clinical examination, the patients included in the study were followed up for a period of 4 weeks every 2 weeks. On each visit the patients were assessed for any adverse effects observed which was reported spontaneously by the patients. The patients were also administered Morris Medication Adherence Scale (MMAS-8) during each visit at 2 weeks and 4 weeks.

Result: A total of 59 patients completed the study with mean age of 46 ± 18 years with of a total of 39 males and 20 females completed the study duration. The Mean Morisky's Medication Adherence Scale (MMAS-8) score at baseline was 4.09 ± 1.33 (Mean ± SD). The mean MMAS-8 score at baseline significantly ($p < 0.05$) improved to at the end of week 2 and further the scores were improved at the end of 4 week. Although there was no difference between the males and female neither at 2 weeks nor at 4 weeks. A total of 67 adverse events were reported by patients, out of whom 42 adverse events was reported at the end of 2 weeks and another 25 adverse events were reported at the end of 4 weeks.

Conclusion: The results of our study showed that with each follow-up the adherence to therapy of patients increased giving more chance of completion of therapy.

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1. Introduction

Tuberculosis (TB) - a chronic inflammatory disease that is transmitted from patients with active pulmonary tuberculosis with the mode of transmission being through droplets from patient, its recommended treatment involves combination of drugs for a duration of 6–9 months. Long term use of anti-tubercular drugs is associated with various adverse effects and also leads to lack of adherence (non-adherence) by the patients.[1] Medication adherence can be described as degree to which a patient correctly follows medical advice, medical device use, self-care, or therapy sessions. One of the challenges involved during the drug therapy of patients with tuberculosis is lower levels of adherence.[2] Poor adherence can lead to prolonged treatment, higher cost, spurt in newly diagnosed cases and treatment resistance. This results in making treatment more complex and expensive.[3]

For the second time in a row, India stands at first place in the world for deaths from TB: 423,000patients died in year 2016.[3] One of the biggest health problem faced by India is the disproportionately large burden of the world's tuberculosis rates with India being the country with the highest burden of Tuberculosis as per estimates from World Health Organization (WHO) statistics 2016.[4] It is estimated that 40% of population of India is infected with TB bacteria, majority of them have latent TB rather than TB disease.[5] Most of the TB statistics are collected by the government Revised National Tuberculosis Control Program (RNTCP). TB is country's biggest health issue and more worrying is pattern of resistance to the available anti-tubercular drugs. The issue of drug resistance began with multi-drug resistant tuberculosis (MDR-TB) and further more increased pattern of extensively drug resistant tuberculosis (XDR-TB). Gradually the most dangerous form has situated itself in India as Totally drug resistant tuberculosis (TDR-TB).[6] To improve the compliance and adherence of the treatment of tuberculosis, Directed Observed Therapy-Short Term (DOTS) has been in place for the treatment of TB. Patients with TB are expected to have a high adherence level which should be greater than 90% in order to get cured.[7] Thorough literature reviews suggest limited studies on pharmacovigilance monitoring and treatment adherence in tuberculosis.

Aim of the study was Pharmacovigilance Monitoring and treatment adherence of patients suffering from tuberculosis and on Antitubercular Drugs and also to assess factors leading to poor adherence of antitubercular therapy.

2. Materials & methods

This prospective study will be conducted on patients suffering from tuberculosis visiting the Department of Pulmonary Medicine at tertiary care hospital for a period of three months between November 2020 to February 2021. All patients suffering from tuberculosis and on anti-tubercular therapy will be enrolled in the study after approval from the institutional ethics committee. Only those patients will be enrolled in the study that fulfill the selection criteria and give written informed consent. Patients of both sexes above the age of 18 years diagnosed with tuberculosis and on anti-tubercular therapy were included to be a part of this study. Patients who refused to give written informed consent and those females who were pregnant, or lactating were excluded from the study.

2.1. Procedure

All patients visiting pulmonary medicine department and suffering from tuberculosis and on anti-tubercular therapy were included in the study after they gave a written informed consent. The patients underwent thorough clinical examination, and a detailed history was taken, the patients included in the study were followed up for a period of 4 weeks every 2 weeks.

On each visit the patients were assessed for any adverse effects observed which was reported spontaneously by the patients. The reported adverse effects were sent in the form of Pharmacovigilance Program of India and reported to Adverse Drugs reaction Monitoring Center (AMC). The patients were also administered A Morris Medication Adherence Scale (MMAS-8) during each visit at 2 weeks and 4 weeks.

2.2. Outcome

- Spontaneously reported adverse drug reaction sent of AMC
- Adherence assessed by MMAS-8.

2.3. Morisky Medication Adherence Scale (MMAS-8)

The MMAS-8 scale has a total of eight questions with the first seven question has the response of ‘‘yes’’ or ‘‘no’’ and the eighth question is answered on 5-point Likert scale question. The participant is rated with the following scores, a score of complete/full adherence is 8, and lower scores indicate a poorer level of adherence with a lower boundary of zero. For our study the participants were described as adherent if their score was $\geq$6 and non-adherent if they had an MMAS-8 score < 6.[8]

2.4. Statistical analysis

The data was presented as mean ± standard deviation (mean ± SD). The scores obtained from the scales were compared using appropriate non-parametric tests (Chi-Square, Mann Whitney U, Wilcoxon Sign Rank test) and parametric (Student't' test, ANOVA) tests wherever applicable. A $p < 0.05$ was considered statistically significant.

3. Observation and results

This prospective study was conducted on patients suffering from tuberculosis and visiting the Department of Pulmonary Medicine for a duration of three months. All patients suffering from tuberculosis and giving written informed consent were enrolled in the study. A convenient sample of 200 patients was decided to be enrolled in the study but with the outbreak of the Covid-19 Pandemic and our medical college & hospital, so all the services were interrupted. We completed this study

with 59 patients completing the study follow-up for a period of one month, and these participants were included in the result analysis.

4. Demographic characteristics

A total of 59 patients completed the study and there demographic characteristic are presented in Table 1. The mean age of patients was 46 ± 18 years with of a total of 39 males and 20 females completed the study duration. All these patients had a predominant symptom of cough as the main presenting symptom. Out of all the patients 20 patients had a previous history of one month of antitubercular treatment (ATT). The Mean Morisky's Medication Adherence Scale (MMAS-8) score at baseline was 4.09 ± 1.33 (Mean ± SD). The baseline demographic characteristics are presented in table below (Table 1).

5. Baseline demographic characteristic based on sex distribution of patients

The patients were divided into males (n = 39) and females (n = 20), the baseline demographic characteristics are shown in Table 2. The mean age of male participants was 48 ± 18 years whereas, that of female participants was 42 ± 16 years. The number of participants who had earlier taken one month ATT was 12 males and 8 females. Mean MMAS-8 score in males was 4.0 ± 1.49 while that of females was 4.25 ± 0.97. There was no statistical difference ($p > 0.05$) at baseline in the scores of males and females.

6. Morisky's Medication Adherence Scale – 8 (MMAS-8 scores)

The mean MMAS-8 score at baseline was 4.09 ± 1.33 which significantly ($p < 0.05$) improved to 6.32 ± 0.84 at the end of week 2 and further the scores were improved to 6.78 ± 0.77 at the end of 4 week. A statistically significant ($p < 0.05$) improvement in adherence from baseline at week 2 and week 4 was observed as depicted in Fig. 1. The scores at week 2 and 4 were statistically ($p < 0.05$) significantly higher as compared to baseline.

The mean MMAS-8 score at baseline in males and females was 4.0 ± 1.49 and 4.25 ± 0.97 respectively which significantly ($p < 0.05$) improved to 6.20 ± 0.90 in males and 6.50 ± 0.69 in females at the end of week 2 and further the scores were improved to 6.70 ± 0.83 in males and 6.95 ± 0.61in females at the end of 4 weeks. There was a significant improvement in adherence from baseline at week 2 and week 4 as represented in Fig. 2 which was statistically ($p < 0.05$) higher. There was significant difference in the scores at week 2 and 4 statistically ($p < 0.05$) higher. Although there was no difference ($p > 0.05$) between the males and female neither at 2 weeks nor at 4 weeks.

Table 1 – Baseline demographic characteristic of patients.

Parameters	Patients (n = 59)
Age in Years (Mean ± SD)	46 ± 18
Sex Distribution (Male: Female)	39:20
Previous History of One Month ATT (Yes: No)	20:39
MMAS – 8 (0 Week) (Mean ± SD)	4.09 ± 1.33

Table 2 – Baseline demographic characteristic based on sex.

Parameters	Male (n = 39)	Females (n = 20)
Age (Years) (Mean ± SD)	48 ± 18	42 ± 16*
Previous History of One Month ATT (Yes: No)	12:28	8:11[#]
MMAS – 8 (0 Week) (Mean ± SD)	4.0 ± 1.49	4.25 ± 0.97*

*$p > 0.05$ using student 't'test: Both groups comparable at baseline.
[#]$p > 0.05$ using Chi Square Test: Both groups comparable at baseline.

7. Adverse effects noted in patients on antitubercular treatment

All the 59 patients completed the criteria of one month follow up for this study and the drugs were well tolerated by all the patients. None of the patients withdrew from the study due to adverse effects. There were no serious adverse events reported in any of the patients enrolled in the study. Some of the adverse effects reported by the patients were nausea, vomiting, metallic taste, flu like symptoms and abdominal pain. The adverse effects were reported at week 2 and week 4 of the study. A total of 67 adverse events were reported by patients, out of whom 42 adverse events was reported at the end of 2 weeks and another 25 adverse events were reported at the end of 4 weeks (Table 3). The most frequent adverse events reported by patients was nausea which was reported by 25 patients followed by abdominal pain in 8 patients, vomiting in 4 patients, flu-like symptoms in 4 patients and one patient reported of metallic taste at the end of 2 weeks. The number of adverse events reported by patients reduced at the end of 4 weeks as some tolerance developed to these adverse events. The number of adverse events reported at 4 weeks was nausea in 17 patients, followed by metallic taste in 7 patients and one patient came with complaint of flu-like symptoms. There was no statistically significant difference in reporting pattern of the patients.

8. Factors affecting the compliance of patients

One of the objectives of our study was to assess the factors affecting the compliance of patients for adherence to treatment. The patients gave a record of the factors that were responsible for poor adherences which included the distance travelled by patients to visit the centre, the cost of medication, duration of therapy, timing of therapy, lack of caregivers, lack of family support, lack of social support, stigma associated with the disease and non-availability of medicine as the reasons for non-compliance. The responses of patients are shown in Table 4 and distribution of the factors across both sexes is shown in Fig. 3. There was no significant difference among these factors in both sexes.

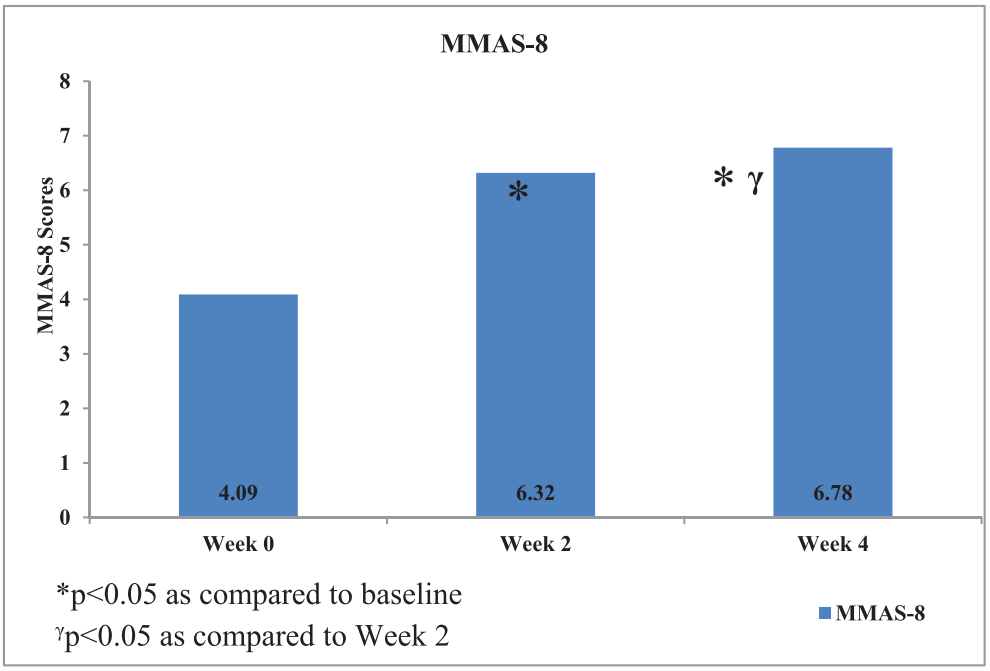

Fig. 1 – MMAS-8 score.

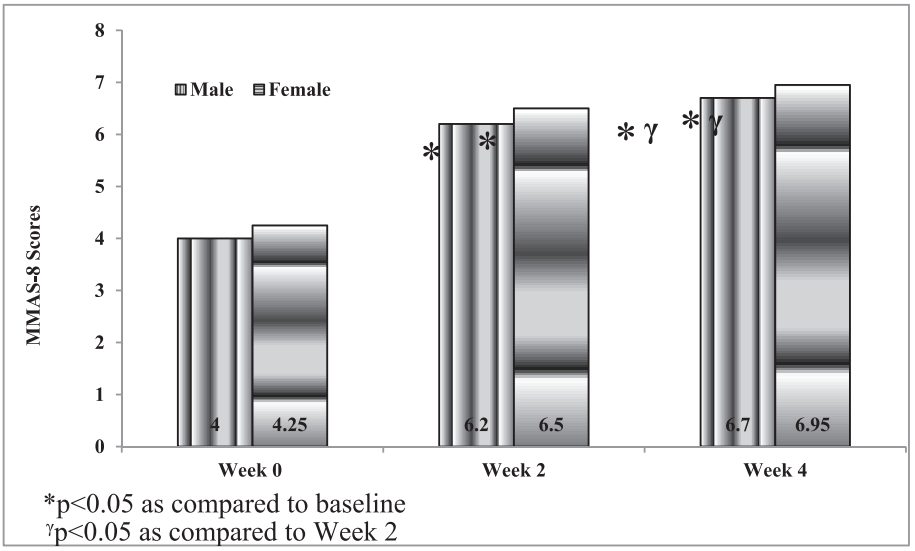

Fig. 2 – MMAS-8 scores in males and females.

Table 3 – Frequency of Adverse events (n) reported by patients at week 2 and 4.

Adverse Events	Week 2	Week 4
Nausea	25	17
Abdominal Pain	8	0
Vomiting	4	0
Metallic Taste	1	7
Flu-like Symptoms	4	1
Total Adverse events reported	42’	25

9. Discussion

As per the estimates of WHO, 95% of the world TB cases are mostly from developing countries creating an economic burden in the development with added challenge of reduced effectiveness of the antibiotics. Abrupt withdrawal and discontinuation may help in development of resistance and decreased response.[9] Withdrawal or resistance leads to multidrug resistant TB (MDR-TB) which takes longer to treat

Table 4 – Factors responded by patients (n) as reason for poor compliance.

Factors	Number of Responses
Distance travelled by patients	43
Cost of Medicine	46
Duration of Therapy	43
Timing of Therapy	40
Lack of caregivers	16
Lack of family support	40
Lack of Social support	36
Stigma associated with disease	42
Non-availability of medicine	38

with second-line drugs, is costly and associated with adverse effects and greater mortality. This is associated with higher cost to the patient and community, higher pill burden, low compliance rates and patient inconvenience.[10] We conducted this study to find out the burden of adverse effects following drug therapy and assess factors for adherence to therapy of anti-tubercular drugs. Our study demonstrated that with each follow-up the adherence to therapy of patients increased giving more chance of completion of therapy. The adverse events were encountered with treatment to which the patients developed tolerance with due course of time, the most frequent adverse event reported by patients was nausea which was tolerated. All patients followed the duration of therapy till the time they were enrolled in the study.

A study done at to assess the rate of prevalence of ADR during intensive phase of treatment in Tirunelveli demonstrated that the most common adverse effect associated with anti-tubercular drugs was nausea followed by hepatitis is similar to the result observed in our study were the most common adverse effect noted was nausea. Our study differs from this study as we also took into account the adherence to the medication.[9]

Another study done in Himachal Pradesh to find out adverse drug reactions (ADRs) in patients on anti-tubercular treatment (ATT) demonstrated that the most common ADRs in patients were related to CNS, followed by Gastrointestinal system; while patients on category I treatment had ADRs involving Gastrointestinal system followed by CNS. The results of our study are similar as this study also showed that gastrointestinal system was most commonly involved. Though, our study also differs from this study as this study also included MDR –TB patients.[10]

One more study done in Amritsar to determine incidence of ADR's in TB patients, on DOTS category I showed that the most common ADR was GI upset followed by hepatitis is similar to the result observed in our study were the most common adverse effect noted was nausea. Our study differs from this study as we also took into account the adherence to the medication.[11]

One study for demonstrating interest of integration of pharmacovigilance in Moroccan Tuberculosis Control Program showed that pharmacovigilance integration not only improved the management of ADRs but helped in detection of new signals of antituberculosis drugs is quite similar with our study as we also demonstrated that with due course of time and adherence follow up improved the overall treatment adherence of patient of anti-tubercular therapy.[12]

Another study done in Kosovo to investigate the anti-tubercular treatment adherence rate showed that age and place of residence effected treatment adherence, along with this other parameters like daily dosage of drugs, adverse effects and knowledge of the treatment prognosis, along with duration of treatment also has a role. The results of our study showed that poor adherences included the distance travelled by patients to visit the centre, the cost of medication, duration of therapy, timing of therapy, lack of caregivers, lack of family support, lack of social support, stigma associated with the

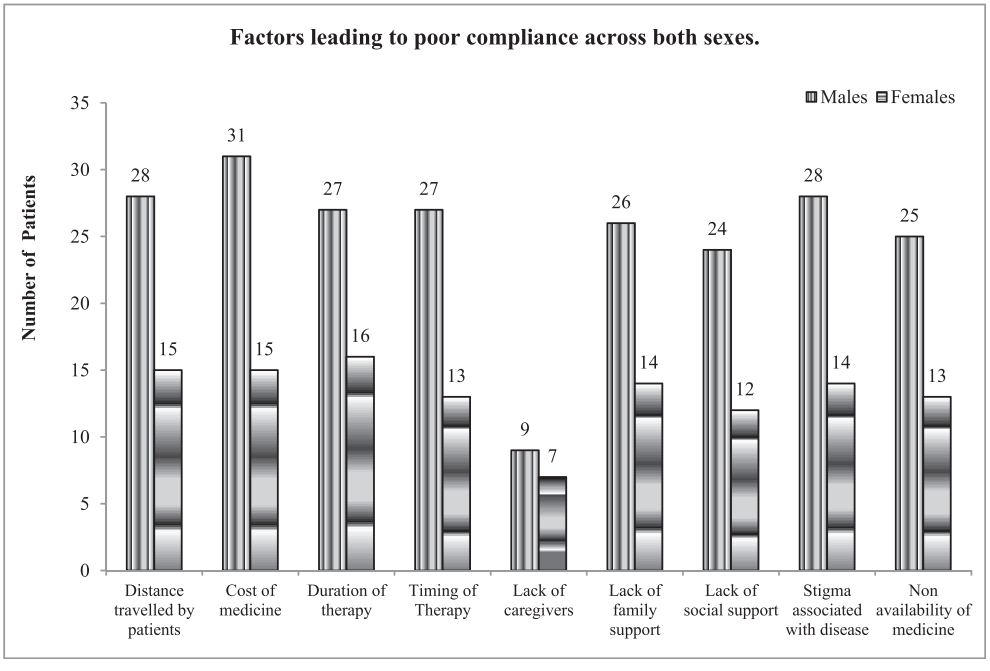

Fig. 3 – Distribution of factors across both sexes.

disease and non-availability of medicine as the reasons for non-compliance, which is quite similar. Our study also monitored the ADRs encountered by the patients.[13]

One more study for the spectrum of Adverse Drug Reaction with the intake of anti-tubercular drugs along with the assessment of severity, causality, and predisposing factors in Mandya showed that gastrointestinal (GIT) adverse effects and hepatotoxicity were the most frequently observed Adverse Drug Reaction and counseling of patients regarding their lifestyle along with early detection and management will minimize the occurrence of Adverse Drug Reaction and improve the treatment adherence. The results are quite similar to our study which showed improved adherence to treatment and tolerance to ADRs in the follow-up.[14]

A meta-analysis done to assess the effectiveness of medication adherence enhancing interventions in TB patients demonstrated that non-adherence factors are patient specific, and hence personalized interventions are required to enhance the impact of a programme to improve medication adherence in TB patients is similar to our study which showed that poor adherences included the distance travelled by patients to visit the centre, the cost of medication, duration of therapy, timing of therapy, lack of caregivers, lack of family support, lack of social support, stigma associated with the disease and non-availability of medicine as the reasons for non-compliance, which is quite similar. Our study also monitored the ADRs encountered by the patients.[15]

There are certain limitations in our study, firstly, the follow-up of patients should have been for the complete duration of treatment of tuberculosis, but this was not possible as the study was for short duration and had to be completed in three months. Secondly, the number of patients in the study had to be reduced due to the current ongoing pandemic and conversion of our tertiary care hospital into a dedicated Covid Center. Thirdly, our study showed that awareness of therapy could improve the adherence to treatment, but we did not give any active counseling for the need of completing the therapy, so this could further change the results if counseling along with therapy is given to the patients for the need to complete the therapy.

To conclude the results of our study showed that with each follow-up the adherence to therapy of patients increased giving more chance of completion of therapy. The adverse events are encountered with treatment to which the patients develop tolerance with due course of time, the most frequent adverse event reported by patients was nausea which was tolerated. All patients followed the duration of therapy till the time they were enrolled in the study. For complete course of therapy for tuberculosis, there is need for regular follow up. One of the major hindrance was the cost of medicine, which can be taken care by guiding the patients for DOTS centre, so that they complete the therapy.

Source of support

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Ethical considerations

Present study was approved by Institutional Ethics Committee, Teerthanker Mahaveer University, Moradabad.

Conflicts of interest

The authors have none to declare.

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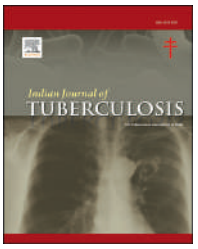

Original article

Programmed cell death ligand 1 (PD-L1) expression in non-small cell lung cancer: Findings from a tertiary care institute in western part of India

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ABSTRACT

Background: Immune checkpoint inhibitors targeting either programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1) have been established as a novel target for immunotherapy in non-small cell lung cancer (NSCLC). Prevalence of PD-L1 expression in NSCLC varies from 13% to 70%, with sparse data from the Indian subcontinent. In this study, we looked at PD-L1 expression and its association with demographic, clinical, radiologic and pathologic parameters in NSCLC patients.

Methods: This was an observational study carried over a period of 18 months in which 65 patients of NSCLC were included. Immunohistochemistry (IHC) for PD-L1 was done using an automated IHC stainer and testing was performed using PD-L1 IHC CAL10. For statistical analysis, unpaired t test, Chi square test, Fisher's exact test and binomial logistic regression were used. $P < 0.05$ was taken to be statistically significant.

Results: Mean age of the patients was 62.9 ± 9.2 years, and majority (87.3%) of them were males. Seventeen (26.2%) patients expressed PD-L1, among whom 10 had high PD-L1 expression (≥50%) and 7 had low PD-L1 expression (1–49%). PD-L1 expression was seen in 13 out of 43 cases of squamous cell carcinoma (SCC) and 4 out of 15 cases of

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adenocarcinoma. On applying binomial logistic regression analysis, association between smoking and PD-L1 expression was found to be insignificant.

Conclusion: Almost a quarter of NSCLC cases were PD-L1 positive without any difference in expression between SCC and adenocarcinoma. PD-L1 status was not associated with any specific demographic, clinical or radiologic parameter including the histologic subtype.

1. Introduction

Platinum-based systemic chemotherapy has been the mainstay of treatment for advanced stage lung cancer for the last forty years, providing a median survival of less than 12 months.[1] Among NSCLC patients, 40% have oncogenic mutations as therapeutic targets. Specific targeted drugs against identified driver mutations like EGFR, ALK and ROS-1 have shown better clinical outcomes compared to chemotherapy.[2] In the recent years, blockade of programmed death receptor 1 (PD-1) and its ligand, programmed cell death ligand 1(PD-L1) has been established as a novel target for NSCLC immunotherapy. PD-1 and PD-L1 are immune checkpoints. PD-L1 is expressed by tumor cells while PD-1 is expressed by activated T cells. Increased expression of these molecules help in escape of tumor cells from T cell-mediated immunity, leading to progression and tumor spread.[3] Formulation of monoclonal antibodies that act against these molecules form the basis of immunotherapy in NSCLC patients. As per the National Comprehensive Cancer Network (NCCN) guidelines, in metastatic NSCLC patients with negative driver mutation status and PD-L1 expression ≥50%, anti PD-1 monoclonal antibody, namely pembrolizumab, can be used as first line single agent therapy.[4] Prevalence of PD-L1 expression in patients vary from 13% to 70%, with sparse data from the Indian subcontinent.[5] A recent meta-analysis has shown differential rates of PD-L1 expression in patients according to varying clinical characteristics, tumour and histological gradation.[6] In this study, we performed immunohistochemistry (IHC) investigation for PD-L1 expression in 65 patients of NSCLC and looked for its association with demographic, clinical, radiologic and pathologic parameters.

2. Methods

This cross-sectional study was carried out in a tertiary care teaching institute in western India over a period of 18 months. We included adult patients diagnosed with non-small cell lung cancer who had never received any prior lung cancer therapy. Patients whose tissue samples were inadequate for IHC were excluded from the study. We screened 110 patients suspected with lung cancer. After considering inclusion and exclusion criteria, 65 NSCLC patients were finally enrolled in the study. The flow chart is given in Fig. 1.

Detailed clinical history was taken and physical examination done in all enrolled patients. Baseline blood and radiologic investigations were performed as mentioned in Table 1. Method of tissue sampling was done as per the decision of treating physician. This included singly or combinations of endobronchial biopsy (EBB), transbronchial lung biopsy (TBLB), brush cytology, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), ultrasound (US) guided biopsy, computed tomography (CT) guided biopsy and peripheral lymph node biopsy. After biopsy results, staging was done according to the NCCN guidelines for NSCLC.[4]

Biopsy samples were fixed in 10% formalin and the H&E stained sections were examined for identification of tumor. The tumor was subtyped into small cell or non-small cell carcinoma, and IHC for further subtyping was put up, if required. IHC for PD-L1 was done in cases of non-small cell carcinoma using the automated IHC stainer (Leica bondmax). PD-L1 IHC testing was performed using PD-L1 IHC CAL10 antibody (concentrated & prediluted rabbit monoclonal antibody from Biocare Medical). This IHC was scored by two pathologists, based on the intensity of staining and the percentage of cells showing positivity (tumor proportion score, TPS) as follows: Intensity score- 0 (negative expression), 1+ (weak positivity), 2+ (moderate positivity), 3+ (strong positivity), and TPS (Tumor proportion score) - <1% (Negative), 1–49% (Low) and ≥50% (High). All cases with PD-L1 positivity (intensity 1+ or more) in ≥1% of the cells were considered positive.

2.1. Statistical tests

Data was entered in Microsoft excel and analyzed by SPSS V.23 software. Continuous variables were summarized as mean and standard deviation. Nominal/categorical variables were summarized as proportions (%). For continuous variables, unpaired t test was used. For categorical/nominal data, Chi square and Fishers exact test were used. For multivariate analysis, binomial logistic regression was applied. P value of less than 0.05 was taken to be significant.

3. Results

The demographic and clinical characteristics of study population is depicted in Table 1. Univariate analysis between PD-L1 expression and these parameters are shown in Table 2. Relationship between the parameters and high or low expression of PD-L1 is depicted in Table 3.

PD-L1 expression was seen in 17 (26.15%) patients. Ten (15.4%) patients had high PD-L1 (TPS ≥50%) and 7 (10.8%) had low PD-L1 (TPS <50%) expression. Among 57 male patients, 13 (22.8%) showed PD-L1 expression, with 6 (10.5%) showing high PD-L1 expression. Out of 8 females, 4 (50%) cases showed PD-L1 expression who all had high PD-L1 expression. Forty-three

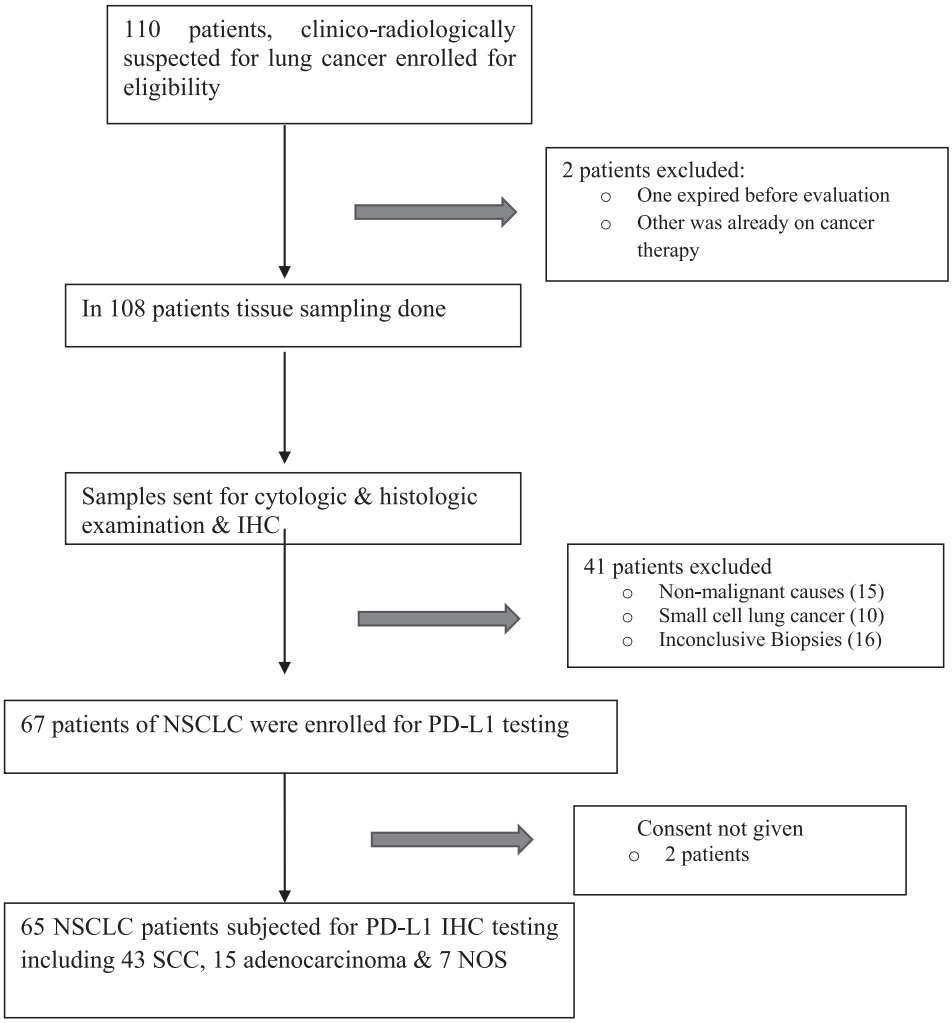

Fig. 1 – Study flow chart.

(66.2%) patients had a histologic diagnosis of SCC, 15 (23.1%) had adenocarcinoma and 7 (10.8%) were not otherwise specified (NOS) NSCLC. Among 43 cases of SCC, 13 cases showed positive PD-L1 expression with 7 of them showing high PD-L1. Out of 15 cases of adenocarcinoma, 4 cases showed PD-L1 expression, with 3 showing high PD-L1. Centrally located primary lung tumour was present in 37 (56.9%), out of which 8 (21.6%) patients had PD-L1 expression. In the remaining 28 (43.1%) patients with peripherally located tumour, 9 (32.1%) expressed PD-L1. With regards to TNM staging, out of 43 SCC patients, 25 (58.14%) were in TNM stage IV, 17 (39.53%) were in stage III and 1 (2.33%) was in stage II. In these patients, PD-L1 positivity was seen in 9 patients in stage IV and 4 patients who were in stage III. In 15 adenocarcinoma patients, 7 (46.67%) were in TNM stage IV, 7 (46.67%) in stage III and 1 (6.67%) in stage II. Among these, 3 (42.9%) in stage IV, 1 (14.3%) in stage III were positive for PD-L1.

On univariate analysis, the smoking index (SI) of patients showing PD-L1 positivity was significantly lower than that of patients who were PD-L1 negative ($p = 0.02$). On applying binomial regression analysis with PD-L1 status as outcome variable and age, gender and smoking index as dependent variables, it was observed that smoking did not show any significant association with PD-L1 status as age and gender were found to be the confounders. This difference also disappeared when comparison was done between patients with high and low PD-L1 expression ($p = 0.38$). Similarly, smoking status did not show any association with PD-L1 expression in our patients.

Four out of the eight female patients (50%) in our study were PD-L1 positive and all of them expressed PD-L1$\geq$ 50%. They all had a histopathologic diagnosis of squamous cell carcinoma and were never smokers. On further analysis, it was observed that out of the 37 patients with stage IV, 10 (27%) were in stage IVB while the rest 27 (72.9%) were stage IVA. PD-L1 expression was seen in 4 (40%) patients in stage IVB and 8 (29.6%) in stage IVA.

4. Discussion

Our study looked for the expression of PD-L1 in NSCLC patients and compared this with patients' clinical characteristics, radiologic findings and histologic subtypes.

Table 1 – Demographic and clinical characteristics of the study participants.

Parameters	Value
Age (in years), mean ± SD	62.9 ± 9.2
Gender, n (%)	
Male	57 (87.3%)
Female	8 (12.7%)
Smokers, n (%)	49 (75.4%)
Mode of diagnosis, n (%)	
Endobronchial biopsy	22 (33.8%)
CT guided biopsy	18 (27.7%)
USG guided biopsy	12 (18.5%)
EBUS TBNA	9 (13.6%)
Pleural biopsy	3 (4.8%)
Peripheral lymph node biopsy	1 (1.5%)
Lung cancer TNM stage, n (%)	
I	0
II	2 (3%)
III	26 (40%)
IV	37 (57%)
Histopathological diagnosis, n (%)	
Squamous cell carcinoma	43 (66.2%)
Adenocarcinoma	15 (23.1%)
Not otherwise specified (NOS)	7 (10.8%)
Clubbing, n (%)	14 (21.5%)
Cavitation, n (%)	6 (9.2%)
Location of primary tumour	
Central, n (%)	37 (57%)
Peripheral, n (%)	28 (43%)
Sites of metastasis in stage IV patients, n (%)	
Liver	10 (15,4%)
Adrenal	10 (15.4%)
Pleura	9 (13.8%)
Brain	8 (12.3%)
Bone	5 (7.7%)
Contralateral lung	5 (7.7%)
Retroperitoneal lymph nodes	1 (1.5%)
Number of sites of metastasis	
None, n (%)	28 (43.1%)
Single, n (%)	27 (41.5%)
Two, n (%)	7 (10.8%)
Three, n (%)	3 (4.6%)

Seventeen (26.2%) patients expressed PD-L1 in the primary tumour or metastatic sites in our study. PD-L1 prevalence of 27% and 33.66% was found in two studies from India.[7,8] Analysis of PD-L1 distribution according to histological subtypes in the study population showed that among 43 cases of SCC, 13 (30.3%) cases showed PD-L1 expression and out of 15 cases of adenocarcinoma, 4 (26.7%) cases showed PD-L1 expression. All cases of NOS lung cancer were negative for PD-L1 expression. We did not find any statistically significant difference in PD-L1 expression between different histologic subtypes. There were similar observations by other investigators who did not find any difference in PD-L1 expression between SCC & adenocarcinoma.[7–9]

Few studies have found that smoking is associated with increased PD-L1 expression.[10,11]

This is likely due to the higher mutational burden associated with smoking.[12,13] On univariate analysis, we found an inverse association between smoking and PD-L1 expression. However, this association was not found to be statistically significant on binomial logistic regression. Age and gender acted as confounders, and all female patients who expressed PD-L1, were non-smokers. Smoking status is an important clinical parameter which affects the efficacy of antiPD-1-/PD-L1 therapy. In a meta-analysis consisting of 24 randomized control trials (RCTs), immunotherapy was shown to significantly prolong the overall survival compared to chemotherapy in smokers but not in never-smokers.[14] However, ambiguity still persists regarding the efficacy of immunotherapy in patients actively smoking during therapy. The landmark KEYNOTE-024 trial showed that pembrolizumab, a humanized monoclonal antibody against PD-1, was more effective among former smokers than among current smokers.[15] The hazard ratios for disease progression or death for current and former smokers were 0.68 (95%, 0.36–1.31) and 0.47 (95% CI 0.33 to 0.67) respectively. Increased clearance of this drug in smokers is one of the suggested reasons for this observation.[16] PD-L1 expression has a correlation with tumor stage and size of the primary lung tumour.[6] However, this was not observed in the results of our study.

In 9 (13.6%) of our patients, EBUS TBNA was the mode of diagnosis and IHC was done on the cell block made from the needle aspirate. The sample quality of cell block is often inferior to that of CT or USG guided true cut biopsy.[17] The yield of IHC for PD-L1 is also found to be dependent on the sample quality, wherein small sample size is associated with the possibility of non-representative tissue.[18] IHC results have shown to vary even in large specimens showing intratumour heterogeneity. Studies have shown abnormally low expression of PD-L1 in cells with lepidic pattern in adenocarcinoma, whereas sarcomatoid cells in adenocarcinoma express relatively high levels.[19] In another study that compared PD-L1 expression rates in diagnostic biopsy samples and resected tissue samples in patients with NSCLC, there was as high as 15% discordance in the IHC reports.[20] For all practical purposes however, sample adequacy for PD-L1 is defined as at least 100 viable tumour cells per sample, irrespective of the sample type.[21] Site of tissue sampling is also an important predictor of PD-L1 expression. Thirteen (20%) of our patients were diagnosed from sites of metastasis either by TBNA, pleural or lymph node biopsy, out of whom PD-L1 expression was seen in 3 (23%) patients. Rest of the patients were diagnosed from biopsies of primary lung tumour site in whom PD-L1 expression in was found in 26.9% cases. However, we did not look at differences in PD-L1 expression between primary and metastatic sites in the same patient. In a study on NSCLC patients who had not received neoadjuvant therapy, PD-L1 expression was found to be discordant between lymph nodes and the primary tumour in 22% of patients, even though both of them were resected at the same time. Ten percent of patients had IHC-immunoreactivity in the primary tumour with a negative lymph node while 12% patients showed PD-L1 immunoreactivity in the LN metastasis with negative IHC reactivity in the primary tumour.[22]

In our study, the PD-L1 IHC was scored by two pathologists to optimize the overall percentage agreement (OPA). Although, technical steps of handling commercially available PD-L1 IHC kits are well standardized, inter operator variation still occurs. Variation reaches up to 20% in tumour cells with expression of 1%, while it decreases to about 5% in tumour cells with 50% PD-L1 expression.[23]

Table 2 – Univariate analysis between parameters and presence of PD-L1 expression.

Parameter	PDL1 positive (n = 17)	PDL1 negative (n = 48)	P value	Test used
Age (in years) mean ± SD	63.3 ± 11.1	62.0 ± 8.5	0.28	Student t test
Gender				
Female, n (%)	4 (50%)	4 (50%)	0.101	Chi square test
Male, n (%)	13 (22.8%)	44 (77.2%)		
Smoking status				
Current/former smokers, n (%)	10 (20%)	40 (80%)	0.04	Chi square test
Never smokers, n (%)	7 (46.6%)	8 (53.3%)		
Smoking Index (SI) mean ± SD	370.6 ± 331.2	637.5 ± 425.6	0.022	Student t test
TNM staging				
II + III, n (%)	5 (17.9%)	23 (82.1%)	0.19	Chi square test
IV, n (%)	12 (32.4%)	25 (67.6%)		
Histological diagnosis				
Squamous cell, n (%)	13 (43.3%)	30 (56.7%)	0.79[a]	Chi square test
Adenocarcinoma, n (%)	4 (26.7%)	11 (73.3%)		
NOS, n (%)	0	7 (100%)		
Location of primary tumour				
Central, n (%)	8 (21.6%)	29 (78.4%)	0.34	Chi square test
Peripheral, n (%)	9 (32.1%)	19 (67.9%)		
Cavitation				
Present, n (%)	3 (50%)	3 (50%)	0.16	Fishers exact test
Absent, n (%)	14 (23.7%)	45 (76.3%)		
Clubbing				
Present, n (%)	4 (28.6%)	10 (71.4%)	0.81	Chi square test
Absent, n (%)	13 (25.5%)	38 (74.5%)		
Number of sites of metastasis				
None, n (%)	5 (17.9%)	23 (82.1%)	0.55[b]	Chi square test
One, n (%)	8 (29.6%)	19 (70.4%)		
Two, n (%)	2 (28.6%)	5 (71.4%)		
More than 2, n (%)	2 (66.7%)	1 (33.3%)		

[a] Chi square test has been calculated between Squamous cell and Adenocarcinoma.
[b] Chi square test has been calculated after removing non-metastasis and by clubbing two and more than 2 sites of metastasis.

Table 3 – Univariate analysis between parameters and presence of high or low PD-L1 expression.

Parameter	PDL1≥ 50% (n = 10)	PDL1 1–49% (n = 7)	P value	Test used
Age (in years) mean ± SD	65.2 ± 11.1	61.1 ± 11.6	0.24	Student t test
Gender				
Female, n (%)	4 (100%)	0	0.10	Fishers exact test
Male, n (%)	6 (46.2%)	7 (53.8%)		
Smoking status				
Current/former smokers, n (%)	5 (50%)	5 (50%)	0.6	Fishers exact test
Never smokers, n (%)	5 (70.1%)	2 (29.9%)		
Smoking Index (SI) mean ± SD	310.0 ± 341.4	457.1 ± 320.0	0.384	Student t test
TNM staging				
II + III, n (%)	3 (60%)	2 (40%)	1.00	Fishers exact test
IV, n (%)	7 (58%)	5 (42%)		
Histopathological diagnosis				
Squamous cell, n (%)	7 (53.8%)	6 (46.2%)	0.60	Fishers exact test
Adenocarcinoma, n (%)	3 (75%)	1 (25%)		
Location of primary tumour				
Central, n (%)	4 (50%)	4 (50%)	0.63	Fishers exact test
Peripheral, n (%)	6 (66.6%)	3 (33.3%)		
Cavitation				
Present, n (%)	8 (57.1%)	6 (42.9%)	1.00	Fishers exact test
Absent, n (%)	2 (66.6%)	1 (33.3%)		
Clubbing				
Present, n (%)	1 (25%)	3 (75%)	0.26	Fishers exact test
Absent, n (%)	9 (69.2%)	4 (30.8%)		
Number of sites of metastasis				
None, n (%)	3 (60%)	2 (40%)	1.00[a]	Fishers exact test
One, n (%)	5 (62.5%)	3 (37.5%)		
Two, n (%)	1 (50%)	1 (50%)		
More than 2, n (%)	1 (50%)	1 (50%)		

[a] Fishers exact test has been calculated after removing non-metastasis and by clubbing two and more than 2 sites of metastasis.

4.1. *Strengths and limitations*

Ours is one of the initial studies from India which looks at PD-L1 expression and its association with demographic, clinical, radiologic and pathologic parameters in NSCLC patients. Subjectivity in PD-L1 IHC reporting was minimized by getting the analysis done by two pathologists. Limitations of our study are worth mentioning. There was under representation of female patients. We are unable to generalize our findings to all stages of NSCLC patients as we had fewer patients in TNM stages 1–2. Multicentric studies are needed to increase the sample size that shall better represent lung cancer patients in the country. Findings from larger studies will help us in defining a rationale for upfront use of immunotherapy in advance stages of NSCLC patients which otherwise have dismal prognosis.

5. Conclusion

PD-L1 positivity was seen in almost one fourth of NSCLC patients without any difference in expression between SCC and adenocarcinoma. PD-L1 status did not alter with demographic, clinical and radiologic parameters including the histologic subtypes.

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Conflicts of interest

The authors have none to declare.

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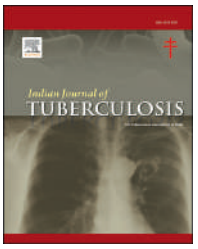

Original article

Spectrum of abdominal tuberculosis presenting as acute surgical emergency: Relevance in 21st century, a case series

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ABSTRACT

Background: Abdominal tuberculosis presenting as acute surgical emergency continues to be a major issue in developing countries including India. Being an indolent disease with varied presentation, there is a need to describe the epidemiology, clinicopathological nature of the disease. Hence, this series was conducted with the aim of describing our institutional experience in the management of abdominal tuberculosis presenting as acute surgical emergency, outlining the epidemiology, management aspects and the analysis of risk factors for poor outcome in our population.

Methods: This was a descriptive series of patients operated for abdominal tuberculosis presenting as acute surgical emergency at a tertiary care hospital in Eastern India from January 2021 to January 2022. All consecutive patients presenting with intestinal obstruction or peritonitis who underwent laparotomy with intra operative and histopathological finding suggestive of tuberculosis were taken for the study.

