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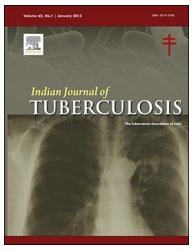

Editorial

Epidemiological paradigm: Tuberculosis in HIV, diabetes, and smoking in North East India: An impact greater than sum of its parts

In addition to the well-known risk factors contributing to the rise of tuberculosis cases in India, such as human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), poor nutritional status, and young age, realization that other emerging factors like diabetes mellitus, indoor air pollution, alcohol abuse, tobacco smoke are also fuelling the epidemic, is adding more complexities to tuberculosis control and making the task onerous. Individually and in combination these risk factors tend to increase the burden two to three times. Unless addressed concurrently these numbers are likely to overwhelm the tuberculosis control programme and annul its efforts. The eight states of the North Eastern region characterized by hilly, forested area, sparsely inhabited mainly by tribal populations also share high prevalence of emerging risk factors for tuberculosis. India has set itself a target of elimination of tuberculosis by 2025.[1] To achieve the target in the North Eastern states special resources would be needed to be put in place for controlling these risk factors as well. A comprehensive integrated approach taking help of other departments in health sector and beyond is critical.

In this editorial, we share our concern on two risk factors viz. diabetes mellitus and tobacco smoking which impact a larger section of North-Eastern population and accelerate progression of tuberculosis disease.

Some of the states here have highest prevalence of HIV in India, notably Manipur (1.15%), Nagaland and Mizoram (0.7–0.8%).[2] India has an average of 0.26%. People with HIV have a 20–30 times higher risk of developing active tuberculosis, which is more of the extra pulmonary type and throws up challenges of diagnosis and management. Tobacco consumption is highest in this region of the country. On an average people in NE smoke more tobacco than rest of India. Mizoram and Meghalaya have a prevalence of over 60%, Tripura follows at 40%. India's average is around 26%. One in four Mizo women smokes, whereas average for India is 1 per hundred.[3] Smokers are two-three times at higher risk of developing tuberculosis than non-smokers. The disease is more severe. A regular smoker has twice the risk of getting the disease again, recurrences are more often. If an HIV infected individual also smokes, the risk increases three folds. Diabetes is the third risk factor. Results of an India-wide study (Indian Council of Medical Research-India Diabetes Study, ICMR-INDIAB) show high prevalence of pre-diabetes especially amongst the urban poorin the states of Arunachal Pradesh, Manipur, and Meghalaya which is of major concern.[3] Diabetes again increases the risk of tuberculosis to three folds and the risk of multi drug resistant (MDR) among diabetics who get TB is 2–8 times higher. The progression of the disease is rapid. And it develops more frequently when the diabetes control is poor.

What does all this mean to the tuberculosis elimination programme?

Elimination of TB means stopping transmission. That is reach every person suspected to have tuberculosis, get diagnosis confirmed, and if positive put on appropriate treatment and help to complete the therapy. Given the difficult terrain and hard to reach population, more resources would be needed. Health workers may have to travel long distances to bring one patient under treatment successfully. Finding TB cases is critical. Modelling studies have shown that if the case detection is increased by 25%, it can translate in to about 40% reduction in mortality, the prevalence decreases by about 30% and the reduction of incidence cases is by more than 20% in 10 years.[4]

For persons with chest symptoms, sputum examination for acid-fast bacilli (AFB) is the recommended test. Acid fast staining of sputum for AFB performs poorly as a screening test. Its sensitivity is poor. The cartridges based nucleic acid amplification test (Cartridge Based Nucleic Acid Amplification test, CB-NAAT) is now available at the district level as it needs a controlled temperature and dust free environment. A nucleic acid amplification test (*TrueNat MTB*), a chip based test has been developed by an Indian company. It is reported to have good sensitivity and specificity.[5] It has recently been validated in 100 designated microscopy centres in 50 districts in 10 states in which 18,000 samples have been tested. This battery operated test takes around an hour to give the result whether

a sputum sample is positive for TB, for positive samples rifampicin resistance can be determined in another hour's time. It does not require dust proof air-conditioned environment. It is projected as a test to be used at primary health centre (PHC) level. If it is found to have an acceptable sensitivity and specificity, this test should be deployed in the NE states on a priority basis.

Relying on symptoms-screen alone may be contributing to delayed diagnosis of tuberculosis. Using chest X-rays (CXR) as a pre-screen test can reduce numbers needed to test for each case of tuberculosis. Abnormal CXRs could, therefore, be key to active case finding by identifying cases that otherwise would have not have been diagnosed by conventional, passive case finding, Today, CXR is becoming more accessible in remote settings due to technological advances such as digital imaging instead of film-processed images. The sensitivity of CXR has been shown to increase if a computer-aided diagnosis (CAD) software is used to analyse digital images. It gives a probability percentage consistent with TB. It could possibly be used as a 'filter' in TB screening to identify who gets tested by CB NAAT (*GeneXpert*).[6,7] We need a locally available and economic version of the CAD4TB which would help in improving diagnosis especially in areas where a radiologist is not available to interpret the CXR.