Results: A total of 30 patients with acute abdominal tuberculosis were included in the study. 56.7% of patients were males; the mean age of presentation was 43 years with majority of patients in the younger to middle age groups. Most (80%) patients were from rural areas with limited access to healthcare. One patient had co-infection with HIV. Five patients had diabetes and six patients had hypertension as co-morbidities. 73.3% of patients had primary intestinal tuberculosis. Majority (76.7%) presented with acute intestinal obstruction. All patients had colicky abdominal pain as a consistent feature. 40% of patients were anaemic and 70% had low serum albumin levels. The most common site of affection was Ileo-cecal region (73.3%) with stricture as the pathology. Segmental resection with end to end anastomosis was the most common procedure performed (46.7%). 26.7% of patients had an adverse post operative complication, and 23.3% had surgical site infection (SSI). The mortality rate in our series was 6.7%. Although coexisting SSI, co-morbidities were associated with increased mortality, it was not found to be statistically significant ($p = 0.08$). 16 patients were lost to follow up.

Conclusion: Abdominal tuberculosis presenting as acute abdomen continues to challenge surgeons even in the 21st century. Majority in the developing countries present late with

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varied complications. A high index of clinical suspicion is required for timely diagnosis to reduce the mortality and morbidity of the disease.

1. Introduction

The history of medicine is not complete without the mention of Tuberculosis (TB), a communicable, infectious disease caused by the organism *Mycobacterium tuberculosis*. From the era of Hippocrates, when tuberculosis was called "phthisis", it continues to scourge mankind. Having earned the name "Captain Among these Men of Death" in the 18th and 19th century,[1] Tuberculosis has stunned physicians and surgeons alike with its toll on human mortality. With the advancement of medical science, we have understood the bacteriology, pathogenesis, and epidemiology behind this disease. With the introduction of many health programmes and improvement in the standard of living, TB has become rare in the developed countries.[2] On the contrary in developing countries like ours, tuberculosis remains to be one of the leading cause of mortality due to poor surveillance, overcrowding and coexistence of diseases like diabetes and HIV.[3] With its capability to colonise in any tissue of the body, pulmonary and abdominal tuberculosis are the most common variants.[4] For the general surgeon, the abdominal variety is of utmost importance, with its affections being bowel, peritoneum, lymph nodes and solid viscera.

The abdomen is involved in 11% of patients with extrapulmonary tuberculosis.[5] Similar such incidences were noted in Indian series.[6,7] Abdominal tuberculosis is an indolent disease with delayed presentations. The intestinal variety has three main forms, ulcerative, hypertrophic or ulcerohypertrophic, and fibrous stricturing form.[8] These often have an acute presentation in the form of impassable obstruction or perforation peritonitis warranting a prompt surgical intervention. Although abdominal tuberculosis is a potentially curable disease with anti tubercular therapy (ATT) with or without surgery, its progression to acute surgical emergency still has a high mortality with varied presentations. With the rise of HIV co-infection and diabetes, there is a concurrent increase in tuberculosis, still causing a burden to the healthcare system.

This study was conducted with the aim of describing our institutional experience in the management of abdominal tuberculosis presenting as acute surgical emergency, outlining the epidemiology, management aspects and the analysis of risk factors for poor outcome in our population.

2. Methods

This prospective descriptive series of patients operated for abdominal tuberculosis presenting as acute surgical emergency was conducted in a tertiary care hospital in Eastern India from January 2021 to January 2022. Our institution, the prime referral centre of the state, caters to a population of approximately 30 million. All consecutive patients presenting with intestinal obstruction or peritonitis who underwent laparotomy with intra operative and histopathological finding suggestive of tuberculosis were taken for the study. Exclusion criteria included patients who refused consent and age less than 16 years. The study was approved by the institutional ethics committee and informed consent of the patients was taken.

Initially, all patients were resuscitated at the ER with fluid replenishment, electrolyte correction, nasogastric aspiration, urinary catheterisation and broad spectrum antibiotics. Preoperative evaluation included a complete haemogram, renal and liver function tests, erythrocyte sedimentation rate (ERS) and HIV testing via rapid screening test. Radiological investigations including plain radiograph of abdomen and pelvis, chest, ultrasonography of abdomen and pelvis, and contrast enhanced computed tomography (CT) of the abdomen was performed where warranted. With a documented indication, all patients underwent exploratory laparotomy with a midline incision. The intraoperative findings were noted, tissue samples sent for histopathological study. Patients either underwent a diversion ostomy, resection and anastomosis or band excision with adhesiolysis as necessitated. Post operatively patients were either monitored in the intensive care unit or the surgical care unit until recovery based on institutional protocol. Final diagnosis was confirmed based on intra operative findings and histopathological results. With the confirmation of tuberculosis, all the patients were started on anti tubercular therapy based on Revised National Tuberculosis Control Programme (RNTCP). The drugs included Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. The entire patient's data such as age, sex, socio economic details, symptomatology, investigations, intra operative finding, histopathological study, post operative complications (Clavien-Dindo> 2[9]), length of hospital stay and mortality were recorded.

The cases among the series worth a mention are as follows:

Case 1. An 18 year old lad presented to our ER with complaints of pain in the peri-umblical area with abdominal distension, vomiting, non passage of stool and flatus for 5 days. He was a known case of pulmonary Koch's' on anti tubercular therapy for past 4 months. On examination, he was found to have tachycardia with distended abdomen with visible bowel loops and exaggerated bowel sounds. On plain abdominal radiograph (Fig. 1) and Non contrast CT scan of the abdomen, the small bowel loops were grossly dilated with multiple air fluid levels. With the diagnosis of acute small bowel obstruction, patient underwent exploratory laparotomy under general anaesthesia. Intra operatively, there was multiple adhesions in the distal ileal loops with a stricture noted

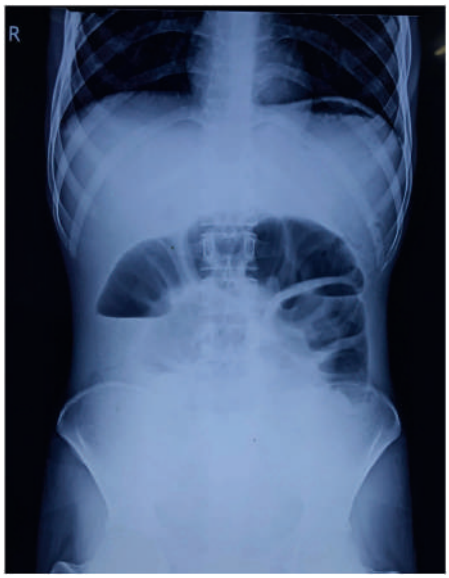


Fig. 1 – Plain abdominal erect radiograph showing small bowel obstruction.

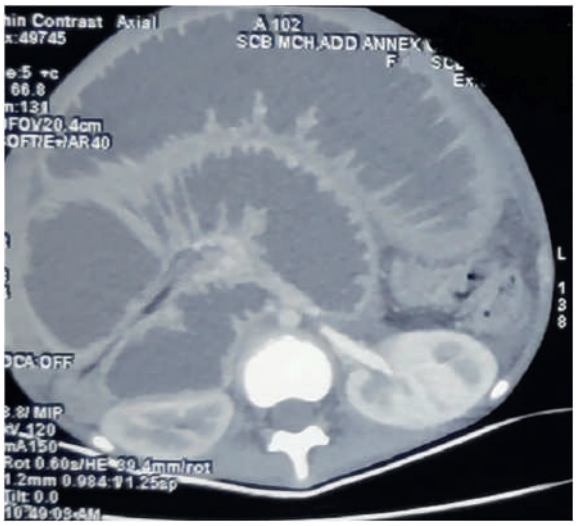


Fig. 3 – Contrast enhanced CT abdomen showing thickening of the bowel wall, grossly dilated small bowel with moderate peritoneal fluid.

30 cm proximal to ileo-cecal junction as shown in Fig. 2. Small segmental resection and anastomosis with adhesiolysis was done. Post operatively patient improved and was started on complete enteral feed by day 7. The histopathological examination of the resected bowel was confirmed to be tuberculosis. He was continued on ATT and was symptom free on follow up for 3 months.

Case 2. 21 year old lady with no co-morbidities presented with pain abdomen, abdominal distension and constipation since past 15 days. On examination, abdomen was distended with visible bowel loops and exaggerated bowel sounds. Contrast enhanced CT of the abdomen revealed thickening of the bowel wall, grossly dilated small bowel with moderate peritoneal fluid as shown in Fig. 3. With the diagnosis of small bowel obstruction, patient underwent exploratory laparotomy to reveal morbidly adhered bowel loops into a cocoon (Fig. 4) with grossly dilated small bowel and 500 ml of straw coloured peritoneal fluid. Three consecutive strictures were noted raging from 70 to 100 cm from duodenal-jejunal flexure as shown in Fig. 5. Resection of the stricturous segment with anastomosis was done. In view of refractory hypotension, patient was monitored in the intensive care unit. The initial 3 post operative days were uneventful. The histopathological examination of the resected bowel was confirmatory of tuberculosis. Subsequently she developed a rise in leukocyte

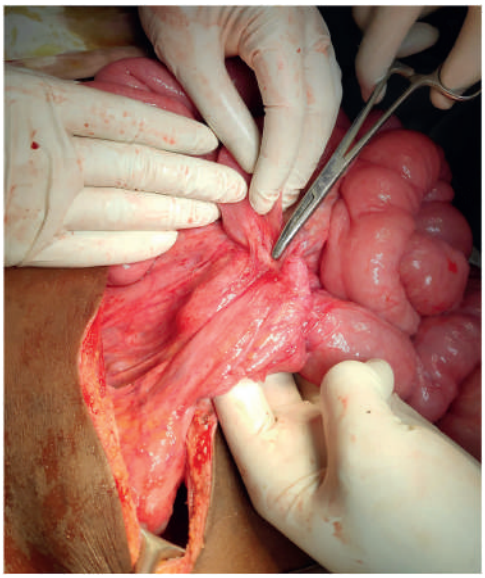


Fig. 2 – Intra operative photograph showing multiple adhesions in the distal ileal loops with a stricture.

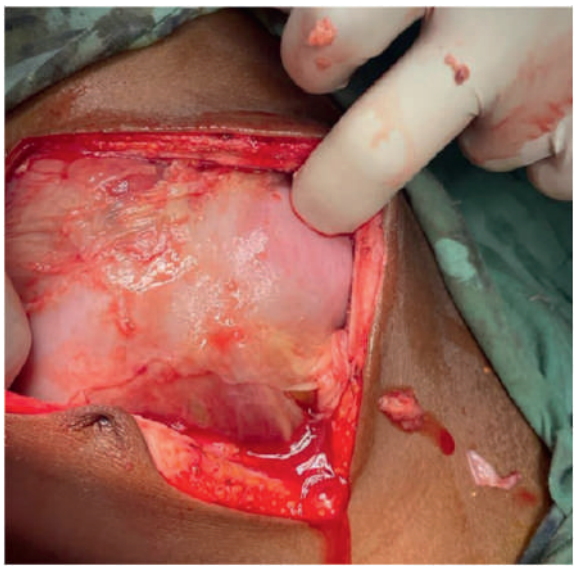


Fig. 4 – Intraoperative photograph showing morbidly adhered bowel into a cocoon.

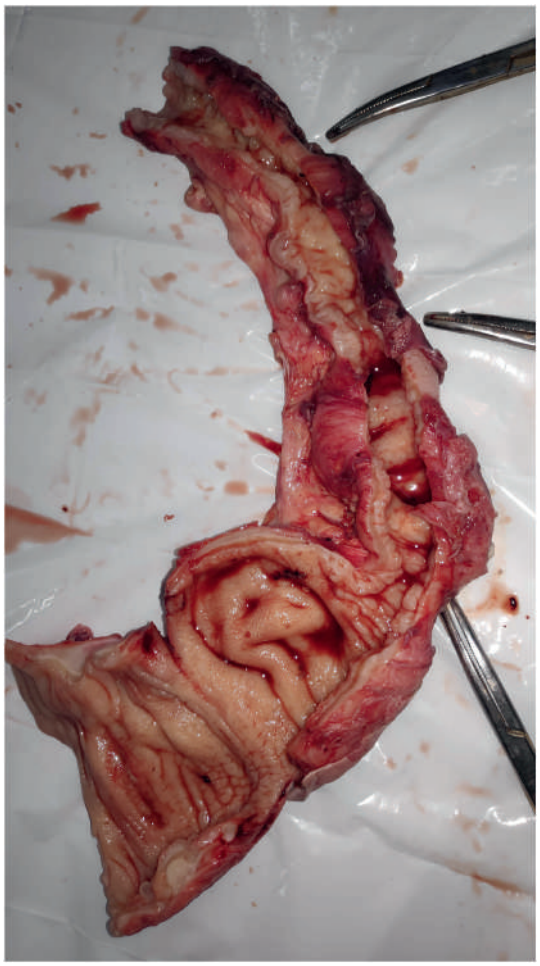


Fig. 5 – Resected specimen showing three consecutive strictures were noted raging from 70 to 100 cm from duodenal-jejunal flexure.

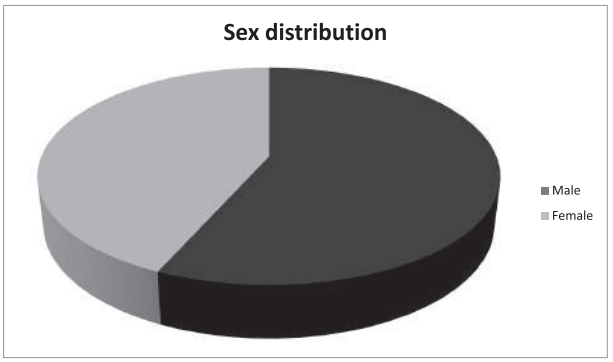
count with deranged renal function tests. Antibiotics were escalated in view of sepsis with multi organ dysfunction syndrome. Despite all efforts patient succumbed on post operative day 7 with cause of death being septicaemia with multi organ dysfunction syndrome.

3. Statistical analysis

The statistical analysis was performed using SPSS version 20.0 (SPSS, Chicago IL, U.S.A). The mean and standard deviations were calculated along with the frequency tables. Chi square test was performed to assess statistical significance with level of significance set at $p < 0.05$.

4. Result

A total of thirty patients were taken into this series during the study period as depicted in Table 4. Of this, seventeen (56.7%)



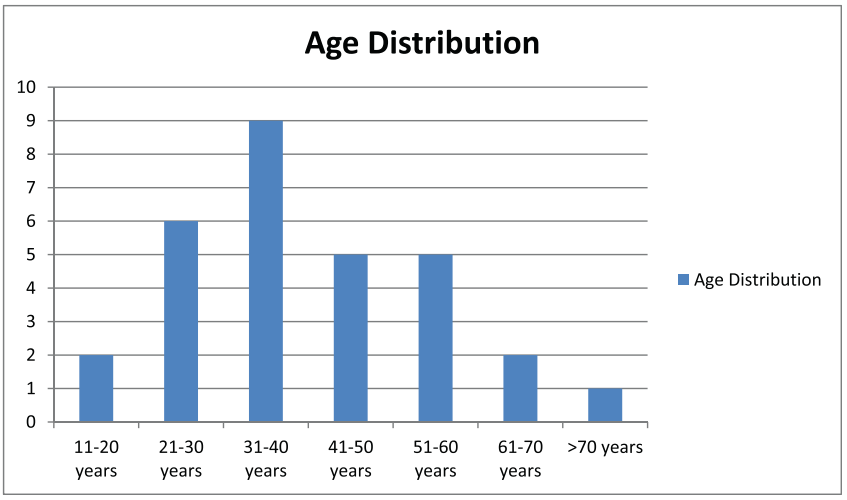
Graph 1 – Pie diagram depicting the sex distribution.

were male and thirteen (43.3%) were female with a male to female ratio of 1.3:1 as shown in Graph 1. The mean age of presentation was 43 years (43 ± 14.9 years), with the youngest patient being 17 years and oldest being 72 years as shown in Graph 2. Most of the patients (80%) were from rural background with limited access to healthcare. Co-infection with HIV was present in one patient (3.3%). Five (16.7%) patients had diabetes and six (20%) patients had hypertension as co-morbidity.

Of the thirty patients, twenty two (73.3%) had primary intestinal tuberculosis and the remaining eight (26.7%) had secondary intestinal tuberculosis (i.e. associated with pulmonary tuberculosis). Four (13.3%) patients who presented were already on anti tubercular therapy. Thirty three (76.7%) patients presented with acute intestinal obstruction, five (16.7%) patients presented with subacute obstruction and two (6.7%) patients presented with peritonitis. In regard to symptomatology, colicky abdominal pain was the most frequent complaint (100%) as depicted in Table 1.

Twelve (40%) patients had a haemoglobin level below 10.0 g/dl. ESR was found to be elevated more than 20 mm in twenty six (86.7%) patients. Serum albumin assessment revealed hypoalbuminaemia in twenty one (70%) of patients. Plain abdominal radiographs was performed pre operatively in all patients which revealed dilated bowel loops with air fluid levels as a consistent feature. All patients underwent exploratory laparotomy with the distribution of intra-operative findings shown in Table 2. The most common site of involvement was ileo-cecal junction with a frequency of twenty two (73.3%). The surgical procedure carried out is depicted in Table 3, with segmental bowel resection with end to end anastomosis as the most common (46.7%) followed by segmental resection with ileostomy (23.3%).

Eight (26.7%) patients had an adverse post operative complication (Clavien Dindo score more than 2). Surgical site infection (SSI) was present in seven (23.3%) patients, all limited to superficial SSI. The mean length of hospital stay was 11.6 ± 3.5 days. Two patients died in the post operative period with a mortality rate of 6.7%. Although both the patients who died had coexisting SSI, and one of them had diabetes and hypertension, this was not found to be statistically significant ($p = 0.08$). Of the 28 patients discharged from the hospital, 12 were available for follow up till 3 months and no complications were noted. 16 patients were lost to follow up.



Graph 2 – Bar diagram depicting the age distribution.

Table 1 – Distribution of clinical presentation.

Clinical presentation	Occurrence (number)	Percentage (%)
Abdominal pain	30	100
Constipation	28	93.3
Weight loss	12	40
Fever	14	46.7
Abdominal distension	25	83.3
Peritonism	5	16.7
Abdominal Mass	3	10

5. Discussion

This case series considered 30 patients with abdominal tuberculosis who presented as acute surgical emergency and underwent exploratory laparotomy. The study showed that the peak incidence of presentation of such patients is in the young to middle age groups. Similar observations were noted by other authors.[10,11] Since the affection is more in the productive years of life, the economic and social impact of tuberculosis is devastating, more so in the developing countries. We also found that the incidence of abdominal tuberculosis is higher in male with a male to female ratio of 1.3. Similar such finding was observed in other studies,[10] while Homan et al[12] noted that the disease is more common in males in western countries and in females in developing countries. The explanation behind such an occurrence is little understood. It was also found that the incidence of this disease is more common in patients from rural areas with a low socio-economic status. Accessibility to healthcare and social factors such as overcrowding, lack of awareness could be the possible reasons for such an occurrence.

Abdominal pain usually of prolonged duration is the most common clinical presentation, with other studies[13,14] validating this point. Obscure and non specific clinical features leads to a delay in the diagnosis, further leading to complications such as intestinal obstruction. The presence of co-existing medical illnesses such as diabetes has been shown to have a poor outcome in patients with tubercular intestinal obstruction.[15] Although we found that the mortality and complication rate are higher in patients with co-morbidities, it was not statistically significant. The prevalence of co-infection with HIV was present in 3.3% of cases. This rate seems to be low in comparison to other studies where HIV seroprevalence is around 20%.[10,16] It is expected that patients with HIV infection are prone to more severe and disseminated disease of tuberculosis manifesting with complications having a poor outcome. Studies have shown that HIV co-infection with low CD-4 counts have a higher incidence of surgical site infections[17] and multi drug resistance.[18]

In our study, 40% of the patients were anaemic and 70% had hypoalbuminaemia, which further affect the prognosis. Patients with acute abdominal tuberculosis can have either

Table 2 – Distribution of patients based on intra operative findings.

Operative finding	Occurrence (number)	Percentage (%)
Small bowel strictures	23	76.7
Band adhesions	5	16.7
Perforation	1	3.3
Ileocecal mass	2	6.7
Mesenteric lymphadenopathy	3	10

Table 3 – Distribution of patients based on surgical procedure carried out.

Surgical procedure	Frequency (number)	Percentage (%)
Right hemicolectomy	2	6.7
Segmental bowel resection with end to end anastomosis	14	46.7
Adhesiolysis	5	16.7
Ileostomy	7	23.3
Ileo-transverse bypass	2	6.7

Table 4 – Details of cases in the series.

Case no.	Age/sex	Predominant symptoms	Comorbidity	Intra-operative pathology	Surgical procedure performed	Outcome
1	18/male	Abdominal Pain, constipation distension		Ileal stricture	Resection and anastomosis	Survived
2	21/female	Abdominal pain, fever, distension, constipation		Ileal stricture	Resection and anastomosis	Death
3	34/male	Abdominal pain, distension, constipation		Ileal stricture	Ileostomy	Survived
4	38/male	Abdominal pain, constipation, distension		Ileal stricture	Resection and anastomosis	Survived
5	37/female	Abdominal pain, constipation, distension	Hypertension	Band adhesion	Adhesiolysis	Survived
6	49/female	Abdominal pain, fever, constipation, distension		Ileal stricture with mesenteric lymphadenopathy	Resection and anastomosis	Survived
7	72/male	Abdominal pain, constipation, distension, weight loss	Hypertension	Ileal stricture	Ileostomy	Survived
8	58/female	Abdominal pain, constipation distension, weight loss	Diabetes	Band adhesions	Adhesiolysis	Survived
9	50/male	Abdominal pain, distension, fever, peritonitis	Diabetes, hypertension	Ileal Perforation	Ileostomy	Death
10	28/female	Abdominal pain, mass constipation, fever		Ileocecal mass	Right hemicolectomy	Survived
11	37/male	Abdominal pain, distension, constipation, weight loss		Ileal stricture	Resection and anastomosis	Survived
12	48/male	Abdominal pain, distension, constipation, weight loss, fever	Hypertension	Ileal stricture with mesenteric lymphadenopathy	Resection and anastomosis	Survived
13	57/female	Abdominal pain, constipation, distension	Diabetes	Ileal stricture	Resection and anastomosis	Survived
14	70/female	Abdominal pain, distension, constipation, weight loss	Hypertension	Ileal stricture	Ileo-transverse bypass	Survived
15	55/male	Abdominal pain, constipation, distension, fever		Ileal stricture	Ileostomy	Survived
16	29/female	Abdominal pain, constipation, distension		Ileal stricture	Resection and anastomosis	Survived
17	39/male	Abdominal pain, constipation, distension, fever		Band adhesions	Adhesiolysis	Survived
18	43/male	Abdominal pain, weight loss, constipation, fever, mass		Ileocecal mass	Right hemicolectomy	Survived
19	59/male	Abdominal pain, distension, constipation, peritonitis		Ileal stricture	Ileo-transverse bypass	Survived
20	38/female	Abdominal pain, distension, constipation, weight loss, fever		Ileal stricture	Resection and anastomosis	Survived
21	29/male	Abdominal pain, constipation, peritonitis, fever		Ileal stricture with adhesions	Adhesiolysis	Survived
22	17/female	Abdominal pain, constipation, distension		Ileal stricture	Resection and anastomosis	Survived
23	34/male	Abdominal pain, constipation, distension	HIV co-infection	Band adhesions	Adhesiolysis	Survived
24	29/male	Abdominal pain, constipation, weight loss, fever, peritonitis with distension		Ileal stricture	Ileostomy	Survived
25	68/female	Abdominal pain, constipation, distension, weight loss peritonitis		Ileal stricture with mesenteric lymphadenopathy	Ileostomy	Survived
26	49/male	Abdominal pain, constipation, distension	Diabetes	Ileal stricture	Resection and anastomosis	Survived
27	38/female	Abdominal pain, fever, constipation, weight loss, mass		Ileal stricture with adhesions	Ileostomy	Survived
28	27/male	Abdominal pain, constipation, distension, fever		Ileal stricture	Resection and anastomosis	Survived
29	44/male	Abdominal pain, constipation, distension, weight loss	Diabetes	Ileal stricture	Resection and anastomosis	Survived
30	60/male	Abdominal pain, weight loss, fever	Hypertension	Ileal stricture	Resection and anastomosis	Survived

one of these four manifestations: intestinal obstruction, peritonitis, acute mesenteric lymphadenitis or acute tubercular appendicitis.[19] Patients presenting with obstruction tend to require surgery as the standard treatment.[14] The most common pathology encountered was small bowel strictures followed by band adhesions and mesenteric lymphadenopathy. The surgical treatment of which depends on the intraoperative pathology and the distance of the small bowel stricture from the ileo-cecal junction. The most common procedure carried out was segmental bowel resection with end to end anastomosis, followed by ileostomy and adhesiolysis. Although other studies demonstrated that the right hemicolectomy with ileo-transverse anastomosis was the most common surgical procedure performed,[10] this varies from institutions based on the prevailing surgical guidelines. Katariya et al[20] introduced the method of stricturoplasty for easy and quicker way of relieving the obstruction, and this is now being practiced all over. All patients must be started on anti-tubercular therapy with the confirmation of tuberculosis for a minimum duration of 6–12 months as per the prevailing guidelines.

We found that 26.7% of patients had an adverse post operative complication and 23.3% had surgical site infection. Presence of these complications put the patients at increased risk of mortality. The mean length of hospital stay was 11.6 days which was lower in comparison to a study by Chalya et al[10] where the duration of hospitalisation was 24 days. This depends on the presence of post operative complications with a direct association. The mortality rate in our series is 6.7%, which could be related to delayed presentation, associated co-morbidities, presence of post operative complications, although this was not statistically significant in our series.

Our study has a few limitations. Firstly, the sample size of the series is limited; hence the definitive representation of the population is not established. Secondly, poor socio economic conditions of the study population, delayed presentation, loss to follow up and lack of certain investigations lead to delayed diagnosis and poorer outcomes. Having a targeted approach to management of abdominal tuberculosis based on the socio economic factors and patient related conditions, a good outcome can be achieved.

6. Conclusion

As very correctly said by Tom Frieden "A vaccine that prevented tuberculosis would merit a Nobel prize, but it's just very difficult to develop". Abdominal tuberculosis presenting as acute surgical emergency is a challenge to surgeons even in the 21st century. The disease is more common in male and younger age group, having a devastating social impact in developing countries. Most of the patients affected are from a rural background having limited access to healthcare. As the prevalence of abdominal tuberculosis is very less in developed countries, there is least interest to develop a standardised guideline for the treatment of this grievous entity. Till today, no single test is available with high specificity and sensitivity to diagnose the devastating disease. Still we need a high clinical index of suspicion in patients with a past history of tuberculosis, subacute onset and the demographic profile with proper radiological and biochemical pre-operative evaluation for patient optimisation prior to definitive surgery. With the rise in HIV, multi drug resistance and co-morbid medical illnesses, there is a need to revisit our multidisciplinary approach towards this disease to achieve the least morbidity and mortality. More studies with higher sample size is the need of the hour to formulate a concrete guideline for this treatable disease.

Conflicts of interest

The authors have none to declare.

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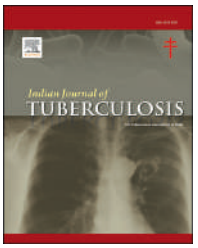

Original article

Ligand-based pharmacophore modelling, virtual screening and docking studies to identify potential compounds against FtsZ of *Mycobacterium tuberculosis*

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ABSTRACT

Background and introduction: Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*M.tb*) which is the most common cause of death from bacterial illness. Millions of victims of TB infections have been recorded including 20,800 deaths amongst HIV positive individuals. Hence, there is a rising need for new and active compounds against *M. tb* protein targets especially as there is a persistent resistance to the current drug treatment regime.
Aim: This study identifies new potential compounds against the *M. tb* target protein ftsZ via pharmacophore modelling, QSAR analysis and docking studies.
Method: Inhibitors with known PIC_{50} were used as a training set and the pharmacophore features (1 aromatic center, 2 hydrophobic, 2 hydrogen bond acceptors and 1 hydrogen bond donor) were validated against four test set compounds. The identified hits were subjected to rigorous ADMET properties and docked using PyRx. DS visualizer was used in binding interactions study. Stability was measured based on the total number of interactions and preference given to the number of hydrogen bond interactions.
Results: Based on the number of interactions, hydrogen bonds, extensive virtual screening and ADMET filtration, 40 compounds have been identified as potential inhibitors of ftsZ with only 3 considered to be the best leads.

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Significance of research: The identified compounds have potential of being drug candidate against *Mycobacterium tuberculosis* and may possess a novel mechanistic route in inhibiting the resistant strains.

1. Background and Introduction

Tuberculosis (TB) was found in 1882 to be caused by *Mycobacterium tuberculosis (M.tb)*, and it is the primary cause of death from bacterial infection. Approximately 10 million people have been infected with tuberculosis (TB) worldwide in 2019, with men accounting for 56 percent of infections, women for 32 percent, and children under the age of 15 accounting for 12 percent. 8.2 percent of the 10 million persons who have been infected, are HIV positive. Approximately 1.4 million people have died as a result of tuberculosis infections including 20,800 deaths amongst HIV positive individuals.[1] Smoking, type 2 diabetes, alcoholism, malnutrition, air pollution and alcoholism have been described as some of the risk factors of this infection.[2]

Transmission of *M. tb* usually occur through the inhalation of particles released into the atmosphere by TB infected patients. Alveolar macrophages engulf these particles in the lungs, where the organism survives by blocking the effects of phagocytosis. FtsZ of *M. tb*, known for its GTPase activityis regarded to have a critical role in cell division within the bacteria. Studies have shown that inhibition of FtsZ can result in a blockage of cell division and can therefore serve as a target for drug development.[3]

Over the decades, various efforts have been put in place to totally curb the spread and menace of tuberculosis. Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, Rifabutin and Rifapentine were approved as first-line oral agents while streptomycin, kanamycin, ofloxacin, capreomycin, cycloserine, amikacin and ethionamide as second line drugs and many of the oxazolidine antibiotics as third-line treatment.[4] Also, significant number of drugs have been subjected to clinical trials and are in various stages. However, the prevailing drug-resistant tuberculosis, emergence of slow acting drugs, poor patient compliance, persistent and latent occurrence of the bacterium and undesirable toxicity have necessitated the further development of antituberculosis drugs.

In silico approach to drug discovery has consistently offered support to *in vitro* and *in vivo* experimentations by reducing the number of compounds used in a multi compound screening.[5] Ligand based pharmacophore modelling and QSAR provide useful properties in identifying lead compounds of interest. In order to determine compounds that actively work against FtsZ, a number of different tools *in silico* tools have been employed. Pharmacophore modelling maps the major elements of drug design to create a 3-dimensional arrangement which defines the type of interactions. Pharmacophores are used as queries for retrieving potential leads from structural databases, for designing molecules with specific desired attributes (lead optimization), and for assessing similarity and diversity of molecules using pharmacophore fingerprints.[6] Further QSAR analysis is applied to develop relationships between physicochemical properties of chemical substances and their biological activities. The statistically reliable model obtained is used for prediction of the activities of newly identified chemical entities and validation of our model.[7] A high-throughput virtual screening is done to obtain potential hits and is then correlated for drug–likeness properties. Molecular docking is performed to model the interactions between small molecules and proteins at the atomic level.[8] In this research, we have employed ligand-based pharmacophore, QSAR modelling, docking, visualization and ADMET studies to identify potential inhibitors of *Mycobacterium tuberculosis (M.tb)* based on the pharmacophore features of pyridopyrazine and pyrimidothiazine derivatives that have exhibited good activity against *ftsZ* in an *in vitro* assay.[9]

2. Materials and methods

The presented research aims to find new potential lead compounds against the *ftsZ* protein. It is based on virtual screening of compounds using pharmacophore modelling and QSAR analysis. The compounds are further docked to increase their credibility. Fig. 1 highlights the methodology adopted in this study.

3. Protein collection, binding site evaluation and preparation

FtsZ is a critical protein for the initiation of cell division of *Mycobacterium tuberculosis*. To achieve our main objective, profound analysis and preparation of this protein is essential to finding the appropriate leads. The target protein was obtained from RCSB PDB (https://www.rcsb.org/),[10,11] a database for the three-dimensional structural data of large biological molecules The structure of *M. tb ftsZ* protein with PDB ID 1RQ7 was retrieved[12–14] is shown in Fig. 2. Prankweb online server was used to identify the active pockets with the corresponding residues of the dimeric protein[15].

Chain A of the homo-2-mer protein was chosen due to its reported activity[12] and prepared using UCSF Chimera. Water molecules, non-standard residues, lone pairs and non-polar hydrogens were removed. Molecular visualizations and analyses were carried out using UCSF Chimera, a program developed by the University of California, San Francisco's Resource for Biocomputing, Visualization, and Informatics with NIH P41-GM103311 support (https://www.rbvi.ucsf.edu/chimera/).[16]

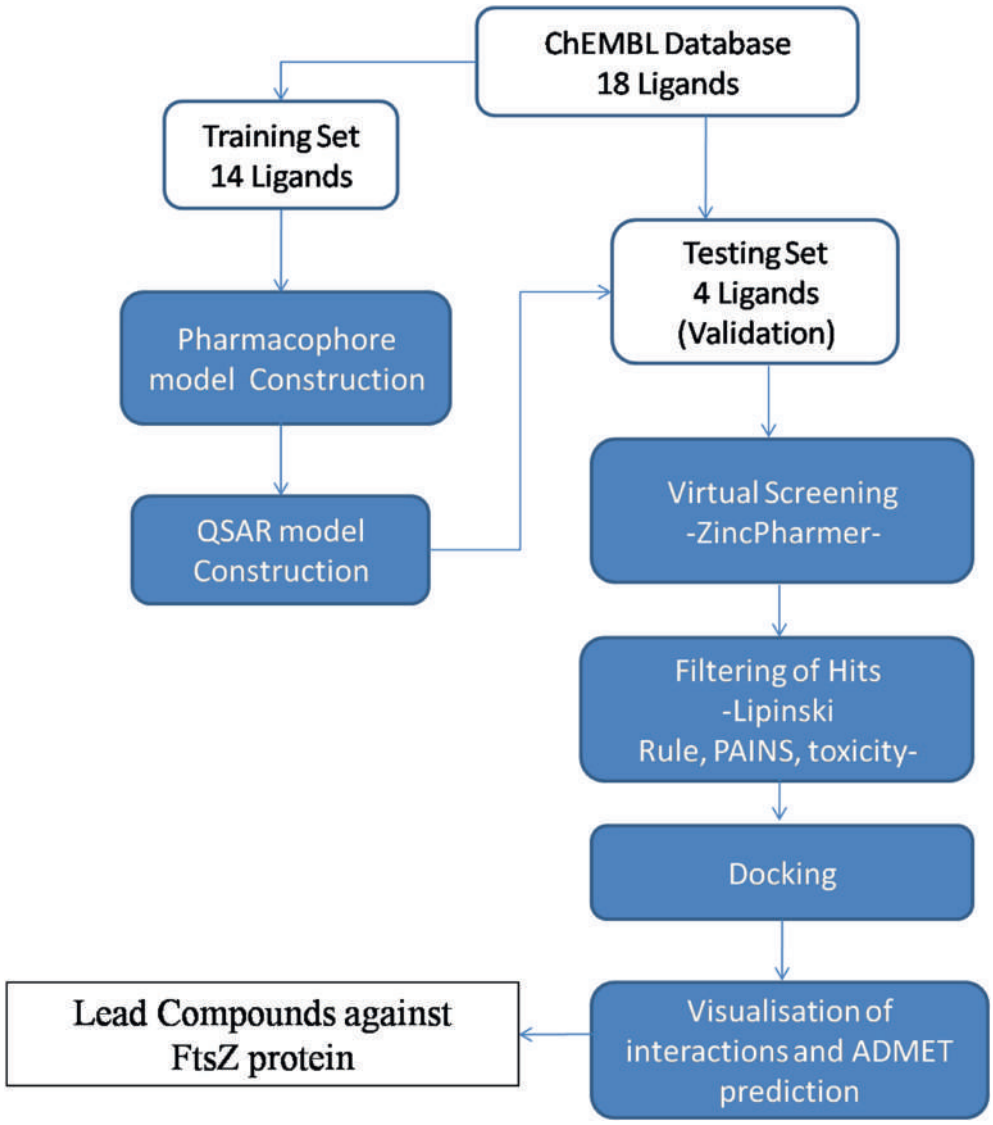


Fig. 1 – Schematic representation of workflow.

4. Ligand collection

ChEMBL is a database of bioactive compounds with drug-like characteristics that has been carefully selected. It combines chemical, bioactivity, and genetic data to aid in the translation of genomic data into new medications that are efficacious.[17,18] A series of pyridopyrazine and pyrimidothiazine derivatives showing activity against ftsZ in *in vitro* assay was obtained from the database.[9] The list of 18 ligands with their IC50 values is provided in Table 1.

The 3D structures of the ligands were downloaded from PubChem[19] as sdf files and converted to mol2 chemical format using OpenBabel, an open-source chemical toolbox that helps in interconversion of chemical data to over 110 formats.[20]

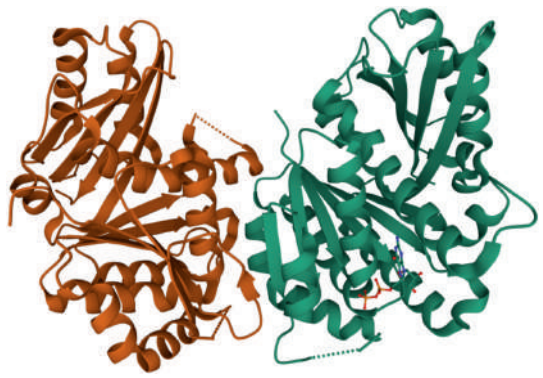


Fig. 2 – Structure of ftsZ (PDB ID: 1RQ7).

5. Pharmacophore modelling

A pharmacophore model is a collection of steric and electronic properties that represent a three-dimensional (3D) molecular interacting ecosystem of ligands and receptors (protein). Chemical properties such as hydrogen bond (H-bond), ionic charges, lipophilic and aromatic contacts are described by a 3D pharmacophore. They are responsible for the functional

Table 1 – Initial set of ChEMBL Ligands.

S.No.	ChEMBL ID	IC50 (nM)
1	CHEMBL1651196	6210
2	CHEMBL1651202	7690
3	CHEMBL1926717	16,400
4	CHEMBL1926724	18,600
5	CHEMBL1926719	21,000
6	CHEMBL1929425	26,800
7	CHEMBL1926722	27,300
8	CHEMBL1926725	29,500
9	CHEMBL1926723	34,100
10	CHEMBL110168	34,200
11	CHEMBL1929426	34,300
12	CHEMBL1926720	34,800
13	CHEMBL106808	38,100
14	CHEMBL1929427	45,700
15	CHEMBL1926726	46,000
16	CHEMBL1926727	46,200
17	CHEMBL457349	52,000
18	CHEMBL1926721	54,600

and biological response resulting from receptor–ligand association. Consequently, molecules with desired chemical features from the chemical space of millions of compounds stored in the form of databases[21] are retrieved using a 3D pharmacophore model.

Before pharmacophore modeling, an initial random division of training set and test set was done within our set of 18 ligands in the ratio of 80:20. The selection was done in a manner that all range of activity was included within the training set. PharmaGist, a free web server efficient in pharmacophore detection was employed in building of the model.[22,23] The method employed in this study was ligand based, where the input was the training set of structures of drug-like molecules that are known to bind to and inhibit the receptor ftsZ. By multiple flexible alignments of input ligands, well computed candidate pharmacophores were provided as outputs.

Table 2 – Distance matrix of pharmacophore features.

Distance in Å	AR	DON	ACC1	ACC2	HYD1	HYD2
AR	0	2.699	3.987	2.701	7.296	5.133
DON	2.699	0	2.343	4.655	4.632	6.611
ACC1	3.987	2.343	0	6.521	4.178	5.786
ACC2	2.701	4.655	6.521	0	9.162	7.111
HYD1	7.296	4.632	4.178	9.162	0	9.898
HYD2	5.133	6.611	5.786	7.111	9.898	0

6. QSAR analysis

The quantitative structure–activity relationship (QSAR) method, which is based on mathematical and statistical relationships, predicts the biological effect of chemical substances. It predicts the biological activities of compounds through the analysis of quantitative characteristics of structural features. It takes a complementary approach, while attempting to determine how structural changes in a group of molecules are linked to their activity.[24]

Computation of descriptors was the first step for QSAR model building. A molecular descriptor is a mathematical representation of a structural or physicochemical property of a molecule that is generated by algorithms.[24] ChemDes, a free web-based platform for the calculation of molecular descriptors and fingerprints was used for generation of descriptors for our training set.[25] This was used to build a QSAR model in continuation to the pharmacophore model and analyze the results. ChemoPy (chemoinformatics in Python) descriptors which includes topological, constitutional, Moreau-Broto autocorrelation, connectivity indices, E-state descriptors and more were used within ChemDes.[26] Two descriptor pre-selection steps were performed before applying them to model construction – (1) Only descriptors that provided value for all the compounds in training set were selected and (2) descriptors with identical values in more than 50% of the compounds were removed.

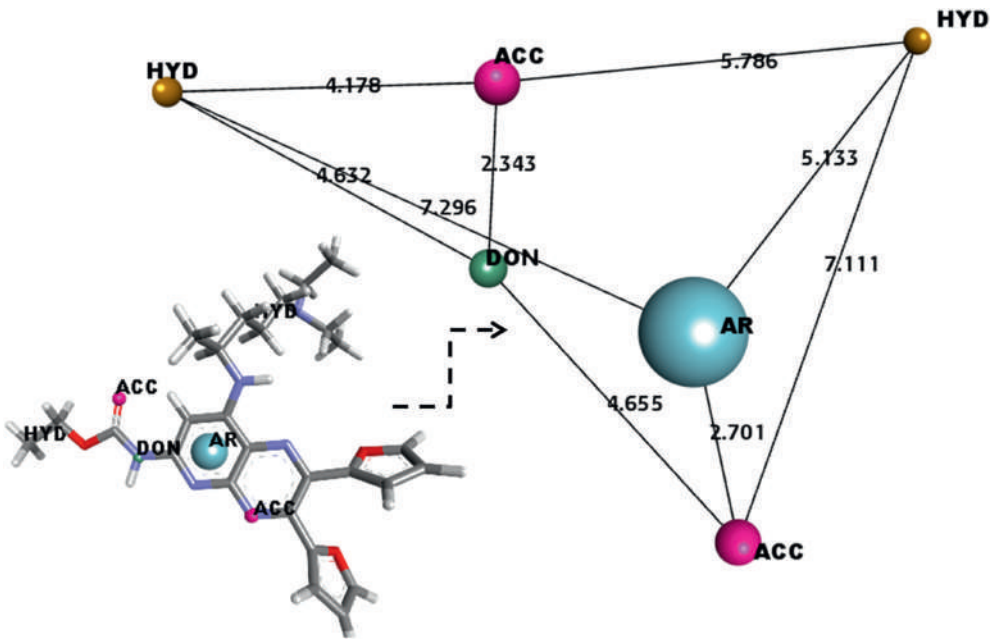


Fig. 3 – Pharmacophore model.

Table 3 – QSAR top 4 models.

Model No.	Descriptor	R^2	Q^2	F	Spress	SDEP
1	Chi10	0.8373	0.7561	61.74	0.1477	0.1419
2	Chi8	0.8264	0.7238	57.12	0.1572	0.151
3	Chi9	0.813	0.7278	52.17	0.156	0.1499
4	ATSe7	0.8091	0.7231	50.87	0.1574	0.1512

Table 4 – Experimental activity vs. Predicted activity.

SET	ChEMBL ID	Y (obs)	Y (calc)	Y (res)
training set	CHEMBL1651196	5.207	5.069	−0.138
	CHEMBL1651202	5.114	5.213	0.099
	CHEMBL1926717	4.785	4.554	−0.231
	CHEMBL1926719	4.678	4.591	−0.086
	CHEMBL1929425	4.572	4.539	−0.033
	CHEMBL1926722	4.564	4.637	0.073
	CHEMBL110168	4.466	4.359	−0.107
	CHEMBL1929426	4.465	4.539	0.074
	CHEMBL1926720	4.458	4.591	0.133
	CHEMBL106808	4.419	4.359	−0.06
	CHEMBL1929427	4.34	4.359	0.019
	CHEMBL1926726	4.337	4.359	0.022
	CHEMBL1926727	4.335	4.375	0.039
	CHEMBL1926721	4.263	4.458	0.195
test set	CHEMBL1926724	4.73	4.945	0.214
	CHEMBL1926725	4.53	4.945	0.415
	CHEMBL1926723	4.467	4.637	0.17
	CHEMBL457349	4.284	4.375	0.091

BuildQSAR version 2.1.0.0 was used for the construction of QSAR model.[27] It is a free QSAR program that helps to construct and analyze quantitative models through regression analysis. Multiple regressions performed by using BuildQSAR correlate the physicochemical descriptors and the inhibitory activity of pyridopyrazine and pyrimidothiazine derivatives. The dataset with descriptors (X values - independent variables) and the known inhibitory activity (pIC50 = -logIC50) values (Y values - dependent variables) were uploaded in BuildQSAR. The model was selected based on R^2, Q^2 and p-values. Leave-one-out cross validation was performed to verify the robustness of the model. It involved excluding each sample once, constructing a new model without this sample and predicting the value of its dependent variable.[28]

7. Virtual screening

Virtual screening is a computational approach that analyses large databases or collection of compounds in order to identify potential hit candidates with similar structural features. Based on the pharmacophore model generated from PharmaGist, virtual screening was done using ZincPharmer[29] to search for similarly structured compounds matching the pharmacophore query model from 'ZINC purchasable database'. An initial filtering was done in ZinPharmer to obtain hits with molecular weight less than or equal to 500 and rotatable bonds less than or equal to 10. Amongst the 2638 hits provided by the webserver, 1128 unique compounds were taken for further analysis by removing the repeats.