Diabetes triples the risk for active tuberculosis, thus the increasing burden of type 2 diabetes will further burden the TB elimination programme. An epidemiological model in India indicates that diabetes mellitus may account for 15% of TB cases.[8] The International Diabetes Federation has predicted an increase in diabetes prevalence to 10% world wide by 2035. Modelling exercises have predicted that if such an increase does happen it could undercut the decrease in new cases of tuberculosis by about 3%. Some believe that increase in the prevalence of diabetes in India has contributed in part to a negligible reduction in new cases of tuberculosis between 1988 and 2008.[9] Diabetic tuberculosis patients have a higher risk of treatment failure, death, and recurrent tuberculosis as compared to non-diabetic tuberculosis patient. Poorly controlled diabetes increase the risk of tuberculosis and leads to unfavourable tuberculosis treatment outcomes. Researchers have long known that diabetes patients have higher blood sugar levels making their disease difficult to control and putting them at greater risk of developing complications. A bidirectional screening for tuberculosis and diabetes mellitus at hospital and community level has been shown to be feasible and effective.[10,11] Such a screening should be piloted at hospital and community level and scaled-up. This presents a unique opportunity to capture persons presenting with either of these two conditions as potential targets for screening and treatment. Patients with diabetes often present with atypical symptoms and pose hurdles in diagnosing tuberculosis. Clinical management of patients with both diseases can be difficult. Tuberculosis patients with diabetes have a lower concentration of tuberculosis drugs and a higher risk of drug toxicity than tuberculosis patients without diabetes. Besides drug treatments for tuberculosis and diabetes, other interventions, such as education, intensive monitoring, and lifestyle interventions, might be needed, especially for patients with newly diagnosed diabetes or those who need insulin. Modelling study analysed the potential effect of diabetes on tuberculosis epidemiology in 13 countries with high TB burden. The study estimated the tuberculosis burden that can be reduced by alternative scenarios of diabetes prevention. Lowering the prevalence of diabetes by an absolute level of 6.6–13.8% could accelerate the decline of tuberculosis incidence by an absolute level of 11.5–25.2% and tuberculosis mortality by 8.7–19.4%. If interventions reduce diabetes incidence by 35% by 2025, 7.8 million tuberculosis cases and 1.5 million tuberculosis deaths could be averted by 2035.[12]

The evidence for an regular tobacco smoking increases risk of TB in active smokers is well established.[13] There is also some evidence that second hand smoking (passive smoking) is a risk factor for developing tuberculosis especially in children 0–5 years.[14] When exposed to second hand smoke, household/environmental factors (crowding, biomass fuel burning) may increase risk for developing tuberculosis. In addition, smoking has been associated with cavitary lesions, bacillary load, smear conversion delay, and high risk of reactivation and death during or after treatment. Smoking rates are high among men in North Eastern states, and, together with rising rates of diabetes, the risk of progression to tuberculosis disease will also increase. Interventions such as smoking cessation and early screening for tuberculosis can be advocated, but the impact of these interventions in reducing TB risk remains negligible at population level. Both active and passive smoking increase susceptibility to TB infection, progression to active TB disease and the risk of adverse anti-TB treatment outcomes. Systematic reviews suggest that the risk of TB disease among smokers is increased two to threefold compared with people who have never smoked.[15] Tobacco control and smoking cessation among people with diabetes and tuberculosis can therefore play an important role in limiting the burden of TB. It is also known that diabetic smokers have more than 5-fold increased risk of pre-treatment positive smears than do non-diabetic non-smokers. This is a remarkable joint effect of diabetes and smoking that increased risk of tuberculosis transmission.

Against the background of risk factors fuelling the epidemic of tuberculosis in India, a critical assessment of the tuberculosis control programme (like strengths, weaknesses, opportunities and threats (SWOT) analysis) especially in the North-Eastern region would be helpful in identifying the areas that need strengthening to deal with these risk factors, and the resultant possible increase in number of active tuberculosis patients. From a health systems point of view, issues such as delays in diagnosis, initiation of appropriate treatment and its successful completion would be crucial. Experience from the combating combined HIV/TB disease would be helpful. But more operational research would be needed to tackle diabetes and tobacco smoking. The Revised National Tuberculosis Control Program (RNTCP) would need to solicit assistance from other programmes within and outside the health sector to develop integrated comprehensive approach in meeting the targets of tuberculosis elimination in the North Eastern region.

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Viewpoint

Strategy and way forward for TB elimination☆

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ABSTRACT

India's National Strategic Plan (NSP) for TB Elimination 2017-25 looks ambitious in terms of targets of TB notification aiming to reach 35 lakh TB patients annually, i.e. double that of current status. Strategies and interventions designed under the Plan with patient centered approaches, with synergistic public-private-patient partnership can make it possible to achieve real aim of reaching the unreached, by extending patient support systems and social protection to affected communities. In this review point, these strategies and commitments are summarized as future plan.

Till 19th century, every seventh of whole human race on this planet was eaten up by Tuberculosis. This white plague continued its menace, but after that TB started declining due to development especially in western world. But, in India despite of all efforts TB continues to be a major public health challenge today also remains a major killer among the infectious diseases.

India is one of the first countries to invest in TB control after independence and the national TB Programme is in place since 1962. Sometime, experts do talk about failures or shortcomings of NTP and also RNTCP. It is important to review the strategies and evaluate the programme. In the recently developed National Strategic Plan, all inputs have been taken from various stakeholders.

Setting the target of reaching to 35 lakh TB patients in a year, by 2020 looks ambitious, and difficult to achieve for many. Yes, it is ambitious. The issue is that TB should be eliminated? If yes, then there is need to aim bigger. Without reaching to all TB patients in the country, the issue cannot be addressed. Complete surveillance is a key to control of any disease and in particular, the infectious diseases like TB in which early identification and ensuring complete treatment is the cornerstone of control and prevention of disease.

To reach to almost the double number of TB patients currently being notified in the country, there is need to first believe. Gathering here had believed in 1997 that, all TB patients in the country can be supported for six months of treatment by Directly Observation. It was believed in 2007 that drug resistant TB patients can be diagnosed at the time of diagnosis and can be managed under programmatic conditions for 2 years duration. Now, is the time to believe that 20 lakh TB patients in the country can be brought under surveillance and ensure quality of care. In addition to 15 lakh patients notified by the programme each year, aim is to reach out to 20 lakh more patients by forging partnership with the private sector. To overcome issues of access and ensure early diagnosis, active case finding efforts will be scaled up to screen 12 crores mapped high risk population every year. This will yield 2–3 lakh additional cases.

Patient has to be at the centre to any strategy to reach to them, to support them for completion of care, to prevent any catastrophe to his/her social life. For the first time, World

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Health Organization has advocated to bring down Catastrophic Costs to TB Affected families to Zero as one of the top three high level indicators and part of the End TB Strategy globally. For achieving TB targets and milestones of End TB Strategy and Sustainable Development Goals (SDGs), provision of TB care and prevention is required within the broader context of Universal Health Coverage (UHC) along with addressing social and economic determinants and consequences of TB. In India, the patients support services have been incorporated right from inception of systematic approach to address the disease. The sanatoria approach was covering comprehensive care and ensuring prevention of any financial hardship to TB patients. With introduction of ambulatory care, the public health programme has been ensuring free diagnosis, free drugs, patient support for transportation; cash transfers to TB patients in tribal area and for adherence, the providers are being incentivised through cash transfer.

The patient support will include a range of approaches from policy to eliminate discrimination to social protection schemes and on a broader scale, the poverty alleviation, housing support, job security etc. Cash transfer is one such intervention, to address multiple facets of the disease, not only patient support or social protection but also from the provider's perspective, it gives opportunity to deliver public health measures. The Cash Transfer aimed at fulfilling nutrition need, can improve notification and hence surveillance; enables providers to ensure treatment compliance of patient and hence, ensure cure; to cover household expenditure which is critical when no point of care diagnostic test available, specialized care of drug resistant TB, preference to seek care in private sector.

It is equally important to have targeted delivery of the cash transfer to those who are eligible and need the most. That is where leverages cab be on Direct Benefit Transfer (DBT) Mission driving across different social sectors, bringing in the efficiency and transparency of cash transfer given to the beneficiaries. So far, 119 Crore Aadhaar numbers have been generated. Pradhan Mantri Jan-Dhan Yojana brings about financial inclusion through access to banking facilities. NIKSHAY – a case based web based TB notification system under RNTCP maintains record of TB patients reported to the Programme. As a step forward to establish DBT system for an individual eligible to receive the benefit under the RNTCP, a notification has been published through the Extraordinary Gazette of India. Through this Gazette, an individual eligible to receive the benefit under the RNTCP required to furnish proof of possession of Aadhaar number or undergo Aadhaar authentication. The integration has been established among Aadhaar (UIDAI), Bank Account of Beneficiary through Public Finance Management System (PFMS) and NIKSHAY. The System has been implemented to ensure that TB patients and their providers who are eligible for cash transfer under RNTCP, get the benefit without fail. Three schemes (1) for TB patients, (2) treatment supporter, and (3) private providers are on-board on DBT Bharat Mission Portal which is directly monitored by the PMO. Cash transfer is also provided to TB patients from individual States as part of Social Welfare Schemes for priority populations in the form of social protection scheme, pension benefits, and for nutrition support. Extension of Direct Benefit Transfer System for these State specific patient support services will bring more efficiency and accountability to the State Government.