8. Filtration of hits

For a hit to qualify as a lead, its drug-likeness, pharmacokinetics and ADMET properties were examined. Determination of drug-likeness involves a qualitative assessment based on the structural and physicochemical inspection of a molecule and identify if it is sufficiently advanced to be considered as an oral drug candidate. ADMET refers to the 'absorption, distribution, metabolism, excretion and toxicity' properties and describes the deposition of pharmaceutical compounds within an organism. In this study, three levels of filtration were done.

1. *Lipinski rule of 5*[30] – This rule of thumb, proposed by Christopher A. Lipinski is used to verify if a chemical compound with a particular pharmacological or biological activity has properties that are likely to make it an orally active drug in humans. Compounds with more than one violation of the rules – no more than 5 hydrogen donors, no more than 10 hydrogen acceptors, molecular weight under 500 g/mol, logP less than 5 – were eliminated in this stage.
2. *PAINS alert* – Pan-assay interference compounds (PAINS) are chemical compounds that have an increased probability of giving false positive results due to non-specific binding in high-throughput screens. Therefore, these false-positive compounds were also eliminated in this

Table 5 – Ligand Docking scores.

Ligand	Binding Energy (kCal/mol)	Ligand	Binding Energy (kCal/mol)
CHEMBL1651196	−7.5	CHEMBL110168	−6.7
CHEMBL1651202	−6.8	CHEMBL1929426	−6.5
CHEMBL1926717	−7	CHEMBL1926720	−7
CHEMBL1926724	−6.9	CHEMBL106808	−7
CHEMBL1926719	−6.4	CHEMBL1929427	−6.7
CHEMBL1929425	−6.8	CHEMBL1926726	−6.4
CHEMBL1926722	−6.6	CHEMBL1926727	−6.9
CHEMBL1926725	−6.6	CHEMBL457349	−6.8
CHEMBL1926723	−7.2	CHEMBL1926721	−7.7

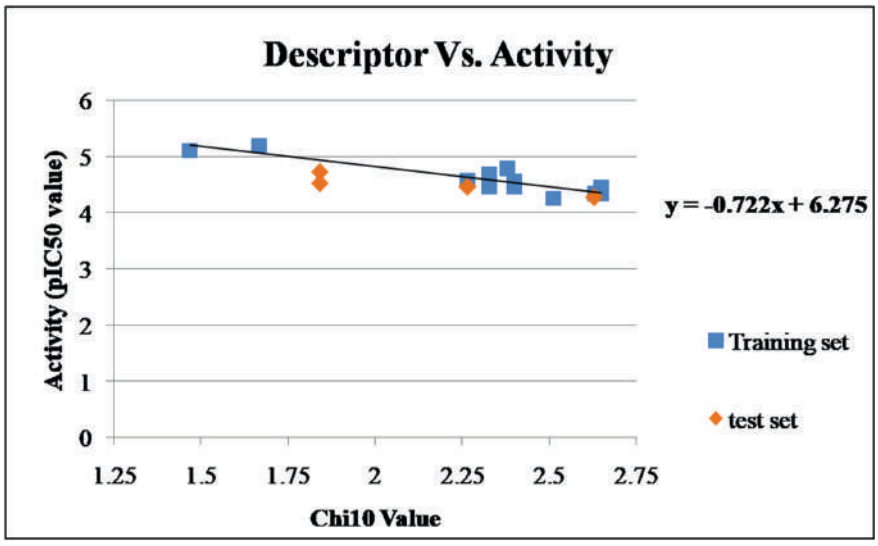


Fig. 4 – QSAR linear regression plot.

stage. SWISSADME[31] was used for this first two filtration stages using SMILES of the obtained hits.

3. *Toxicity* –Toxicity is the ability of a chemical substance to harm or produce injury once it reaches a particular site within the body. Three main toxicity evaluations – AMES test, hepatotoxicity and carcinogenicity were conducted. AMES test predicts whether a chemical can cause mutations in the DNA of the test organism itself. Hepatotoxicity measures the ability of a compound to injure the liver due to adverse reaction in the specified site. PkCSM[32] which uses graph-based signatures to predict toxicity was used to evaluate whether the hits passed the AMES test and hepatotoxicity. Further, CarcinoPred-EL[33] which predicts carcinogenicity using molecular fingerprints and ensemble learning methods was used. Ensemble XGBoost was employed as the model has a high average accuracy of 70.1 ± 2.9%, sensitivity of 67.0 ± 5.0%, and specificity of 73.1 ± 4.4% in five-fold cross-validation and an accuracy of 70.0%, sensitivity of 65.2%, and specificity of 76.5% in external validation.

9. Docking

Molecular docking approach is used to understand the interaction between a ligand of specific orientation and a protein to form a stable complex. It generates different possible adduct structures that are ranked and grouped together using binding scoring function. PyRx,[34] a widely used virtual screening software was utilized to find potential lead compounds based on docking score. The AutoDockVina software[35] which has a high accuracy of the binding mode predictions was used within PyRx.

The 3D structure of hits were obtained from databases such as PubChem[19] and ChemSpider[36] using the SMILES obtained from Zinc12 directed by ZincPharmer.[29] However, MolView, which is an open-source web server was used to search through databases and find the appropriate structure of compounds whose structures were not available in the database. Also, for structures directly downloaded from MolView, chemical structures were drawn and MMFF94 energy minimization was executed using the jmol menu.

Initially, the 18 ligands which were obtained from ChEMBL database were docked to find a potential threshold (Table 5). The hits whose docking score crossed the threshold energy (which was the average docking score of the 18 ligands – –6.8 kCal/mol), were considered to be potential molecules that can inhibit ftsZ.

Visualization of the non-bond interactions between protein and hits was done using BIOVIA, DassaultSystèmes, Discovery Studio Visualiser, v21.1.0.20,298, San Diego: DassaultSystèmes, 2021.[37] The hits with maximum number of interactions were considered as the most stable, as stability of protein-ligand complex increases with increase in number of interactions.

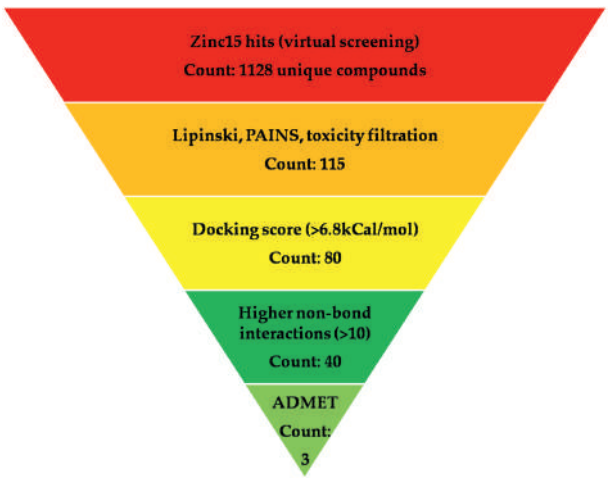


Fig. 5 – Virtual screening and filtration workflow for identification of potential compounds against ftsZ.

Table 6 – Combined result table of the 40 potential compounds.

Molecule	Zinc ID	Binding Energy (kCal/mol)	Total no. of interactions	No. of hydrogen bonds	No. of electrostatic bonds	No. of hydrophobic interactions	other interactions	predicted activity (μM)
1	**ZINC10157410**	**-10**	**15**	**8**	**1**	**6**	**0**	**4.8**
2	**ZINC05013094**	**-9.7**	**12**	**7**	**1**	**4**	**0**	**4.79**
3	ZINC18323255	-9.6	10	5	0	5	0	4.34
4	**ZINC18203893**	**-9.5**	**12**	**6**	**1**	**5**	**0**	**4.11**
5	ZINC13692508	-9.3	13	5	0	8	0	2.75
6	ZINC13734748	-9.2	12	5	0	7	0	2.75
7	ZINC13693261	-8.8	13	5	0	8	0	2.63
8	ZINC09668767	-8.4	14	10	0	4	0	5
9	ZINC32810183	-8.2	11	6	0	5	0	3.4
10	ZINC36695182	-8.1	13	12	1	0	0	4.65
11	ZINC03205972	-8.1	15	7	0	3	$6^{(1*)}$	2.24
12	ZINC04991736	-8	13	13	0	0	0	1.81
13	ZINC12243567	-8	10	4	0	6	0	18.18
14	ZINC12149115	-8	13	8	1	4	$1^{(1*)}$	7.15
15	ZINC12241893	-8	14	10	1	3	$2^{(2*)}$	11.09
16	ZINC12243568	-7.9	12	5	2	5	0	18.18
17	ZINC11791113	-7.9	10	7	0	3	0	10.76
18	ZINC13745298	-7.8	12	7	3	2	$2^{(2*)}$	1.19
19	ZINC02808097	-7.8	12	7	0	3	$3^{(1*)}$	1.69
20	ZINC71812724	-7.8	11	7	1	3	0	2.21
21	ZINC02075313	-7.7	10	7	0	1	$2^{(2*)}$	2.15
22	ZINC14885187	-7.7	10	5	0	5	0	11.28
23	ZINC01049053	-7.6	11	8	1	2	$2^{(2*)}$	1.2
24	ZINC08693946	-7.6	18	8	0	2	$13^{(5*)}$	1.99
25	ZINC12098508	-7.6	10	8	1`	2	0	8.1
26	ZINC03205710	-7.6	13	4	0	2	$8^{(1*)}$	2.12
27	ZINC10100753	-7.5	10	7	0	3	0	2.16
28	ZINC76094327	-7.4	10	7	0	3	0	0.82
29	ZINC13153662	-7.4	13	8	0	5	0	2.13
30	ZINC04264321	-7.4	12	11	1`	1	0	3.5
31	ZINC2756924	-7.4	11	6	0	5	0	2.08
32	ZINC36695204	-7.3	11	4	0	7	0	2.26
33	ZINC03205173	-7.3	16	4	0	8	$5^{(1*)}$	1.99
34	ZINC01796392	-7.3	21	11	0	3	$13^{(6*)}$	1.99
35	ZINC03205786	-7.2	17	5	0	8	$6^{(2*)}$	2.09
36	ZINC04330948	-7.1	10	7	1`	3	0	2.2
37	ZINC04292215	-7.1	14	7	0	7	0	2.26
38	ZINC06377901	-7.1	13	7	1	3	2	1.22
39	ZINC54858084	-7	10	6	1	3	0	1.25
40	ZINC02845675	-7	10	7	0	0	$5^{(2*)}$	1.49
$^{(n*)}$ denotes hydrogen-halogen interaction					1` denotes hydrogen-electrostatic interaction			
Molecules with hydrogen, electrostatic and hydrophobic interactions								

Table 7 – ADMET properties of identified potential compounds.

Mol. No.	Consensus Log P	ESOL Class	GI absorption	BBB permeant	Pgp II inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Total Clearance	Oral Rat Acute Toxicity (LD50) mol/kg
1	**3.5**	**Moderately soluble**	**High**	**No**	**Yes**	**Yes**	**Yes**	**0.378**	**2.936**
2	**3.18**	**Moderately soluble**	**High**	**No**	**Yes**	**Yes**	**Yes**	**0.406**	**2.73**
3	3.24	Moderately soluble	High	No	Yes	Yes	Yes	0.175	3.363
4	**2.85**	**Moderately soluble**	**High**	**No**	**Yes**	**Yes**	**Yes**	**0.362**	**2.946**
5	3.34	Moderately soluble	High	No	No	Yes	Yes	0.349	2.617
6	3.36	Moderately soluble	High	No	Yes	Yes	Yes	0.322	2.593
7	3.04	Soluble	High	No	No	Yes	Yes	0.318	2.619
8	2.57	Moderately soluble	Low	No	Yes	No	Yes	0.403	2.422
9	3.35	Moderately soluble	Low	No	Yes	No	Yes	0.397	2.49
10	3.89	Moderately soluble	High	No	Yes	Yes	Yes	-0.087	2.109
11	5.66	Poorly soluble	Low	No	Yes	Yes	No	-0.666	2.323
12	3.73	Moderately soluble	High	No	No	No	Yes	0.288	2.491
13	3.33	Moderately soluble	High	No	Yes	Yes	Yes	1.39	2.671
14	3.82	Moderately soluble	High	No	Yes	Yes	Yes	0.844	2.402
15	2.93	Moderately soluble	High	No	Yes	Yes	Yes	1.047	2.447
16	3.35	Moderately soluble	High	No	Yes	Yes	Yes	1.39	2.671
17	3.53	Moderately soluble	High	No	Yes	Yes	Yes	0.987	2.31
18	2.33	Moderately soluble	Low	No	No	No	Yes	0.043	2.023
19	1.15	Soluble	High	No	No	No	No	0.687	2.841
20	2.66	Moderately soluble	High	No	No	No	Yes	1.123	1.912
21	3.75	Moderately soluble	High	No	Yes	No	Yes	0.364	2.797
22	3.87	Moderately soluble	Low	No	Yes	Yes	Yes	1.005	2.238
23	4.57	Moderately soluble	High	No	No	No	Yes	-0.132	2.223
24	4.76	Moderately soluble	Low	No	Yes	Yes	No	0.127	2.211
25	2.15	Soluble	High	No	Yes	Yes	Yes	1.17	2.464
26	5.72	Poorly soluble	Low	No	Yes	Yes	No	0.319	2.274
27	3.41	Moderately soluble	High	No	No	No	Yes	0.247	2.453
28	1.38	Soluble	High	No	No	No	No	0.499	2.338
29	0.6	Soluble	High	No	No	No	No	0.503	1.805
30	4.63	Moderately soluble	Low	No	Yes	No	Yes	0.438	2.472
31	4.94	Poorly soluble	Low	No	Yes	No	Yes	0.046	2.656
32	4.2	Moderately soluble	High	No	Yes	No	Yes	0.316	2.445

Table 7 continued

33	4.82	Moderately soluble	Low	No	Yes	Yes	No	-0.331	2.283
34	5	Moderately soluble	Low	No	Yes	Yes	No	0.134	2.262
35	5.02	Moderately soluble	Low	No	Yes	Yes	No	0.196	2.394
36	4.09	Moderately soluble	High	No	Yes	No	Yes	0.335	2.439
37	4.1	Moderately soluble	High	No	Yes	No	Yes	0.337	2.434
38	3.72	Moderately soluble	High	No	Yes	Yes	Yes	0.223	2.173
39	3.01	Soluble	High	No	No	No	Yes	0.401	2.01
40	4.86	Moderately soluble	Low	No	Yes	No	No	0.165	2.617

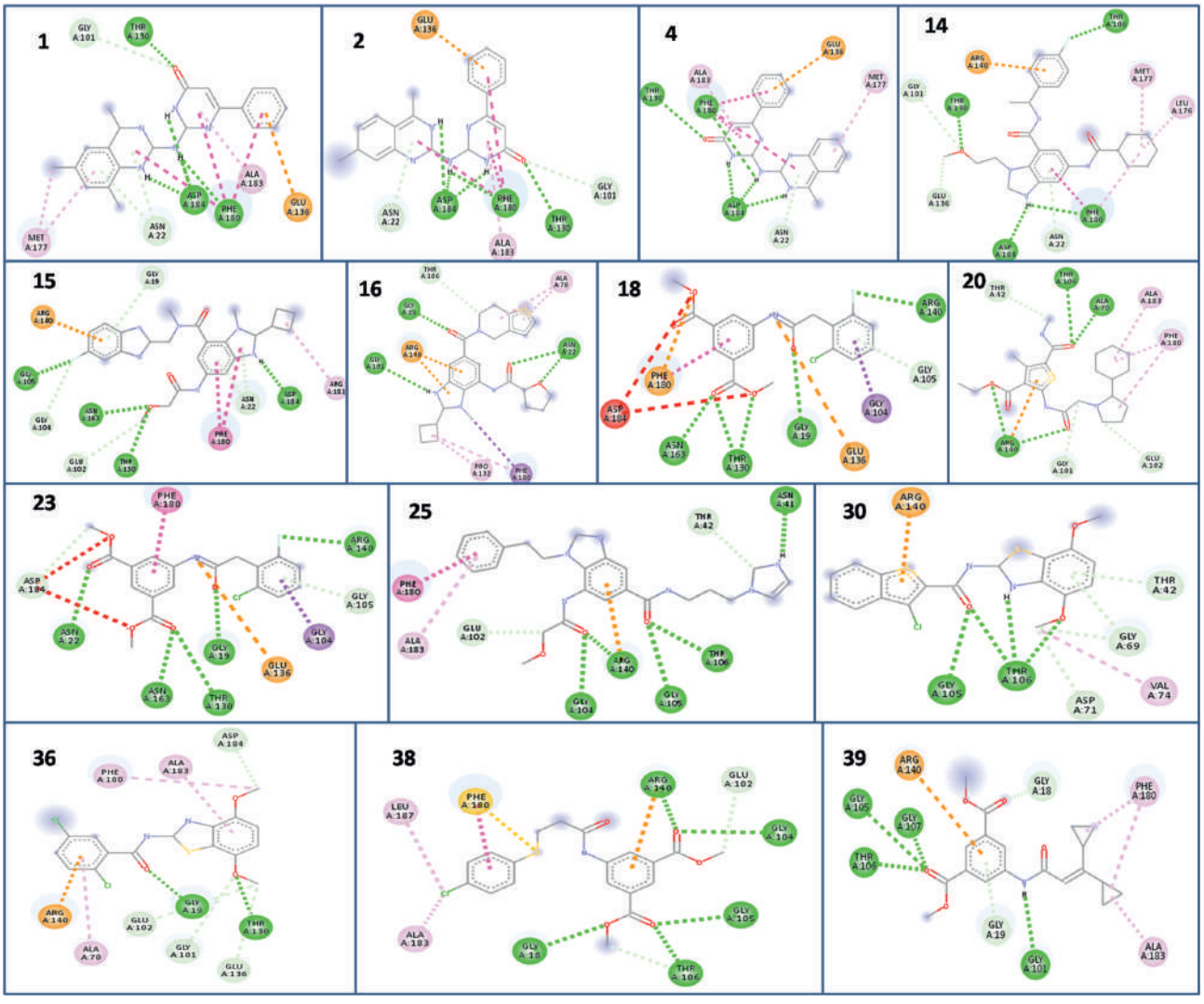


Fig. 6 – 2D visualistaion of protein-ligand complex of hits.

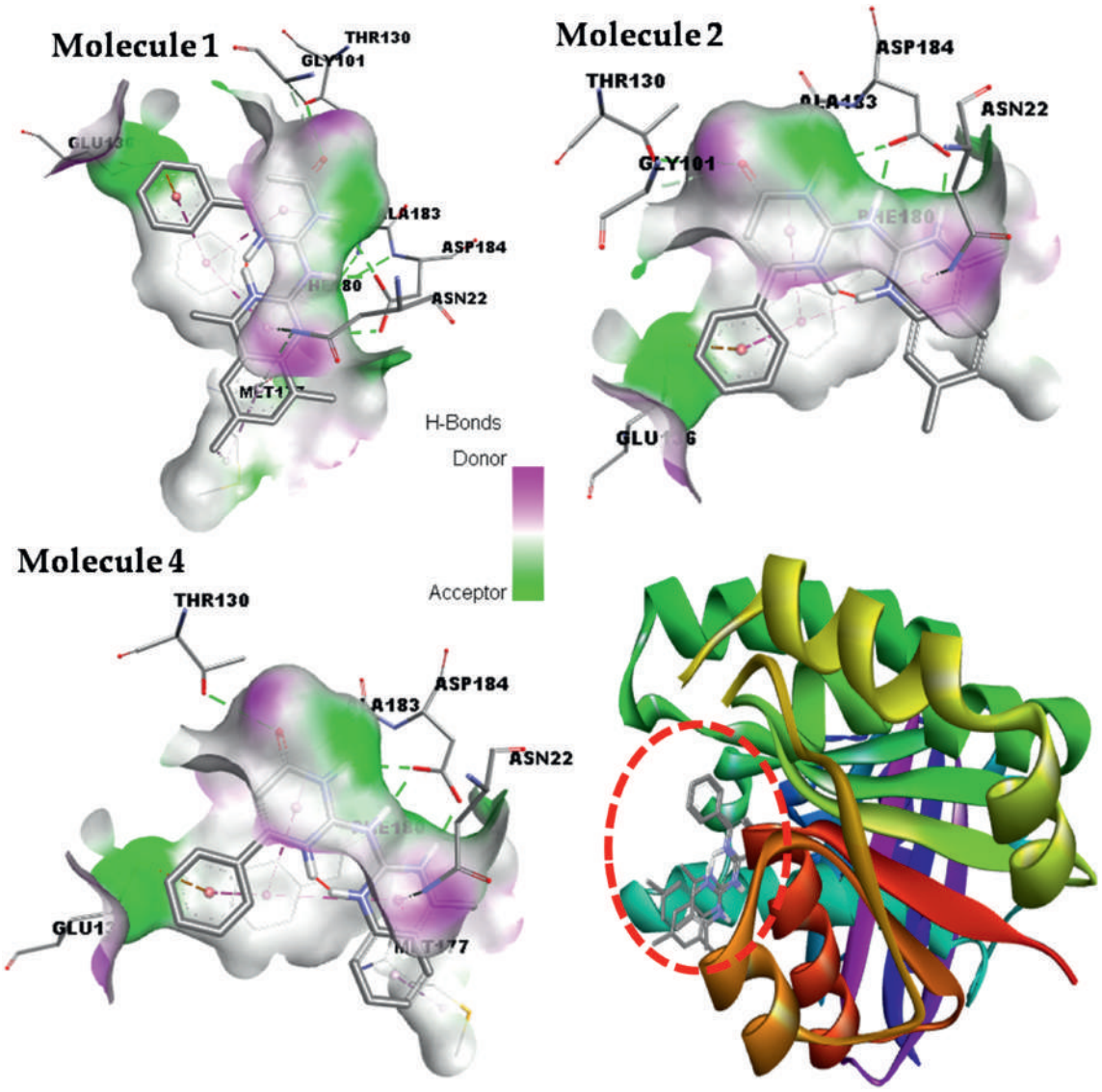


Fig. 7 – 3D visualization of top 3 candidates docked with ftsZ.

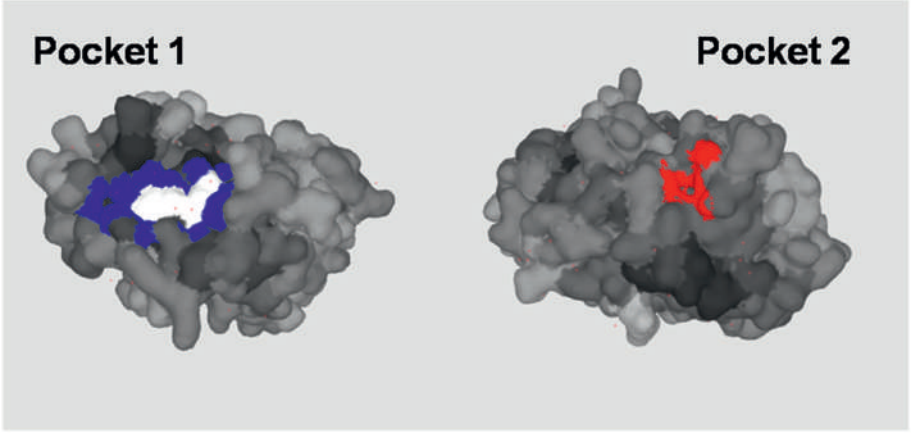


Fig. 8 – Predicted binding pockets of chain A protein target.

10. Results and discussion

i. Pharmacophore model:

For the 14 ligands aligned from the training set, 10 models were provided. The model with top score (32.400) was selected for further investigation. The model consisted of 6 spatial features – 1 aromatic, 2 hydrophobic, 1 donor and 2 acceptors. The visualization of the obtained pharmacophore model seen in Fig. 3 was done using BIOVIA, DassaultSystèmes, Discovery Studio Visualiser, v21.1.0.20,298, San Diego: DassaultSystèmes, 2021.[37] The distance between the spatial features (in Å) were measured to understand the orientation of the model. The distance matrix is shown in Table 2.

ii. QSAR analysis:

A QSAR model was built in BuildQSAR using the ChemoPy descriptors.[26] The activity value IC50 was converted to pIC50 by taking negative logarithmic value of the IC50 values in Molar. pIC50 is a dimensionless quantity. This conversion helped to get a linear regression plot.

$$pIC50 = -\log(IC50) \quad (1)$$

The top 4 models obtained using the training set is listed in Table 3. The model with the best R^2, Q^2 value was selected. The R^2 value, known as the coefficient of determination indicates the percentage of response variable variation. R^2 value greater than 0.7 is found to indicate that the data are close enough to the fitted regression line. Q^2 value determines the predictive capability of the model and a value higher than 0.6 and close to R^2 is considered as a good model.

Model 01 which used the descriptor chi10 denoting the atom connectivity index was chosen with regard to R^2 value = 0.837 and predictive capability Q^2 value = 0.756. The linear equation obtained was:

$$y = -0.722x + 6.275 \quad (2)$$

Here, y denotes the activity (dependent variable) and x the descriptor (independent variable). The above Eq. (2) is used to predict activities of training set and test set. The closeness of prediction can be seen in Table 4 along with the low residual value which denotes the difference of calculated and observed value.

$$Y\ (\text{residual value}) = Y\ (\text{calculated value}) - Y\ (\text{observed value}) \quad (3)$$

The linear regression plot obtained (training and test set) along with the Model 01 fitting parameters is shown in Fig. 4.

Leave-one-out cross validation was done using BuildQSAR[27] and it was found that for all n predictions with (n-1) samples, the R^2_{LOO} and Q^2_{LOO}were greater than 0.7 and 0.6 respectively.

iii. Hits analysis:

Using the pharmacophore model as template, 2638 hits were obtained from 'Zinc Purchasable Database'. Amongst the 2638 hits provided by the web server, 1128 unique compounds were taken for further analysis by removing the repeats. The RMSD values were lower than 0.8 indicating a good precision of the hits obtained. After the three stages of filtration based on Lipinski rule, PAINS and toxicity, 115 hits which had passed all these filters were obtained. Fig. 5 depicts the numerical workflow and filtering hierarchy implemented.

The 115 hits were further docked with ftsZ to predict the orientation and type of interactions between the protein and molecule. Only hits that crossed the threshold docking score which was obtained using average score of initial known 18 ligands were further analyzed (Table 5). A count of 80 hits was obtained crossing the threshold value of 6.8 kCal/mol. More so, the non-bond interactions between protein and ligands such as hydrogen bonds, hydrophobic, electrostatic bonds were visualized using Discovery Studio.[37] The hits with larger number of interactions were considered as potential compounds to inhibit ftsZ and the number of hydrogen bond interactions were taken as the basis of stability measurement. This arises from the fact that a greater number of interactions confer a higher stability on the protein – ligand interactions and hydrogen bond is stronger than other covalent and hydrophobic interactions. Hits with more than 10 non-bond interactions were considered to be most stable and were visualized. As a result, a set of 40 compounds were found to be

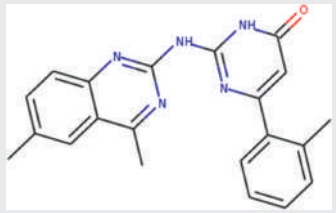
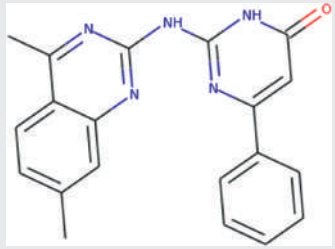
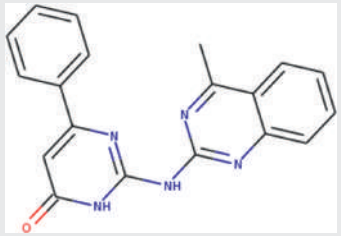
Table 8 – Active Pockets and residues of ftsZ A chain.

Pockets	Score	Residues
pocket1	30.87	A_101, A_102, A_104, A_105, A_106, A_107, A_130, A_132, A_136, A_140, A_16, A_163, A_17, A_18, A_180, A_183, A_184, A_187, A_19, A_22, A_41, A_42, A_63, A_69, A_70, A_74
pocket2	0.79	A_157, A_214, A_215, A_218, A_255, A_256, A_258, A_288, A_308, A_310

Table 9 – ADMET properties of top 3 molecules.

Mol. No.	Molecular formula	Mol. Wt	Bio-availability	iLOGP	Max. tolerated dose (human)	BBB permeability	Intestinal absorption (human)	Oral Rat Acute Toxicity (LD50) mol/kg	Total Clearance
1	C21H19N5O	357.417	0.55	2.68	0.766	−1.167	95.013	2.936	0.378
2	C20H17N5O	343.39	0.55	2.5	0.74	−1.023	94.55	2.73	0.406
4	C19H15N5O	329.363	0.55	2.24	0.79	−1.162	95.287	2.946	0.362

Table 10 – Spatial features of top 3 compounds.

Molecule No.	Structure	Spatial features	Aromatic	Hydrophobic	H-bond acceptors	H-bond donors
1		15	4	5	4	2
2		13	4	3	4	2
4		11	4	1	4	2

potential inhibitors of ftsZ. Using Eq. (2) obtained from QSAR linear regression model, the activity was predicted and tabulated for these compounds as shown in Table 6.

A potent lead molecule must be in sufficient concentration in its target while at the same time retaining its supposed bioactivity within the expected duration required to elicit biological action so as to be effective. Consequent upon this, the ADMET properties of the compounds were also tabulated using SWISSADME[31] and pKCSM[32] to quantify and qualify the lipophilicity, GI absorption capacity, total clearance, Pgpsubstrate, BBB penetrating capacity and acute oral rat toxicity (Table 7). Partition coefficient P of a molecule between two immiscible solvents has been described as a significant descriptor of its lipophilicity.[38] The reported Log P_o/w is a consensus value that represents the average of other five predictions that are based on different predictive method; ILOGP is based on an inhouse physics, XLOGP3 makes use of atomistic and knowledge-based method as formulated by Shanghai institute of Organic chemistry, WLOGP is only based on atomistic method while MLOGP applies topological method. A hybrid fragmental/topological method calculated by FILTER-IT program is applied in SILICOS-IT lipophilicity prediction.[31] Generally, Lipinski's rule has recommended that logP value less than 5 result in optimal ADME and physicochemical properties.[39] It was found that 36 out of 40 compounds analyzed abide by this rule concerning logP value. Water solubility is essential for drug targeted for oral solubility as it influences absorption, and drugs requiring parenteral administration should have high water solubility to enable a sufficient distribution of the pharmaceutical active ingredient from a small volume of the dosage to the target sites. GI absorption determines the rate of gastric emptying of drugs which also influences the plasma concentration of orally administered drugs and hence necessitates its measurement in drug discovery.[40] Blood brain barrier consists of structural and chemical barrier resulting from network of vessels which restricts the movement of substances from systemic circulation to the brain so as to protect the brain from harmful substances. However, some drugs and other useful and harmless substances do not permeate through this medium due to the restriction. Drugs used for treatment of brain disease should have good BBB permeability.[41] Permeability glycoprotein often described as multi-drug resistant protein is a vital component of the cell membrane which pumps various foreign substances away from the cells. The resulting ATP-dependent efflux pump is substrate specific which is significant in drug discovery and development.[42] Out of the existing over 50 CYP450, only CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5 are involved in the metabolism of about 90 percent of administered drugs and even though they occur in kidneys, placenta, small intestine and lungs, they are predominantly expressed in the liver cells.[43]

iv. Potential compounds identification:

Hydrogen bonding plays a significant role in protein-ligand binding due to the ubiquity and flexibility of hydrogen bonds between donors and acceptors in systems of biomolecules. Hydrophobic interactions that occur between non-polar amino acid side chains of protein and lipophilic groups of ligand influence binding in a solvent system and also proper folding and stacking of protein to be functional. Electrostatic interactions are strong bonds and influence protein-ligand complex interaction in a system due to frequent protonation state changes while binding. Therefore, a cumulative effect of all the three interactions helps the protein-ligand complex to be bound in several different environments and remain stable. It was observed that 14 amongst the 40 molecules show all the three interactions and a 2D visualization of the binding site was done using Discovery Studio[37] as seen in Fig. 6.

Based on the binding score, predicted activity as well as number of interactions between protein and ligand with having at least 50% of hydrogen bonds, molecules 1 (**ZINC10157410**), 2 (**ZINC05013094**) and 4 (**ZINC18203893**) were considered as best lead compounds through *in silico* approach. 3D visualization of the compounds is shown in Fig. 7. It is seen that the molecule 1 bind with residues Phe180, Asp184, Glu136, Asn22, Met177, Ala183, Thr130, Gly101; molecule 2 binds with Phe180, Asp184, Glu136, Asn22, Ala183, Thr130, Gly101 and molecule 4 binds with Phe180, Asp184, Glu136, Asn22, Met177, Ala183. These molecules were found to be on the high confident score pocket as predicted through prankweb online server (Fig. 8 and Table 8).

The lead molecules also have good ADMET properties, bioavailability score besides following Lipinski rule, passing PAINS alert, AMES test, hepatotoxicity and carcinogenicity. The properties and the spatial features of the top 3 molecules are listed in Table 9 and Table 10 respectively. This shows that the molecules that we selected are capable of being potential oral drug candidates for the inhibition of ftsZ.

11. Conclusion

In this ligand-based pharmacophore modelling, 14 FtsZ inhibitors were used as a training set and the resulting 1 aromatic center, 2 hydrophobic, 2 hydrogen bond acceptors and 1 hydrogen bond donor were validated against four test compounds. Based on the number of interactions, hydrogen bonds, extensive virtual screening and ADMET filtration, 40 compounds have been identified as potential inhibitors of ftsZ with only 3 molecules; ZINC10157410, ZINC05013094 and ZINC18203893 considered to be the best leads due to their high binding affinity (above −9.0 kCal/mol), lower IC50 value (~4 μM) and considerable ADMET properties. These molecules interacted with the active sites that have been both experimentally verified and predicted by prankweb online server. The study being based on pyridopyrazine and pyrimidothiazine derivatives have validated a previous report by Mathew et al[9] on the FtsZ inhibitory potencies of synthesized pyridopyrazine and pyrimidothiazine derivatives. These identified molecules have potential of being drug candidate against *mycobacterium tuberculosis* and may posses a novel mechanistic route in inhibiting the resistant strains, hence the findings in this work may be useful in further design and identification of novel drug candidates against FtsZ of *Mycobacterium tuberculosis*.

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Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2023.03.003.

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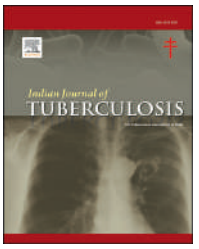

Original article

Comparison of gastric lavage/sputum and stool specimens in the diagnosis of pediatric pulmonary tuberculosis- A pilot study

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ABSTRACT

Background and Objective: Global TB report 2021 mentions 11 % prevalence of pediatric TB, whereas 5.65% of the cases were reported from India in 2020. India features in the list of TB high burden countries, HIV-TB high burden and MDR-TB high burden countries. The diagnosis of pulmonary tuberculosis in children is difficult as they tend to swallow the sputum, invasive techniques of gastric aspirates needs to be followed and the disease itself is paucibacillary. The disease progresses rapidly in young children and hence rapid diagnosis is needed. Obtaining appropriate respiratory samples for diagnosis is difficult especially in primary care settings. Stool sample is easy to obtain and since children swallow sputum, it can be used to diagnose pulmonary tuberculosis. With this background, a pilot study was planned to evaluate the accuracy of the Xpert MTB/RIF assay for the detection of MTB in stool specimens obtained from pediatric pulmonary TB patients confirmed either by gastric lavage(GL) or sputum(SP) Xpert MTB/RIF assay. In addition, the results of microscopy of stool specimen were compared with that of gastric lavage/ sputum (GL/SP) specimen by Ziehl-Neelsen (ZN) and fluorescent light-emitting diode (LED) staining.

Material and methods: A prospective study was carried out on 50 GL/SP Xpert MTB/RIF assay positive children (0-14 years). Stool specimens from these children were processed for Xpert MTB/RIF assay. The GL/SP and stool specimens were processed for ZN and Auramine O fluorescent microscopy as well.

Results: Fluorescent staining detected acid fast bacilli (AFB) in 24 GL/SP and 16 stool specimens as compared to 20 GL/SP and 10 stool specimens by ZN staining. Stool Xpert MTB/RIF assay was positive in 29 out of 50 children. Rifampicin resistance was detected in 13 of the 50 (26%) GL/SP specimens. Of these 13 children, rifampicin resistance was detected in 7 stool specimens, rifampicin indeterminate resistance was detected in one specimen and in the remaining 5 children, M.tuberculosis was not detected in stool.

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Conclusion: Stool is a good non-invasive specimen for the detection of pulmonary TB in children, especially in remote areas, where invasive techniques cannot be performed for sample collection.

1. Introduction

World Health Organization (WHO) announced the end tuberculosis (TB) strategy with a target of reducing TB deaths by 90%, and incidence by 80% by 2030.[1] The Indian Government has set a target to eliminate TB by 2025.[2] In 2020, in India, 5.65% of the total TB cases were in children (0–14 years).[3] In 2019, around 1,51,286 (only 44% of estimated) cases of pediatric TB were notified in India.[4] In 2018, TB infection was reported in about 11 lakh children, and deaths due to TB were reported in about 2,50,000 children globally.[4] It has been one of the top 10 causes of childhood mortality.[4]

The diagnosis of pulmonary tuberculosis in children is difficult as the disease itself is paucibacillary in nature. Children tend to swallow the sputum (SP), hence gastric lavage (GL) needs to be collected. Induced sputum, and nasopharyngeal aspirates, are also used to diagnose tuberculosis in children.[5] However, obtaining these samples is difficult especially in primary care settings as these samples need to be collected under the supervision and need some intervention. As children tend to swallow the sputum produced instead of coughing it out, bacilli enter in the gastrointestinal tract and are excreted in the stool. As stool sample is easy to obtain they can be used to diagnose pulmonary tuberculosis. Stool specimen collection can be done at home and a visit to the health care facility for specimen collection is not required.[5]

The disease progresses rapidly in young children and hence rapid and early diagnosis is needed. Microscopy by Ziehl-Neelsen (ZN) staining is a rapid and specific method for the diagnosis of tuberculosis. In 2011 WHO recommended light-emitting diode (LED) based fluorescent microscopy using Auramine O in place of ZN stain.[6,7] The advantage of LED-based fluorescent microscopy is that it is cheap, can be used in routine laboratories, and has a longer battery and bulb life. It is 5% more sensitive and 1% more specific than conventional fluorescent microscopy.[6] It has shown 84% sensitivity and 98% specificity as compared to culture on pulmonary specimens.[6] The added advantage of LED-based fluorescent microscopy is that screening of smears can be done in almost half the time and with minimal eye strain.[6,8,9]

In 2011 WHO recommended Cartridge Based Nucleic Acid Amplification test (CBNAAT) using Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA).[6,7] The Xpert MTB/RIF assay is a fully automated cartridge-based nucleic acid amplification test that simultaneously detects *M.tuberculosis* (MTB) and rifampicin resistance within 2 h.[10] WHO has approved this test for the detection of tuberculosis using various specimens except for stool. The novelty of the study is that it may provide information on the utility of non-invasive specimens in the detection of pediatric TB.

In this pilot study, we evaluated the accuracy of the Xpert MTB/RIF assay for the detection of MTB in stool specimens obtained from pediatric pulmonary TB patients in comparison with gastric lavage/sputum (GL/SP) Xpert MTB/RIF assay. In addition, the results of microscopy by ZN and LED fluorescent staining of stool specimens were compared with that of GL/SP specimens.

2. Methodology

A prospective cross-sectional study was planned in the Department of Microbiology at tertiary care teaching hospital in Mumbai after getting Institutional Ethics Committee approval (EC/OA- 105/2017). For sample size calculation, the prevalence was considered 10%,[11] the precision of 5%, and the confidence level of 95%. The sample size by Cochran's formula was 138. However, due to cost constraints, we restricted the sample size to 50.

The study was carried out on 50 pediatric patients positive for pulmonary tuberculosis from February 2017 to January 2021. Children less than or equal to 14 years of age, of any gender, who submitted GL/SP to this lab for Xpert MTB/RIF assay and in whom MTB was detected were included in this study. Parents/children not willing to give consent/assent and those already started on anti-TB treatment were excluded from the study. The demographic and clinical details of the enrolled children were recorded from the records in the file. Based on the positive Xpert MTB/RIF assay report, a sterile container was provided and instructions were given for the collection of a spoonful of a stool specimen. The stool was processed immediately for both microscopy techniques and Xpert MTB/RIF assay.

GL/SP and stool specimens were processed for microscopy as per the standard procedure for ZN staining and LED-based fluorescent microscopy.[12,13] Two smears were prepared from each specimen on separate new, clean grease free slides. One smear was stained with the ZN staining and another with Auramine O fluorescent staining technique. The ZN-stained smears were observed under an oil immersion (100×) objective lens. The Auramine O-stained smears were observed under high power (40×) objective lens. Results were recorded as per guidelines.[12,13] The GL/SP specimens were processed for Xpert MTB/RIF assay as per WHO guidelines,[7] whereas the stool specimens were processed as described by Nicol et al.[5]

Processing of stool specimen for Xpert MTB/RIF (Xpert) assay:

A small amount (0.15g) of stool specimen was picked up by a wooden stick and placed in 2.4 ml sterile phosphate-buffered saline (PBS) in a Falcon tube and vortexed briefly. It was kept on the table undisturbed for 20 min at room

temperature. 1–1.5 ml of supernatant was removed in a centrifuge tube and centrifuged at 3200g for 15 min. After centrifugation, the supernatant was removed with a pipette, and 1 ml of PBS was added to the sediment. The centrifuge tube was vortexed again. This was used as a specimen for Xpert assay and the test was performed as described by Nicol et al.[5]

The results of the Xpert assay of GL/SP specimens were considered standard and the results of the Xpert assay of stool specimens were compared with it.s.

3. Results

Consecutive 50 children up to the age of 14 years having positive results on Xpert assay of pulmonary specimens (GL/SP) were included in the study. Of these, 9 children were in the age group of 0–5 years and submitted gastric lavage as a specimen. 41 children were in the age group of 6–14 years and submitted sputum specimens for Xpert assay. Of the 50 children, 18 were male and 32 were female.

44 children were from economically backward classes. 47(94%) children stayed in urban areas while 3 children stayed in rural areas. 47(94%) children had a history of recent weight loss or failure to gain weight in the last three months. Cough was present in 40 children (80%), 20(50%) of whom had no complaint of any expectoration. 12(30%) children had white expectoration, 7(17.5%) had yellow expectoration and 1(2.5%) had green expectoration. Hemoptysis was seen in only one child, and that too in a single episode prior to giving the sample. Fever was present in 46(92%) children, 8(17.4%) of these children had a high-grade fever as per the parent's assessment. Household contacts of tuberculosis in the previous 3 months were reported in 12(24%) children. A tuberculin skin test was performed on 16 children. Of these, 10 showed positive (>10 mm) tuberculin skin test.

Chest radiograph results were available in 48 children. Of these, 31(64.6%) were suggestive of tuberculosis. 24 of these had consolidation, 2 had consolidation with pleural effusion, 5 had cavitary lesions and 1 had bilateral peri-lobular infiltrates. In 17(35.4%) children, a chest radiograph was not suggestive of tuberculosis. Of these 17 cases, the bacillary load in GL/SP Xpert assay was low in 10 and very low in 7. Stool Xpert assay could detect only 5 of these cases, the bacillary load of which was 1 medium, 1 low, and 3 very low. 1 stool Xpert was invalid and 11 failed to detect MTB.

The gross appearance of stool showed the following colors – 6(12%) (black), 3(6%) (brown-black), 25(50%) (brown), 7(14%) (brown-yellow), 7(14%) (yellow), 1(2%) (brown-orange), 1(2%) (brown-orange with mucus). The consistency was formed in 35(70%), semi-formed in 14(28%), and liquid in 1(2%). There was no gross appearance of blood in 48 stool samples. However occult blood was positive in 2 samples.