Notification of TB patients by chemists is also opening up and also patients will be allowed to self-notify. Call centres in 12 local languages are being established to ensure hassle free and convenient notification and follow-up of each patient.

Research is equally important for making appropriate changes in the strategies from time to time. And programme is coordinating with Indian Council of Medical Research (ICMR), Department of Health Research which has formed India TB Research Consortium in partnering with Department of Biotechnology (DBT), Defence Research and Development Organization (DRDO), Council of Scientific& Industrial Research (CSIR), Department of Science and Technology (DST), Department of Pharmaceuticals, World Health Organization (WHO), Bill & Melinda Gates Foundation (BMGF), Tata Trusts, The Union and the Tuberculosis Association of India.

Programme is also committed to validate and use appropriate newer diagnostics, novel drugs and regimens and vaccines that will be recommended by Expert group of India TB Research Consortium. While addressing drug resistant TB, in addition to diagnosis and treatment of DR-TB, focus will be equally on its prevention.

Coming back to the difference from 15 lakh and 35 lakh TB patients. At the implementation level, the current cash transfer benefits to TB patients are offered only to those who seek care from public sector. Even, the state specific schemes are not being extended to TB patients who seek care from private sector. Need is to reach every one and ensure that all TB patients irrespective of where they seek care, are benefited with equal quality of care as well as patient support schemes including cash transfer.

Momentum should continue to keep the drive to reach to all TB patients, ensure them equitable care and extend cash transfer through direct benefit transfer.

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Viewpoint

Koch's postulates – Pitfalls and relevance in the 21st century

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Koch's postulates, described by Robert Koch and Friedrich Loeffler in 1882-1984 in relation to tuberculosis (TB) and cholera laid the foundation of microbial etiology of infectious diseases.[1,2] The postulates were partly based on earlier concepts of Koch's teacher, Jacob Henle (known for the discovery of Loop of Henle in the kidney) who had previously proposed microbes as the cause of infectious diseases. Koch's postulates had changed the scene of clinical diagnosis and management of infectious diseases, in particular the TB.

In summary, the Koch's postulates required that the following criteria should be present to prove the causative relationship between the microorganism and the disease:

i. The microorganism must be found in abundance in the diseased tissues and should be present in the healthy;
ii. The microorganism must be isolated by pure culture from the diseased tissue;
iii. The cultured microbes should cause the disease in the healthy; and,
iv. The microbe must be isolated from the diseased experimental host in whom the inoculation has been done.

The logical explanation of microbial-disease association, which won Robert Koch the Nobel Prize in Physiology and Medicine in 1905, was clearly irrefutable in theory and also demonstrable in clinical practice. Limitations and pitfalls however, were soon recognized by clinicians as well as by Koch himself. Some of the following important observations were made by different people:

i. Several of the microbes are routinely present in asymptomatic carriers who are not diseased. The presence of microorganisms does not always imply the presence of disease;
ii. Microorganisms may not be always grown in pure cultures;
iii. Inoculation of microorganisms may not always cause the disease, especially in the healthy-host factors are equally or sometimes more important;
iv. Microorganisms may not be necessarily isolated and cultured from the inoculated, experimental host.

Some of the limitations were attributable to the generalization of the postulates to all the infectious diseases which were originally described only with reference to TB and cholera.

In the subsequent decades, the clinicians started relying less upon the postulates than upon their own clinical judgments (based on other criteria) for the diagnosis of infectious diseases, including TB. This change in attitude got further multiplied as the number of clinicians expanded worldwide. There followed a large-scale under- and over-treatment of infections. This proved to be particularly dangerous for a disease such as TB which required significantly longer duration of treatment. In the absence of a definite microbial etiology, people started treating TB based on

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their own understanding. There was gross misuse of drugs as well as inappropriate choice of drugs in inadequate dosages and duration. All these major clinical errors were subsequently responsible for the emergence and spread of drug-resistant TB which soon turned to become multi-drug resistance and extensive drug-resistant TB.

By the end of the 20th century, other investigators made attempts to modify and redefine the postulates to take care of the scientific and clinical limitations with reference to the microbial demonstration, isolation, culture and inoculation, etc. Alternate concepts based on molecular structure of microbes were suggested. The revised version of Koch's postulates was proposed which replaced 'microorganism' with 'nucleic acid-sequences' and the number of their copies associated with the disease (TB).[3] In summary, the revised postulates propose that the nucleic acid sequences of the causative organism (TB) should be present; the number of copies should decrease with disease resolution; fewer or no copies should be there in the healthy tissue; the copy number correlates with disease-severity; nucleic acid sequence should correlate with diseased tissue at cellular level, and; the sequence based evidence should be reproducible.[3]

The nuclei-acid sequences constitute the structure of deoxyribo nucleic acid (DNA) – the basic unit of 'life', including the microbes. In case of TB therefore, the presence of mycobacterial DNA automatically implies the presence of the mycobacteria and therefore, TB – at least theoretically. Polymerase chain reaction (PCR) based tests are used to multiply the DNA several fold for easy detection. But the number of copies of DNA in the tissues are usually very few and hard to detect. Of the several available PCR tests, cartridge based Gene XpertTB test from sputum appears to be most relevant because of the ease of testing, availability of quick results within a few hours, higher sensitivity and specificity, in spite of some reservations and limitations.[4,5] However, the sensitivity and specificity of the test from other tissue sample and secretions remains to be established. Recently, there is a limited report on finding mycobacterial DNA from even oral swabs of patients of pulmonary TB.[6]

Clinical tests apart, there are difficulties in accepting the postulation of '*presence of DNA as the proof of disease-diagnosis*'. There are problems of techniques and standardization for DNA testing. There are large variations in the sensitivity and the specificity of tests. The test results also depend upon the primer used for DNA detection. More importantly however, the problem of distinguishing the presence of a mere asymptomatic infection or latent disease remains almost the same with the nucleic acid sequences as with the microbes.

There is hardly any doubt that the Koch's postulates will be redefined (or refined) in the near future. In clinical practice, the DNA-based criteria are likely to replace the 'microbe' for a better understanding of the microbial etiology and pathogenesis. But it will require significant further inputs by clinicians to select DNA-based criteria for clinical diagnosis and treatment of TB especially for pulmonary TB.

Conflicts of interest

The author has none to declare.

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Review Article

Research on tuberculosis in tribal areas in India: A systematic review

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ABSTRACT

Background: Tuberculosis (TB) remains a major public health problem in resource-poor countries including India. Scientific knowledge is used to guide policy and practice. There is however, a limited, systematically collected data required for guiding the scale-up of interventions particularly amongst vulnerable populations including tribal groups in the country. In view of this, a systematic review of the TB research studies carried out in tribal areas of different parts of the country was undertaken.

Objective: To undertake a systematic review of the TB research studies carried out in tribal areas of India between 1996 and 2016.

Methods: A systematic review of English articles published between 1996 and 2016 on any aspect of TB was done through internet searches using Literature search EndNote programme. The words used for searching were tuberculosis, India, tribal, indigenous, disadvantaged, adivasi. The most common topics classified as annual risk of tuberculosis infection (ARTI), prevalence of TB, laboratory studies, clinical symptoms of TB, risk factors for TB, knowledge attitude practice, community Directly Observed Treatment (DOT) providers, performance of Revised National Tuberculosis Control Programme (RNTCP), and drug resistant TB. Classification was also done on the basis of the type of tribe studied and place of study conducted. A total of 47 studies identified through the search were included in the review.

Results: Of the 47 studies reviewed, 12 were on TB prevalence, 7 were laboratory studies, four on ARTI and 5 on performance of RNTCP in tribal areas. Among these, majority (23 studies) of the tribal studies did not mention the type of tribe. Ten studies were conducted among Saharia, a particularly vulnerable tribal group in the Indian state of Madhya Pradesh mainly by the National Institute for Research on Tribal Health, five were among the mixed tribes and very few on other tribes.

Conclusion: The systematic review indicates that the research studies on TB among tribal population are very few. There is a need to invest and encourage researcher to work on the research plans for the control of TB in tribal areas.

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1. Introduction

Tuberculosis (TB) remains a major public health problem in India, accounting for a quarter of the 8.6 million cases of TB that occur worldwide. Despite, drugs are available to cure most TB patients since the 1950s, yet TB remains still huge burden, in resource-poor settings and the world's most important cause of death especially in India. When scientific knowledge is used to guide policy and practices, evidences are ranked according to the relative merits of different data.

Over the past few decades, several new interventions in TB control have been developed and recommended in WHO guidelines and implemented into India's TB control programme. While recommendations for new interventions are usually based on evidence from general population, little is known from tribal areas. Owing to limited evidence, along with other factors, this might have hindered wide-scale use of tribal specific evidence based interventions. One of the ways is to take evidence from at least one good systematic review. Its aim is to reduce large quantities of information to usable dimensions. Some claim that doing so is an efficient scientific technique, one that is less time consuming and more reliable then conducting new studies. The use as well as usefulness of systematic reviews is one powerful mechanism for improving the evidence available to inform population health decision making.[1] Moreover, they contend that the diverse circumstances in which individual studies are carried out permit a reviews' results to be generalized across different contexts and become more significant than individual studies. There are currently rare, systematically collected data on the availability of evidence for scale-up of newly recommended interventions for TB control in tribal areas. Therefore, we conducted a systematic review of published study reports on TB amongst tribal population in India over a decade.