3.1. *Microscopy*

Fluorescent staining detected acid-fast bacilli (AFB) in 24 GL/SP (pulmonary) specimens as compared to 20 by ZN staining. All GL/SP specimens positive by ZN were also positive by fluorescent microscopy. Of the 24 pulmonary positive specimens in LED microscopy, AFB was detected in 13 (54.2%) stool specimens. AFB was detected in 3 (11.6%) additional stool specimens which were negative in pulmonary specimens. Of the 20 pulmonary positive specimens in ZN microscopy, AFB was detected only in 8 (40%) stool specimens. AFB was detected in 2 (6.7%) additional stool specimens which were negative in pulmonary specimens. (Table 1), (Table 2)

5. Xpert MTB/RIF assay

5.1. *Gastric lavage/sputum*

50 specimens positive by Xpert MTB/RIF assay were included in the study. These 50 specimens had bacillary loads ranging from high (6), medium (14), low (19), to very low (11). Rifampicin resistance was detected in 13(26%) specimens.

5.2. *Stool*

MTB was detected in 29 (58%) specimens. Bacillary load varied from medium (4), low (16) to very low (9). None of the stool specimens showed high bacillary load. Of the 13 children having rifampicin resistance in GL/SP specimens, 7 stool specimens showed rifampicin resistance. Of the remaining 6 specimens, one showed indeterminate rifampicin resistance and MTB was not detected in 5 stool specimens. No additional rifampicin resistance was detected in any stool specimen. One stool specimen showed an invalid Xpert MTB/RIF assay result **(Table 3).**

6. Discussion

Early diagnosis of TB is very important to start appropriate treatment, especially in children. For accurate microbiological diagnosis, good quality and a sufficient quantity of specimen is the basic need. But the collection of such respiratory specimens in children is a task. Collection of gastric lavage in a child less than 5 years of age needs preparation like keeping the child nil by mouth for the required period, putting the Ryle's tube with a lot of resistance from the child, and rapid

Table 1 – Comparison of Gastric lavage/Sputum and Stool specimens by LED Fluorescent microscopy.

		Gastric lavage/ Sputum LED Fluorescent Microscopy		Total
		Positive	Negative	
Stool LED Fluorescent Microscopy	Positive	13	3	16
	Negative	11	23	34
	Total	24	26	50

Sensitivity: 54.2% [95% CI: 32.8%–74.5%].
Specificity: 88.5% [95% CI: 69.9%–97.6%].
Positive Predictive Value: 81.3% [95% CI: 58.4%–93.0%].
Negative Predictive Value: 67.6% [95% CI: 57.0%–76.8%].
Accuracy: 72% [95% CI: 57.5%–83.8%].
P value: 0.001 (Significant).

Table 2 – Comparison of Gastric lavage/Sputum and Stool specimens by ZN microscopy.

		Gastric lavage/ Sputum ZN Microscopy		Total
		Positive	Negative	
Stool ZN Microscopy	Positive	8	2	10
	Negative	12	28	40
	Total	20	30	50

Sensitivity: 40% [95% CI: 19.1%–63.9%].
Specificity: 93.3% [95% CI: 77.9%–99.2%].
Positive Predictive Value: 80% [95% CI: 48.6%–94.4%].
Negative Predictive Value: 70% [95% CI: 61.7%–77.2%].
Accuracy: 72% [95% CI: 57.5%–83.8%].
P value: 0.009 (Significant).

Table 3 – Comparison of bacillary load in gastric lavage/ sputum and stool Xpert MTB/RIF assay(n = 50).

		Gastric lavage/Sputum Xpert MTB/Rif assay load				
		High	Medium	Low	Very low	Total
Stool Xpert MTB/ RIF assay load	High	0	0	0	0	0
	Medium	3	0	0	1	4
	Low	2	10	3	1	16
	Very low	1	1	7	0	9
	Negative	0	3	8	9	20
	Invalid	0	0	1	0	1
	Total	6	14	19	11	50

transport of the specimen to the lab to prevent the action of gastric acid. Stool sample collection rules out all such inconvenience to the patient as it is easy to collect in sufficient quantity and there is no need to carry a sick child to the laboratory. Hence this study was performed to compare the performance of stool specimens in comparison with gastric lavage/sputum specimens in the diagnosis of pediatric pulmonary tuberculosis.

Of the 50 children enrolled in the study, weight loss/failure to gain weight, fever, and cough were the primary presenting symptoms in the majority of children. These symptoms have been reported in other studies as well[14,15,16,17,18]. History of close contact with a case of tuberculosis was reported in about one-fourth of the cases. Various studies reported contact with a TB patient in the family in 12%–56% of the cases[15,16,17,18,19,20,21,22].

In 64.6% (31/48) of patients, a chest radiograph was suggestive of tuberculosis. Chest radiographs failed to detect tuberculosis in 35.4% (17/48) of the cases. It may be due to the low bacillary load and lung pathology not being sufficient enough to be detected on chest radiographs. However, amongst stool Xpert-positive patients, 81.5% (22/27) had an x-ray chest suggestive of tuberculosis. Walters et al.[15] reported radiologically severe disease in 100% of the stool Xpert-positive cases.

The stool processing for Xpert MTB/RIF assay was done as per Nicol et al.[5] A similar method has been followed in a few other studies.[17,19] In the present study, the reference standard used was MTB detected by Xpert MTB/RIF assay in GL/SP specimens. It was observed that Xpert MTB/RIF assay detected a lower number of cases with stool specimens (29/50) compared to pulmonary specimens (50) in the same patient. Banada et al.[20] in their study compared stool Xpert with gastric wash/induced sputum Xpert. Few other studies compared stool Xpert with gastric aspirate/induced sputum/ sputum culture and Xpert.[5,15–19,21,22]

LED Fluorescent microscopy reported an 8% and 20% increase in positivity for GL/SP and stool specimens as compared to ZN staining. A similar increase in positivity was reported in sputum samples in various studies.[8,23,24]

The Xpert assay could detect MTB in 29 (58%) stool specimens of the 50 cases positive by Xpert assay in GL/SP specimen. One specimen gave invalid results even on repeat testing. All 6 patients having a high bacterial load in GL/SP were detected by stool specimen. Xpert assay failed to detect 20 cases of having medium (3), low (8), and very low (9) bacteriologic load in GL/SP specimens. It may be due to the small amount (0.15g) of stool mass considered for processing.[21] The second reason may be that a single stool specimen was collected from each patient. In low/very low bacillary load GL/SP Xpert cases, the bacilli may be shedded intermittently due to which stool Xpert may have failed to detect the TB bacilli.[21] The use of a second sample has shown an increase in the detection of cases in a few other studies.[21,25,26] Moussa et al.[21] have reported 83.3% sensitivity of stool Xpert in confirmed (Sputum/Induced sputum culture positive) TB patients. Their study used 2 cm^3 of stool mass and paired stool samples from each patient. Banada et al.[20] suggested using a stool mass of 0.6 gm and Xpert testing thrice to increase the sensitivity. In the present study, multiple-sample testing was not carried out due to cost constraints. The third reason may be the level of detection for respiratory samples is about 131 CFU/ml[27] and that for experimental Macaque stool was determined to be approximately 1000 CFU/ml.[15,20] The fourth reason may be interference by PCR inhibitors present in stool specimens. The PCR inhibitors in stool can be inactivated by pre-treatment with stool processing buffer.[20] This was not undertaken in the present study.

Rifampicin resistance was detected in 13 GL/SP specimens and the remaining 37 specimens showed no Rifampicin resistance. Concordant results were obtained in 7 stool specimens for the presence of Rifampicin resistance and in 21 stool specimens for the absence of Rifampicin resistance.

The objective of the study was to evaluate the accuracy of the Xpert MTB/RIF assay for detecting MTB in stool specimens. But since the GL/SP Xpert assay variable has only one category i.e. they were all positive, statistical tests could not be applied. There is a significant association between Xpert MTB/RIF assay performed on the stool and GL/SP specimens. Based on data from 29 positive samples (both by stool and GL/SP Xpert assay), the chi-square test was applied and there is a significant association between the two variables ($p < 0.0001$).

One of the limitations of this study is that due to cost constraints, the sample size was restricted to 50 and only one sample per patient was tested.

6.1. *Conclusion*

Stool is a good non-invasive specimen in children for the detection of pulmonary tuberculosis by using Xpert MTB/RIF assay in situations where pulmonary specimens cannot be collected. However, the sensitivity is low as compared to pulmonary specimens. Fluorescent microscopy is more sensitive as compared to ZN staining in both GL/SP and stool specimens. A study with a large number of samples needs to be conducted for generalizing the findings.

Conflicts of interest

The authors have none to declare.

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Original article

One-pot synthesis, spectral characterization, biological evaluation, molecular docking studies and *in silico* ADME/Tox profiling of new 2,4,5 triaryl imidazole derivatives as anti tubercular agents

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ABSTRACT

Background: Tuberculosis still looms large on the global epidemiological radar and warrants continuous effort in the direction of developing new anti TB drugs to battle evolving resistance mechanisms of the causative agent *Mycobacterium tuberculosis*.

Methods: In the present paper, synthesis of n has been attempted. All the synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, IR and Mass spectroscopy. Anti TB profile of the synthesized compounds were tested by MABA assay employing M.*tb* H37Rv strain.

Results: Two compounds namely N-(2-acetoxy)-N-methyl-4-(4,5-diphenyl-1H-imidazole-2-yl) benzenamine and 2-(N-(4-(4,5-bis(4-methoxyphenyl)-1H-imidazole-2-yl)phenyl)-N-methylamino) ethanol exhibited impressive anti TB inhibitory potential with an **MIC** of 3.125 μg/mL. To visualize the binding interactions of the active compounds molecular docking studies were carried out on putative target M. *tuberculosis* Glutamine synthetase (MtGS) in complex with a trisubstituted imidazole. To ascertain their drug likeliness and safety profile *in silico* ADME/T prediction was performed on all the synthesized compounds.

Conclusion: Three compounds **1a**, **2g** and **2c** exhibited good inhibitory potency against M.*tb* H37Rv and all the synthesized compounds also show promising antifungal activity.

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1. Introduction

The war between the microbes and human beings is as old as the history of mankind on this planet. Tuberculosis (TB) is one of such battles the human race is waging since very long and even today TB remains as one of the major health threats globally.[1,2] TB is caused by *Mycobacterium tuberculosis* (M.*tb*) that primarily infects lungs and the infection if uncontained may subsequently spread to the other parts of the body which may be lethal.[3] In 2019 alone TB related death toll is 1.2 million and what is more alarming is registry of nearly 0.4 million new multidrug resistant (MDR) TB cases. Besides this WHO estimates, one fourth of world's population to be reservoirs of M*tb* but are clinically asymptomatic as well as non-infectious and this condition is marked as latent TB. In People with lowered or compromised immune response latent TB flares up to active TB and this is most common in HIV positive people with compromised immune system. In HIV/TB co-infection the mortality rate is very high and in 2019 around 0.2 million HIV positive people died due to HIV/TB co-infection. High mortality rates of TB complexed with emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) TB calls for an urgent need to step up the volume of novel anti TB drug candidates.[4]

Imidazole moiety has long been known to display diverse biological activity against variety of microbes like bacteria, virus and fungus.[5–7] Quite a good number of imidazoles are reported to be highly active against M*tb* and for this reason a scaffold built on imidazole was conceived.[8,9] The following Fig. 1, depicts some highly potent anti TB agents with imidazole moiety incorporated in their structural framework.

2. Experimental

2.1. Materials and methods

All starting materials, reagents, and solvents were commercially available and used after purification. Melting points of the compounds were determined in open capillary tubes using melting pointing apparatus. Infrared (IR) spectra of the compounds were recorded on a perkin Elmer FTIR 400 Spectrometer as KBr pellets (for solids) and the IR data is reported in wave numbers (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra of the compounds were determined in $CDCl_3$ and DMSO solutions on 400 and 100 MHz Bruker Ultra shield Arance spectrometers respectively (Instrument Bruker Ultra shield Arance III Nano. Chemical shift signals are provided in δ (parts per million) relative to TMS, and coupling constants (J) are expressed in Hertz (Hz). Flash column chromatography was performed using silica gel (Merck, 80–100 Mesh). All reactions were carried out in oven-dried glassware. All the synthesized compounds are characterized by spectroscopic (^{1}H NMR, ^{13}C NMR and ESI-MS) data and were found to be in good agreement with their corresponding structures (Spectral data can be viewed in supplementary Information).

3. Synthesis of 2,4,5 triaryl imidazole derivatives

On the synthesis front, literature is replete with variety of ways to synthesize triaryl imidazoles and a noteworthy one is

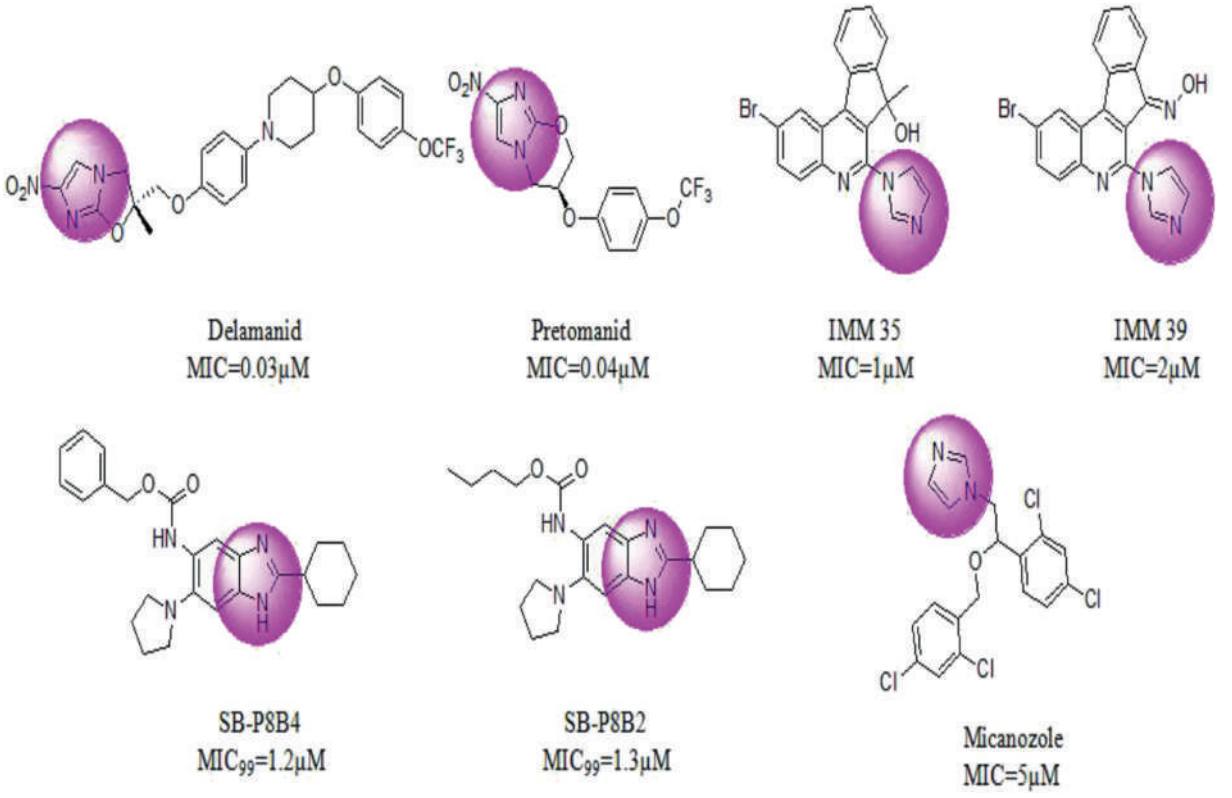

Fig. 1 – Potent molecules against *Mycobacterium tuberculosis* featuring an imidazole moiety.

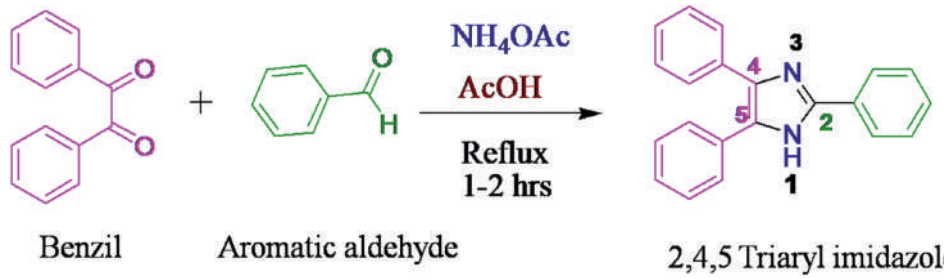

Fig. 2 – A general scheme of Radiszewski Synthesis.

Radiszewski Synthesis (Fig. 2), a multi component one-pot synthesis with relatively high yields.[10–14] Triaryl imidazoles with reasonably high hydrophobicity would likely enhance better penetration through lipid rich Mycobacterial cell wall and hence present an attractive avenue to start with.[15–17] Furthermore, keeping in view the ability of hydroxyl and substituted amine groups in enhancing hydrogen bonding ability of the compounds with the amino acid residues at the binding site of target protein, triaryl imidazoles with these moities have been explored.[18]

The following paragraph describes the typical synthesis procedure adopted for compounds 1a-1d and 2a-2d. A mixture of Benzil (2mmol), benzaldehyde (2mmol) and ammonium acetate (6mmol) was dissolved in 20mL glacial acetic acid taken in 100mL round bottom flask. The reaction mixture was then refluxed at 150 °C for 5 hours and completion of the reaction was established using TLC. The reaction mixture was cooled to room temperature and 50 mL of distilled water was added. The pH of the solution was adjusted to 7 by dropwise addition of dilute ammonia solution. The details of the scheme have been presented in Fig. 3.

The solid precipitated was filtered and washed with water. The dried compound was then subjected to column chromatography to obtain the compound of interest. Mobile phase of 6–8% ethyl acetate in n-hexane (v/V) was used and the purity of the final compound was verified by TLC. The compounds 2a-2d are esters and are prodrugs. One millimole of ester(2a-2d), was taken in a 50 mL round bottom flask. To it 20 mL of 20% HCl was added and the mixture was reflued for 2 hours. The hot mixture was poured into ice cold water to crystallize solid of interest.The compound was filtered, dried and its purity was established by using TLC. The compound obtained was pure and needed no futher purification.

3.1. *Spectral data of synthesized compounds*

3.1.1. *5-(diethylamino)-2-(4, 5-diphenyl-1H-imidazole-2-yl) phenol (1a)*

White solid; Yield: 75%, Mp.138–140 °C,IR (KBr) $\bar{\nu}$ = 1212(C–N), 1562(C=C), 1630(C=N),2926(C–H), 3232(O–H);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (t, J = 7.03 Hz, 6H), δ3.35 (q, J = 7.03 Hz, 4H), δ6.23 (d, J = 8.78, 1.25 Hz, 1H), δ6.32 (d, 1H), δ7.26–7.37 (m, 7H), δ7.56 (d, J = 7.28 Hz, 4H) ^{13}CNMR (100MHz, $CDCl_3$): 12.67, 44.42, 99.2, 100.82, 103.62, 124.95, 127.57, 127.78, 128.60, 132.04, 146.84,149.99,158.94; MS(ESI) for $C_{25}H_{25}N_3O$ $[M+1]^+$, calcd: 384.2, found: 384.

3.1.2. *2-(4,5-bis(4-bromophenyl)-1H-imidazole-2-yl)-5-(diethylamino) phenol (1b)*

Pale yellow solid; Yield: 80%, Mp.235–236 °C,IR (KBr) $\bar{\nu}$ = 1219(C–N), 1558(C=C), 1635(C=N),2970(C–H), 3226(O–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16 (t, J = 6.02 Hz, 6H), δ 3.33 (d, J = 6.53 Hz, 4H), δ 6.23 (d, J = 8.53 Hz, 1H),), δ 6.28 (S, 1H), δ7.32–7.38 (m, 5H), δ7.41–7.46 (m, 4H) ^{13}CNMR (100MHz, $CDCl_3$):12.67, 44.8, 99.03, 103.66, 121.65, 124.69, 129.22, 131.89, 147.44, 149.99, 158.92; MS(ESI) for $C_{25}H_{23}Br_2N_3O$ $[M+1]^+$, calcd: 540.02, found: 540.

3.1.3. *2-(4,5-bis(4-methoxyphenyl)-1H-imidazole-2-yl)-5-(diethylamino)phenol (1c)*

Pale brown solid; Yield: 76%; Mp.125–128 °C, IR (KBr) $\bar{\nu}$ = 1242(C–N), 1562(C=C), 1630(C=N),2924(C–H), 3330(O–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16 (t, J = 7.15 Hz, 6H), δ3.33 (q, J = 6.94 Hz, 4H), δ 3.81 (S, 6H), δ 6.21 (d, J = 8.78 Hz, 1H), δ6.32 (d, J = 2.26 Hz, 1H), δ6.86 (d, J = 8.53 Hz, 4H), δ7.34 (d, J = 8.78 Hz, 1H), δ7.46 (d, J = 8.53 Hz, 4H) ^{13}CNMR (100MHz, $CDCl_3$): 12.71, 44.46, 55.28, 99.12, 99.12, 103.45, 114.07, 124.87, 128.96, 146.14, 159.06; MS(ESI) for $C_{27}H_{29}N_3O_3$[M+1]$^+$, calcd: 444.22, found: 444.35.

3.1.4. *2-(5-(2-chlorophenyl)-4-(3,4-dimethoxy phenyl)-1H-imidazole-2-yl)-5-diethylamino)phenol (1d)*

Pale yellow solid; Yield: 74%, Mp:128–133 °C, IR (KBr) $\bar{\nu}$ = 1257(C–N), 1508(C=C), 1630(C=N),2931(C–H), 3311(O–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ1.19 (t, J = 7.01 Hz, 6H), δ3.37 (q, J = 6.98 Hz, 4H), δ3.68 (S, 3H), δ 3.87 (S, 3H), δ 6.27 (d, J = 8.64 Hz, 1H), δ6.34 (s, 1H), δ 6.81 (d, J = 8.00 Hz, 1H), δ7.05 (s, 1H), δ7.24–7.37 (m, 4H), δ 7.47 (d, J = 8.00 Hz, 1H), δ 7.52 (d, J = 8.00 Hz, 1H); ^{13}CNMR (100MHz, $CDCl_3$): 12.67, 44.49, 55.51, 55.81, 99.11, 103.59, 109.91, 111.12, 118.99, 124.72, 127.02, 129.78, 130.16, 132.77, 134.11, 146.66, 148.24, 148.67, 149.87, 158.93; MS(ESI) for $C_{27}H_{28}ClN_3O_3$ $[M+1]^+$, calcd:478.18, found:478.35.

3.1.5. *N-(2-acetoxy)-N-methyl-4-(4,5-diphenyl-1H-imidazole-2-yl) benzenamine(2a)*

Pale yellow solid; Yield: 72%, Mp:177–180 °C, IR (KBr) $\bar{\nu}$ = 1215(C–N), 1499(C=C), 1612(C=N), 1740(C=O), 2959(C–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ1.97 (S, 3H), 2.98(S, 3H), δ 3.59 (t, J = 6.02 Hz, 2H), δ 4.22 (t, J = 6.02 Hz, 2H), δ6.70 (d, J = 8.78 Hz, 2H), δ7.21–7.31 (m, 6H), δ7.50 (d, J = 7.03 Hz, 4H), δ7.74 (d, J = 8.78 Hz, 2H); ^{13}CNMR (100MHz, $CDCl_3$): 20.90, 38.59, 50.86, 61.46, 111.95, 118.33, 126.66, 127.11, 127.84, 128.20, 128.47, 133.2, 146.90, 149.24, 171.16; MS(ESI) for $C_{26}H_{25}N_3O_2$ $[M+1]^+$, calcd:412.19, found: 412.35.

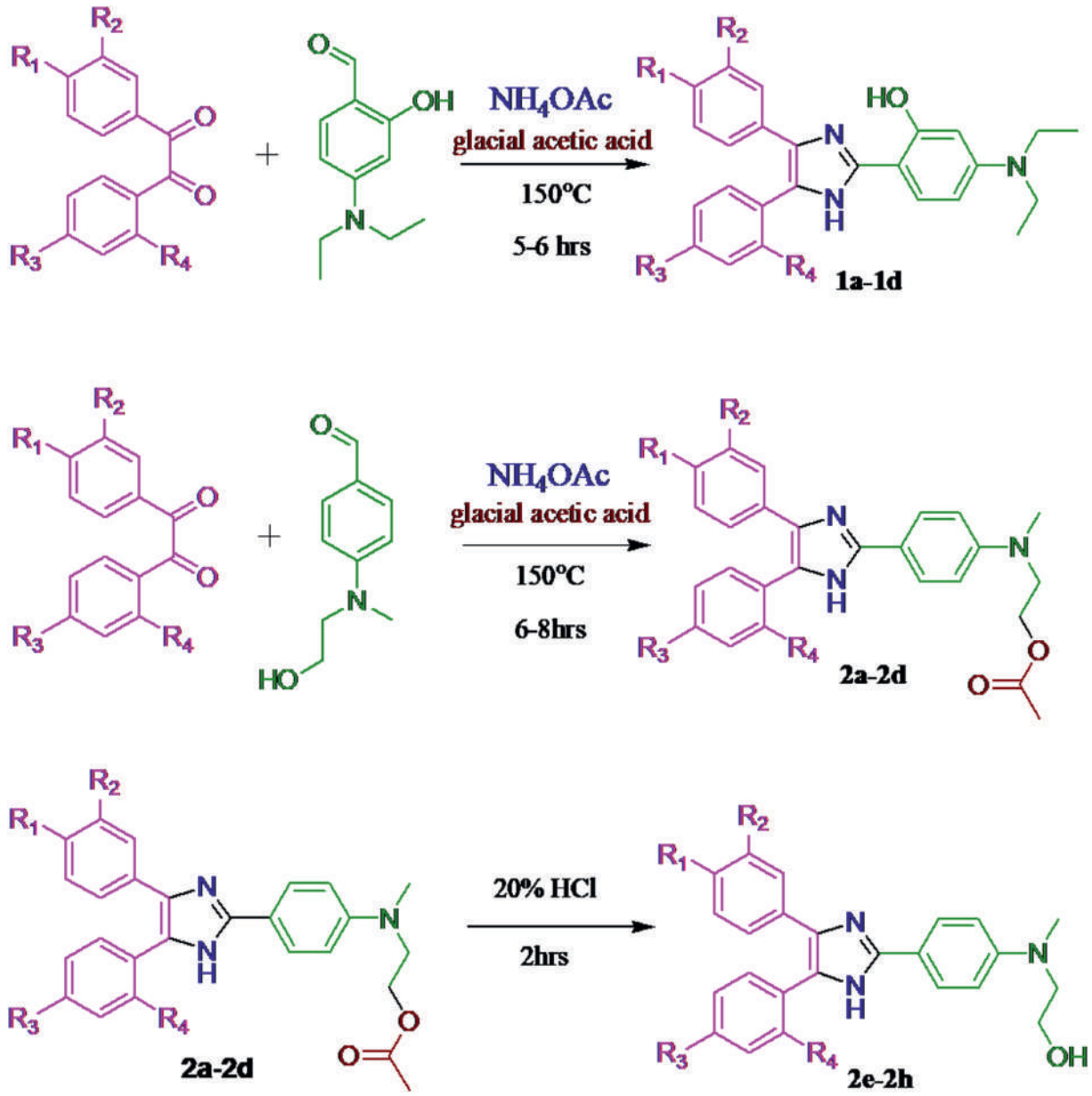

Fig. 3 – Scheme for synthesis of compounds reported in the present work. Details of substituents and activity and be seen in Table 1.

3.1.6. *N-(2-acetoxyethyl) -N-methyl 4-(4,5-bis(4-bromophenyl)-1H-imidazole-2-yl)-benzenamine (2b)*
Pale brown solid: Yield: 78%, M.p 218–222 °C, IR (KBr) $\bar{\nu}$ = 1218(C–N), 1498(C=C), 1613(C=N), 1737(C=O), 2959(C–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ2.01 (S, 3H), 3.03(S, 3H), δ 3.64 (t, J = 6.02 Hz, 2H), δ 4.26 (t, J = 6.02 Hz, 2H), δ6.74 (d, J = 9.03Hz, 2H), δ7.25–7.46 (m, 8H), δ7.74 (d, J = 9.03 Hz, 2H); ^{13}CNMR (100MHz, $CDCl_3$): 20.93, 38.66, 50.83, 61.43, 111.88, 117.86, 121.19, 126.79, 129.38, 131.63, 147.60, 149.37, 171.18; MS(ESI) for $C_{26}H_{23}Br_2N_3O_2$, calcd:570.29, found 570.15.

3.1.7. *N-(2-acetoxyethyl) -N-methyl 4-(4,5-bis(4-anisyl)-1H-imidazole-2-yl)-benzenamine (2c)*
Pale yellow solid; Yield: 73%, Mp. 152–154 °C, IR (KBr) $\bar{\nu}$ = 1244(C–N), 1502 (C=C), 1614(C=N), 1730(C=O), 2925(C–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ2.00 (S, 3H), 3.02(S, 3H), δ 3.63 (t, J = 6.02 Hz, 2H), δ3.81(S, 6H), δ 4.26 (t, J = 6.02 Hz, 2H), δ6.74 (d, J = 8.78 Hz, 2H), δ6.85 (d, J = 8.78 Hz, 4H) δ7.45 (d, J = 8.53 Hz,4H), δ7.76 (d, J = 8.78 Hz, 2H); ^{13}CNMR (100MHz, $CDCl_3$):20.90, 38.61, 50.85, 55.25, 61.44, 111.90, 113.91, 126.85, 129.13, 132.69, 145.99, 149.35, 158.83, 171.07; MS(ESI) for $C_{28}H_{29}N_3O_4$ $[M+1]^+$, calcd:472.22, found: 472.35.

3.1.8. *4-(4-(2-chlorophenyl)-5-(3,4-dimethoxyphenyl)-1H-imidazole-2-yl)-N-(2-acetoxyethyl)-N-methylbenzenamine (2d)*
Pale brown solid; Yield: 68%, Mp. 115–118 °C, IR (KBr) $\bar{\nu}$ = 1250(C–N), 1502 (C=C), 1614(C=N), 1737(C=O), 2924(C–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ2.00 (S, 3H), 3.01(S, 3H), 3.60–3.65 (m, 5H), δ3.83 (S, 3H), 4.25 (t, J = 5.90 Hz, 2H), 6.75 (m,3H), 6.69 (d, J = 4.00 Hz, 2H), δ7.25–7.30 (m, 2H), δ7.40 (d, J = 7.28 Hz,1H),

Table 1 – Antitubercular activity of synthesized molecules along with substituents.

Entry	R_1	R_2	R_3	R_4	*M. tuberculosis* H37Rv MIC(μg/mL)
1a	H	H	H	H	**3.125**
1b	Br	H	Br	H	25
1c	OMe	H	OMe	H	25
1d	OMe	OMe	H	Cl	25
2a	H	H	H	H	25
2b	Br	H	Br	H	>25
2c	OMe	H	OMe	H	**6.25**
2d	OMe	OMe	H	Cl	25
2e	H	H	H	H	25
2f	Br	H	Br	H	25
2g	OMe	H	OMe	H	**3.125**
2h	OMe	OMe	H	Cl	25
Standard	Isoniazid				0.059
	Rifampicin				0.411

δ7.45 (d, J = 8.03 Hz, 1H) δ7.78 (d, J = 8.03 Hz, 2H); ^{13}CNMR (100MHz, $CDCl_3$): 20.89, 38.61, 50.83, 55.43, 55.73, 61.44, 110.04, 111.01, 111.82, 111.93, 118.14, 118.97, 126.70,126.83, 129.86, 132.75, 134.07, 146.76, 147.87, 148.57, 149.24, 171.13; MS(ESI) for $C_{28}H_{28}ClN_3O_4$ [M+1]+, calcd: 506.18, found: 506.35.

3.1.9. 2-(N-methyl-N-(4-(4,5-diphenyl-1H-imidazole-2-yl) phenyl)amino)ethanol (2e)

White solid; Yield: 72, Mp. 198–202 °C, IR(KBr) $\bar{\nu}$ = 1253.73(C–N), 1512.19 (C=C), 1612.49(C=N), 2935.66(C–H), 3313.71(O–H);^{1}H NMR (400 MHz, DMSO-d_6): δ3.02 (S, 3H), δ3.48 (brS, 2H), δ3.59 (brS, 2H), δ6.82 (d, J = 8.53 Hz,2H), δ7.32–7.42 (m, 7H), δ7.50–7.54 (m, 5H), 7.92 (d, J = 8.53 Hz, 2H) ^{13}CNMR (100MHz, $CDCl_3$): 38.62, 55.40, 58.07, 111.34, 118.19, 126.54, 127.35, 129.62, 133.02, 145.83, 148.29; MS(ESI) $[M+1]^+$ for $C_{24}H_{23}N_3O$, calcd:370.18, found: 370.30.

3.1.10. 2-(N-(4-(4,5-bis(4-bromophenyl)-1H-imidazole-2-yl) phenyl)-N-methylamino)ethanol (2f)

Yellow solid; Yield: 76%, Mp. 204–207 °C, IR (KBr) $\bar{\nu}$ = 1200.37(C–N), 1492.90(C=C), 1616.35(C=N), 2891.30(C–H), 3147.63(O–H); ^{1}H NMR (400 MHz, DMSO-d_6): δ2.99 (S, 3H), δ3.45 (brS, 2H), δ3.58 (brS, 2H), δ6.77 (d, J = 8.53Hz,2H), δ7.42–7.47 (m, 4H), 7.52–7.61 (m,4H), 7.96 (d, J = 8.53 Hz, 2H) ^{13}CNMR (100MHz, $CDCl_3$): 38.62, 54.00, 58.07, 111.28, 117.30, 120.50, 126.46, 129.12, 129.97, 131.15, 131.51, 147.17, 148.25; MS(ESI) $[M+1]^+$ for $C_{24}H_{21}Br_2N_3O$, calcd: 526.01, found: 526.20.

3.1.11. 2-(N-(4-(4,5-bis(4-methoxyphenyl)-1H-imidazole-2-yl) phenyl)-N-methylamino)ethanol (2g)

White solid; Yield: 82%, Mp. 208–210 °C, IR (KBr) $\bar{\nu}$ = 1251.60(C–N), 1519.91 (C=C), 1608.63(C=N), 2943.37(C–H), 3369.64(O–H); ^{1}H NMR (400 MHz, DMSO-d_6): δ3.05(S, 3H), δ3.52 (brS, 2H), δ3.61 (brS, 2H), δ3.80 (S, 6H), δ6.89 (d, J = 9.03Hz,2H), δ7.04 (d, J = 8.78Hz, 4H), 7.85 (d, J = 8.78 Hz, 4H), 7.96 (d, J = 9.03 Hz, 2H) ^{13}CNMR (100MHz, $CDCl_3$): 38.28, 55.23, 58.07, 61.05, 111.57, 114.20, 120.34, 126.73, 127.91, 129.79, 131.46, 146.90, 149.21, 159.45; MS(ESI) for $C_{26}H_{27}N_3O_3$ $[M+1]^+$, calcd: 430.21, found:430.40.

3.1.12. 2-(N-(4-(5-(2-chlorophenyl)-4-(3,4-dimethoxyphenyl)-1H-imidazole-2-yl)phenyl)-N-methylamino)ethanol (2h)

White solid; Yield: 76%, Mp. 178–181 °C, IR (KBr) $\bar{\nu}$ = 1203.58(C–N), 1496.76 (C=C), 1612.49(C=N), 2943.37(C–H), 3314.33(O–H); ^{1}H NMR (400 MHz, DMSO-d_6): δ3.00(S, 3H), δ 3.43–3.49 (m, 2H), δ3.55 (S, 3H), δ 3.56–3.60 (m, 2H), δ3.72 (S,

Table 2 – The antimicrobial activity results for synthesized compounds.

Entry	Bacillus (G + ve)	*Escherichia coli*(G-ve)	Sclerotium *rolfsii*	Macrophamina *phaseolina*
Zone of Inhibition(cm)				
1a	0.1	0.5	**1.1**	**1.1**
1b	0.4	0.4	**1.1**	0.4
1c	0.1	0.3	0.9	0.6
1d	0.3	0.5	0.8	0.7
2a	0.3	0.3	**1.1**	**1.3**
2b	0.4	0.5	0.5	0.8
2c	0	0.5	**1.1**	**1.4**
2d	0.4	0.5	0.8	**1.3**
2e	0	0.5	0.4	**1.5**
2f	0	0.5	0.8	**1.2**
2g	0.1	0.5	0.9	0.8
2h	0	0.5	0.7	**1.8**
Standard	Streptomycin		Indofil M-45	
	1.1	1.0	1.0	1.3

Table 3 – Dock scores and Binding free energy of synthesized molecels and Co-Crystallized Ligand (CCL).

S.No	Entry	Dock Score (KCal/mol)	ΔG(MM/GBSA) (Kcal/mol)
1.	CCL	5.30	−56
2.	1a	4.19	−48
3.	2g	3.86	−31

CCL, Co-Cystallied ligand.

3H), δ6.78 (d, J = 8.53Hz,2H), δ6.86–6.99 (m, 3H), δ7.42–7.62 (m, 4H), 7.85 (d, J = 8.53 Hz, 2H) ^{13}CNMR (100MHz, $CDCl_3$): 38.62, 54.02, 54.98, 55.42, 58.08, 109.69, 111.35, 111.75, 118.19, 126.53, 127.34, 129.61, 130.06, 133.02, 133.78, 145.85, 147.63, 148.30, 148.41; MS(ESI) for $C_{26}H_{26}ClN_3O_3[M+1]^+$, calcd:44.30, found: 464.30.

3.2. *Biological evaluation of synthesized compounds*

The synthesized compounds post spectral and mass characterization were evaluated for their anti TB activity employing MABA assay.[19] Additionally antimicrobial activity of the synthesized compounds was also evaluated using agar gel diffusion assay.[20]

3.3. *Molecular docking studies*

To understand the mode of interaction of most active compounds molecular docking studies were carried out on Mycobacterium Tuberculosis Glutamine Synthetase (MtGS)

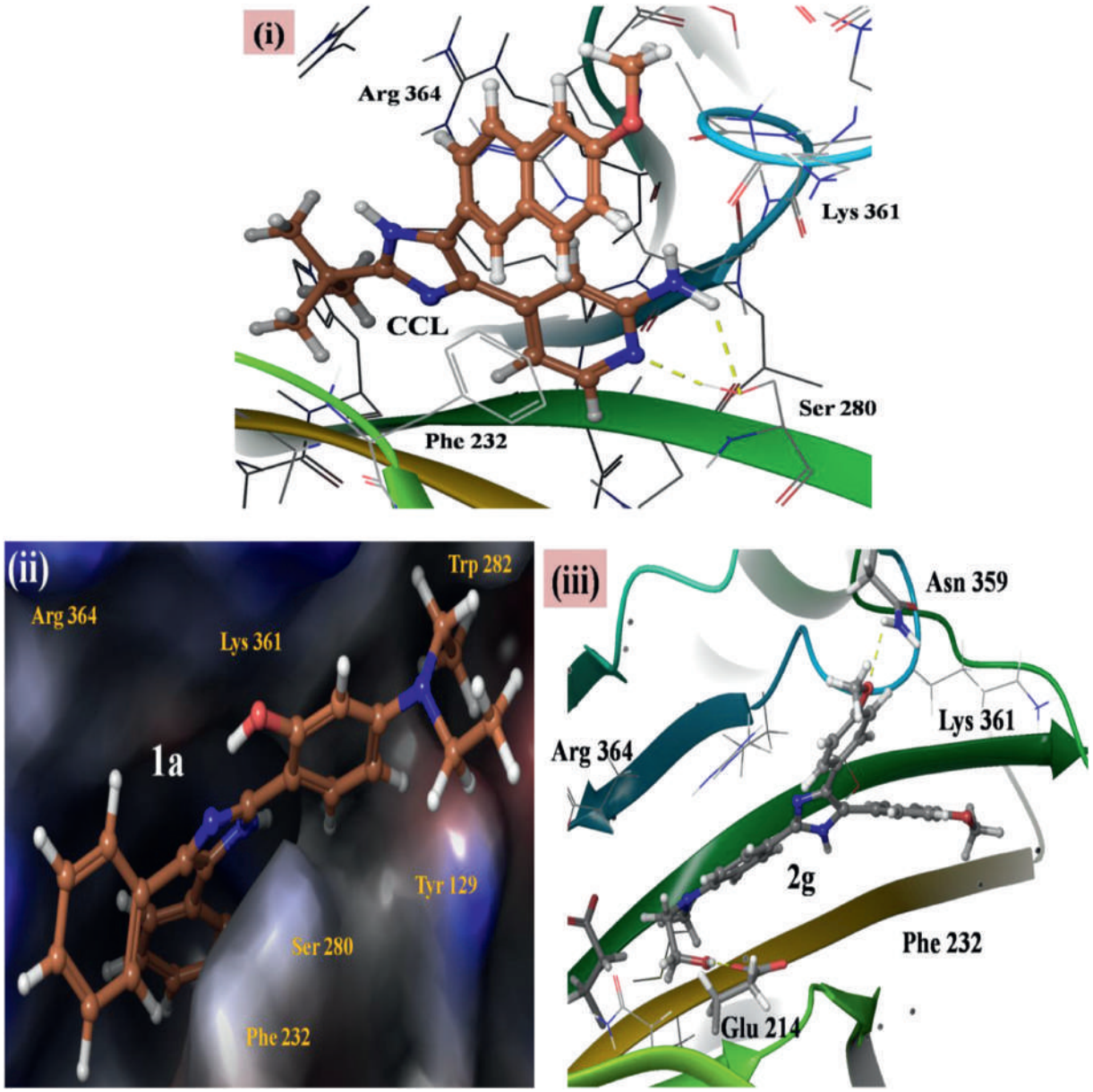

Fig. 4 – (i) Dock pose of Co-Crystallized ligand (CCL) showing hydrogen bond interactions with Ser-280 (ii) Synthesised molecule 1a in the binding pocket of MtGS2g (iii) Synthesized molecule 2g showing hydrogen bond interactions with Asn 359 and Glu 214 in the ligand binding domain of MtGS (PDB ID: 3ZXV).

Table 4 – Qikprop ADME Prediction for Synthesized molecules.

Entry[a]	QPlogHERG[b]	QPPCaco[c]	QPlog(P_o/W)[d]	%HOA[e]	Number of violations	
					Lipinski's rule of five[f]	Jorgensen's rule of three[g]
1a	−7.0	3139	6.0	100	1	1
1b	−6.5	3348	7.0	100	2	1
1c	−6.7	3298	6.1	100	1	1
1d	−6.5	3441	6.5	100	1	1
2a	−7.4	1477	6.0	100	1	1
2b	−7.2	1461	7.1	100	2	1
2c	−7.3	1201	6.1	100	1	1
2d	−7.2	1492	6.7	100	2	1
2e	−7.2	1990	5.1	100	1	1
2f	−7.6	1913	6.7	100	2	1
2g	−6.8	2429	5.4	100	1	1
2h	−6.6	1804	5.6	100	1	1

[a] Synthesized molecules.
[b] QPlogHERG K^+ Channel Blockage: log IC_{50} (concern below −5).
[c] Predicted Caco cell permeability in nm/s (Acceptable range:< 25 is poor and >500 is great).
[d] QPlog(P_o/W), Predicted aqueous solubility in mol/L (Acceptable range −2 to 6.5).
[e] %HOA(Percentage of human oral absorption) (Acceptable range: <25 is poor and >80% is high.
[f] Lipinski Rule of 5: Maximum number of violation in 95% of drugs is 4.
[g] Jorgensen Rule of 3: Maximum number of violation in 95% of drugs is 3.

(PDB ID: 3ZXV) in complex with tri-substituted imidazole inhibitor (4-(2-tert-butyl- 4-(6-methoxynaphthalen-2-yl)-1H-imidazole-5-yl)pyridin-2-amine).[21] MtGS plays an important role in synthesis of L-Glutamine, a key component of pathogenic mycobacterial cell wall. MtGS is also implicated in modulation of ammonia level in the phagosomes of M*tb* which influences phagosomal pH and thus aids in inhibition of phagosome-lysosome fusion. MtGS is an attractive target in developing new inhibitors against M*tb* and has been employed in the present molecular docking studies as synthesized compounds share a common structural frame work with Co-Crystallized Ligand (CCL) that happens to be a trisubstituted imidazole.[22,23] Molecular docking and MM/GBSA calculations were performed on Schrödinger software in accordance with protocols reported earlier.[24,25]

The structures of Co-crystallized Ligand along with most active compounds **1a** and **2g** were built on maestro panel of schrödinger suite and further structural refinements were performed using Ligprep module of Schrödinger software. The X-ray crystal structure of MtGS was retrieved from Protein Data Bank (PDB), a repository of solved protein structures.[26] The downloaded structure is not suitable for direct utilization and has to be processed before loading into Glide molecular docking portal of Schrödinger software. The protein was prepared using protein preparation wizard by following well documented procedures and Grid was generated around Co-Crystallized Ligand.[27,28] ADME properties of the synthesized compounds were predicted using QIKPROP module of Schrödinger suite. In silico toxicity profiling of synthesized compound was carried out using freely accessible pkCSM pharmacokinetics online server. The physicochemical descriptors of query compound are pitched against large experimental data set to predict different toxicity descriptors. Hepatotoxicity and oral rat acute toxicity LD_{50} values for the synthesized compounds were predicted using the pkCSM Web tool.[29]

4. Results and discussion

4.1. *Analysis of biological activity*

The anti TB activity values obtained are presented in Table 1, wherein isoniazid and rifampicin were used as standards. Out of twelve synthesized compounds, two compounds **1a** and **2g** showed promising activity of 3.125μg/mL against *M. tuberculosis* H37Rv. The compound 2c which is a prodrug (ester form) of compound **2g** also showed reasonably good activity of 6.25μg/mL. This means 3 out of 12 synthesized compounds that amount to 25 percent are shown to be active against M.*tb*.

Furthermore, the synthesized compounds showed promising antimicrobial activity and especially against fungus Sclerotium *rolfsii* and Macrophamina *phaseolina*. The synthesized compounds **1a**, **1b**,**2a** and **2c** showed a higher zone of inhibition of 1.1cm against the standard Indofil which exhibited a zone of inhibition of 1.0 cm against fungus Sclerotium *rolfsii*. As shown in Table 2, Compounds **1a**, **2a**, **2c**, **2d**, **2e**, **2f** and **2h** showed very good activity against fungus Macrophamina *phaseolina* with zones of inhibition of 1.1, 1.3, 1.4, 1.3, 1.5, 1.2 and 1.8 cm in comparison to the standard Indofil with a zone of inhibition of 1.3cm. This promising anti fungal activity of synthesized triaryl imidazole derivatives have a precedence with some of the most celebrated antifungal agents featuring an imidazole moiety in their structural frame work.[30]

4.2. *Discussion of molecular docking studies*

Extra precision (XP) variant of Glide module was used for molecular docking and the dock poses were then subjected MM/GB SA (Molecular Mechanics Generalized Born model and Solvent Accessibility method) analysis to evaluate in-silico binding free energy of the protein-ligand complexes. The

molecular docking and MM/GBSA scores are presented in Table 3.

The Nitrogen of pyridine ring and amine substituent on the pyridine ring of Co-Crystallied Ligand (here on **CCL**) exhibited two hydrogen bond interactions with Ser-280 and imidazole ring of the **CCL** showed π-π stacking interactions with amino acid residue Arg 364 (Fig. 4). The **CCL** exhibited a dock score of **5.30** and binding free energy of **56 Kcal/mol**. The synthesized compound **1a** did not show any hydrogen bonding interaction but was seen well positioned in a hydrophobic pocket exhibiting a dock score of **4.19** and a bind free energy of **48 Kcal/mol**. Dock pose of compound **1a** exhibited multiple π-π stacking interactions with residues Tyr 129, Trp 282 and Phe232. The synthesized compound **2g** exhibited two hydrogen bond interactions with Asn 359 and Glu-214. Compound **2g** also exhibited π-π stacking interactions with Arg 364. The dock score of 2g showed a dock score of 3.86 and a binding free energy of **31 Kcal/mol.** Both the synthesized compounds with MIC of **3.125 μg/mL** exhibited lower dock scores and binding energies compared to CCL with an MIC of **2 μg/mL**.

4.3. *In-silico ADME/Tox profiling*

The Qikprop module of Schrödinger suite processed all the compounds and none was skipped indicating reasonably good ADME profile. The synthesized compound have slightly high values of log(P_o/W) owing to triaryl groups which are hydrophobic besides which all the synthesized compounds pass the ADME filter satisfactorily. Results of ADME prediction are presented in Table 4.

Predicted LD_{50} values from pkCSM Web tool, ranged from 2.39 to 2.42 mol/kg and among the most active compounds, **1a** was predicted to be non-toxic whereas compound **2g** was predicted to be hepatotoxic.

5. Conclusion

All in all, a set of 12 compounds were designed and synthesized Three compounds **1a**, **2g** and **2c** exhibited good inhibitory potency against M.*tb* H37Rv and this is fair degree of success as three out of twelve compounds that accounts for about 25% of designed compounds have shown good anti TB profile. The molecular docking studies were performed to understand the mode of interaction of these compounds at the interface of putative target, MtGS binding site. The synthesized compounds have shown reasonably good ADME profile. These compounds have scope for further structural modifications especially at the first nitrogen of the imidazole ring where in hydrogen can be substituted. Different substituents on the aryl groups can also be incorporated to generate more number of substituted triaryl imidazole derivatives and evaluated for their anti TB profile. In addition, the synthesized compounds also show promising antifungal activity yet again showing the prominence of imidazole class of compounds as potent antifungal agents.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2023.01.005.

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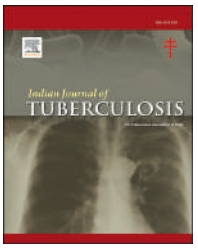

Review article

Addressing the challenges in implementing airborne infection control guidelines and embracing the policies

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ABSTRACT

Airborne pathogens not only lead to epidemics and pandemics, but are associated with morbidity and mortality. Administrative or managerial control, environmental control and use of personal protective equipments are the three components in airborne infection control.

National and international guidelines for ideal airborne infection control (AIC) practices are available for more than a decade; however the implementation of these need to be looked into, challenges identified and addressed for effective prevention of airborne disease transmission. Commitment of multiple stakeholders from policy makers to patients, budget allocation and adequate fund flow, functioning AIC committees at multiple levels with an inbuilt reporting and monitoring mechanism, adaptation of the AIC practices at various health care levels, supportive supervision, training and ongoing education for health care providers, behaviour change communication to patients to adapt the practices at health care facility level, by health care personnel and patients will facilitate health system preparedness for handling any emergencies, but will also help in reducing the burden of persisting airborne diseases such as tuberculosis. Operational research in this least focused area will also help to identify and address the challenges.

1. Introduction

Globally, there has been a steady rise in transmission of diseases which spread by airborne route. Some of these spread in a rapid manner for varied reasons, affecting masses of people across various geographical locations and impacting their lives in many different ways. Airborne transmission of disease causing pathogens could occur direct route or indirectly through droplets of various sizes and fomites.[1] Examples of airborne viral illnesses include but not limited to Severe Acute

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Respiratory Syndrome (SARS) infections, Middle East Respiratory Syndrome viruses (MERS), mumps, measles, varicella, Respiratory Syncytial Virus (RSVs) etc.[2] Similarly bacterial illnesses such as tuberculosis (TB), diphtheria, etc. also spread by droplet transmission. Factors influencing the air borne transmission of disease causing pathogens include seasonal patterns,[3] weather,[4] environmental pollution, socio-economic,[5] poor living conditions, literacy level etc. However, during pandemics, factors such as malnutrition, poor living conditions and comorbidities contribute to serious debilitations and mortality[6] in both the developing and developed nations equally. Pandemics due to airborne pathogens such as Spanish flu in 1918, Asian flu (H2N2) in 1957 and Hong Kong flu (H3N2) in 1968, pandemic influenza (pH1N1) in 2009, MERS between 2012 and 2019, not only affected millions of people but were associated with mortality as well.[7–11] The 21st Century COVID 19 pandemic is a reminder that airborne pathogens could impact not only the health status, but also other vital factors such as economy, citizens livelihood, international travel etc.[12]

The health care providers' (HCP) not only play a major role in providing routine health care but also are the frontline warriors in any public health emergencies including epidemics/pandemics. They are at increased risk of exposure to infectious pathogens, thereby getting infected and transmitting it to other HCPs and patients. The risk of annual latent tuberculosis infection ranged from 0.5% to 14.3% and annual incidence of TB disease was between 69 and 5780 per 100,000 population among HCPs.[13] The associated risk factors included but not limited to the place of work (inpatient TB ward, emergency care, laboratories, internal medicine units etc.) and occupational categories such as doctors, radiology technicians, nurses, paramedics and ward attendants.[13]

The guidelines for environmental infection control in health-care facilities were released by Centre for Disease Control and Prevention (CDC) in 2003. This was later updated over a period of time addressing specific diseases.[14] World Health Organization (WHO) released the "Hospital Infection Control Guidance for Severe Acute Respiratory Syndrome (SARS)" on 24 April 2003, interim guidelines promoting non-pharmacological practices and principles for infection prevention and control for epidemic and pandemic acute respiratory diseases in healthcare in 2007, and later updated it in 2014 following H1N1 influenza pandemic (2009). The Government of India released National airborne infection control (AIC) guidelines in 2010 aimed at improving the health system preparedness to curb the spread of airborne infections as well as provide insights about household precautions. In addition to the universally recommended standard precautions, the guidelines project the hierarchy of AIC namely administrative control, environmental control and personal protective equipments. The guidelines were developed with the consideration of TB as the prototypic disease transmitted via airborne route, but also apply to other airborne infectious diseases as well.[15] The National Guidelines for Infection Prevention and Control in Healthcare Facilities was released in India in January 2020. Though various national and international AIC guidelines are available, awareness and extent of implementation of these guidelines still remain poorly understood. P.T. James et al assessed the awareness of AIC practices among HCPs in a tertiary care centre in Kerala, South India.[16] Despite reasonable awareness among HCPs, there were lacunae in the practices. A panel of multidisciplinary experts conducted site visits to document the AIC practices and review the resource capacity across varied health settings in selected states in India. Based on their observations, they provided facility specific recommendations to improve the awareness and practice of the National AIC guidelines and showed them to be effective.[17] Several obstacles and limitations for implementation of adequate infection control practices include but not limited to managerial support, poor funding, shortage of manpower, limited awareness and lack of institutional commitment.[18] Here we reviewed the key pillars of AIC (administrative control, environmental control and personal protective equipments) as per WHO and National guidelines with an objective to understand the challenges in implementation and propose possible solutions for addressing these challenges.

1.1. *Administrative control key features*

The National AIC guidelines 2010, recommends considering the following key elements in administrative control.

- Availability of infection control committee, infection control plan and training documents in every healthcare setting.
- Screening of respiratory symptomatics immediately upon arrival.
- Segregation and fast-tracking the respiratory symptomatics.
- Provision of masks, patient education on cough etiquette and safe disposal of sputum for symptomatic patients.
- To have designated personnel to implement and monitor the practices.

1.1.1. *Challenges in administrative control*

The administrative control has the largest impact in AIC prevention control strategies and acts as the first line of defence against transmission of airborne pathogens. The protocol calls for prompt triage of persons with presumed TB based on respiratory symptoms. Screening of these patients requires an understanding by the HCP about identification of a potentially infectious person. Segregation of the respiratory symptomatics need availability of separate space in health care facilities designated for the purpose. In case, such space is not available, respiratory symptomatics could be segregated in an open well ventilated place. Fast-tracking of patient services for respiratory symptomatics decreases the exposure time of others. Expediting respiratory symptomatic services at the expense of others could be difficult. Also, attention should be paid to the type of health care facility that could benefit from fast-tracking of respiratory symptomatics. For instance, this feature might not be effective in a health care centre meant for respiratory diseases, rather this could be useful in general hospitals and OPDs. Implementation of administrative control measures doesn't require huge materialistic investments or resources. Successful implementation of administrative measures is achieved by prioritizing training and education of human resources (Table 1).

1.2. *Environmental control key features*

The key component of environmental control is dilution of infectious agents in the air and achieving maximum air exchange. This is achieved by

- Attaining the minimal (about 6–12) air changes per hour (ACH) to eliminate or decrease the infectivity in the air.
- Ensuring effective unrestricted ventilation by following proper operations in facilities which are dependent on natural ventilation.
- Verifying the design, operation and maintenance of the system, where mechanical ventilation is utilized.
- Adapting the most favourable seating arrangements between the staff and patient to reduce the risk of disease transmission.
- Opting for special devices like high efficiency particulate air (HEPA) filters and ultraviolet germicidal irradiation (UVGI) filters in high risk settings where adequate air exchange by natural ventilation is not feasible, and if the funds permit.
- Assigning staff for monitoring environmental control activities.

1.2.1. *Challenges in environmental control*

Environmental control forms the second line defence factor in stopping the spread of TB in health care settings, after administrative control. The methods to achieve environmental control include ventilation (natural and mechanical), UVGIs, filtration and other methods of air purification.

Table 1 – Addressing challenges in implementation of administrative control.

Commonly faced administrative control challenges	Possible ways of tackling the administrative obstacles
Non-availability/non-functioning of facility infection control committees[17,18]	Constitution of facility infection control committee with the staff which oversee/implement AIC practices at primary level centres. Constitution of a committee including the staff and experts for secondary as well as tertiary level centres. Development of e-platform for submission of risk assessment report, minutes of the meeting etc. and monitoring by the higher level committees.
Non-availability of facility AIC plan[17–20]	Standardized AIC plan for each level of health care facilities drafted by experts/programme managers in district/state Infection control committees for adaptation and customization by individual facilities. Inclusion of budget in the programme implementation plan and allocation of adequate annual funds for implementation.
Screening,[21] identification, segregation[19] and fast-tracking of services for respiratory symptomatic patients not widely done[17]	Appropriate training of concerned HCPs and supervision of implementation by the allotted HCP/facility-in-charge. (or) Self-identification[22] by the patients with proper signage placed at the entrance, in the event of staff shortage. Fast-tracking of services by having special stamps that could serve as an indicator for the staff.
Inadequate AIC practices followed by the patients visiting the health facility[23]	An onerous task, which solely can be achieved by repeated patient education and sustainable demonstration by the staff in adhering to the practices.
Lack of patient education[20,23] and display of Information Education and Communication materials[17]	Rigorous motivation and encouragement of the staff to educate the patients. Preparation of patient educational materials such as posters for display in health care facilities and pamphlets for distribution to patients by the Programme managers.
Lack of designated staff to overlook the AIC practices	Designation of all the staff on rotational basis to ensure the implementation of practices, promoting a sense of responsibility among all the staff equally.
Lack of adequate awareness of the guidelines[20] and Institutional Commitment	Providing repeated education to the staff by a member of the infection control committee/facility in charge, insisting on the benefits of AIC practices.
Lack of adequate staff training in infection control policies.	Incorporation of AIC training programme in the Programme implementation plan and regular annual training of HCPs.
Lack of motivation among the staff for implementation of AIC practices.	Awards to the staff who are actively involved in AIC implementation at the facility/district level.[24] Motivation by the facility-in-charges/programme managers by provision of appropriate support in the form of weekly briefings, incentives etc. for implementation.
Frequent turnover of HCPs which affects the knowledge and routine practices to be followed.	Sensitisation of newly recruited HCPs along with proper orientation, delegation of their responsibilities by the senior staff or the facility-in-charge.
Inappropriate infrastructure resulting in lack of space for segregation, ventilation etc.[25]	To involve AIC experts/engineers from planning to completion so that infrastructure can be in accordance with the guidelines ex. place for segregation, appropriate cross ventilation etc.

Some forms of environmental control measures are simple and economical while some others might be complex and costly. Ex. UVGI and HEPA filter, air-conditioners, air-purifiers etc. Parmar et al showed that minor renovations in existing natural ventilation helped to achieve the minimal required ACH.[17] Researchers from Peru estimated the effectiveness of natural ventilation by assessing different hospital room types constructed in the old fashioned way before 1950s and in the modern era between 1970 and 1990 using the Wells-Riley equation. They found that higher rates of air exchange, absolute ventilation (which is the amount of air inflow per unit time (m^3/h)) was achieved by opening the windows and doors, especially in old buildings. They noticed that the calculated risk of respiratory infection contagion was slightly higher in mechanically ventilated rooms (39%), even with the recommended air changes, compared to the rooms with natural ventilation (11%) when exposed to a source of infection. Factors such as heat loss, culture, tradition or security pose as barriers in practicing natural ventilation. Given that isolation of an infected person is often difficult, it is safer to keep the windows and doors open to reduce the risk of transmission. The protective effects of ventilation could be achieved with half open windows as well.[26] Installation of negative pressure rooms are costly with operational challenges and require maintenance. However, mechanical ventilation will be required wherever there are implementation inadequacies in natural ventilation due to local conditions.[27] Improving the ventilation was found to be the most effective and efficient solitary initiative by a modelling study conducted in South Africa. It modelled improved natural ventilation, use of mechanical ventilation systems such as fans, and the impact of HEPA and UVGI filters for prevention of XDR TB transmission. Improvement in natural ventilation alone and mechanical ventilation prevented 33% and 12% of XDR TB transmission respectively. Addition of HEPA filters to mechanical ventilation reduced the infectivity by further 10%, while there was a 32% decline in transmission rates with the combined use of UVGI and mechanical ventilation.[28] Installing split air conditioners and keeping doors and windows closed indicates a complete lack of air-exchange (see Fig. 1). Therefore particular attention must be given to ensure adequate ventilation when installing measures for climate control[15] (Table 2).

1.3. *Challenges in PPE*

PPE is the third important defence mechanism in improving AIC. It should be used together with proper hygienic practices. PPE includes gloves, gowns, masks, goggles, N95 particulate respirators etc. HCPs are required to take adequate precautions such as hand hygiene before and after providing care

Table 2 – Addressing challenges in implementation of environmental control.

Commonly faced environmental control challenges	Possible ways of tackling the difficulties in implementing environmental control
Lack of awareness about the right environmental practices among the staff in the facility	Providing ongoing education to the staff by a member of the infection control committee/facility in charge, insisting on the benefits and vital role of environmental control in achieving AIC. Incorporation of AIC training programme in the Programme implementation plan and regular annual training of HCPs.
Closed doors and windows restricting the air-exchange	Opening of the windows and doors[26] to its maximum capacity. Assigning a staff to take charge of the same.
Improper seating arrangement between the HCP and staff	Minor modifications in the seating arrangements as per NAIC guidelines (Fig. 2) to ensure direction of airflow between the patient and the staff and not from patient to staff (see Fig. 1). Picture (A) shows potential air transmission between the health care worker and patient. Picture (B) depicts ideal seating arrangement between the health care worker and patient. Staff seating to be placed near a clean air source and patient seating to be placed near an exhaust.[15]
Imprecise handling of the additionally installed ventilator systems	Proper training and education of the staff about the right methods of operating the mechanical ventilator systems during installation and also to have a manual for reference in future.
Closing doors and windows in a room with split air conditioners, which doesn't allow air-exchange	Following proper instructions in installation of air-conditioners as per the National Guidelines. Ensure location of air conditioners near the door, away from the exhaust fan. Exhaust fan to be installed on the opposite end allowing adequate air-exchange. Provision of space under the door for easy air-entry.[15]
Poor hygiene and pest infestations around the facility surroundings as the reason for keeping the openings closed.	Maintenance of good hygiene and sanitation with adequate help from the local government and multi sectoral/inter departmental coordination.
High cost of mechanical ventilation systems, costs involved in their installation and maintenance, need for prior planning and designing of the infrastructure for their placements.	Appropriate budgeting and allocation of funds by the program managers to allow proper maintenance and functioning of appliances appropriately.[29]

Table 3 – Addressing challenges in the implementation of PPE.

Commonly faced challenges in PPE usage	Possible ways of managing the issues surrounding PPE
Non-availability/Limited availability[33] of the required PPE materials	Allocating adequate funds and ensuring supply based on the needs by the facility in charge/programme managers. Routine budgeting for funds in programme implementation plan. Educating and encouraging the staff for the rational use of PPE.[34] (appropriate method of using the PPE, scenarios where PPE is a mandate, optimal usage and re-use of appropriate PPE etc.)
Lack of knowledge and usage of incorrect PPE (over protecting/ under protecting)[30]	Proper educational sessions on correct PPE selection. Overseeing the appropriate use of PPE and reducing its wastage and prevention of cross contamination by a monitoring/infection control committee.[34]
Lack of compliance among healthcare workers handling patients with presumed/ diagnosed airborne infections[30,35]	Repeated education to the healthcare workers, reinforcing the importance of using the PPEs in appropriate situations. Monitoring the staff on proper use of PPE, by the infection control committee/governing body/officer-in-charge
Improper donning/doffing techniques leading to impaired protection[36]	A short educational session (using videos etc.) about the proper techniques of wearing and removal can be conducted/circulated at a regular frequency.
Perceived difficulties in usage of PPEs such as face-mask or N95[33,35]	These are perceptions and not facts, therefore can be removed by constant re-assurance.

to patients. Full PPEs are required to be worn in situations such as nebulization, endotracheal intubation, manual ventilation, oral/air-way suctioning, cardiopulmonary resuscitation, bronchoscopy etc. Aerosol generating procedures strictly recommends the usage of N95 particulate respirator.[15] Knowledge and practice of appropriate donning and doffing off the PPE are vital in prevention of cross contamination.[25]

Inconsistent/inappropriate use of PPE increases the risk of self/environmental contamination. Another factor that is responsible for cross contamination while using PPE is improper donning and doffing methods.[30] Also inappropriate use of PPE leads to wastage and shortage thereby exposing the needy to the risk of infection[31] (Table 3).

Sterilizing PPE similar to other medical devices for recycling/reuse is questionable as there is limited knowledge on its efficacy post sterilization. Also using radiation to sterilize them could reduce the functionality as it might affect the material. Damage to the material and reduced performance is expected in gas sterilization using hydrogen peroxide.[32]

2. Discussion

Airborne transmission of respiratory infectious agents could be controlled by prevention of pathogen release from the source, its transport through air or touching the contaminated surfaces and protection of the susceptible contacts.[1] There are global and national guidelines for improving the AIC practices since more than a decade, however the awareness and practice of these guidelines is still limited.

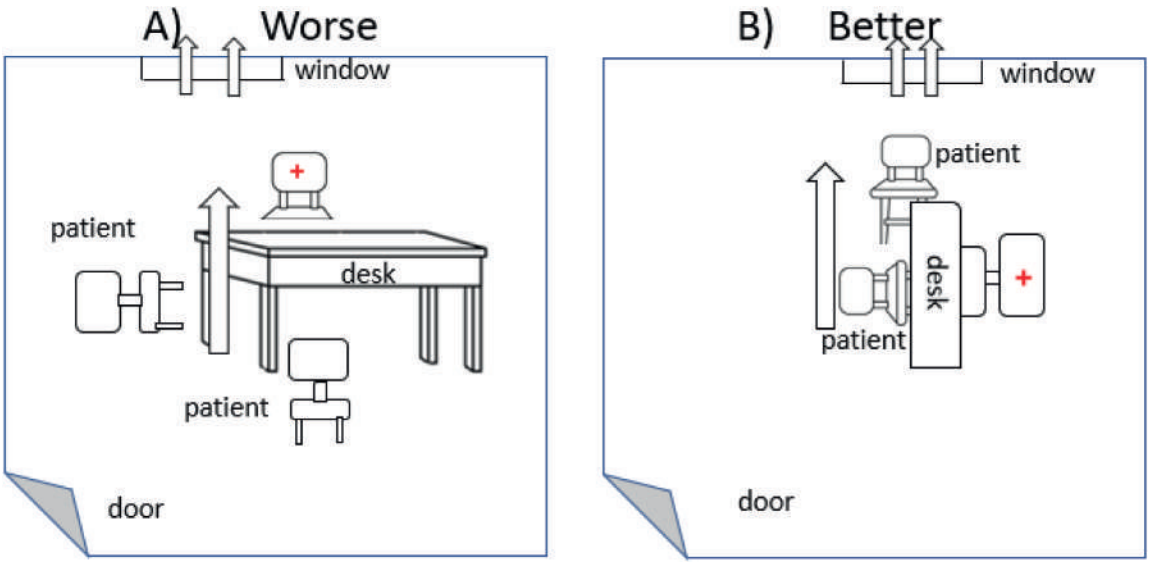

Fig. 1 – Roles and responsibilities for implementation of AIC practices in a health care facility.

Frequent staff turnover, training needs for the newly hired staff, heavy patient load, staff and patient perceptions of using appropriate PPE are some of the barriers for infection control in a health care setting where as ample infection control supplies, adequate funds, integration of infection control team with the clinical team, adequate cleaning team, staff knowledge etc. act as facilitators.[35] A study in a large medical centre in United States of America during the COVID 19 pandemic showed that rigorous implementation of comprehensive AIC plan covering various aspects could prevent the nosocomial transmission of COVID 19 which is known to be highly transmissible. One COVID 19 transmission was attributed to be hospital acquired among 8370 patients who were hospitalized for non COVID reasons.[37] Multipronged interventions including proactive screening, isolation, masking of patients and HCPs, monitoring PPE usage and HCP education were shown to be effective in prevention of COVID 19 transmission among HCPs in Hong Kong.[38]

Key stake holders including policy makers, programme managers and HCPs need to be sensitized regarding the necessity for successful implementation of AIC guidelines in disease prevention and transmission by multiple advocacy meetings. Advocacy for financial plan and budget allocation not only for implementation of infection control practices but also for sustaining these activities at national, state and district level by the programme managers is vital.[39] Building a system for monitoring mechanisms at multiple levels, creation of reporting mechanisms with specific timelines through electronic applications could facilitate review of the implementation. National and international level conferences, seminars, expert meetings and webinars with a focus on AIC could play a role in sensitizing the scientific committee in AIC implementation and its role in prevention of disease

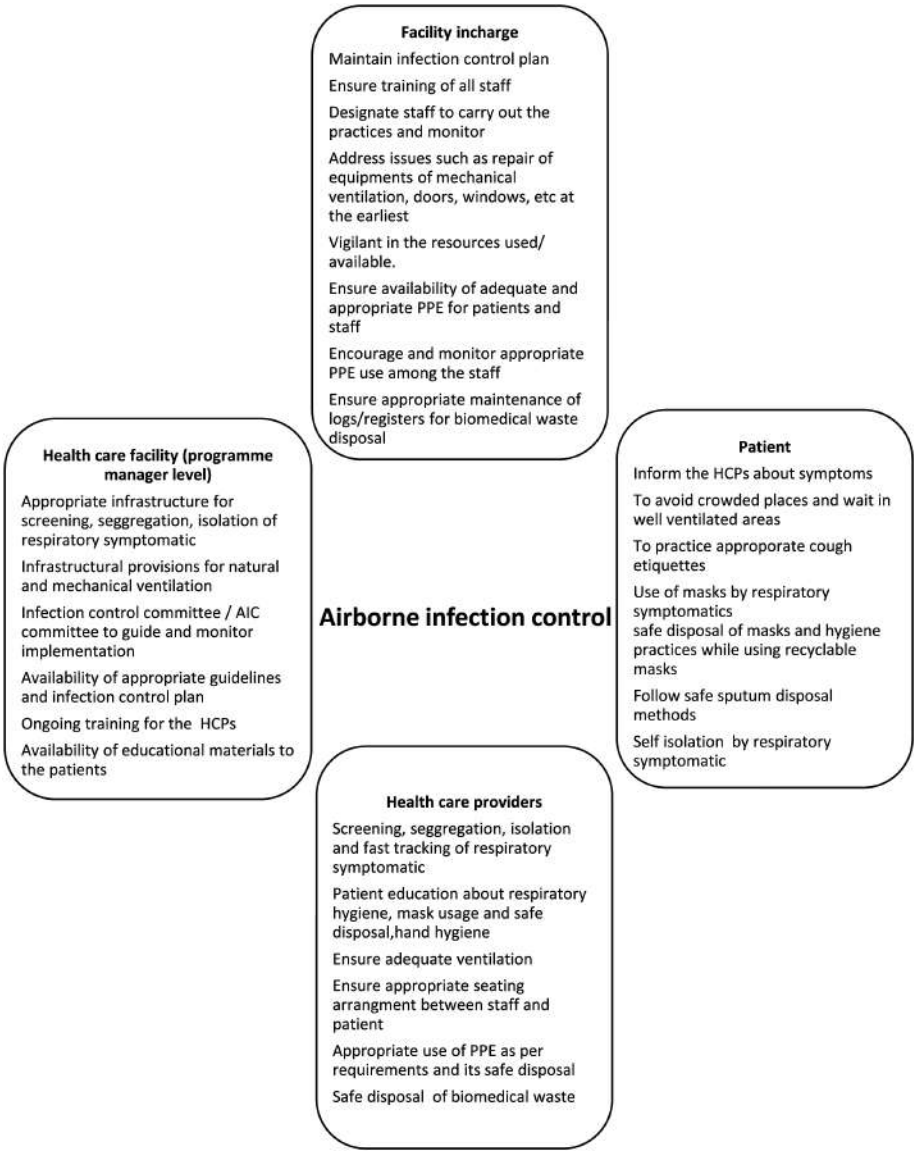

Fig. 2 – Ideal seating arrangement between health care provider and patient for appropriate airflow as per Indian National AIC guidelines.

transmission. Operational research to understand the strengths and gaps and identify solutions is the need of the hour to strengthen the implementation.

At the field level, multi-pronged approach is essential for the successful implementation of AIC practices (Fig. 2). Routine and continuing education of HCPs in AIC practices as per the guidelines need to be included in the programme implementation plan, implemented and monitored as well. Role of functional AIC committees including experts, clinicians, public health personnel, community representatives, engineers at multiple level (national, state, district, tertiary, secondary and primary health facility level etc.) to update the guidelines, train the HCPs and community and monitor the implementation of these guidelines is vital. Multisectorial coordination including AIC experts from the planning stage till completion would help to address the infrastructural issues. Adequate stock of PPEs and its appropriate use is essential for protection of HCPs as well as prevention of spread of nosocomial infections. Community preparation including behaviour change communication regarding cough etiquette, isolation of respiratory symptomatic, seeking early care and treatment in case of sickness, safe disposal of sputum, appropriate hand hygiene practices, ensuring adequate ventilation in the residence to the level feasible while suffering from respiratory illness should be continuously given even in times without epidemics or pandemics. Children should be educated in schools regarding respiratory and hand hygiene practice. Development and use of patient friendly educational materials including videos, skits, catchy slogans, identifying a celebrity ambassador for campaign etc. will help to raise awareness and encourage the practice. Improvement of awareness among the community might lead to self-discipline, eliminate stigma associated with some of the respiratory diseases ensuring appropriate AIC behaviour by the patients.

3. Conclusion

Effective implementation of the AIC practices is of utmost importance in this era of pandemics and epidemics due to transmission of pathogens which spread by airborne or droplet route. The guidelines and policies are in place; however its practice is very limited especially due to poor awareness. It is the need of the hour to focus on the implementation of these guidelines and policies at every level to ensure adequate health care system preparedness to tackle any health system emergency. The challenges in implementation of these guidelines and its appropriate solutions need to be looked into at the each level of health care system. Commitment at the Programme/managerial level, every level of HCP cadre and community is essential for the effective implementation of AIC practices which will pave way for prevention and control of airborne diseases.

Authors contribution

Bella Devaleenal Daniel, (a) conception, design and/or analysis and interpretation of data and to (b) drafting the article or revising it critically for important intellectual content and on (c) final approval of the version to be published.

Abinaya Baskaran, (a) Design, analysis and interpretation of data and to (b) drafting the article, revising it critically for important intellectual content (c) final approval of the version to be published.

D. Baskaran, (a) Design (b) revising it critically for important intellectual content and on (c) final approval of the version to be published.

Hephzibah Mercy, (a) analysis and interpretation of data and to (b) drafting the article (c) final approval of the version to be published.

C. Padmapriyadarsini, (a) Design (b revising it critically for important intellectual content and (c) final approval of the version to be published.

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Declaration of competing interest

The authors have none to declare.

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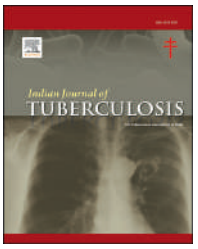

Review article

TB diagnostic insights, progress made on point of care diagnostics and bioinformatics as an additional tool for improvement

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ABSTRACT

Despite major efforts made to control tuberculosis disease (TB), this disease continues to present a major global health challenge and drug resistance is continuously growing. TB is caused by *Mycobacterium tuberculosis* and spreads exclusively via human-to-human contact transmission. Therefore, early detection and diagnosis for proper treatment with active TB have a great impact on public health. Regardless, most people in developing countries with TB or TB-associated symptoms do not have access to an adequate initial diagnosis. Available bacteriologic-based techniques are either inefficient or may require a longer turnaround time from the laboratory. Contemporarily, non-bacteriologic based methods have both questionable sensitivity and specificity and while others cannot distinguish between active and latent TB. Thus, additional efforts have been made to find accurate diagnostic tests for TB. Herein, we review the available methods used for TB diagnosis, and in addition, we explore point of care (POC) diagnostics as an alternative way to develop TB diagnostic tests and further evaluate whether bioinformatics can be used as an additional screening tool for identification of possible TB biomarkers for the development of POC TB diagnostics, which is part of our research focus.

1. Introduction

Tuberculosis (TB) is a contagious, chronic infectious disease that is caused by a bacterium, *Mycobacterium tuberculosis*, and can infect a variety of organs including the lungs (pulmonary TB) or organs other than the lungs (extrapulmonary TB).[1] The Global Tuberculosis report of 2020 reported that almost 10.4 million people were infected with TB in 2019 and approximately 1.2 million TB-related deaths were reported in HIV-negative individuals, while an estimated 250 000 accounted for HIV-positive individuals. The majority of these cases were reported in the developing world including Asia (44%), Africa (25%), Western Pacific (18%), and Eastern Mediterranean

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(8.2%), while the developed regions accounted for a minority of the cases, Americas (2.9%), and finally Europe (2.5%).[2]

TB is curable and preventable under the right conditions.[3,4] People who develop TB disease can be successfully treated with a 6-month antibiotic regimen. Although the cost of treatment is reasonably cheap and highly effective, this disease has remained the leading cause of illness and death for many years before the COVID-19 pandemic. TB is mostly associated with poverty, with the majority of cases (95%) and deaths (98%) reported in developing countries.[5] Moreover, the HIV/AIDS pandemic in the majority of these regions has been shown to increase the risk of infection proceeding to overt disease.[5]

TB is primarily spread through human-to-human transmission and thus the disease must be diagnosed early and followed by appropriate treatment in individuals with active TB.[6] Several issues have been associated with the elimination of TB globally. One of the major aspects of the hindrance is the availability of better diagnostic tools to enhance early detection.[7] Many developing countries still employ traditional methods such as Tuberculin skin testing (TST), sputum smear microscopy (SSM), and evaluation of clinical symptoms as a way of early detection.[8,9] The sensitivity and specificity of these tests have been questionable, particularly in patients with HIV and extrapulmonary infection.[10,11] Thus, there is a need to improve current diagnostics which will ultimately improve cure rates, and to achieve this relies primarily on the implementation of point of care (POC) diagnostics.[11]

Almost two-thirds of TB cases remain undiagnosed or reported to health authorities.[12] Thus, if the goal is to eliminate TB, several tools must be put in place. For example, new vaccines against TB, the development of new treatment regimens that can be administered for a shorter period, and lastly, new diagnostic tools that are easier to use and do not require specialized infrastructure.[12] Central to TB control and possibly elimination is the development of rapid diagnosis. Improved diagnosis does not rely entirely on sensitivity or specificity and drug resistance, but the test should be cost-effective, and user-friendly, i.e., useable in remote areas with no or lack of infrastructure development and can be used by health workers with minimal training.

Advancements in TB POC diagnostics have been made in recent years. For example, molecular tests such as the Xpert MTB, loop-mediated isothermal amplification (LAMP) assays, lipoarabinomannan (LAM) urine strip test, and molecular line-probe assay have been used and shown improvements in early TB diagnosis.[13] However, these are costly and there is still limited access to these diagnostic tools in most developing countries, thus a need for improvement and accessibility. This review aims to provide insights on POC diagnostics and highlight other technologies that can be used to further improve this diagnostic test for better use and improved sensitivity and specificity.

2. Current TB diagnostic methods

There are numerous methods used for screening and diagnosing TB. Diagnosis is primarily dependent on initial clinical assessment, chest radiography or chest x-ray (CXR), and subsequent laboratory confirmation by various bacteriologic studies. The most commonly used TB screening and diagnostic methods include purified protein derivative (PPD), TST or Mantoux test, SSM, TB culture, and various nucleic acid amplification tests (NAATs).[14,15]

2.1. *Rapid tests*

The TST is a subcutaneous delivery/injection of a purified protein derivative protein. This test has been successfully used for rapid TB screening, but it has since failed to discriminate between active and latent TB infection. Moreover, a high percentage of false positive results have been reported in individuals with preceding Bacillus Calmette–Guérin (BCG) vaccination.[14] It is used as a standard test to diagnose latent TB but has the potential for a high number of false positives. The high percentage of false positives cannot only be not limited to previous BCG vaccination but infection with non-tuberculosis mycobacteria and incorrect interpretation of the results[15,16]

2.2. *Microscopy*

The SSM is a simple test used to identify MTB acid-fast bacilli and remains the primary method for diagnosis of pulmonary TB in low- and middle-income countries with limited settings. It is affordable and can be performed in basic laboratories at primary healthcare clinics.[17] It has low sensitivity and requires a minimum of 5000 acid-fast bacilli per milliliter (AFB/ml) for detection which is a challenge to pulmonary TB diagnosis in children and immunocompromised individuals with HIV/AIDS comorbidities that have low bacilli count and those with extrapulmonary TB.[18] Furthermore, it cannot distinguish between dead or live bacilli. Since all mycobacteria are acid-fast, the SSM cannot distinguish between tuberculous and non-tuberculous mycobacteria, hence it cannot be used for species differentiation and drug sensitivity testing (DST).[19]

TB culture is the gold standard for diagnosing TB and has high sensitivity and specificity and can also be used for DST. MTB is a slow-growing and fastidious bacterium that requires specialized media and approximately 2–6 weeks to detect growth on culture media, and these cultures are kept for up to 12 weeks before they can be reported negative.[15] TB culture has its limitations, for instance, it requires specialized infrastructure such as Biosafety level 4 (BSL4) laboratories which may not be always available in resource-limited settings, poses a high risk to laboratory personnel, and therefore requires highly trained staff to perform tests, and the turnaround time for results is long.[20]

2.3. *Point of care diagnostics*

Recent innovations in POC diagnostic technologies for TB have resulted in early treatment initiation. For instance, the roll-out of GeneXpert MTB/RIF devices offers the potential for rapid and effective diagnosis of TB, especially in resource-limited countries with a high burden for TB/HIV co-infection.[21–23] Currently, Xpert MTB/RIF assay (Cepheid, Inc., Sunnyvale, CA, USA) is the recommended NAAT for rapid detection of TB and rifampicin resistance by WHO in people with suspected pulmonary TB[24–26] The NAAT can rapidly detect the genetic

material of MTB from the respiratory specimen; however, it cannot be used to monitor treatment response because it detects both viable and non-viable tuberculous mycobacteria.[15] The Xpert MTB/RIF also has limitations associated with it, for example, resistance to rifampicin (RIF) is used as a marker for multi-drug resistance TB (MDR-TB), however, other bacterial strains may exhibit only mono-resistance to RIF which may not necessitate full line MDR therapy, thus resulting in false positive RIF diagnosis. Other drawbacks include high costs for testing, appropriate infrastructure, and annual calibration of the instrument.[27] Ensuring an uninterrupted supply of cartridges remains a challenge as the global demand for Xpert cartridges increases.[24]

3. Bioinformatics

The introduction of high throughput sequencing technologies has allowed clinical laboratories to extract a large amount of data about the genome, transcriptome, metabolome, and phenome of various patients.[28] This information allows screening and early detection of diseases as well as therapeutic targets in drug discovery. Bioinformatics is a discipline in the field of biology that uses computational techniques for analysis and extracting information from biomolecules.[28] This review will be mainly focused on the role of transcriptomics in the identification of possible biomarkers for TB diagnosis.

3.1. *Transcriptomics*

In recent years transcriptomic analysis of human samples has provided insights into understanding genes that are expressed in different patient samples. Thus, comparisons of gene expression profiles from test samples (i.e. those with disease) to those without, have made it possible to identify genes that differ in their expression between groups, thus giving a disease signature. Identification of disease signatures is beneficial for diagnostic tool development and drug discovery, thus understanding pathways that are affected during the disease.[29] For example, de Araujo et al. (2019) showed differential expression of four major classes of small non-coding RNAs in peripheral blood from patients with different stages of TB infection. This study reported that in addition to microRNA (miRNAs), which are known to be highly regulated in blood cells of TB patients, there was a significant expression of PIWI-interacting RNA (piRNA) and small nucleolar RNA (snoRNA) in both latent and active TB, thus yielding promising biomarkers. While the functions of small nuclear RNA (sncRNA) have not been fully elucidated, their results strongly suggested that at least piRNA and snoRNA populations may represent potential biomarkers of TB diagnosis in the different stages of TB infection.[30] Currently, there is a plethora of data generated for biomarker development using microarray platforms. In another study, bioinformatic tools including gene ontology (GO) analysis, and KEGG pathway analysis for gene function, and enrichment was used to analyze differentially expressed genes from pulmonary TB from the Gene expression Omnibus (GEO) database. Overall, 7 genes namely C–C motif chemokine ligand 20 (*CCL20*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), Intercellular adhesion molecule 1 (*ICAM1*), TIMP metallopeptidase inhibitor 1 (*TIMP1*), matrix metalloproteinase 9 (*MMP9*), C-X-C motif chemokine ligand 8 (*CXCL8*) and Interleukin-6 (*IL6*) were associated.[31] Using a similar approach, a transcriptional regulator factor 1 (IRF1) was up-regulated in TB-infected individuals when compared to healthy controls and thus making it a potential biomarker for TB.[32] Another study investigating host-blood derived biomarkers using blood-derived transcriptomes for the GEO database has revealed that Ubiquitin/ISG15-conjugating enzyme E2 L6 (UBE2L6), Basic leucine zipper transcriptional factor ATF-like (BATF2), Plasma protease C1 inhibitor (C1-IMH), and vesicle-associated membrane protein 5 (VAMP5) could potentially be used for diagnosis of active TB and possibly be used to differentiate active and latent TB.[33] Another study showed that a protein marker, C-X-C motif chemokine ligand 1 (CXCL1) was successfully used in discriminating between active pulmonary TB from non-pulmonary TB, latent TB, and healthy controls.[34] A recent study was conducted using whole-blood transcriptome data obtained from the GEO database. This data was collected from children with active and latent TB. The data revealed that genes associated with neutrophil activation and degranulation, neutrophil-mediated immunity, and defense response were significantly differentially expressed. Amongst the cohort was Toll-like receptor 2 (TLR2), Formyl peptide receptor 2 (FPR2), Matrix metallopeptidase 9 (MMP9), Myeloperoxidase (MPO), carcinoembryonic antigen-related cell adhesion molecule 8(CEACAM8), Elastase, neutrophil Expressed ELANE (Fc fragment of IgG receptor 1A), Fc fragment of IgG receptor 1A (FCGR1A), Selectin (SELP), Arginase 1 (ARG1), G protein subunit gamma 10 (GNG10), Haptoglobin (HP), Lipocalin 2 (LCN2), Lactotransferrin (LTF), Adenylate cyclase 3 (ADCY3) were able to differentiate between latent and active TB in children, thus, presenting potential gene markers for developing non-sputum diagnostic tools for childhood tuberculosis.[35] These are just a few examples of studies performed on host transcriptomics to search for potential biomarkers for the diagnosis of different stages of TB. Many studies available on biomarker development for diagnosis and treatment have mainly focused on the host transcriptome and little has been reported on the pathogen differential expression at different stages of the disease. Perhaps, future studies should also consider the differential expression of the TB pathogen in the host in conjugation with the host immune response to better understand the host-pathogen interaction. These findings may be useful in searching for additional biomarkers for TB diagnostics.

4. The application of photonics in TB diagnostic test development

Photonics-based diagnostic platforms have shown a great potential to be adapted for rapid and accurate diagnosis of TB, nonetheless, these have not been extensively explored and thus, have since emerged as a promising technology for diagnosis. For example, more advanced biophotonics-based methods such as Raman spectroscopy (RS), surface plasmon resonance (SPR), and biosensors-based approaches for TB diagnosis will be discussed in more detail in this review.

4.1. *Surface plasmon resonance*

Surface plasmon resonance (SPR) is defined as a label-free, high-throughput, optical test that can be used to analyze protein-protein interactions. It is highly sensitive and uses small numbers of samples for real-time measurement of biomolecular interactions. In addition to studying the thermodynamics of biomolecular interactions, it can also be used to determine study binding kinetics, specificity, and affinity. The phenomenon of SPR occurs when photons of an incident light source hit a thin metal surface (for example, gold, and silver) at a specific angle, these metal surface electrons get excited and start resonating. These resonating electrons generated at the boundary of the metal surface are referred to as surface plasmons.[36] A study by Malabi et al. (2019) showed that SPR can be used to detect biological analyte.[37] Other studies showed that SPR was able to detect TB-specific antigens or antibodies. For example, Hong et al. (2010) showed that a tissue-specific antigen found in most TB patients, CF10 could be detected using SPR. In this study monoclonal antibodies (anti-CF10) were immobilized on a gold surface to specifically bind to CF10 antigen. This assay was able to detect up to 100 ng/μl of TB antigen (CFP-10 antigen) in tissue fluid.[38] The sensitivity of the SPR system was further enhanced by using TB antigen CFP-10 in sandwich assay and coupling the secondary sandwich antibodies with nanoparticles ([39]). Another study by Sun et al. (2017) showed that a localized SPR was able to detect TB- specific antibodies. In this study, a fusion protein CFP10-ESAT6 antigen was immobilized to gold nano-rods by using chemical modification. The functionalized gold nanorods-antigen complex was incubated with serum collected from patients with active TB, non-tuberculous pulmonary disease, and their healthy controls. The test successfully detected TB CFP10-ESAT6 with a specificity and sensitivity of 79% and 92%, respectively.[40] Kimuda et al. (2018) showed that SPR can also be used to characterize antibody avidity in patients with active pulmonary TB, and latent TB when compared to the healthy controls.[41] SPR has also been used to detect TB-specific sequences, for example, Duman et al. (2010) showed that the *M. tuberculosis* complex (MTBC) was successfully detected using SPR based multichannel system. Herein the probe was designed to have a spacer that would bind directly to the target sequence. The sensor was prepared by direct coupling of the thiolated probe with a gold surface and blocked with mercapto-1-hexanol to prevent non-specific binding. This assay was able to detect up to 30 ng/μl of the target.