2. Methods

2.1. Searching

PubMed is a free search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. EndNote programme was used to search research papers published in peer reviewed scientific journals. We also included few of accepted manuscripts of National Institute for Research in Tribal Health, ICMR, Jabalpur. The search was limited in manuscripts published in English language during 1996 to 2016 only. The search strategy included the following key words: tuberculosis, India, tribal, Indigenous, disadvantaged and adivasi.

2.2. Selection

Studies were included if they focused on any aspect of TB. The most common topics classified are: annual risk of tuberculosis infection (ARTI), prevalence of TB, laboratory studies, clinical symptoms of TB, risk factors for TB, knowledge attitude practice, community DOT providers, performance of RNTCP, drug resistant TB. Classification also done on the basis of type of tribe studied, place of study conducted and year of publication.

2.3. Data extraction

The final set of manuscripts were assessed with the researcher and accumulated information on objective of the study, salient findings and implications or recommendation from that study findings.

3. Results

Of the 47 publications on TB research conducted in tribal areas, majority were on prevalence of pulmonary TB (12),[2–13] 7 on laboratory studies,[14–20] 5 on RNTCP in tribal areas,[21–25] 4 on ARTI,[26–29] 5 on identifying risk factors,[30–34] 6 about knowledge, attitude and practice,[35–40] two studies about community DOT providers,[41,42] two on diabetes and TB,[43,44] three studies on drug resistant TB[45–47] and one on clinical symptoms of TB[48] (Fig. 1).

Fig. 2 describes TB studies conducted among different tribes in India. Most published studies did not mention the type of tribe. Of the 12 studies conducted to find out the burden of PTB, 6 mentioned the tribes without specifying the names. Three studies were conducted among Saharias and only one study covered Bhils and Bhilalas; one study among Nicobarese and one study among Baiga tribal population.

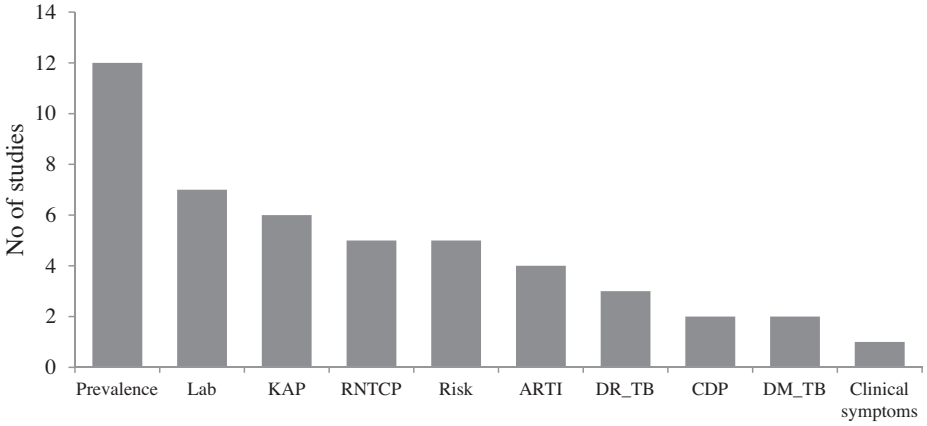
Publications in the peer reviewed journals on TB research conducted in tribal areas shown in Fig. 3. It was observed that there was an increasing trend over a period.

Among studies to find out the ARTI, Saharia has high ARTI (4%) as compared to Khammam, Bhils & Bhilalas and other tribal population of Madhya Pradesh (Fig. 4). Similarly, the prevalence of pulmonary TB was high among Saharia population as compared to other tribes (Fig. 5). It was reported that constantly high from 1996, 2010 and 2014. Among studies conducted to find out risk factors for pulmonary TB, it was reported that aged more than 45 years, male sex, tobacco smoking, alcohol consumption and being a tribal population are significant risk factors for pulmonary TB (Fig. 6).

Performance of RNTCP in tribal areas studied in Odisha and found that the case detection parameters were better in the tribal areas and treatment success was almost equal in the tribal as compare to non-tribal district. It was also reported that half of the symptomatics need to travel more than 5 km for TB diagnosis and waiting time to meet doctor was around 30 min (Box 1).