4.2. *Raman Spectroscopy*

Raman Spectroscopy has been used as a preferred method for qualitative analysis and it is often used for the characterization of biological samples owing to its high specificity in determining and differentiating chemical bonds in molecules.[42] It has become a powerful non-invasive and non-destructive molecular identification tool.[42] RS has been used for TB detection using sputum sample.[43] The analysis done on Raman spectra showed the presence of a TB biomarker compound in the sputum sample and this biomarker created variations of Raman peaks. These variations included intensity fluctuation and wavenumber shift of coinciding peaks, furthermore, the signal detected was weak. As a result, RS was coupled to a machine learning algorithm, Principal component analysis (PCA) to accurately differentiate TB-negative and TB-positive specimens using cell-free supernatant of sputum samples. Thus, the inclusion of PCA on the acquired Raman data resulted in a more rapid and accurate detection system for TB.

5. Biomarkers and biosensors

Studies on the identification of TB biomarkers for the development of biosensors that can be used for effective TB diagnosis have been described. For example, Liu et al. (2018) developed a multiplex TB biomarkers detector coupled with an optical sensor. This optical diagnostic platform was based on silicon photonic microring sensors and asymmetric isothermal amplification technique (SPMS-AIA). These biphotonic sensors did not require labeling and were successfully used for the detection of a single MTBC biomarker (IS6110) and as a multiplex for the detection of two biomarkers (IS6110 and IS1081) in clinical sputum specimens. The assay was rapid and label-free, and results were obtained in real-time.[44] In another study, Crawford et al. (2017) used a Surface-enhanced Raman scattering (SERS)-based immunoassay approach for the detection of mannose-capped Lipoarabinomannan (ManLAM), an antigenic marker for active tuberculosis infection in human serum. The platform used a combination of gold nanoparticle (AuNP) labels, ManLAM monoclonal antibodies (mAbs), and surface-enhanced Raman scattering (SERS) detection. The limit of detection was also evaluated in human serum samples that were spiked with ManLAM antigen. Furthermore, the sensitivity was further enhanced by adding a layer of Cyanine 5 to a layer of gold capture surface, thus this complex chemically interacts with the substrate to form the Surface-enhanced Resonance Raman Scattering (SERRS).[45] Other studies showed that the fabrication of biosensors has improved with the coupling of photonic sensor chips into polymer microfluidic cartridges. For instance, Gonzalez-Guerrero et al. (2016) developed a novel photonics-based platform for the detection of TB in human urine samples.[46] This platform consists of the label-free detection of Lipoarabinomannan (LAM) in unprocessed urine. The photonic sensor chip is based on a Mach–Zehnder Interferometer (MZI) transducer which was combined with an on-chip spectral filter.[46,47] A broadband light source was used as a light source whereas a complementary metal oxide semiconductor (CMOS) sensor was employed as a signal detector. The sensor chip surface was functionalized with specific monoclonal antibodies against LAM. The shift in the wavelength induced by the refractive index changes on the surface of the sensor was used to evaluate the sample diagnosis in real-time. TB has detected in human urine samples in less than 15 min and the limit of detection was as low as 475 pg/mL for the direct detection in spiked urine samples. The results obtained using the Mach-Zehnder interferometer biosensor correlated to those obtained with GeneXpert. The platform showed up to 100% sensitivity and specificity when compared to rapid tests commercially available for TB detection.[47]

Novel devices that utilize immunosensor and bio-opticals have been developed to diagnose pulmonary Tuberculosis (TB) in patients.[39,48] These devices use prisms whereby the surfaces are coated with *Mycobacterium* tuberculosis-specific antibody and fluorescent peptide determinants.[48] In the presence of TB bacterium, the fluorescent-coated determinants are displaced by TB bacterium and the diode laser of the measuring device interrogates this biochemical process. The amount of the antigen present on the surface of the prism is determined by evanescent wave fluorimetry on the device.[39,48] Results are obtained within a few minutes and the assay was able to detect up to 50–75 CFU/mL of Mycobacterium tuberculosis cells[48]

All these studies show that POC diagnostics offers more advantage in TB diagnosis as they are rapid, easy to handle, and operate. In particular, biosensors are portable, rapid, and sensitive and they can be used even in clinical settings outside the laboratory which is advantageous, especially in the developing world where there is limited access to specialized laboratories.[39]

6. Conclusions

TB disease still affects millions of people globally and achieving complete eradication will depend primarily on the availability of proper diagnostic tools for early detection. Herein, we reviewed POC diagnostic tools that are reliable, robust, and easily accessible even in the most decentralized areas that offer a better alternative. Most of these tests are performed on samples that are easily accessible and results are usually obtained in real-time. Thus, the availability of these tests will increase treatment turnaround time and consequently reduce the risk of patient loss-to-follow-up. Although many of these tests including the Xpert MTB/RIF, LAM, and NAAT have been proven effective in recent years, there is also a major drawback associated with them, thus a need for improvement. Biophotonics-based techniques have somewhat shown increased sensitivity and specificity in TB diagnostics and thus can ultimately be used to complement available diagnostic tools. Regardless, there is still limited data available on the biomarkers that can be effectively used for the definitive diagnosis of TB in different stages of infection (active vs latent), different patient samples (healthy vs immunocompromised), and in adults vs children. The advancement of next-generation sequencing technology or bioinformatic tools has enabled the identification of possible TB biomarkers in the host. Similarly, these technologies can also be used for searching for additional biomarkers of TB *ex vivo* in different stages of TB infection, which is our research focus. These biomarkers can be effectively used for the development of biphotonic-based POC diagnostics tools.

Author's contribution

Dr. Mabotse A Tjale: Concept of the paper, literature search, acquisition of the information, and drafting of the article.

Dr. Saturnin Ombinda-Lemboumba: Literature search, acquisition of the information, and editing of the article.

Mr. Charles Maphanga: Literature search, acquisition of the information, and editing of the article.

Dr. Patience Mthunzi-Kufa: Revision of article for intellectual content, editing of the article.

Conflicts of interest

The authors have none to declare.

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Review article

Relationship between smoking and tuberculosis recurrence: A systematic review and meta-analysis

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ABSTRACT

Introduction: Of the problems in tuberculosis (TB) control program is the recurrence of this disease. In some studies, smoking has been reported as the most important risk factor. Therefore, the present study aimed at examining the association between smoking and tuberculosis recurrence using meta-analysis.

Methods: To report the findings of this meta-analysis, we used PRISMA. The protocol of this study has been recorded in PROSPERO. The research question has been formulated based on PICO, and the search was performed using both MeSH and non-MeSH keywords. After screening and selecting the articles and evaluating their quality using the NOS checklist, the overall estimate of the odds ratio of tuberculosis recurrence in smokers was assessed with a 95% confidence interval.

Results: Fourteen studies met the inclusion criteria. The total number of samples in the group of patients with tuberculosis recurrence was 1988 with 855 (43%) smokers, and in the group of patients affected by tuberculosis without recurrence, it was 27,226 with 7503 (27.56%) smokers. In 13 studies, the odds ratio of tuberculosis recurrence was higher in smokers; this difference was statistically significant in 12 of them. Combining the results of these 14 studies, the odds ratio of tuberculosis recurrence in smokers was 2.10 times higher, using the random effects model (95% CI:1.69, 2.61).

Conclusion: Based on the results of study present, smoking increases the risk of tuberculosis recurrence. Therefore, to eradicate tuberculosis by 2030, more serious interventions should be taken to quit smoking, which in turn reduces the incidence of tuberculosis.

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1. Introduction

Tuberculosis (TB) is one of the main reasons of death in the world.[1] Based on the 2022 report of World Health Organization (WHO), approximately 10.6 million persons were infected with TB worldwide in 2021.[1] The COVID-19 pandemic caused fewer reports of newly detected TB cases,[1] thus the statistics might be underestimated. TB recurrence following the treatment for active TB occurs in 2–3% of patients properly treated with the four-drug regimen[2]; this is one of the TB control problems that requires retreatment.[3] It can be due to the relapse of the previous strain of Mycobacterium TB (M. TB) or exogenous reinfection due to another strain of the bacterium.[4]

Relapse is the most important cause of recurrence in areas with a low prevalence of TB, and reinfection occurs less frequently compared to areas with a high prevalence of TB.[5] Diagnosis of exogenous relapse and reinfection is important in evaluating the effectiveness of control programs and understanding the immune response to TB.[6] It is performed in each episode of the disease based on the genomic profile of TB.[7]

The cause of many cases of TB is attributed to 5 risk factors, including malnutrition, smoking, alcohol abuse, HIV infection, and diabetes. Among them, the share of smokers in 2018 was estimated at 0.86 million people, of which 0.81 million were men.[8]

In the previous studies, various risk factors have been reported for relapse and reinfection. For instance, in a cohort study,[6] the recurrence rate was found to be higher in HIV-positives, women, drug-resistant individuals, and those who had a positive smear sample during the treatment. In another descriptive study, a significant association was observed between smoking and TB relapse.[9] According to this study, smoking increases the risk of TB relapse more than two times.

Some studies have surveyed the association between smoking and TB recurrence; however, there are inconsistencies between the results of these initial studies. One method that contributes to resolving these discrepancies is meta-analysis. Combining the results of initial studies, this method provides evidence with higher sample size and test power to prove the causal relationship between smoking and TB recurrence. Therefore, the present study aimed at determining the relationship between smoking and TB recurrence.

2. Method

The PRISMA guideline was employed to report the findings in this meta-analysis.[10] To accomplish the study, a protocol was prepared, based on which the study was performed. The protocol of the study has been recorded in PROSPERO (CRD42017071977).

2.1. Inclusion criteria

Criteria for the inclusion of initial studies in this meta-analysis are based on PI(E)COTS. These criteria include studies in which 1) the study population (P) involved patients with recurrence TB, 2) exposure (E) included smoking TB patients, 3) the comparison (C) group encompassed patients with tuberculosis without recurrences, 4) their outcome (O) was the chance of recurrence, 5) the time (T) of publication of the initial studies was from the beginning of 2000 to February 15, 2021, and 6) type of studies (S) was either case-control or cohort. The language of publication of the initial studies was English. Regional constraints were not considered in terms of the location of the study.

2.2. Search process

Databases reviewed included PubMed, Scopus, Cochran library, Web of Science, and Google Scholar search engine. Search strategy comprised of MeSH, Non-MeSH keywords of Tuberculosis, Tuberculosis, Koch's Disease, Relapse, Recurrence, Reinfection, Smoking, Cigarette smoking, Tobacco, Treatment, Outcome, Case-control, and Cohort. Search was done from February 16 to February 25, 2021. In addition, to increase the sensitivity of the research, the research team reviewed the references of the searched articles. Search management was done in Endnote software.

2.3. Study selection

Two people independently selected the studies according to the inclusion criteria. Selection of the articles was done after restrictive the search strategy and eliminating the duplicates. First, the article titles were appraised, and duplicated articles were withdrawn. After reading the summaries, the irrelevant ones were recognized. Full-texts of the remaining articles were received, and after reading them, another group of irrelevant articles was deleted. Finally, eligible studies based on the inclusion criteria were selected. The kappa coefficient was also used to evaluate the full-texts and was interpreted based on the guideline proposed by Landis and Koch. A kappa value between 0 and 0.20 shows a slight agreement, between 0.21 and 0.40 shows a fair agreement, 0.41 to 0.60 specifies a moderate agreement, 0.61 to 0.80 indicates a considerable agreement, and a value higher than 0.80 indicates a perfect agreement.[11]

2.4. Quality assessment

The NOS checklist was used for quality assessment, and studies with a quality assessment score of less than 5 were excluded.

2.5. Extracting the data

Two authors used Excel software to extract the data. Contradictory cases were examined and resolved by the third researcher. Variables intended for data extraction included the name of the first author, year of publication, study design, study location, the sample size in the case group (patients with recurrence TB), the sample size in the control group (patients without recurrent TB), matched variables, sample size determination logic, sampling method, case group definition in each study, control group definition in each study, case group selection source, control group selection source, the mean age of the samples, the number of smokers in the case and control groups, and the number of non-smokers in the case and control groups.

2.6. Data analysis

To analyze the data, we used Stata ver. 14 package (StataCorp, College Station, TX, USA). For each study, a two by two contingency table was made for the case and control groups. Data were given weight and pooled using the inverse variance technique. The heterogeneity Indicator among studies was obtained using Cochran (Q) and I_2 tests. The criteria for heterogeneity was a significance level of less than 0.1. Moreover, according to Higgins et al., low, moderate, high are assigned to I-squared values of less than 25%, 25–75%, and above 75%.[12] Based on the heterogeneity results, a random or fixed-effect model was used to estimate the odds ratio of recurrence in smoker patients with TB. Publication bias was performed with funnel plot and Egger and Begg tests. The impact of each study on the total estimate was evaluated by sensitivity analysis. Moreover, heterogeneity sources were checked by a meta-regression test.

3. Results

Searching the databases mentioned in the method section, 1283 articles were found. After deleting the duplicates, 380 remained. Then, 342 unrelated articles were identified through screening the articles by title and abstract. The full-texts of the remaining articles (number = 38) were evaluated, and 24 cases were excluded due to the lack of either a control group or reporting the required index. Finally, 14 articles entered the process of systematic review and meta-analysis (Fig. 1).[3,9,13–24] Based on the quality evaluation results, all 14 studies met the inclusion criteria (the quality evaluation scores of all 14 articles were above 5). The kappa agreement coefficient between the two researchers who selected the articles was equal to 94.3%.

The articles had been conducted in different years, i.e., from 2005 to 2020. Two studies were conducted in India and one in each of the countries, including Pakistan, Bangladesh, Brazil, China, Congo, Croatia, Hong Kong, Iran, Poland, Vietnam, Tunisia, and Taiwan (Table 1).

The matching between the recurrent and non-recurrent TB groups was mentioned in 12 of the initial studies, though the type of matched variable was relatively diverse. Among them, matching the demographic variables (age and sex) between the case and control groups was only done in seven studies. The total number of samples in the group of patients with recurrent TB was 1988 with 855 (43%) smokers, and in the other group, it was 27,226 with 7503 (27.56%) smokers.

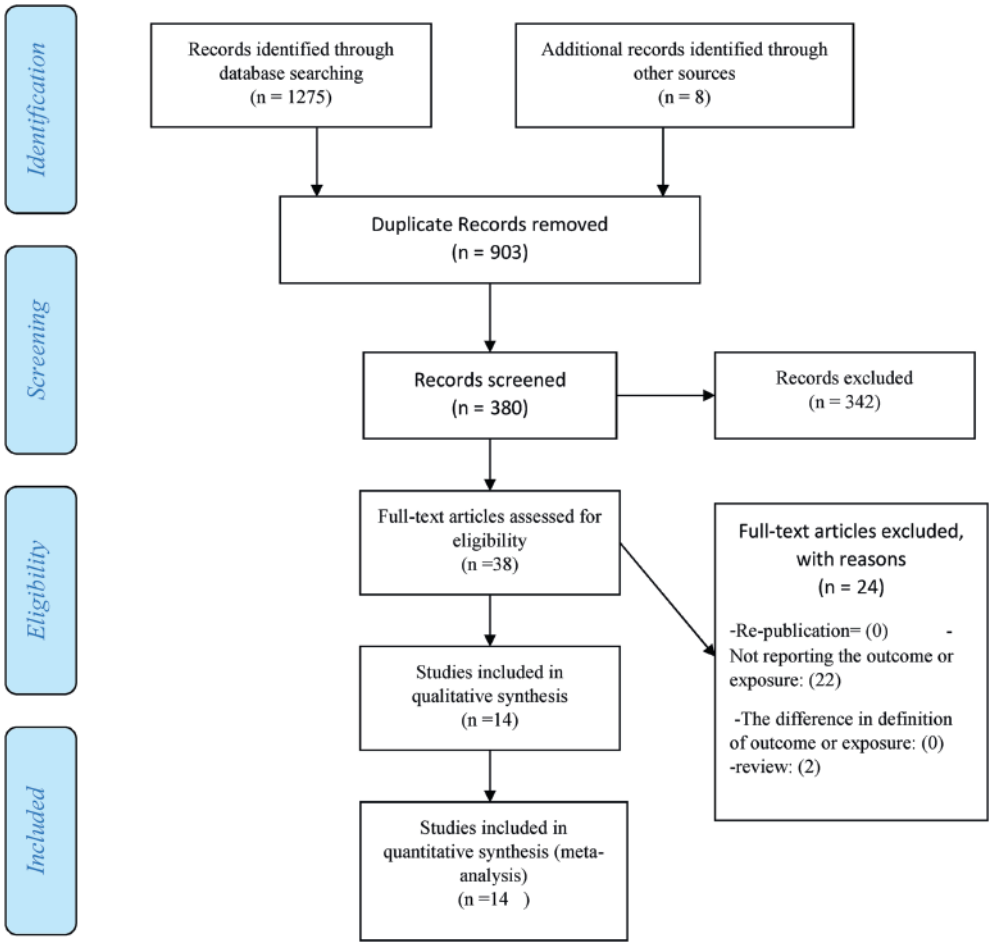

Fig. 1 – Flowchart of study selection.

Table 1 – Characteristics of preliminary studies included in the meta-analysis to estimate the odds ratio of recurrence of tuberculosis in smokers.

id	First author	Publication year	Area study	Type study	Type of reinfection	Number Case (with relapse)		Number Control (without relapse)		Machine (by demographic)
						smoker	Non-smoker	smoker	Non-smoker	
1	Ahmad	2016	Pakistan	case control	recurrence	69	80	49	109	No
2	dArc Lyra Batista	2008	Brazil	cohort	relapse	14	23	138	530	yes
3	Leung	2015	Hong Kong	cohort	relapse	136	166	3085	6351	yes
4	Thomas	2005	India	cohort	relapse	41	19	185	241	no
5	Tian	2014	China	case-control	recurrence	127	33	195	125	yes
6	Yen	2014	Taiwan	cohort	recurrence	21	59	631	4523	No
7	Salam	2020	Bangladesh	case-control	relapse	19	151	12	158	yes
8	Andre	2019	Congo	descriptive	relapse	31	56	899	3895	No
9	Mahishale	2015	India	prospective	relapse	51	69	344	1524	No
10	Bestrashniy	2018	Vietnam	case-control	recurrence	178	327	182	448	yes
11	Lampalo	2019	Croatia	case-control	recurrence	38	34	228	164	Yes
12	Moosazadeh	2015	Iran	Cohort	recurrence	31	75	200	965	No
13	Przybylski	2012	Poland	case-control	recurrence	70	6	1333	607	No
14	Racil	2012	Tunisia	case-control	relapse	29	35	22	83	Yes

The odds ratio of TB recurrence was higher among smokers in 13 studies[3,9,13-22,and 24]; this difference was statistically significant in 12 studies.[3,9,13–22] On the other hand, in one study, the odds ratio of TB recurrence was lower among smokers, though it was not statistically significant.[23] As it is illustrated in Figs. 2 and 3, the results of these 14 studies were combined, and the odds ratio of TB recurrence among smokers was estimated to be 2.10 using a random effect model (95% CI: 1.69, 2.61) and 1.91 using a fixed-effect model (95% CI: 1.71, 2.14). Moreover, based on heterogeneity indices, there was considerable heterogeneity between the initial studies (I-squared: 68.5%, Q: 41.30, $P < 0.001$). Therefore, estimating the odds ratio of TB recurrence in smokers should be done based on the random effect model. TB recurrence was of relapse type in seven studies[3,9,13,15,18,19,and 24]; by combining the results of these studies, the odds ratio of total relapse among smokers was equal to 2.35 (95% CI: 1.82, 3.03). In the other seven studies,[14,16,17,20–23] recurrence was of reinfection type; by combining the results of these studies, the total odds ratio of reinfection among smokers was estimated to be 1.90 (CI 95%: 1.34, 2.69).

According to the funnel plot diagram (Fig. 4), the publication bias to assess the odds of TB recurrence was not significant among smokers. In addition, the results of the Egger test ($\beta = 2.22$, $P = 0.079$) and the Begg test ($Z = -0.82$, $P = 0.412$) show that there is no publication bias. On the other hand, the sensitivity analysis revealed that the effect of each of the initial studies on the overall estimate is not significant (Table 2). The effect of matching demographic variables between the case and control groups on heterogeneity in the initial studies was evaluated using meta-regression. The results displayed that the effect of this variable on heterogeneity was not significant ($\beta = -0.95$, $P = 0.090$).

4. Discussion

In this study, the relationship between smoking and TB recurrence was evaluated. Combining the results of 14 eligible studies based on the inclusion criteria using meta-analysis, the odds of TB recurrence among smokers is estimated to be 2.1 times higher than that of the non-smokers.

Overall, 14 initial studies evaluated the association between TB recurrence and smoking.[3,9,13–24] Among them, seven initial studies examined the effect of smoking on TB relapse.[3,9,13,15,18,19,and 24] In all seven studies, the odds of relapse were higher among smokers, although the results were not statistically significant in one study.[24] However, in the same study, second-hand smokers and past smokers were significantly associated with TB relapse. In the present study, being the current smokers or not at the time of the study was one of the entry criteria.

A significant relationship has been observed between smoking and reinfection in 6 of 7 studies that entered the meta-analysis examining the effect of smoking on TB reinfection,[14,16,17,20–23] and it was revealed that reinfection has been higher among smokers.[14,16,17,20–22] In one study, smoking was suggested as a protective factor, although this effect was not statistically significant.[23]

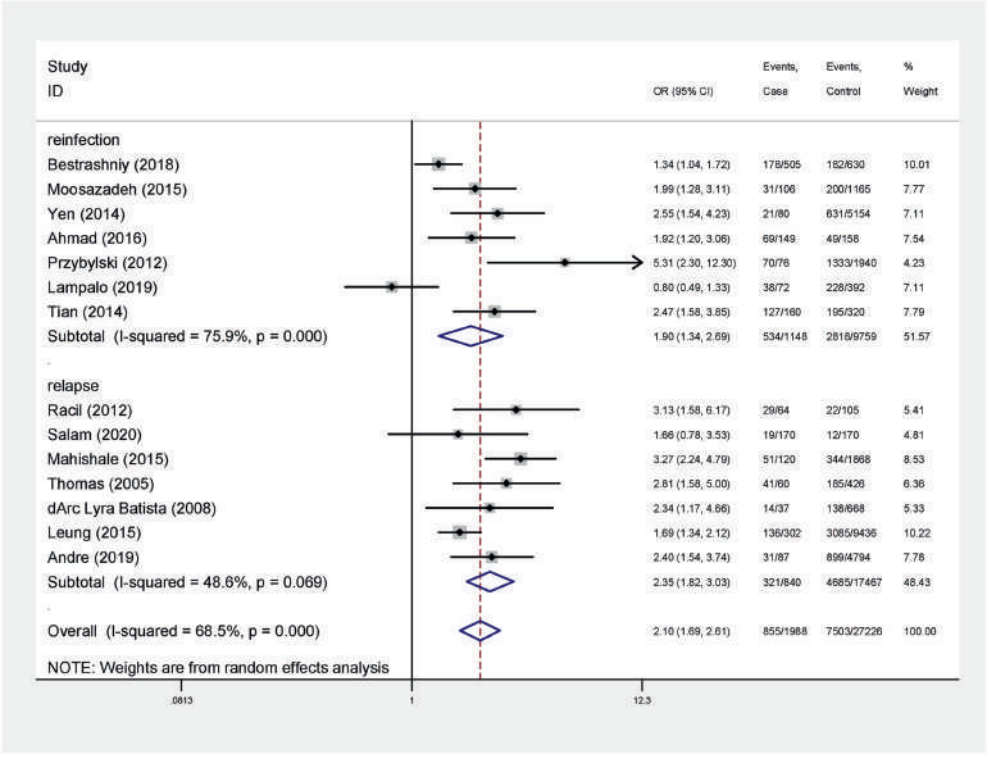

Fig. 2 – Overall odds ratio of tuberculosis recurrence in smokers with 95% confidence interval in each of the initial studies and overall estimation using a random effect model.

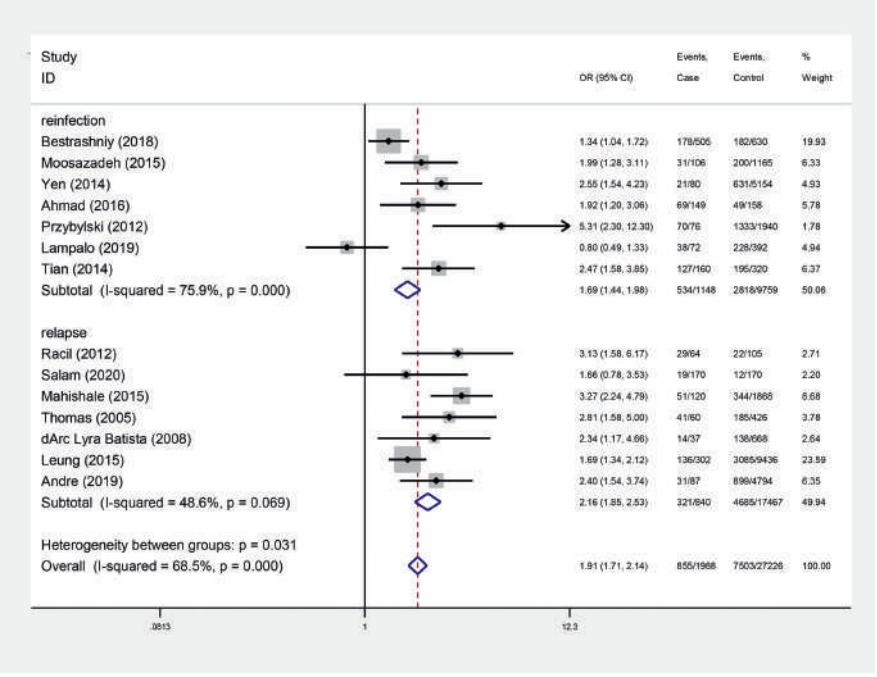

Fig. 3 – Overall odds ratio of tuberculosis recurrence in smokers with 95% confidence interval in each of the initial studies and overall estimate using the fixed effect model.

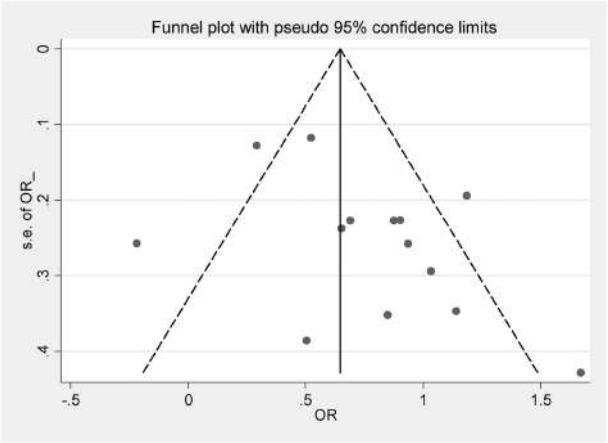

Fig. 4 – Funnel plot figure to check the publication bias.

Table 2 – The effect of each of the primary studies on the overall estimate using sensitivity analysis.

Study omitted	Estimate	[95% Conf.Interval]
Thomas (2005)	2.0636735	1.646778–2.5861092
Salam (2020)	2.1327853	1.7001543–2.6755064
dArc Lyra Batista (2008)	2.094198	1.6685152–2.628484
Tian (2014)	2.0793915	1.650436–2.6198344
Andre (2019)	2.0849183	1.6535723–2.6287839
Lampalo (2019)	2.2342556	1.8339767–2.7218986
Leung (2015)	2.1682279	1.6918238–2.7787836
Mahishale (2015)	2.0063388	1.6209254–2.4833932
Ahmad (2016)	2.1256025	1.682374–2.6856015
Yen (2014)	2.0756254	1.650925–2.6095796
Bestrashniy (2018)	2.2074785	1.7720541–2.7498944
Racil (2012)	2.0564463	1.645772–2.5695972
Przybylski (2012)	2.0146482	1.6327412–2.4858851
Moosazadeh (2015)	2.1195233	1.6763498–2.6798575
Combined	2.1036984	1.6934707–2.6132999

The findings of this meta-analysis are consistent with the systematic qualitative review study[25]; among the 24 reviewed articles, a significant relationship was observed between smoking and TB in 22 articles (including new cases and TB recurrence). Of course, a study conducted by Jee al. in South Korea examined the risk of TB recurrence independently in men and women smokers.[26] It was concluded that in contrast to men, the recurrence risk in women was not significant.

Smoking damages various organs, especially the lungs. Affecting the immune system, smoking makes a smoker more vulnerable to TB.[27] Smoking affects the components of the immune system both locally and systemically, including reducing the defense mechanism of the lungs. It is also associated with an increase in the number of neutrophils and a decrease in their function.[28] It also increases the synthesis and decreases anti-apoptotic proteins in macrophages, leading to an increase in the number of macrophages that do not function properly. This number of macrophages has been observed in the Bronchoalveolar Lavage (BAL) samples of smokers.[29–31] Since M. TB must enter the macrophages in the lungs to cause infection, smoking provides the appropriate conditions for germs to multiply and survive; this can be one of the causes for the increased risk of TB recurrence.[32,33] Further evidence for this claim is that animal studies have revealed that the reduction of alveolar macrophages has a protective effect against M. TB infection.[34]

Due to the presence of iron in cigarette tobacco, a smoker or second-hand smoker inhales some iron with each inhalation of cigarette smoke and increases the iron load in alveolar macrophages.[35] Increased iron surges the intracellular growth of M. TB and aggravates the disease leading to death.[36] There is evidence when there are cavities in the lungs, an increased risk of TB recurrence is due to increased bacterial load and less access of anti-tuberculosis drugs to these areas.[37] Moreover, smokers have shorter cilia which reduce mucociliary clearance in the airways. As a result, the risk of lung infections increases in these people.[38]

Another issue is that smoking harms other TB control indicators such as positive sputum smear after two months of aggressive treatment, absence from treatment, etc. Studies have demonstrated that smokers are more likely to be smear-positive two months after the treatment. They are also more likely to miss treatment by default, increasing the risk of treatment failure.[18,39]

Smokers are also more likely to drink alcohol[40]; this can lead to more severe manifestations of lung disease. Although low to moderate alcohol consumption is not associated with an increased risk of TB, high alcohol consumption surges the risk of pulmonary TB.[40,41] This can be another reason for the higher incidence of TB recurrence among smokers. In short, combating tobacco use should be a serious and practical assurance of governments.

The strategic goal of the end TB is to reduce TB-related deaths by 90% and TB incidence by 80% by 2030 compared to 2015.[8] One of the problems of TB control programs is reinfection or relapse TB cases that need to retreat.[3] Therefore, in addition to smoking, other risk factors related to TB recurrence should be recognized.

One of the limitations of this study is that we have considered being a smoker and non-smoker only at the research time. Thus, non-smokers encompassed people who had never smoked or used to smoke; This factor may affect the findings of the study. Another limitation of this meta-analysis is that it was not possible to examine the relationship between the smoking index and the recurrence of tuberculosis.

According to the results of this study, smoking significantly increases the risk of TB recurrence. Therefore, to achieve the goal of end TB by 2030, it is necessary to take more serious interventions to quit smoking, which will subsequently reduce the incidence of TB.

Authors' contributions

MM and FP done the search process, data extraction, screening, statistical analyses, Data interpretation, and drafted and revised the manuscript for important intellectual content and approved the final version. MK, SA, FR and SB interpreted data, reviewed the analyses and accepted the final version. All authors have read and approved the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

This study has been approved by the Ethics Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1397.35).

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no Competing interests.

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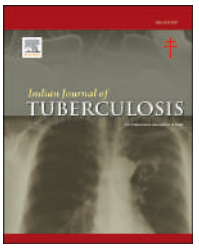

Review article

Community-friendly tool to assess patient satisfaction in tuberculosis programme in the covid pandemic period; Bhubaneswar; India

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ABSTRACT

National Tuberculosis Elimination Programme (NTEP) is a priority programme for India, given that India is one of the 20 countries with high burden of TB. Odisha (a state in Eastern India) in 2017 reported 159/lakh/year cases as against a national average incidence of 138.33/lakh/year. Thus, the state, under an encouraging political milieu went to vigorously implement the newer initiatives outlined in the National Strategic Plan 2020–25, the result of which in 2021 Odisha was ranked second in the country for its efforts on TB elimination. The current article attempts to take community feedback on the programmatic endeavours, by using a tool for client satisfaction.

350 consecutive subjects, adults aged 18 years and above consented among the 465 who were diagnosed and started on treatment between 5/4/21 to 5/4/22. The selected subjects were interviewed after confirmation of diagnosis at one DOTS centre in an urban city, using a pre-designed and pretested tool after taking requisite ethical permission from the institute as well as after consent from the participating subjects.

The tool had 10 items on structure; 10 items on the process and 3 on outcome each rated on a Likert scale of 1–5 (very satisfied to very dissatisfied) and lastly a score on 10 scale for overall satisfaction. For all the 24 items; alpha Cronbach coefficient was 0.928 (bootstrap 95% CI); for subscales infrastructure, process and outcome isolatedly was 0.931, 0.912 and 0.959 respectively. This shows that the questionnaire had very good reliability.

Infrastructure mean score for all 10 questions was above 4.5; for processes, it was <4.05 for a few questions and mainly these referred to Out of pocket expenditures and waiting time; outcome again for all three questions mean score was near or above 4.4. The overall score was between 5 and 10; maximally at 8. This simple tool gave clear-cut hints at the best picture scenario, as the study was done at a single DOTS service centre in the capital city of the state, which ran effectively even during the pandemic. However, it brings out the weak points in the processes like the cost incurred to come to the centre and

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communication with ancillary staff. No difference in satisfaction levels was reported among pulmonary and extrapulmonary cases (ratio 8.4:1.5) in this study in the covid period; with overall satisfaction being 4.45 ± 0.44 and 4.41 ± 0.25 respectively.

The promptness in the programmatic services at the DOTS centre under study is encouraging but warrants conformity with DOTS centres in rural and far-to-reach areas. Best evaluation of achievements of programme can be determined by word of mouth of the beneficiaries. Hence, this tool if replicated at all service centres can help programme managers plug any disconnects in service delivery and assure good satisfaction from all quarters.

1. Introduction

India has one of the highest burdens of Tuberculosis among all countries in world, with largest incidence of 2,590,000 cases at rate of 188/100,000 population in 2020.[1] Since 1962, with the conception of National TB control programme, India's effort to curb this menace had begun, however due to several constraining factors including lack of funds, it was then limited to introducing BCG vaccination to prevent TB infection in infants and children. Only in 1997, Revised National TB Control Programme (RNTCP) was formulated that adopted the internationally recommended Directly Observed Treatment Short-course (DOTS) strategy, as the most systematic and cost-effective approach to revitalise the TB control programme in India. Political and administrative commitment helped to turnaround the redundancy of the core programme and smear microscopy was a feasible lab procedure for early diagnosis and to initiate treatment. Ready availability of drugs in a supervised manner also was an enabling factor. It also ensured regulated systems and infrastructure by establishment of a sub-district supervisory unit, known as a TB Unit, with dedicated RNTCP supervisors posted, and decentralization of both diagnostic and treatment services. Given the large burden of disease, such developments led to nationwide expansion and covered the whole country under RNTCP by the year 2005. Phase II of RNTCP (2006–2015) enforced quality and addressed newer challenges being posed by HIV/TB comorbidity and Multi-Drug Resistance TB.[2,3] With a gradual yet steadfast progression, now India plans TB elimination by 2025 and hence this public health initiative is being addressed by christening the programme as National TB elimination Programme and the country is working towards this by strategies under the broad themes of "Prevent, Detect, Treat and Build pillars for universal coverage and protection".[4]

History gives evidence that control of any endemic disease in a developing nation like India, calls for a government-owned programme to maximize reach and the best way to assure quality is to get direct feedback from the patients regarding their satisfaction with all facets of the programme.[5] This client/patient satisfaction offers pertinent suggestions to improve small gaps in the programme and make the big objectives achievable.

Odisha figured among the top-ten TB incidence states in the country yet given its favourable political milieu, it ranked now second in its efforts targeting TB elimination, in spite of the rage of the pandemic of Covid 19.[6] This encouraging achievement provoked the study team to access patient satisfaction with all aspects of the programme ie infrastructure, processes and outcomes to derive finer details for further improvement.

2. Objective

To validate a community-friendly patient satisfaction score among patients who received treatment under NTEP in a tertiary care DOTs clinic in Bhubaneswar city.

To determine any difference in the satisfaction score (as a whole) among the pulmonary and extrapulmonary TB cases in the selected sample.

3. Methods

The study was planned between March 2020 to Aug 2021, in the midst of shutdowns and lockdowns in the state and even countrywide due to Covid 19 pandemic. Thus, the study team adds the disclaimer that the inferences in the study are limited to data from one DOTs centre in a private hospital, which caters to a large slum population in the city and has a good case reporting record. As the study was undertaken during the active Covid 19 pandemic waves, the study data could not involve more centres for data collection due to limitations of travel as well as stringent norms to enter any other health institution for data entry. The inclusion criteria were the study participant should have tested positive for TB disease by sputum microscopy or molecular diagnostics and seeking treatment services from the centre under NTEP. Adult subject 18 years of age and above, regardless of gender and type of TB and willing to reply to the study tool were taken up for the study. The study proposal and its tool were duly approved by the IEC of the hospital. Given the incidence of 26% in India,[7] at 95% CI, the precision of 5% and non-response rate of 10%, a sample of 320 and more were considered statistically adequate for the study. Newly diagnosed cases of TB, having completed at least 4 or more months of their TB treatment were taken up so that reviews on TB outcomes can be obtained.

The study tool was an adapted version of a study done in Ethiopia[8] and the questions were adapted as per the local

Table 1 – Mean scores of Likert scale on the Satisfaction Tool for TB programme.

Scoring Variables	Very satisfied	Satisfied	Neutral	Dissatisfied	Very dissatisfied	Mean ± SD
Infrastructure (Are you satisfied with) n (%)						
Availability of necessary equipment, drugs, laboratory reagents to treat TB Disease	179 (51.1)	166 (47.4)	4 (1.1)	0 (0.0)	1 (0.3)	4.49 (0.55)
Easy access of the HCPs as you need	247 (70.6)	99 (28.3)	3 (0.9)	1 (0.3)	0 (0.0)	**4.69** (0.5)
Easy access of HCPs to re fill your medication	189 (54.0)	159 (45.4)	2 (0.6)	0 (0.0)	0 (0.0)	4.53 (0.51)
Wheelchair friendliness of the environment	193 (55.1)	152 (43.4)	5 (1.4)	0 (0.0)	0 (0.0)	4.54 (0.53)
The waiting area, registration and treatment room comfortableness and availability of seats	217 (62.0)	124 (35.4)	8 (2.3)	1 (0.3)	0 (0.0)	4.59 (0.55)
The safeness of the facility for the patients	206 (58.9)	136 (38.9)	8 (2.3)	0 (0.0)	0 (0.0)	4.57 (0.54)
Availability of signage/directions guidance where to go in the health	198 (56.6)	141 (40.3)	10 (2.9)	1 (0.3)	0 (0.0)	4.53 (0.57)
Facility						
Cleanness, goodness and working order of the latrine	201 (57.4)	141 (40.3)	5 (1.4)	3 (0.9)	0 (0.0)	4.54 (0.57)
Treatment room keeping your privacy	187 (53.4)	156 (44.6)	6 (1.7)	1 (0.3)	0 (0.0)	4.51 (0.55)
Availability of safe water to take a medication	217 (62)	122 (34.9)	10 (2.9)	1 (0.3)	0 (0.0)	4.59 (0.56)
Process (Are you satisfied with) n (%)						
Explanation and response of HCPs about your questions	162 (46.3)	176 (50.3)	10 (2.9)	2 (0.6)	0 (0.0)	4.42 (0.58)
HCPs ability of the diagnosis, treatment and care of TB	151 (43.1)	182 (52)	12 (1.1)	4 (1.1)	1 (0.3)	4.37 (0.64)
The cost you paid for TB diagnosis and treatment	129 (36.9)	153 (43.7)	35 (10.0)	33 (9.4)	0 (0.0)	**4.08** (0.92)
Obligation of costs beyond my ability to pay for transport	140 (40)	132 (37.7)	35 (10)	42 (12)	1 (0.3)	**4.05** (1.00)
Carefulness and allotted time of HCPs to check my clinical condition	149 (42.6)	161 (46)	33 (9.4)	7 (2.0)	0 (0.0)	4.29 (0.72)
HCP welcoming, respect, friendly treatment and courteous	166 (47.4)	159 (45.4)	23 (6.6)	1 (0.3)	1 (0.3)	4.39 (0.65)
HCPs considered you are unwise	172 (49.1)	151 (43.1)	25 (7.1)	2 (0.6)	0 (0.0)	4.41 (0.65)
appointment system for follow up	172 (49.1)	150 (42.9)	24 (6.9)	4 (1.1)	0 (0.0)	4.4 (0.67)
So long waiting time with registration process to get TB care	159 (45.4)	157 (44.9)	25 (7.1)	9 (2.6)	0 (0.0)	4.33 (0.72)
HCP uses medical terms/jargon without explaining what they mean	179 (51.1)	165 (47.1)	6 (1.7)	0 (0.0)	0 (0.0)	4.49 (0.53)
Outcome (Are you satisfied with) n (%)						
Your TB symptoms reduction rate	192 (54.9)	135 (38.6)	21 (6)	1 (0.3)	1 (0.3)	4.47 (0.65)
Physical wellbeing acquired due to TB treatment	181 (51.7)	146 (41.7)	20 (5.7)	2 (0.6)	1 (0.3)	4.44 (0.66)
Psychological	196 (56)	132 (37.7)	20 (5.7)	1 (0.3)	1 (0.3)	4.49 (0.65)
Wellbeing						

The values in bold depict the highest or lowest mean scores.

programmatic context. Since the tool had to be introduced in an interview mode in community settings, it was translated and back-translated into Odia, ie the local language. A team trained in the tool were deputed in the DOTs clinic to select and interview subjects as per the inclusion and exclusion criteria. The tool was pilot tested before use. Besides the client satisfaction questions, there were questions on socio-demography, income, living conditions and details on health-seeking behaviour. In this article, we present the validation and suitability of the tool used in the study.

4. Results

Out of the 1170 suspect cases who appeared for first-time testing in the centre, 465 tested positive for TB in the DOTs centre under the tertiary care hospital and 350 consented to participate in the study and take the interview as well as the scoring.

The study tool comprised of a total of 24 items of which 23 items reported subscales for structure (10 items), process (10 items) and outcome (3 items) on a Likert scale ranging from 1 to 5 (Very dissatisfied to Very satisfied). Almost everyone could complete the questionnaire within 30–40 minutes and could understand the Odia translation. The questionnaire structure proved valid as all inter-scale correlations were significant moderately hinting that the scales were assessing distinct components of the programme service delivery and were as per the Standards of care prescribed under the programme. The reliability of the scale as a whole as reflected by Cronbach alpha was 0.94 (bootstrap at 95% CI based on 1000 samples) and even for the subscales was 0.93, 0.91 and 0.95 for infrastructure, process and outcome respectively. Thus, the tool translated into Odia proved to be both valid and reliable and can be recommended for use in the state.

Some population characteristics of the sample were that Males are 62.9% and we have nearly 18% representation in all age groups above 18 years, barring higher age groups above 45 years. Subjects from varied socioeconomic strata are present though over 66% belong to urban slum which explains the Mean family income being 21344.29(SD 9644.00; range 3000 to 50,000 Indian Rupees). Nearly 26% of the cases had only primary level education or below and 38% were unemployed. 48% of the respondents habitually seek service from a public facility.

Table 1 shows the mean scores of the Likert choices of the total sample for each item in the questionnaire. The comforting finding is that all means of each item are above 4, compared to as per the scale the maximum mean could be 5, hinting at a very high satisfaction level.