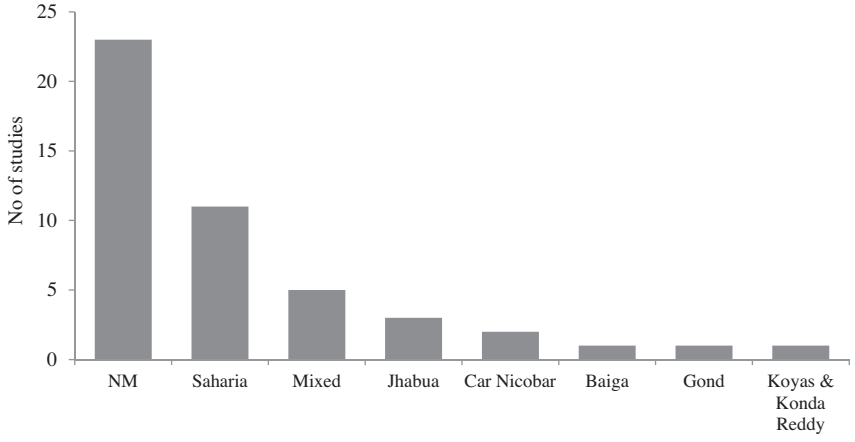
4. Discussion

The number of published systematic reviews is growing rapidly, and such reviews are receiving increased attention from research community.[49,50] It also widely acknowledged as a key component of the policy and guideline development process.[51] To reduce duplication of research and ensure that scarce resources address the priority research needs. One key component in this process of synthesizing the evidence and tailoring information can be conveyed in a manner that policy



Note: CDP → Community DOT Providers

Fig. 1 – TB studies conducted in tribal population of India. Note: CDP: community DOT providers.



Note: NM → Not mentioned

Fig. 2 – TB studies conducted among different tribes in the country. Note: NM: not mentioned.

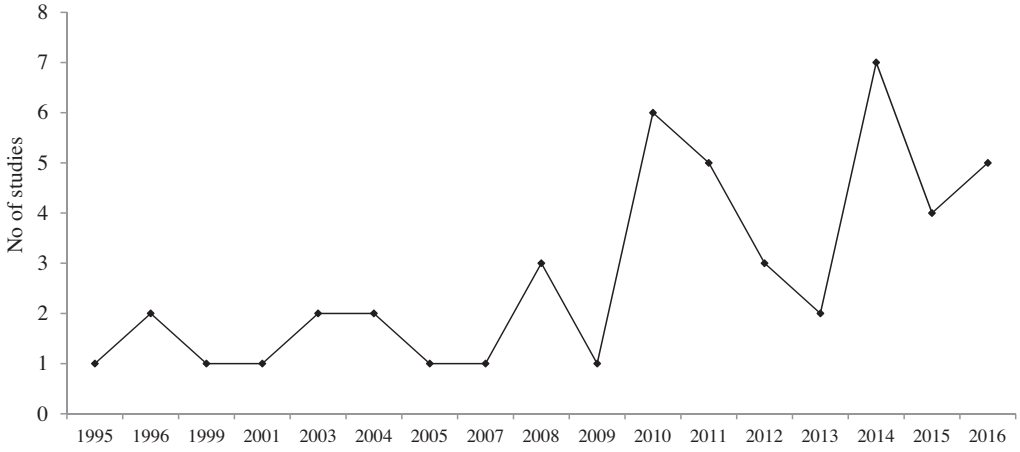


Fig. 3 – Publications over the years.

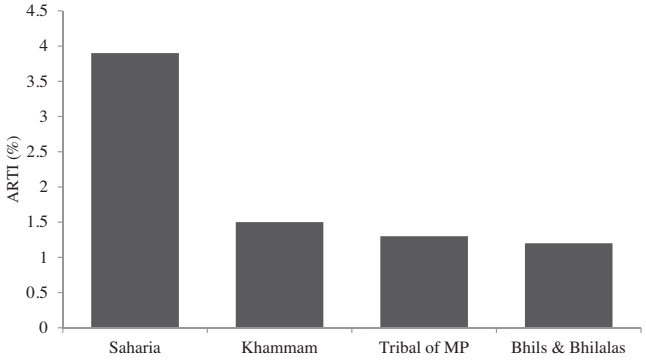


Fig. 4 – Annual risk of TB infection (ARTI).