Under infrastructure, satisfaction for the ability to access Health Care provider (HCP) scored 4.69, which is the highest

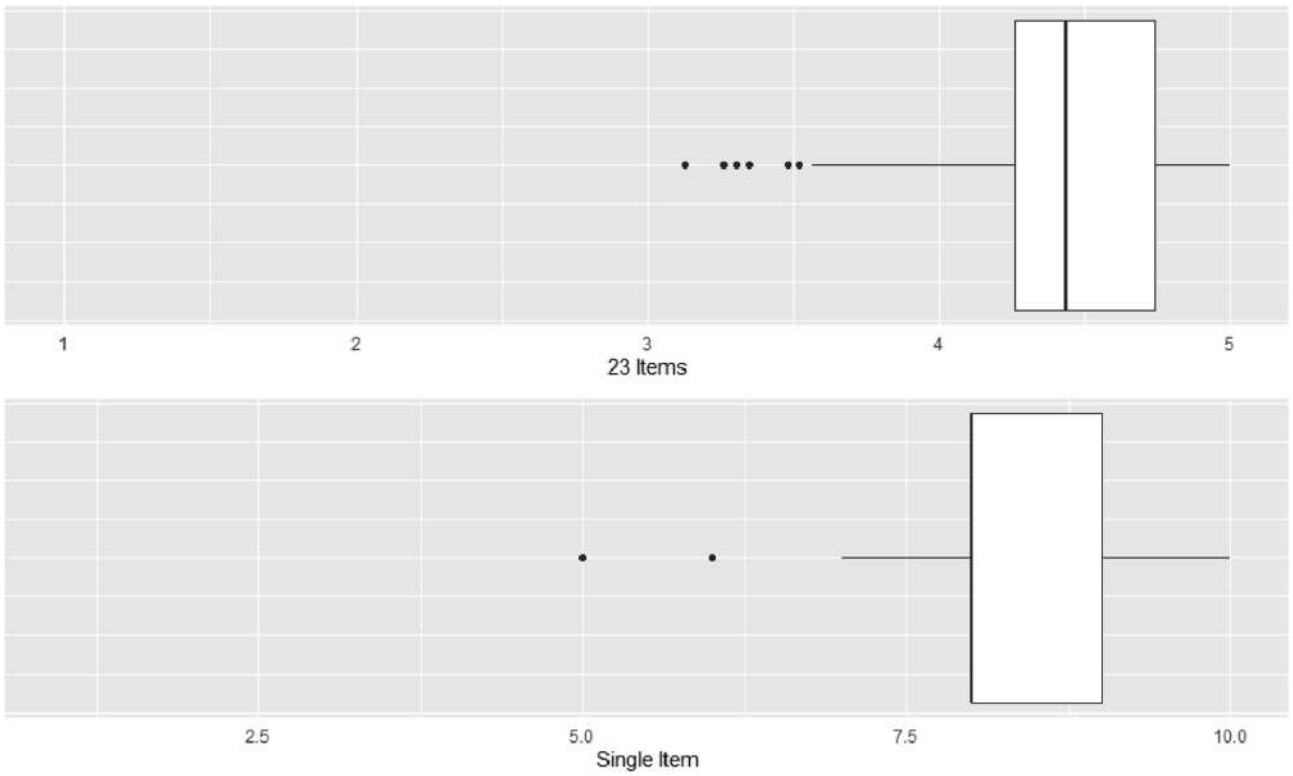

Fig. 1 – Comparison of Satisfaction scores from the single item and 23 items of the questionnaire.

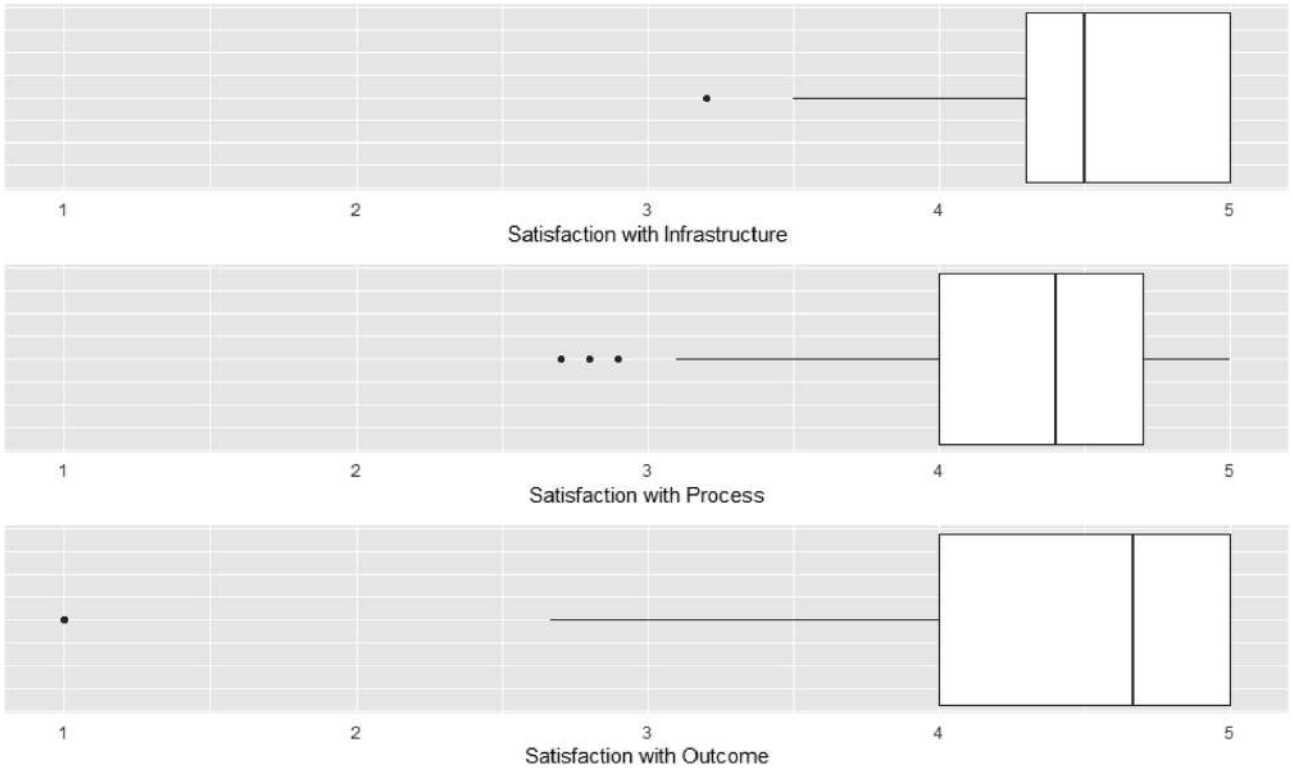

Fig. 2 – Satisfaction scores stratified by infrastructure, process and outcome subscales as per Likert scale.

Table 2 – Satisfaction (overall and subscale wise) in case of Pulmonary and Extra Pulmonary TB cases (Fig. 3).

	Pulmonary TB (N = 295)	Extra-Pulmonary TB (N = 54)
Infrastructure	4.57 ± 0.42	4.5 ± 0.33
Process	4.32 ± 0.54	4.38 ± 0.36
Outcome	4.51 ± 0.60	4.25 ± 0.56
Total	4.45 ± 0.44	4.41 ± 0.25

mean score, but this could be because of the location of DOTS centre in a tertiary care centre and the timing of data collection was during Covid 19 pandemic when health services were on red alert. The same could be the reason for the above 4 mean scores for all items under infrastructure where TB care was more demand-driven and people were eager to rule out TB diagnosis in wake of the pandemic.

Under process subscale, 12% of subjects quoted dissatisfaction with transport costs, making a mean score of 4.05 ± SD 1.00 and also 9.4% dissatisfaction with cost for diagnosis and treatment of TB , the mean being 4.08 (0.92). The highest mean of 4.49 (0.53) was reported under this subscale for HCP uses medical terms/jargon without explaining what they mean.

Under outcome, the highest satisfaction mean score of 4.49 (0.65) was reported in terms of Psychological well-being, although amelioration of symptoms and physical well-being too were addressed equally. But psychological satisfaction in the case of chronic illness is a very essential measure of feeling good.

Fig. 1 gives a cumulative graphic representation of the 23-item scoring wherein the mean is evidently skewed towards a much higher satisfaction, closing on to 5, though the ranges can depict some scores a little more than 3 (mean = 4.445; median = 4.435; SD 0.412).

The single item scoring which measures satisfaction on a scale of 0–10, is depicted in the graph as between 7.5 and 8 (mean = 8.371; median = 8; SD 1.158), which again is overall a positive remark on the programme, though we do find scores beginning from scale 5 onwards. This is a more realistic picture of the spectrum of satisfaction across population and the reason for the gaps can be discerned from the subscale scoring in Table 1.

A stringent programmatic review, which aims at achieving the missed targets would focus on the scores which are on the lower side of the scale.

The above Fig. 2 box plots give a clear comprehension of the mean value of scores being pushed from the higher to lower side as we move from outcome to process to infrastructure. This gives us a dissected pragmatic view of the satisfaction levels suggesting that even if scores are good in terms of outcome assessment, but the judgement on infrastructure and processes needs strengthening. The eagerness to overcome the disease helps outcome satisfaction scores to be good, negating the compromises made in infrastructure and processes.

As suggested by the results of sample data in this study shown in Table 2 and Fig. 3, there is no statistically significant difference between the satisfaction scores for the Pulmonary and Extrapulmonary TB. This is because of the very low number of Extra pulmonary cases who agreed to participate in the study suggesting a sampling bias, as well as the very evident limitation of the study of having taken up study in a well-equipped DOTS centre in an urban area, catering to needs of an urban, educated and aware population. This

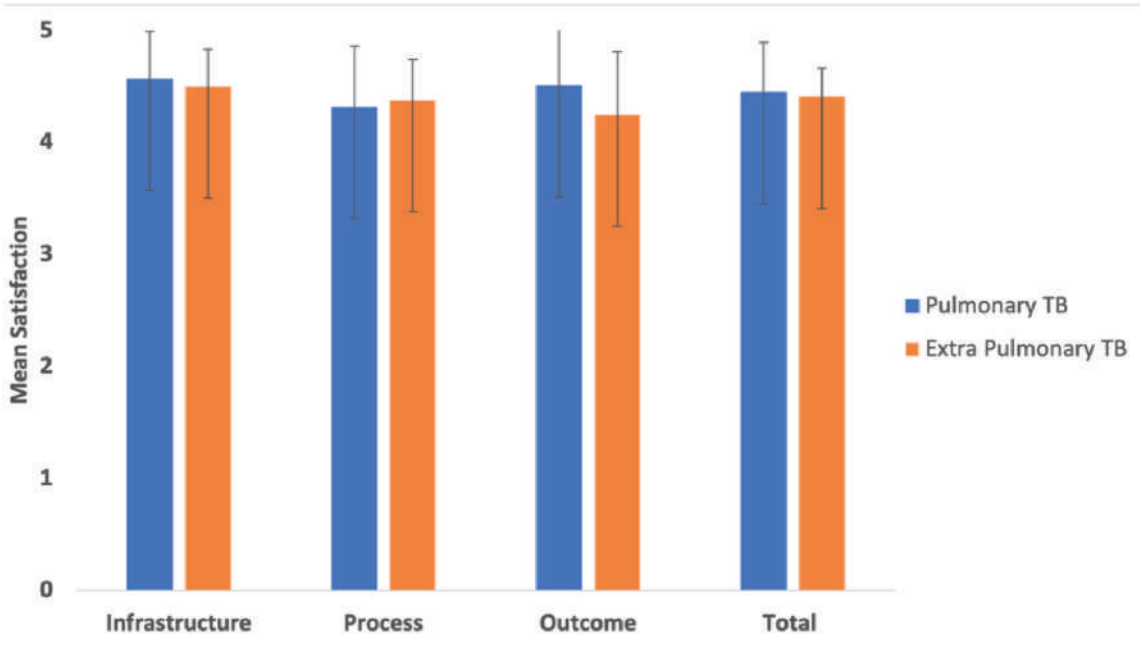

Fig. 3 – Satisfaction of subscales as well as 24 item complete scale in pulmonary vs extrapulmonary TB cases.

warrants a multicentric study with proportionate number of Pulmonary and Extra pulmonary TB cases to get more definitive insight into the satisfaction levels of both groups.

5. Discussion

India has taken up the daunting task of TB elimination with a multitude of strategies with aim to improve case finding and assuring treatment and declaring disease free. A mammoth programmatic drive has enabled 13,000 DOTs clinics in the country offering free NTEP services. However, still we are sceptical of achieving the target of TB elimination by 2025. With the increase in quantitative operations under the program, the most vital yet fragile link of quality gets often compromised. The beneficiary can give the critical judgement on the quality, and this is perhaps the cost-effective tool to find out gaps in service delivery.[9] In this context, use of psychometric tools that are specific, reliable, and valid are valuable for health service improvement.[10–12] In India, given the diversity of health services across the country, satisfaction scores reported 67.5–92%.[9] Such tools have highlighted facets of service delivery which led to patient satisfaction and also has alerted health systems to be sensitive towards unorthodox yet essential needs of patients like warm behaviour, answering to all queries and respect for patient privacy and confidentiality, which some time back was considered the domains of private sector alone.[12,13,14]

This article takes up the validation of a replicable tool that can be used in program assessment. As Active or Passive Case Finding is being prioritized to get best results, a feedback mechanism from the beneficiaries would be very useful.

The limitations of this article are that it explores a best practice situation as only active DOTS centre is chosen for participant selection. The pandemic situation also attributed to prompt service delivery, as healthcare providers were conscious about disease detection and the public was also sensitive about getting treated. It also disabled the team from travelling and interviewing participants from other DOTS centres. Even then, the predictable gaps in the process come out regarding dissatisfaction due to out-of-pocket expenditures as is suggested in many other Indian studies.[15–17] This is more because of rising drug resistance and Extra pulmonary cases in the country, which now calls for molecular diagnostics, and its expansion is gaining momentum under NTEP.

The tool hence deserves replication in all DOTs clinics to get a better comprehension of the expectations of the people.

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Conflicts of interest

The authors have none to declare.

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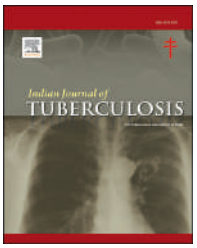

Review article

Anti-tubercular therapy (ATT) induced thrombocytopenia: A systematic review

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ABSTRACT

Introduction: Drug-induced thrombocytopenia is a known adverse event of several drugs. Antitubercular therapy (ATT) is rarely reported but important cause of thrombocytopenia. The present review aimed to understand the profile of thrombocytopenia caused by first-line ATT i.e. isoniazid, rifampicin, pyrazinamide, and ethambutol.

Materials and methods: We screened case reports, case series, and letter-to-editor from databases, like Pubmed/MEDLINE, Ovid, and EMBASE from 1970 to 2021. The PRISMA guidelines were followed in the present systematic review.

Results: Categorical data were expressed as n (%) and quantitative data were expressed as median (IQR). After applying the inclusion/exclusion criteria, 17 case reports and 7 letters to the editor were selected for the present review. Rifampicin was most frequently associated with thrombocytopenia (65%). A median (IQR) drop to 20,000 (49,500) platelets/mm3 was observed. Anti-rifampicin associated antibodies and anti-dsDNA positivity were found in six studies. Except for two, all patients responded to symptomatic treatment.

Discussion: ATT-induced thrombocytopenia can be life-threatening and require hospitalization. Clinicians should be aware of the association of ATT with thrombocytopenia and should take appropriate measures for patient management.

Conclusion: This review provides clinicians a comprehensive picture of adverse effects and their management in ATT induced thrombocytopenia.

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1. Introduction

Thrombocytopenia is characterized by the decline of platelet count below normal limit i.e below 1,50,000/microliter.[1,2] It can be immune-mediated due to bacterial/viral infections, drug-induced, bone marrow suppression, or idiopathic.[1] According to National Organization for rare disorders, the prevalence of drug-induced thrombocytopenia is 9.5 cases per 100,000 in the USA, with the incidence per year of 3.3 per 100,00 in adults.[3]

Several drugs are implicated as causative agents for drug induced thrombocytopenia such as furosemide, nonsteroidal anti-inflammatory drugs (NSAIDs), penicillin, quinidine, quinine, ranitidine, sulfonamides, statins, linezolid,[4] and some of the anti-tubercular drugs used for the treatment of drug-susceptible tuberculosis.[5]

Although drugs remains the most common cause of drug-induced thrombocytopenia but the putative drug often remains unrecognized especially when drugs are prescribed as fixed dose combinations (FDC).[6] Antitubercular therapy constitutes a rare but important cause of thrombocytopenia.[6] Interestingly tuberculosis itself may cause immune-mediated thrombocytopenia.[7]

The pathophysiology of anti-tubercular treatment-induced thrombocytopenia seems to be complicated. Antibodies directed against platelet membrane Gp1b/IX complex are considered to be the main mechanism.[8,9] Various drugs, mentioned above, recognize platelet-reactive antibodies which lead to platelet destruction.[9–15] It has been proposed that sensitizing drugs contain charged molecules that enable them to bind both to platelet surface proteins and antibodies. In this way, the drug binds non-covalently and reversibly to platelets, commonly to sites on GP IIb-III and/or GP Ib-V-IX, and also to the antibody. This further results in the formation of a "sandwich" structure of a tight bond between the platelet epitope and antibody. In general, the formation of antibodies takes 1–2 weeks or longer after exposure to a new drug.[11]

Several case reports and case series enumerate anti-tubercular drugs causing thrombocytopenia.[16–26] Due to use of antitubercular drugs as a fixed drug combination (FDC) it is difficult to identify a single component of ATT responsible for thrombocytopenia in a given patient. The discontinuation of implicated drugs with or without supportive therapy such as Intravenous Immunoglobulin (IVIG), platelet transfusion, use of steroids (prednisolone and methylprednisolone) & antihistamines have been found to be helpful in management of ATT induced thrombocytopenia with variable efficacy.[9,20,24,27–30]

Given the above background, the present review was conducted to understand the profile of thrombocytopenia caused by first line antitubercular drugs isoniazid, rifampicin, pyrazinamide, and ethambutol.

2. Methods

2.1. Ethical approval

This study involved retrieval and analysis of data from already published studies so ethical approval from Institutional Ethics Committee (IEC) for this systematic review is not required.

The conduct, design and reporting of results are in accordance to Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.

2.2. Search strategies and information sources

The PubMed/MEDLINE, Ovid, and Embase databases were searched for published literature from 1970 to 2021. The MeSH terms used for searching the literature were "Thrombocytopenia; antitubercular drugs; Isoniazid; Pyrazinamide; Rifampicin; Ethambutol." The search was conducted independently by three authors: AK, AKP, and RK. Limits such as publication year and language were not set. All studies related to anti-tubercular drugs induced thrombocytopenia were searched and further screened by NS, RK, AK, CM, and AKP. Duplicates searches were removed by Mendeley software. All the eligible studies were included in the systemic review. Reports were further screened for potential eligibility.

2.3. Inclusion criteria

For inclusion of studies in the systematic review, the published reports needed to have documentation of (i) thrombocytopenic event supposedly related to first-line anti-tubercular drugs, (ii), sufficient clinical data, (iii), method(s) of diagnosis, (iv), patient outcomes and (v), details of management of the thrombocytopenic event.

2.4. Exclusion criteria

Criteria for excluding the studies were (i) insufficient clinical data; (ii) thrombocytopenia associated with drugs other than anti-tubercular drugs; (iv) purely mechanistic studies.

2.5. Data extraction

Data was retrieved from text, tables, and figures from each article and noted in a pre-specified data collection form. This customized data form included study characteristics (author's name, year of publication, number of published case report) and patient characteristics (the previous history of exposure to anti-tubercular drugs, mean time to appearance of symptoms, first platelet drop, re-introduction of therapy, additional treatment needed for normalization of platelet count) (Table 1). Data extraction was carried out by two authors independently (CM and AK). Disagreements were addressed by discussion between two authors and consultation with the third author (NS), where necessary. A scoring system was created to account for each of the 30 subitems, according to the Consensus based Clinical Case Reporting Guideline Development (CARE) checklist. Causality assessment was done using Uppsala Monitoring Centre (WHO-UMC) criteria[31] and the adverse drug events were into certain, probable, possible, or unlikely.

3. Statistical analysis

Categorical data were expressed as n (%) and quantitative data were expressed as median (IQR).

Table 1 – Included study characteristics.

S.No.	Study I.D.	Implicated drug/ Offending drug	Age, Gender	Management of the event	Causality assessment of authors
1	Jain VK, 1988	Pyrazinamide	27 Y, M	Pyrazinamide was withdrawn from the regimen; Symptomatic treatment given; patient normalized	Related
2	Ju Lee, 2012	Isoniazid	51 Y, M	Stopped the treatment; Alternate regimen provided; patient normalized	Related
3	Kang, 2010	Rifampicin	68 Y, F	Rifampicin was withdrawn from the regimen	Related
4	Kindelan, 1994	Rifampicin	27 Y, M	Drug withdrawal	Related
5	Kobayashi, 2019	Unclear	82 Y, M	Drug withdrawal; Alternative regimen given; Patient expired after 4 days of drug withdrawal	Unclear
	Lee Ch, 1989	Rifampicin	57 Y, M	Rifampicin was withdrawn from the regimen	Related
			60 Y, F	Drug Withdrawal	Related
8	Leggatpo, 1971	Rifampicin	58 Y, M	Rifampicin was discontinued and thiacetazone was started.	Related
9	Mehta, 1996	Rifampicin	34 Y, F	Rifampicin was withdrawn from the regimen	Related
			43, F	Drug withdrawal	Related
			60, F	Rifampicin was withdrawn from the regimen	Related
10	Mori, 2011	Rifampicin	49 Y, F	Levofloxacin was added in place of Rifampicin	Related
12	Pereira, 2000	Rifampicin	28 Y, M	Drug withdrawal	Related
13	Prasad, 1989	Ethambutol	40 Y, M	Ethambutol was withdraw from the regimen as he tolerated rifampicin and isoniazid.	Related
14	Rabinovitz, 1982	Ethambutol	71 Y. F	Ethambutol was withdraw from the regimen as he tolerated rifampicin and isoniazid.	Related
15	Bansal, 2013	Rifampicin and pyrazinamide	32 Y, M	Drug withdrawal	Related
16	Yakar, 2013	Rifampicin and INH	18 Y. F	Drug withdrawal; Alternative regimen given	Related
17	Agrawal et al , 2012	Rifampicin	32 Y, F	Streptomycin was added to the regimen and rifampicin was removed from the regimen.	Related
18	Blajchman et al., 1970	Rifampicin	56 Y, F	drug withdrawal; symptomatic treatment given; patient normalized	related
19	Burnette et al., 1989	Rifampicin	39 Y, M	ATT discontinued	certain
20	Fahal et al., 1992	Rifampicin	57 Y, M (renal TB)	Rifampicin discontinued	certain
21	Ferguson et al., 1971	Rifampicin	F	ATT discontinued	Not certain
22	Hamad et al., 2019	Rifampicin	55 Y, F	Rifampicin discontinued	Not certain
23	Bhasin DK, 1991	Rifampicin	21 Y, F	Rifampicin withdrawn	related

4. Results

The initial search identified 1705 articles of which 94 were selected for eligibility. Out of 94 studies, 45 were excluded due to various reasons mentioned in the PRISMA diagram. 25 full-text studies were selected for review after abstract screening, out of which 17 case reports were included in the review (Table 1) (see Fig. 1).

4.1. *Quality of case reports*

Of 17 case reports on anti-tubercular therapy induced thrombocytopenia, none of them fully adhered to established CARE guidelines.[32] All case reports (100%) adhered to the patient information item (de-identified the patient information, detailed out primary concerns, therapeutic intervention, adverse events, and important follow-ups results). None of the case reports mentioned the patient(s) perspective(s) on the treatment they received or prognosis (Fig. 2). One of the case reports had discussed diagnostic challenges. Three of the case reports (3/17) included keywords that identified diagnosis or interventions including the word "case report".

Eighty eight percent (88%) of case reports (15/17) mentioned the past interventions with outcomes, while fifty five percent (55%) of the reports discussed the strengths and limitations of the case report, scientifically. Only 3 case

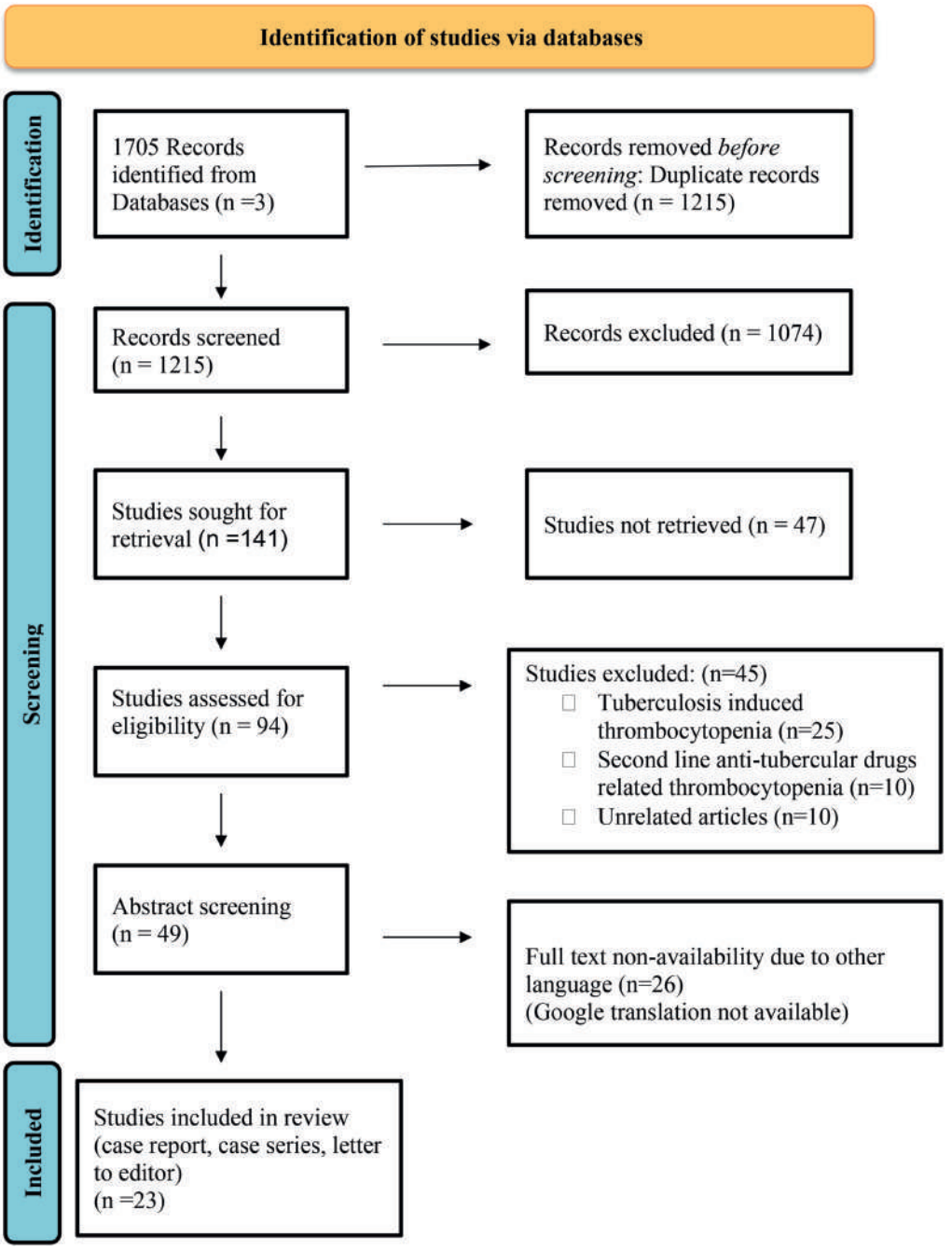

Fig. 1 – PRISMA flow diagram of selection of articles in the review.

reports mentioned that the informed consent was obtained from patient.

4.2. *Profile of implicated drugs*

Rifampicin (65%) was observed to be most common causative agent implicated to cause ATT induced thrombocytopenia followed by isoniazid (10%), ethambutol (10%), and pyrazinamide (5%) (Fig. 3). Only two cases (10%) of thrombocytopenia possibly caused by multiple antitubercular drugs were reported (Table 1).

4.3. *Time to event (TTE) of implicated drugs*

The median time (IQR) between exposure to rifampicin and developing thrombocytopenia was 7 (9) days in all cases where a history of exposure had previously occurred. In contrast, the median (IQR) TTE to event was found to be 13 (42) days in cases where no prior exposure to rifampicin had occurred. There were two cases with rifampicin where the time to event was 270 days and 210 days, respectively. Isoniazid induced events occurred within 21–50 days of initiation of drug, with no prior history of exposure to isoniazid. Whereas for ethambutol, the time to event in two cases was 6 and 4 days, respectively. There was one case of pyrazinamide induced thrombocytopenia reported with TTE between exposure to event as 10 days.

4.4. *Diagnosis of thrombocytopenic events*

The patients presented with varied symptoms with mild to moderate intensity including purpuric or erythematous maculopapular rash, petechiae with or without pruritis, spontaneous gum bleeding, and ulceration in mouth. Three case reports mentioned that severe clinical symptoms of bleeding diathesis, epistaxis, haematemesis and haematuria were observed requiring urgent intervention. Five case reports did not mention the symptoms reported by patients. Routine tests (biochemical and haematological parameters) were the foremost markers for thrombocytopenic events. The median value for (IQR) drop in platelet count was 20,000 (49,500)/mm^3. Out of 13 cases of thrombocytopenia due to rifampicin, 9 (70%) studies mentioned additional investigations to rule out other autoimmune aetiologies including platelet associated IgG and antiplatelet antibody assays. Anti-rifampicin associated antibodies and anti-dsDNA positivity were found in 6 studies indicating an autoimmune response to the drug. In these 6 cases, 3 of them had history of exposure of drug, indicating that these patients might be having autoimmune antibodies before starting ATT therapy.

4.5. *Management of thrombocytopenic events*

The first management strategy for all the included studies on anti-tubercular drugs induced thrombocytopenia was to stop the offending drug (n = 20). Additional treatments were given in those cases where platelet count did not improve after drug withdrawal. Antihistaminics (n = 2) and steroids (n = 3) were administered if the allergic response was associated with thrombocytopenia. Few cases (n = 7) required platelet transfusions to restore the depleted platelet counts. The platelet counts improved after platelet transfusion in all but two patients. One patient was later managed with danazol with limited success followed by Intravenous Immunoglobulin (IVIG) (250 mg 2 times per day for 2 days) which lead to

Fig. 2 – Number of case reports that adhered to each CARE item descriptor.

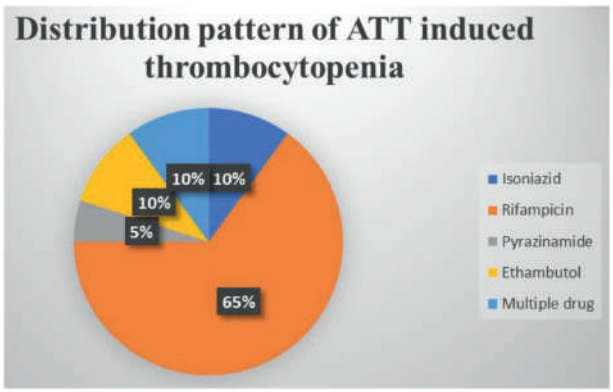

Fig. 3 – Distribution pattern of ATT induced thrombocytopenia.

improvement in platelet count to 122,000/microliter, which further improved to normal levels of 200,000/microliter. The other patient responded to 8 sessions of plasmapheresis in addition to supportive care. In the selected cases, there was one mortality, the patient died after 4 days of stopping ATT, despite platelet transfusion. Drug-induced lymphocyte stimulation test was found to be positive for isoniazid and negative for rifampicin at time of death. The autopsy results showed intra-alveolar haemorrhages, hemosiderin-laden macrophages and hyperplasia of type 2 pneumocytes in lungs, and epithelioid cell granulomas with central necrosis in glomeruli suggestive of military tuberculosis.[33] In addition, the bone marrow autopsy was found be to hyperplastic with increased number of megakaryocytes, probably secondary to thrombocytopenia.

All the reported patients were either put on an alternative regimen for tuberculosis or the implicated drug was withdrawn. The details have been mentioned in (Table 1; Supplementary data). 64% (16 out of 25 studies) of the studies have provided information on the alternative regimen provided after drug withdrawal. Out of these 16 studies, in 9 of them, the implicated drug was withdrawn and for the rest of the studies, the additional anti-tubercular drug was added to the regimen (Table 1; Supplementary data).

4.6. *Causality assessment*

The World Health Organization – Uppsala Monitoring Centre (WHO-UMC) causality assessment criteria were used in assessing the studies. It revealed that in 11/20 (55%) studies causality was certain, while 4 (20%) were in probable and possible category. De-challenge was attempted in all the case reports as part of management. Re-challenge was attempted in 13 studies and in 11 studies of them the platelet counts dropped after reintroduction of suspected drug/s (Re-challenge positive). In 2 case reports there was no drop in platelet counts after reintroduction of the suspected antitubercular drug/s.

5. Conclusion

The current review provides an overview of the clinical outcomes, diagnosis, management of thrombocytopenia associated with anti-tubercular drugs. Although antitubercular drugs induced thrombocytopenia is uncommon clinicians should be aware of the possibilities and have an index of suspicion when patients present with signs and symptoms of thrombocytopenia. This study will guide the clinicians in patient management and to look for autoimmune association in patients presenting with thrombocytopenia before ATT re-introduction. The re-challenge should be avoided in the patients with positive autoimmune antibodies.

There is a need of revival of the database for thrombocytopenia and extend its outreach to include the antitubercular drugs so that the clinicians are aware of the association of thrombocytopenia with antitubercular drugs and stay vigilant for such adverse events.

6. Limitations

Our study included majorly case series or case reports which in itself is a low quality evidence but it is an important signal. There is a need of larger study or database to provide strong evidence and guidance.

7. Discussion

We present a detailed review of the published case reports of thrombocytopenia associated with anti-tubercular drugs. This systematic review of published case reports/series found that among the first line anti-tubercular drugs, rifampicin is the most frequently implicated drug causing anti-tubercular drugs induced thrombocytopenia.

There are concerns about the likelihood of bias and weak interferences related to case reports or case series. However, case reports have an important signal value in bringing forth various important findings related to clinical practice.[34] For rare conditions, the possibility of undertaking evaluation in an RCT is impractical. The current review was undertaken and reported under the PRISMA guidelines, including only cases with thrombocytopenia associated with anti-tubercular drugs. In this review, we have compiled all the information; profile of drugs, quality of reports by CARE guidelines, management of the events, investigations carried out during the event regarding the thrombocytopenia events associated with anti-tubercular drugs. The reason could be that DIT does not have any established criteria for staging.

Although there are no definitive diagnostic criteria for the evaluation of drug-induced thrombocytopenic (DIT) events. The diagnosis relies on clinical features and platelet count. The patients in the reported studies presented with varied symptoms including purpuric or erythematous maculopapular rash, petechiae with or without pruritus, spontaneous gum bleeding, ulceration in mouth. In three case reports patients presented with frank bleeding episodes which included epistaxis, haematemesis and haematuria. The clinical diagnosis was confirmed by platelet count, the fall in platelet drop was variable ranged from <1000/microliter to 128,000/microliter. However, the platelet count did not correlate with the severity of clinical symptoms observed in case reports. De-challenge was performed in all the studies. The

autoimmune antibodies were tested positive in 4 out of 24 studies (Table 1); IgG and IgM rifampicin antibodies were observed in case report by Blajchmen et al.[35] Antiplatelet antibody against rifampicin and INH seen in Mehta et al.[36] Macrophage inhibition factor positivity for ethambutol seen in Robinovitz et al.[37] and drug induced lymphocyte stimulation test for isoniazid was positive in Kobayashi et al.[33] The additional supportive management included steroids, antihistaminics (if allergic component was observed) and platelet transfusion. One patient did not improve despite dechallenge and supportive care and required IVIG treatment. Mortality was observed one patient after 4 days of dechallenge despite platelet transfusion and was found to have positive lymphocyte stimulation test for isoniazid. The autopsy results showed evidence of military tuberculosis and hyperplastic bone marrow with increased number of megakaryocytes. Rest all patients including those which presented with bleeding diathesis improved after treatment and were reported to be discharged in stable condition Re-challenge was attempted in 13 case reports, with positive re-challenge seen in 11 case reports i.e., platelet drop was observed after reintroduction of implicated drug while re-challenge was negative in 2 case reports.

Some of the authors have discussed the inherent nature of producing rifampicin-dependent antibodies as one of its immunological adverse events.[30,38,39] Pereira J et al., 2000 states that there are reported cases of proven rifampicin-dependent anti-platelet antibodies, however detailed description is lacking.[9] As per our findings, we did not find any correlation between the minimum platelet count drop and immune system involvement. The minimum platelet count drop observed is <1000/microliter in a case report and the patient was simply managed by withdrawing the antitubercular drugs given.[40] Whereas, in another case report, the platelet count dropped to 1100/microliter and it took intravenous immunoglobulin to manage the patient despite platelet replacement therapy.[24] This immune-mediated thrombocytopenia was caused by drug-glycoprotein complex antibody formation. Rifampicin acts as a hapten and produces antibodies resulting in platelet destruction.[25] The antiplatelet antibodies form an antigenic complex with rifampicin that interacts with blood cell-membrane proteins.[20,27] This is the reason for the elevated immune markers observed in patients who developed thrombocytopenia with rifampicin.

However, often, the first platelet drop incidence in patients with DIT is not related to the elevated immune markers in the body.

All the patients were given modified alternative regimen after excluding the implicated drug.

The assessment of quality of reports was done by the Consensus-based clinical case Reporting Guideline Tool Adherence to the CARE guidelines was found to be variable across most items, with the highest degree of reporting around the patient's demographics, therapeutic interventions, and diagnostic methods employed.

However, we found some gaps in reporting these cases. The word "case report" was entirely missing in all the included case report titles. Item detailing about the prognosis was also lost from the case reports. The reason could be our subject area which does not include any staging of disease. The checklist also mentioned the term "where applicable". Surprisingly, none of the case reports have taken data from the patient perspective.

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Conflicts of interest

The authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2023.04.029.

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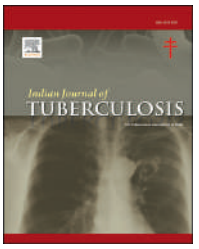

Case report

COVID-19 and pulmonary Tubercuiosis coinfection: three case reports from Iran

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ABSTRACT

Tubercuiosis is still a significant problem worldwide. Until the COVID-19 pandemic, tuberculosis was the leading cause of mortality from a single infectious agent. Pulmonary Tubercuiosis patients are more tending to be co-infected with COVID-19 notably when they have a history of exposure. There are some case reports relating to pulmonary TB and COVID-19 coinfection but the information about TB and COVID-19 was still little. We report three coinfected patients. Case one and two were both middle-aged Iranian mans with history of opium addiction, case one presented with dyspnea and weakness and case two presented with progressive weakness. Case three was a healthy young man with history of progressive dyspnea, productive cough and hemoptysis. Case one and case three were improved. In conclusion, COVID-19 is still an important issue and can coexist with other lung infections such as Pulmonary Tubercuiosis, so we should be aware of the advancement of the Tubercuiosis epidemic after the COVID-19 pandemic.

1. Introduction

Tuberculosis (TB) is still an important issue worldwide even more in the endemic areas like Iran. The pandemy of coronavirus disease of 2019 (COVID-19) has reversely affected global reduction of TB mortality rate and progression in providing essential TB services. Until the COVID-19 pandemic, TB was the leading cause of mortality from a single infectious agent.[1]

Both TB and COVID-19 are communicable respiratory infectious diseases and display similar symptoms.[2] Pulmonary TB patients are more prone to be co-infected with COVID-19 especially when they have a history of exposure[3] and some studies have reported this coinfection, first reported in china.[4–7]

A meta-analysis showed that this co-infection is related to increasing mortality[8] also a recent review mentioned TB as a risk factor for severity and mortality of COVID-19.[9] Information about TB and COVID-19 is still little. Here in Iran, we report three cases of diagnosed pulmonary TB coexisted with COVID-19 and discuss their clinical and radiological presentations and follow up.

Abbreviations: TB, Tuberculosis; FDC, Fixed-dose combination; AFB, Acid- Fast Bacilli; CT, Computed tomogrphy.

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2. Case presentation

2.1. Case one

A 45-year-old Iranian man was refered to our hospital due to dyspnea, severe weakness and myalgia in past 10 days with history of chronic productive cough and significant weight loss and denied fever, night sweating and hemoptysis. He was homeless with addiction to cigarette and opium and history of past IV drugs usage.

On physical examination he was cachectic, apyrexial and normotensive with normal respiration rate and oxygen saturation of 97% under room air, but was tachycard (heart rate of 110 beats/min). Diminished lung sounds in right hemithorax and bilateral finger clubbing was detected.

ECG showed sinus tachycardia only and transthoracic echocardiogram (TTE) was normal. Laboratory findings showed mild anemia (Hb 12 g/dL) with leukocytosis (15,600 cells/mL) and a high neutrophil-to-lymphocyte ratio (NLR) of 8.82 and platelet level was normal. Markers of inflammation were high (C-reactive protein (CRP) 64.2 mg/mL and erythrocyte sedimentation rate (ESR) 63 mm/h)and D-dimer level was high (3694.2 ug/mL). Computed tomogrphy (CT) scan of chest revealed cystic bronchiectasis with fibrosis in right upper lobe due to chronic infection and irregular thick-walled cavitary lesions mostlyin the right lung. Also nodular opacities with tree-in-bud pattern and hilar and mediastinal lymphadenopathy was seen, altogether consistent with active pulmonary TB (Fig. 1).

Based on susceptibility to pulmonary thromboembolism (PTE), CT angiography was done and was ruled out.

Both acid-fast bacilli (AFB) test and nasopharyngeal COVID-19 polymerase chain reaction (PCR) were positive and he was treated with FDC and remdesivir. His symptoms was improved and then he was discharged to continue anti-TB medication. Smear and culture of AFB at the end of the second month of treatment were negative. He was treated and followed for nine months. He had no adverse effects.

2.2. Case two

A 51-year-old Iranian male patient presented with progressive weakness from 1 month ago and significant weight loss and denied fever, night sweating, dyspnea, cough and hemoptysis. Also he was opium addict and HCV positive with history of hypothyroidism and chronic severe anemia. He had no recent travel or contact with ill patients.

On physical examination he was pale and cachectic with normal vital signs. Also chest examination was normal. Laboratory findings showed hypochromic microcytic anemia with Hb 5 g/dL and white blood cell count and platelet level were normal. CRP was 79.8 mg/mL, ESR was 14 mm/h, D-dimer level was 474 ug/mL and LDH was 1234 iU/L. Spiral chest CT scan revealed multiple nodules with random distribution and cavitary formation in both lungs consistent with granulomatous disease including pulmonary tuberculosis (Fig. 2).

Sputum was collected for AFB test and nasopharyngeal COVID-19 PCR were performed, both were positive. He treated with remdesivir and prophylactic anticoagulant. FDC was added to treatment after three days.

His condition worsened with decreased oxygen saturation and loss of consciousness and finally expired on day 5.

2.3. Case three

A 20-year-old Afghan man who was migrated to Iran since 6 month ago, presented with progressive dyspnea, productive cough and hemoptysis from 1 month ago and also noted sore throat, myalgia, weakness and night sweating. His past medical history was negative and he had received one dose Pfizer-BioNTech COVID-19 vaccine only.

On physical examination he was normotensive, afebrile, tachycard (heart rate of 120 beats/min), tachypneic (respiratory rate of 26 breaths/min) with oxygen saturation of 91% under room air. Chest examination revealed diffuse coarse crackles in both lungs.

Laboratory findings showed leukocytosis (12,600 cel, mild anemia (Hb 13 g/dL) and normal platelet level. CRP was 80.4 mg/mL, ESR was 80 mm/h. Spiral chest CT scan revealed

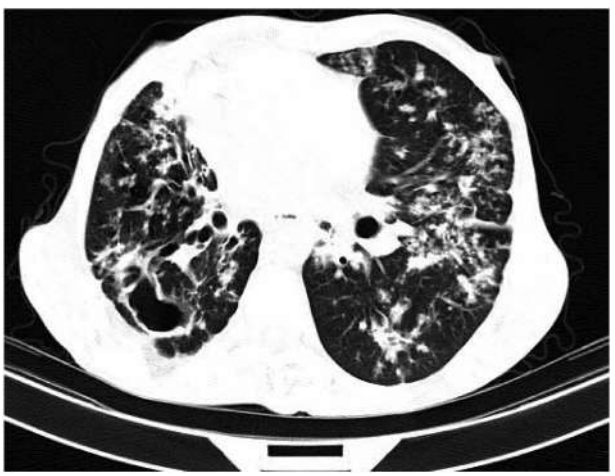

Fig. 1 – Computed Tomography (CT) scan of chest, showing cystic bronchiectasis with cavitary lesions.

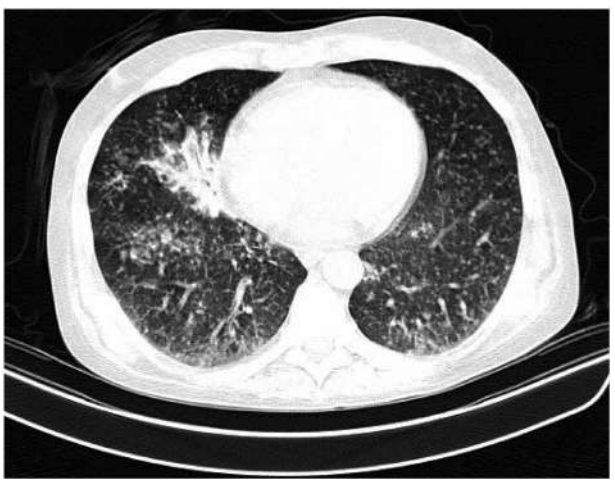

Fig. 2 – Chest CT scan, showing multiple nodular formations in the both lungs.

centrilobular opacities and consolidations in both lungs, especially in the upper lobes consistent with viral and/or bacterial pneumonia and cystic bronchiectasis in left upper lobe (LUL) (Fig. 3).

ECG pointed out only sinus tachycardia.

AFB test and nasopharyngeal SARS-CoV-2 PCR were performed and tested positive. He was treated with remdesivir, FDC and prophylactic dose of anticoagulant. AFB test was negative at the end of the second month of anti tuberclosis treatment. He was treated and followed for nine months and he had no side effects.

3. Discussion

Patients with chronic respiratory infections, such as TB, are more susceptible to hit by the COVID-19 disease. Active pulmonary tuberculosis can afford local reformations in lung immunity and influence host response to COVID-19[8] and also immune suppression related to this virus may cause certain difficulties in the diagnosis and management of TB.[10]

TB usually affects low socioeconomic populations and has a significant effect on public health.[11] In countries with high burden of TB, it is important to maintain the differential diagnosis of TB and COVID-19 to noticing the coinfection and preventing misdiagnosis.[12] So generally, malnutrition and poverty might importantly increase morbidity and mortality of TB and COVID-19 coinfection,[13] in the same way, case one was a homeless addict man who was mostly at risk.

Case two had a history of severe chronic anemia, could play an important role in his mortality. A recent meta-analysis based on risk factors-adjusted effect estimates showed that anemia was independently associated with increased mortality risk inpatients with COVID-19.[14] Increased oxygen demand due to respiratory system damages in COVID-19 patients, in present of low hemoglobin levels, can significantly reduce the oxygen supply, as the disease progresses, constant hypoxic condition may lead to multiple organ failure and even death.[15]

Pulmonary thromboembolism should be considered as a differential diagnosis in patients with acute dyspnea.[16] Additionally, pulmonary embolism is well known as a potential complication that may occur late in the course of COVID-19 cases.[17] As a common standard method, Pulmonary CT angiography is done to evaluate for suspected pulmonary embolism.[18]

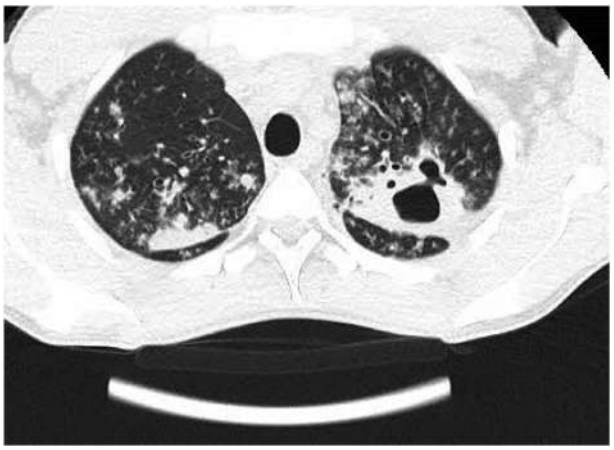

Fig. 3 – Diffuse centrilobular opacities and LUL cystic bronchiectasis.

The clinical manifestations of TB overlap with those of COVID-19 such as dyspnea and cough,[19] both found in case one. On imaging, chest CT scan demonstrate difference between pulmonary tuberculosis and COVID-19. Thick-walled cavitary lesions with pattern of tree-in-bud and consolidations, notably in the upper lobes are suggestive for tuberculosis[20,21] and adversely, multifocal peripheral ground-glass opacities especially in the lower lobes are diagnostic pattern for COVID-19.[21,22]

After all, timely tuberculosis screening and rapidly detection of COVID-19 infection are crucial for public health.[23,24]

Prospective high quality studies are needed to provide an accurate understanding of the correlative effects of these two infections. Future studies should analyse the impact of this coinfection in relation to morbidity and mortality.

4. Conclusion

COVID-19 is still an important problem and can coexist with other lung infections such as TB. We should be conscious of the advancement of the TB epidemic after the COVID-19 pandemic. Both TB and COVID-19 have similar clinical manifestations that require high clinical suspicion for rapid detection. Eventually, clearly documented plans for diagnosis and management of the coinfection should be implemented to improve the outcome.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written consent to publish this case report was obtained from patients.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Authors' contributions

MN and SS participated equally in preparing the manuscript. All authors read and approved the manuscript.

Conflicts of interest

The authors have none to declare.

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Case report

A cross sectional descriptive study of dermoscopic features of clinical variants of cutaneous tuberculosis

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ABSTRACT

Tuberculosis continues to be a major public health concern worldwide with almost 20–40% of the world's population being affected yearly. Cutaneous Tuberculosis (TB) is a rare and underdiagnosed entity that manifests in about 1–1.5% of extrapulmonary tuberculosis cases worldwide. Dermoscopy is a non-invasive tool which will be a useful aid to histopathology in the confirmation of the diagnosis alongside culture, and molecular techniques. This is a cross-sectional descriptive study that was conducted at a tertiary care center in Mumbai, India. A total of 31 patients were enrolled in this study; 14 males and 17 females. The mean duration of disease was 4.3 months and the average age was 31 years. There were 10 cases of lupus vulgaris, 7 scrofuloderma, 5 papulonecrotic tuberculid (PNT), 3 tuberculosis verrucosa cutis (TBVC), and 2 cases each of erythema induratum of Bazin, lichen scrofulosorum and resolved lupus vulgaris. All the lesions demonstrated orange yellow background suggestive of dermal granuloma. Other key dermoscopic features noted include yellowish-white scales, patulous follicles, white structureless areas, milia-like cysts, white streaks, pigment globules, hairpin and linear vessels. Newer findings such as the crown of vessels and perifollicular pallor in lichen scrofulosorum, and radiating white streaks in PNT were also noted. Dermoscopy of infective granulomas such as cutaneous tuberculosis is a less explored field of dermatology. Newer dermoscopic features of each clinical variant of cutaneous TB have been described.

1. Introduction

Cutaneous tuberculosis (TB) is a rare entity, comprising roughly 1–1.5% cases of extrapulmonary tuberculosis.[1–4] Tuberculosis represents a major public health problem, particularly in Southeast Asia where an estimated proportion of 10.4 million infective cases (45% of the global case burden) was listed.[3,5] Despite the availability of modern diagnostic techniques such as polymerase chain reaction for mycobacterial DNA, and interferon-gamma release assay (IGRA) alongside gold standard culture technique, the probability of detection of mycobacterium remains low. Dermoscopy of infective granulomatous conditions is a less explored field of

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Table 1 – Age distribution of patients enrolled in the study.

Age category	n (%)
1–20 years	8 (25.81%)
21–40 years	14 (45.16%)
41–60 years	7 (22.58%)
More than 60 years	2 (6.45%)
Total	31 (100%)

dermatology with mainly descriptions of lupus vulgaris in literature.

2. Report

A total of 31 patients with clinically diagnosed cutaneous tuberculosis, were analyzed for dermoscopic features in lesions. Live images were recorded using Dinolite AM4113ZT dermoscope with a magnification of 50x and 200x with adjustable polarization. Dermoscopic data was used for analysis after histopathological confirmation of cutaneous TB.

Of the 31 patients studied, 14 were males and 17 were females. The mean duration of the disease was 4.48 months and the average age was 31.45 years (with the youngest 6 years old and the oldest 73 years old). According to age distribution (Table 1), there were 8 cases (25.81%) belonging to 1–20 years of age, 14 cases (45.6%) from 21 to 40 years of age, 7 cases (22.58%) from 41 to 60 years of age and 2 cases (6.45%) from above 60 years group. There were total 10 cases (32.26%) of lupus vulgaris, 7 cases (22.58%) of scrofuloderma, 5 cases (16.13%) of papulonecrotic tuberculid, 3 cases (9.68%) of tuberculosis verrucosa cutis (TBVC) and 2 cases (6.45%)each of lichen scrofulosorum, erythema induratum and resolved lupus vulgaris (Table 2). Dermoscopy showed yellowish-orange globules in the background in all the patients (100%) suggestive of dermal granuloma. Other features appreciated include white scales (38.71%), white structureless areas (77.42%%), patulous follicles with plugging (25.81%), white streaks (61.29%), milia-like cysts (45.16%). Consistently observed vascular patterns included hairpins with dotted vessels (22.58%) and linear vessels (35.48%). The frequency distribution for each dermoscopic finding has been tabulated (Table 3).

Lupus Vulgaris (LV) - Out of 10 cases of active LV (Fig. 1), 8 had yellowish-white scales, yellowish to hemorrhagic crusting was present in 6 cases, superficial erosions were seen in 5 patients, and patulous follicles with keratin plugs were seen in 7 cases. The polarized view showed findings such as white structureless areas suggestive of ongoing fibrosis (8 cases), milia-like cysts (9 cases), shiny white streaks corresponding to the orientation of collagen in the dermis, and blue to brown pigment observed at the periphery of lesions in 4 patients. Prominent vascular patterns included hairpin vessels (5 cases) and linear vessels (4 cases). An interesting vascular pattern comprising circumferentially arranged dotted vessels was seen in a case of inoculation TB presenting as LV (Fig. 1f).

2 patients with resolving lupus vulgaris were also enrolled where findings such as white structureless areas, milia-like cysts, irregular pigment globulesand blobs at the periphery with linear vessels were observed (Fig. 1 d,e).

Scrofuloderma – A total of 7 patients with scrofuloderma were studied (Fig. 2). Central sinus with yellowish crust was observed in 5 cases with the other two cases of impending

Table 2 – Tabulation of the frequency distribution of each variant of cutaneous TB.

Diagnosis	n (%)
Lupus vulgaris	10 (32.26%)
Scrofuloderma	7 (22.58%)
Papulonecrotic tuberculid (PNT)	5 (16.13%)
Tuberculosis verrucosa cutis (TBVC)	3 (9.68%)
Lichen scrofulosorum	2 (6.45%)
Erythema induratum of Bazin	2 (6.45%)
Resolved lupus vulgaris	2 (6.45%)
Total	31 (100%)

Table 3 – Dermoscopic features in cutaneous tuberculosis.

Scales	**n (%)**
Concentric	6 (19.35%)
No	13 (41.94%)
White	12 (38.71%)
Crust	**n (%)**
Hemorrhagic	3 (9.68%)
No	16 (51.61%)
Yellow	12 (38.71%)
Erosion	**n (%)**
No	14 (45.16%)
Sinus	7 (22.58%)
Yes	10 (32.26%)
Plugging	**n (%)**
No	23 (74.19%)
Yes	8 (25.81%)
White Structureless Areas	**n (%)**
No	7 (22.58%)
Yes	24 (77.42%)
Milia Like Cyst	**n (%)**
No	17 (54.84%)
Yes	14 (45.16%)
White Streaks	**n (%)**
No	12 (38.71%)
Yes	19 (61.29%)
Pigment	**n (%)**
Brown Globules	13 (41.94%)
No	18 (58.06%)
Vascularity	**n (%)**
Corkscrew	4 (12.9%)
Crown	2 (6.45%)
Dotted	3 (9.68%)
Hairpin	4 (12.9%)
Hairpin, Dotted	7 (22.58%)
Linear	11 (35.48%)
Surface Changes	**n (%)**
Lobulated	4 (12.9%)
Nil	24 (77.42%)
Papillated	3 (9.68%)
Hemorrhagic Blots	**n (%)**
No	27 (87.1%)
Yes	4 (12.9%)
Perifollicular Pallor	**n (%)**
No	29 (93.55%)
Yes	2 (6.45%)

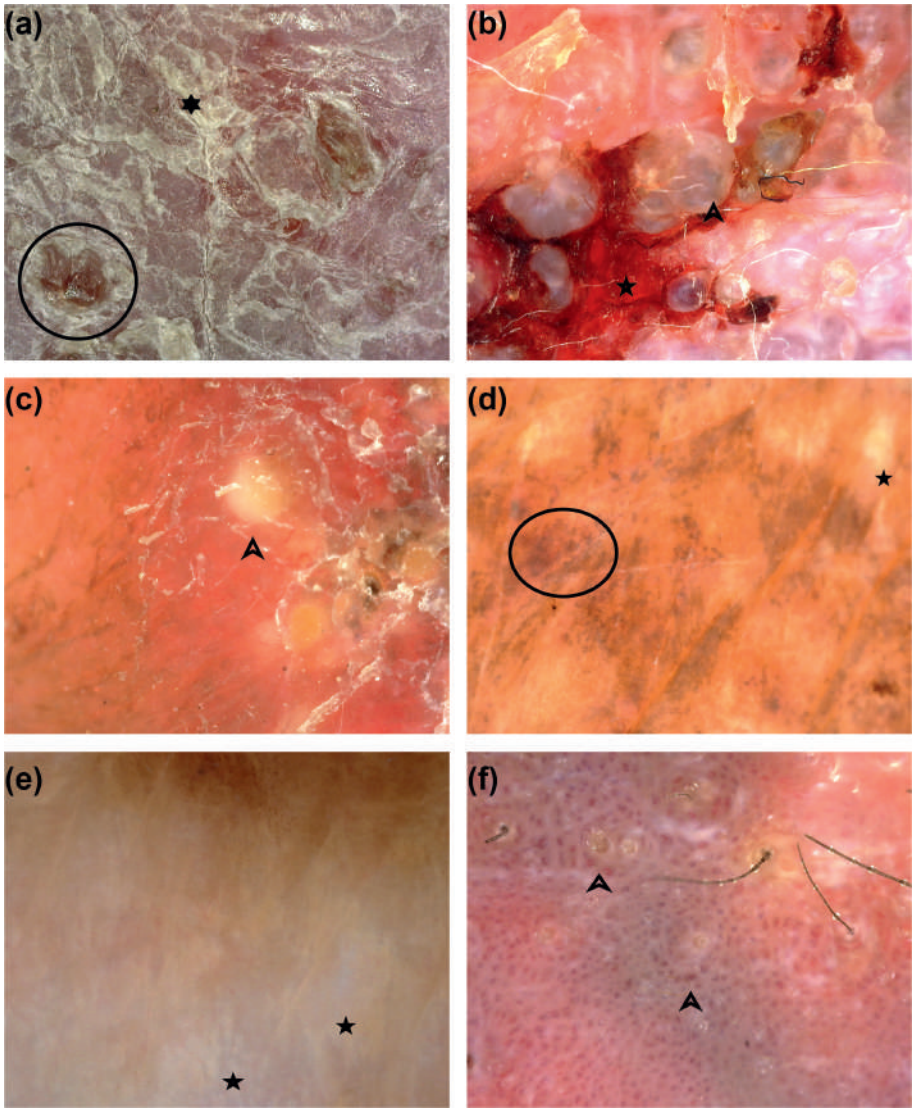

Fig. 1 – Lupus Vulgaris. (a) Patulous follicle with keratotic plug (black circle) and white adherent scales (black star),100x. (b) Hemorrhagic crusting (black star) with bluish pigmented globules (pointed arrow),200x. (c) Milia-like cysts (pointed arrow),100x. (d) Resolved LV showing pigment globules (black circle) and milia-like cysts (black star),100x. (e) resolved LV showing white structureless areas (black star),100x. (f) Inoculation TB with perifollicular circumferential dotted vessels, 100x.

rupture showing orange red background with increased vascularity. Other findings include white structureless areas (6 patients), white streaks (5 cases), brown globules, and pigment blobs at the periphery (3 cases). The predominant vascular pattern observed entangled linear vessels (100%), corkscrew and dotted vessels (2 cases).

Tuberculosis verrucosa cutis (TBVC) – All three cases of TBVC (Fig. 3) showed coarse white scales with papillated surface; white structureless areas and milia-like cysts. Vascular patterns included hairpin vessels and linear vessels along papillae on the surface.

Papulonecrotic tuberculid (PNT) – A total of 5 cases of PNT were enrolled in the study, of which 4 cases showed concentric white scales (collarette sign), yellow to hemorrhagic crusts, and white structureless areas. All 5 lesions demonstrated radiating white streaks (starburst pattern) and hairpin vessels oriented along the white streaks (Fig. 4).

Lichen scrofulosorum (Fig. 5)– 2 patients with lichen scrofulosorum were included in the study; demosctrating perifollicular pallor, white concentric scales, and perilesional hairpin vessels arranged in a crown pattern.

Erythema induratum of Bazin (EI) – Dermoscopy from periphery of EI showed white structureless areas and regularly arranged dotted vessels within the reticular pigment network (Fig. 6).

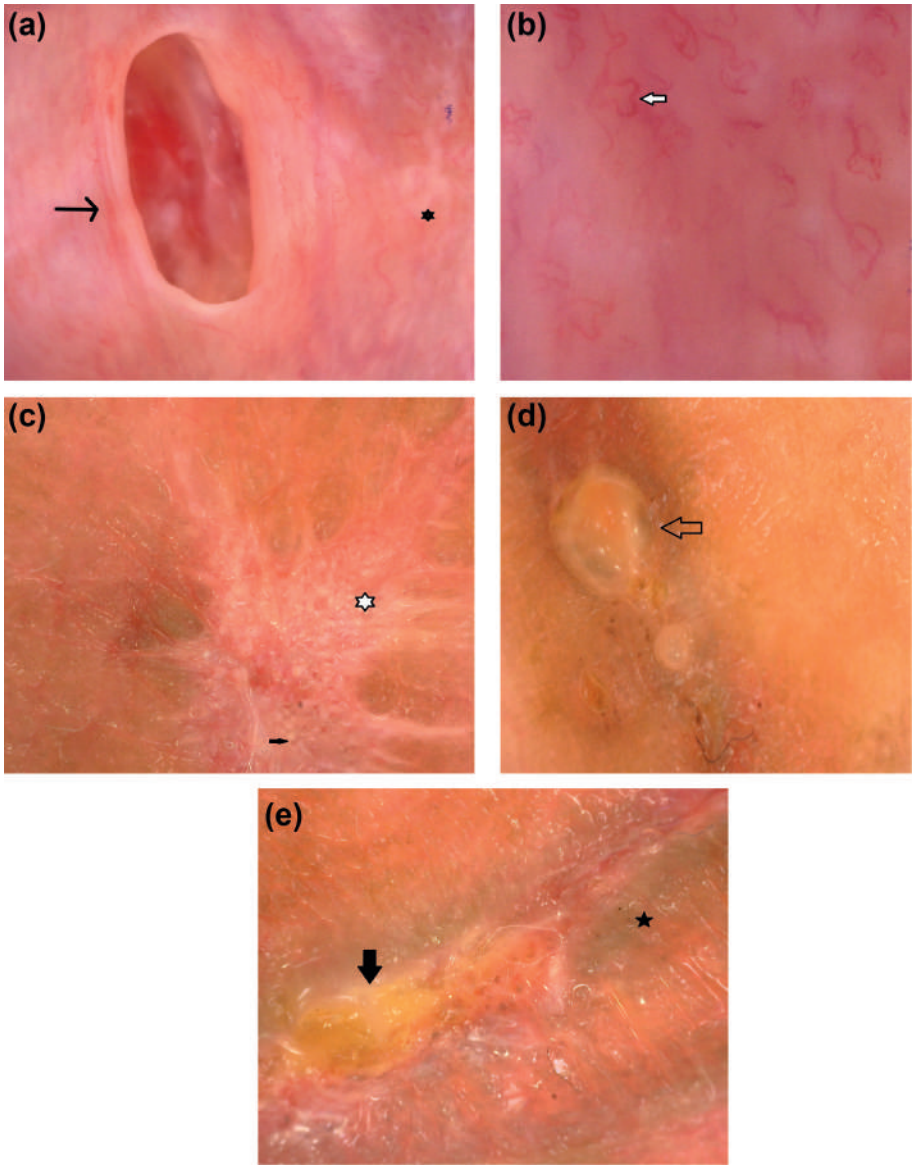

Fig. 2 – Scrofuloderma. (a) Central sinus (black arrow) with a circumferential arrangement of linear vessels (black star),100x. (b) Corkscrew and entangled vessels (white arrow),200x. (c) White streaks (white star) and milia like cysts (black arrow),100x. (d) Greyish brown pigmentation around sinus (black arrow),100x. (e) Greyish brown pigment clods (black star) around sinus containing yellowish purulent material (black arrow),50x.

3. Discussion

Cutaneous tuberculosis (TB) can be divided into true cutaneous TB and tuberculids.[6,7] True cutaneous TB can spread via hematogenous or lymphatic spread, direct inoculation, or contiguous spread. Multibacillary forms include orificial TB, scrofuloderma, acute miliary TB, gumma, and tuberculous chancre.[8] Paucibacillary variants include tuberculosis verrucosa cutis (TBVC) and lupus vulgaris.[4,9]Tuberculids are considered to be immune reactions within the skin due to hematogenous dissemination of M. tuberculosis or its antigens from a primary source.[10] This category includes papulonecrotic tuberculid, lichen scrofulosorum, and erythema induratum of Bazin.[8]

The International Dermoscopy Society proposed a set of 5 dermoscopic parameters to use in general dermoscopy[11,12]: (I) vessels (morphology and distribution), (II) scales (color and distribution), (III) follicular findings, (IV) other structures, (V) specific clues. The findings of each of the variants have been described in accordance with these parameters.

The orange-yellow hue in granulomatous diseases is due to the mass effect of dermal granulomas and is accentuated by applying slight pressure onto the skin, which reduces erythema.[13,14]

In our study, surface epidermal changes such as lobulated or papillated surface, white coarse scales, erosions, and hemorrhagic crusting were more prominent in lupus vulgaris and TBVC. The orange to salmon structureless areas,

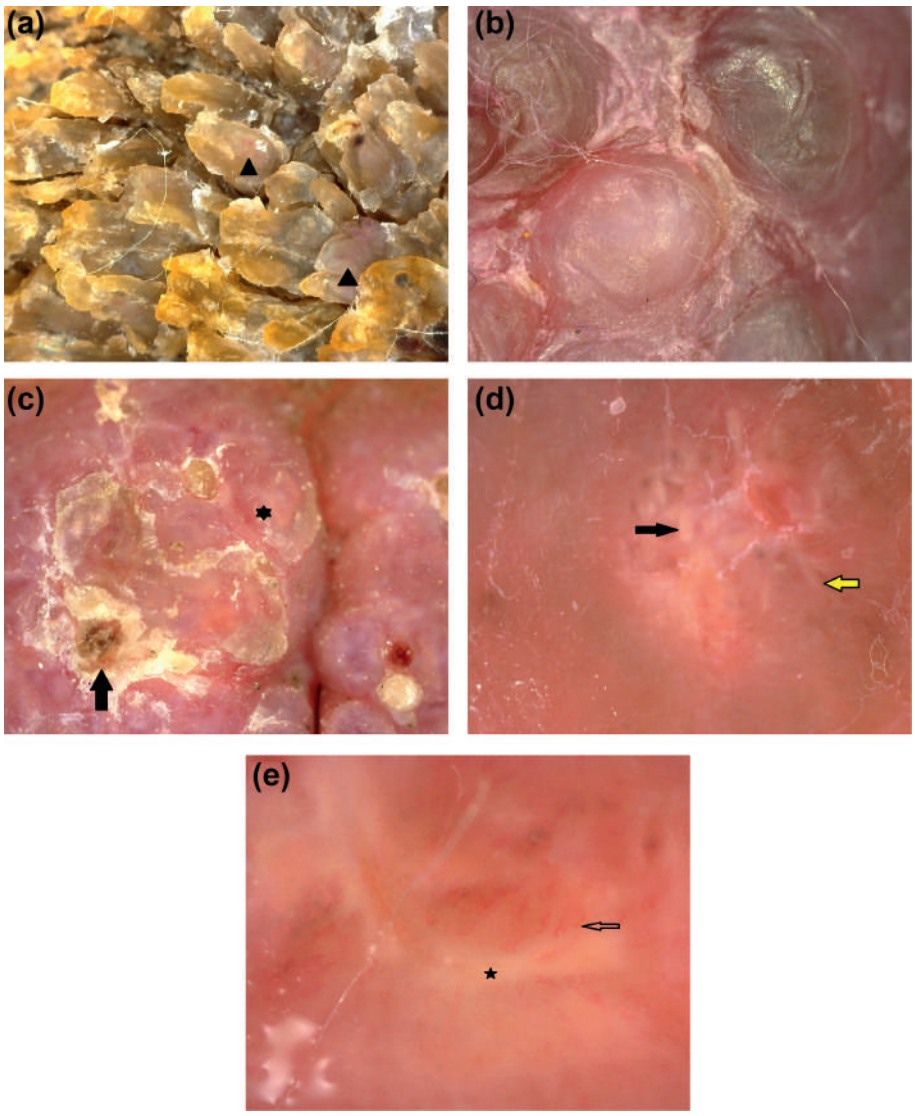

Fig. 3 – TBVC. (a) Papillated surface with linear vessels along papillae (arrowhead),50x. (b) Lobulated surface, 50x. (c) Keratotic plug (black arrow) and milia-like cyst (black star),200x. (d) Milia-like cysts (black arrow) and white streaks (yellow arrow),50x. (e) White streak (black star) with hairpin vessels arranged perpendicular to streak (black arrow),200x

corresponding to underlying dermal granulomas may get masked by these epidermal changes.[14]

Milia-like cysts are yellowish-white structures corresponding to intraepidermal keratin horn cysts. In our patients, milia-like cysts were mainly observed in keratotic lesions of lupus vulgaris. Patulous follicles can be explained by the lateral compression by the underlying dermal granuloma.[13]

White structures can be appreciated under polarized microscopy which is oriented parallel, and sometimes orthogonal to each other, as seen in lupus vulgaris. They correspond to hyperkeratosis and pseudoepitheliomatous hyperplasia.[13] White starburst pattern observed in papulonecrotic tuberculid corresponds to underlying hyperkeratosis. This is appreciated as perilesional concentric white scales innonpolarized view.[13]

Vascular changes are more prominent in the acute phase of the lesion. In our study, scrofuloderma and lupus vulgaris presented polymorphic patterns of vessels such as hairpin, corkscrew, dotted, linear, and entangled vessels. A striking pattern of concentric circumferential arrangement of dotted vessels was observed in a lesion of inoculation TB, presenting as lupus vulgaris. Hairpin vessels arranged in a radial pattern along white streaks were mainly observed in papulonecrotic tuberculids. Linear pattern was the predominant vascularity observed in resolving lesions of lupus vulgaris. Crown vessels originating from the periphery but not crossing the centre of the lesion were observed in lichen scrofulosorum.[13]

Diagnosis of cutaneous TB relies on demosntration of acid fast bacilli in the tissue, culture and molecular techniques. Dermoscopy is a non-invasive tool where correlation with the

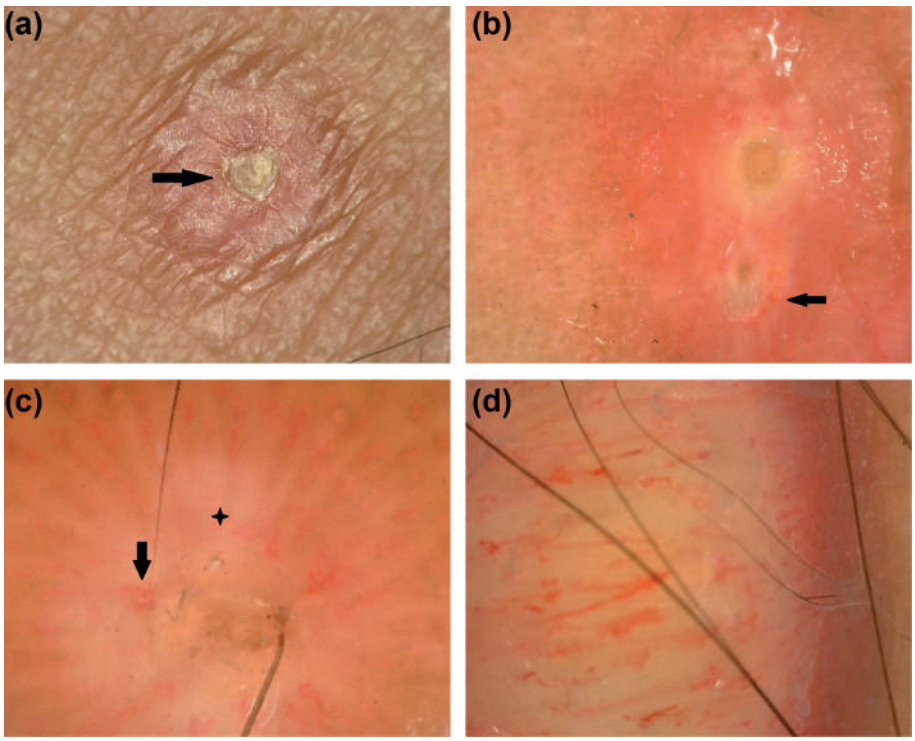

Fig. 4 – Papulonecrotic tuberculid (a) Erythematous papule with necrotic plug, 50x. (b) Hairpin vessels (black arrow) arranged radially around the central plug, 50x. (c) Radially arranged white streak (black star) with hairpin vessels running parallel to streaks (black arrow),50x. (d) Hairpin vessels at 200x magnification.

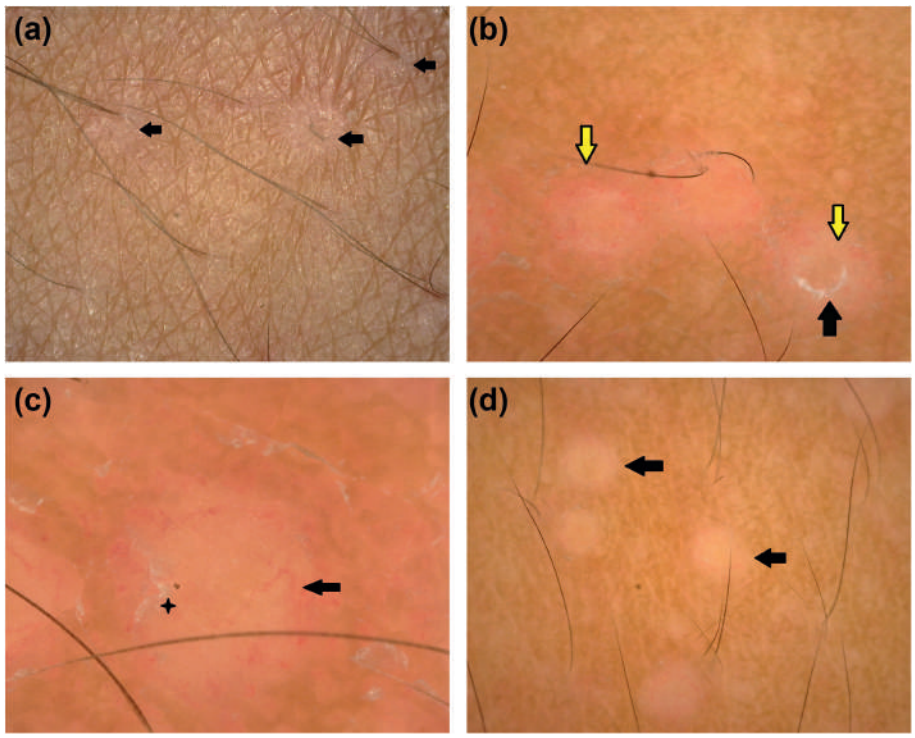

Fig. 5 – Lichen scrofulosorum (a) Perifollicular papules (black arrow),50x. (b) Peripheral collarette of white scales (black arrow) and crown of vessels (yellow arrow), 50x. (c) Crown of vessels (black arrow with scaling (black star) over, 200x. (d) Perifollicular pallor (black arrow) at 50x magnification.

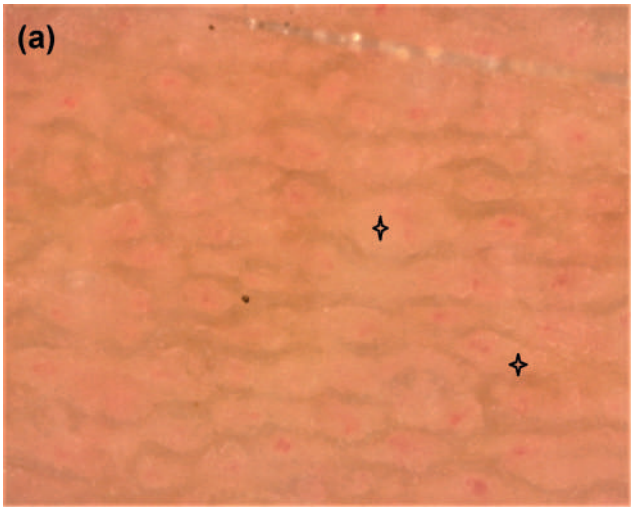

Fig. 6 – Erythema induratum of Bazin: Regular arrangement of dotted vessels within reticular pigment network (black star)(50x magnification).

histopathological findings will aid in early diagnosis and treatment of this condition.

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Declaration of competing interest

None.

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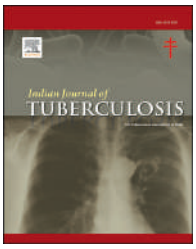

Letter to the Editor

Are we really reducing cost of tuberculosis treatment in private sector: A valuable insight from cost analysis of patient who spend more than 500 dollar (4 lakh rupees)?

India, the country with the world's largest burden of tuberculosis (TB), is an important cause of morbidity and mortality.[1] Although National TB Elimination Program (NTEP) offers free TB diagnosis and treatment, still many patients continue to be treated in the private healthcare sector.[2,3] One of the surveys between 2015 and 16 showed 38.8% while data based on drug sales (2013–16) showed 64% of TB patients treated in the private sector respectively.[4,5] The diagnosis and treatment of tuberculosis are often poor and more cost bear by patients in the private sector.[6] Due to concerns about poor care of TB patients in the private sector, NTEP is pursuing public-private mix (PPM) ventures to optimize TB care and reduce costs in an initiative called Universal Access to TB care.[7] After the pilot project for the involvement of the private health sector in Mumbai and Patna, NTEP involve the private sector and there is massive expansion occurred and TB notifications grew from 7% in 2014 to 28% in 2019.[8] Tuberculosis has huge financial implications for the patient and their family. Although diagnosis and treatment of TB even in the private sector is free, still many patients spend huge money. Understanding the timing and sources of these costs is important to create healthcare delivery models that minimize these costs.

I am sharing a patient who spend more than 500 dollars (4 lakh rupees) on the treatment of drug-sensitive tuberculosis in the private sector despite of drug being provided free of cost through NTEP. It is very important to understand the sources of these additional costs, which is important for the program to minimize these costs. A 52 years lady, belonging below the poverty line and known as diabetic and hypertensive, was diagnosed with pulmonary tuberculosis on clinical-radiological basis by a private doctor and she had been put on an Anti TB drug, which was received free of cost from a government hospital. PatientSputum Genexpert performed in a government facility where she was receiving treatment found to be rifampicin sensitive. The patient was fine for about a year, after which again presented with a fever for which several investigations were performed from blood culture to test for malaria. She has been prescribed several times various courses of antibiotics in parenteral and oral forms. She continues to have a fever and later on developed also breathlessness on climbing up stairs. The patient had also given an oral corticosteroid, Inhaled bronchodilator and corticosteroid to tackle breathlessness. There are several agents prescribed summarised in Fig. 1 without any reason. Sixteen chest Xray and Five CTs chest including one contrast done in the last 2 years. Sputum was not subjected to GeneXpert or acid-fast bacilli microscopy in the last 2 years to detect relapse or drug resistance tuberculosis. We sent sputum for microscopy which showed 3+ AFB positivity and CBNAAT detected MTB and Rifampicin resistance. As told the patient and her attendant spend more than 500 dollars (4 lakh rupees) for investigation and treatment in the last 2 years. As told by the patient attendant they sold their land to bear the cost of treatment

Tuberculosis is considered a social disease, not only affecting the patient physically but also their family financially and socially.[9] There are several initiatives by the NTEP program to support patients suffering from tuberculosis. NTEP aims at supporting the costs incurred by patients toward diagnostics and treatment of TB, which significantly reduces the burden of the disease on the patient as well as reduces the economic impact of the disease on the family. But how to address this poor patient who spends huge money in getting treatment for drug-sensitive tuberculosis? This case undergoes huge investigation and unnecessary treatment in the private sector rather than sending sputum for Genexpert or AFB microscopy to identify drug resistance. To really reduce the impact of tuberculosis on families, there should be some changes in policy to reduce unnecessary investigation and treatment. Although the NTEP program involves the private sector to reduce the cost burden of diagnosis and treatment it requires cost analysis for overall treatment. This is not the one case history, there are several patients struggling financially

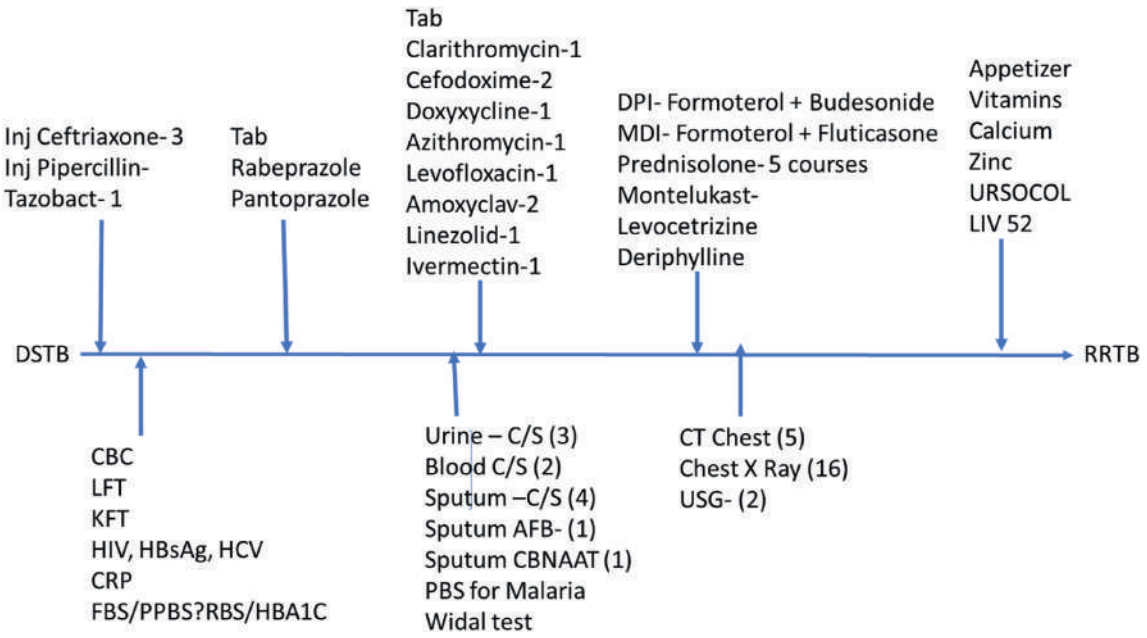

Fig. 1 – Showing spectrum of medication received and investigation done in last 3 years (Number shows courses and investigation). DSTB Drug sensitive TB, RRTB Rifampicin resistance TB, CBC complete blood count, LFT Liver function test, KFT Kidney function test, CRP C-reactive protein, C/S culture & sensitivity, PBS Peripheral blood smear, USG Ultrasonography.

to complete tuberculosis treatment despite the free diagnosis and treatment of tuberculosis in the private sector. Achieving the elimination of tuberculosis by 2025, require addressing such type of economic challenges to reduce the suffering of family.[10]

In conclusion, this case history highlights that despite free TB diagnosis and treatment even in the private sector by NTEP, still, huge money spends by the patient, and that makes the family financially disabled. Cost analysis showed a majority of money spend on unnecessary investigation and treatment. A prospective cohort study may be required to analyse the cost of treatment in the private sector.

Conflicts of interest

The author has none to declare.

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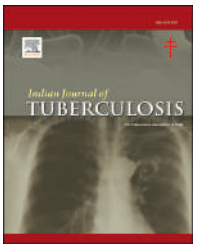

Letter to the Editor

Aviptadil: A promising treatment option for acute respiratory distress syndrome

Dear Editor,

A group of senior doctors with vast experience in the management of respiratory diseases got together on 25th June 2022 under the leadership of Dr. V. K. Arora, Dr. D. Behera, Dr. Agam Vora, Dr. Parthiv Mehta, Dr. Arindam Kar, Dr. A. Jaychandra, Dr. B. P. Singh, Dr. S. K. Katiyar, Dr. Subhankar Kandi, Dr. J. K. Samaria, Dr. Parvaiz Koul, Dr. Naveed Nazir Shah, Dr. Rukhsana Najeeb, Dr. N. K. Jain, Dr. Sadiq Ahmad, Dr. Mir Faisal, Dr. Rayees Najib and Dr. Bhupesh Dewan under the auspices of the Academy of Advanced Medical Education. It was a very useful discussion, bringing out their personal experiences regarding the use of Aviptadil in Acute respiratory distress syndrome (ARDS) a life-threatening respiratory condition. ARDS is a manifestation of acute injury to the lung, associated with sepsis, pneumonia, severe pulmonary infections, aspiration of gastric contents, major trauma and tuberculosis.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread across the globe in no time. In the beginning of the pandemic, people suffered due to the absence of efficacious drugs required to treat ARDS in severely ill patients. There are newer drugs on the horizon that have been recommended, though with very limited experience and devoid of enough data about safety and efficacy. Repurposing may be the most straightforward way to deliver a pharmacological treatment and Aviptadil [a synthetic form of Vasoactive Intestinal Peptide (VIP)] is one of those treatment options. The underlying hypothesis is that VIP defends AT-II cells, prevents cytokine storm, and increases the lung's oxygen concentration and gas exchange.

VIP consists of 28 amino acids, which were first discovered in 1970. Although initially identified in the intestinal tract, human VIP is now known to be produced throughout the body. It is highly localized in the lungs (70%) and binds with ATII cells via VIP receptor type-1 (VPAC1). VIP has shown a multimodal mechanism of action, decreases inflammatory cytokines, preventing cytokine storm syndrome and inhibiting viral replication. Other supporting actions include immunomodulating effect, vasodilating and bronchodilating effects, maintaining surfactant production and preventing the process of apoptosis.[1] Its clinical effect on asthma, chronic inflammatory lung disease, cystic fibrosis, sarcoidosis and primary pulmonary hypertension has already been assessed in the past.[2]

Indian clinical trial data of patients treated with Aviptadil (three successive 12-hour infusions at 0.166/0.332/0.498 mcg/kg/hr) showed rapid recovery in the cases of COVID-19-induced respiratory failure.[3] When Aviptadil was administered to the patients on ventilator, a rapid improvement in PaO_2/FiO_2 was observed within 3 days of starting the treatment.

After reviewing the clinical trial data in April 2022, the drug was approved by DCG(I) in India for the management of ARDS in severe COVID-19 patients. The grant of regulatory approval went through a long process including detailed and step-by-step evaluation of the clinical trial by a subject expert committee designated by the Indian regulatory body Central Drugs Standard Control Organization (CDSCO).

Aviptadil has also been designated as an orphan drug by the US Food and Drug Administration (USFDA) and European Medicines Agency (EMA) to treat respiratory airway diseases such as acute lung injury, ARDS, pulmonary hypertension and sarcoidosis.[4,5]

The group, at the end of the discussion, proposed the following consensus statement:

> Vasoactive Intestinal Peptide (VIP) is produced in the body and is highly localized in the lungs. VIP affects the respiratory and immune systems. It relaxes airway pulmonary vasculature and smooth muscles, inhibits its proliferation, induces bronchodilation and increases pulmonary surfactant production. It has an anti-inflammatory effect and defense mechanism against septic shock.
>
> Aviptadil is a synthetic Vasoactive Intestinal Peptide (VIP) that has been studied in respiratory disease conditions like asthma, acute lung injury, pulmonary arterial hypertension, sarcoidosis and idiopathic pulmonary fibrosis and is designated as an 'Orphan Drug' by USFDA and EMA. Recent clinical trials conducted in USA and India have shown encouraging results in ARDS associated with COVID-19 patients in terms of reduction in mortality rate and hospital stay.
>
> Based on the evidence, the group noted that the ARDS patients treated with Aviptadil have survived longer than those who were given the placebo treatment along with the standard of care treatment as recommended by the Government of India. The patients can be given the benefits of hospital-free survival rather than survival in an ICU.

The group strongly feels that Aviptadil has potential in ARDS but there is a necessity to generate more data on Aviptadil use in ARDS conditions other than COVID-19 etiology.

Conflicts of interest

The authors have none to declare.

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Guide for Authors

Before you begin

The *Indian Journal of Tuberculosis* (IJTB) is a quarterly journal published in January, April, July and October. It publishes original articles on tuberculosis, respiratory diseases, case reports, review articles, and abstracts of articles published in other medical journals and book reviews. Every issue contains editorial sections on contemporary subjects, radiology forum and a forum for readers to express their opinions on published articles and raise questions on subjects appearing in the journal. IJTB is indexed in Medline.

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