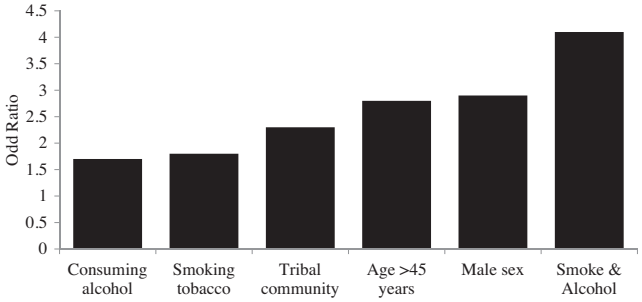


Fig. 6 – Risk factors associated with pulmonary tuberculosis.

makers and stakeholders can utilize effectively. We made an attempt to review the research on TB conducted among tribal areas in India and we found that very minimum number of studies were published in peer-reviewed journals. There is a need for an advocacy among the researchers to include TB research among tribal area that is a top priority and it also encourages them to disseminate their research. Without commitment to and wide support for research on TB in tribal areas, the Stop TB Partnership goal of eliminating TB by 2050 is unlikely to be reached.

Public health research enables us to understand the determinants and distribution of health and diseases in populations. The coupling of this understanding with health systems and public policy is critical for reducing disease burden. However, the component of public health research, amongst overall biomedical research, has been weak in India.[52,53] It was reported by Upendra Bhojani from Institute of Public Health ''Considering the number of academic institutions in the country, India's contribution to health research remains poor''.[54] Dandona et al. reported that the number of original research papers from India indexed in a widely used health related bibliographic database constituted only 1.64% of global health research outputs.[55] He also reported that the proportion of published papers from India in PubMed increased from 0.4% of the global total in 1988 to 1.8% in 2008. However, the proportion of public health research, which is crucial for achieving health care for all in India, continues to be small, at 5% of the total health research published.[56] Tribal health research is not exceptional on this aspect.

National Health Research System (NHRS) is a concept that integrates and coordinates the objectives, structures, stakeholders, processes, cultures and outcomes of health research towards development of equity in health and in national health system. Equity in services and development shall be the cardinal principle under riding the Health Research System. There shall be a special emphasis on vulnerable groups like scheduled castes, tribal populations, and other vulnerable groups. The research system would generate knowledge relevant to these vulnerable groups, appraise the measures available for dealing with health problems, and suggest the actions likely to produce the greatest improvement in health.

Nationally, a great deal of work has been done to assess the capacity and performance of local and state public health systems, but little is known about tribal health. Recognizing

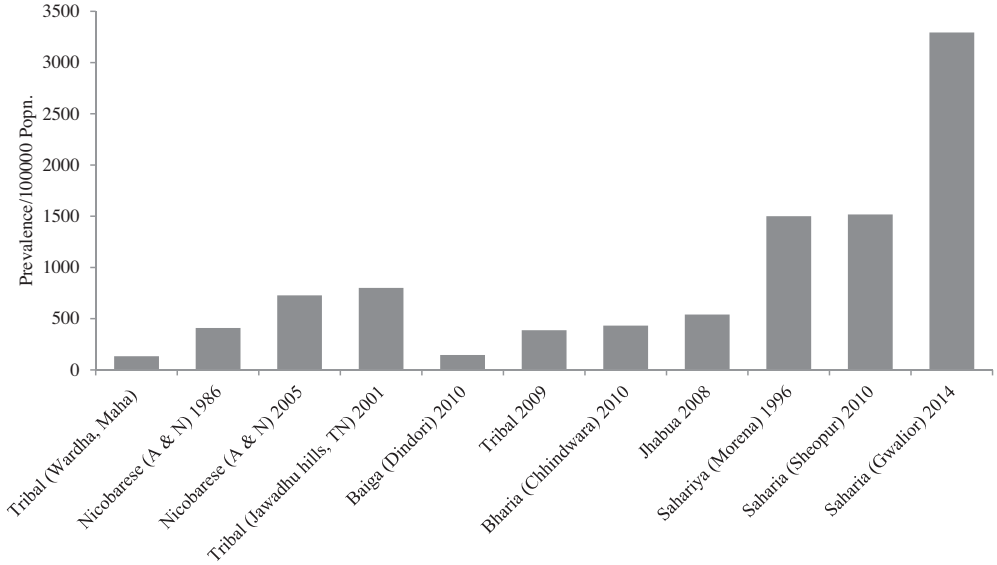


Fig. 5 – Prevalence of pulmonary tuberculosis.

Box 1. RNTCP in tribal areas.

- The case detection parameters were better in the tribal district in Odisha.
- Treatment success was almost equal in the tribal to that of non-tribal district in Odisha.
- Half of the symptomatic reported the availability of diagnosis <5 km.
- 42% of patients received treatment <3 km.
- The average distance to treatment is 4.3 km.
- Waiting time to meet doctor 30 min.
- Time spent by doctor with symptomatic: 17 min.
- 505 community DOT providers (DP) were trained in three blocks.
- Set up sputum collection centres at 10 strategic points.
- 91 active community DPs to supervise medicine intake.

the critical health status of indigenous people in different parts of the country, Indian Council of Medical Research has formulated a Tribal Health Research Forum (THRF) in 2010 with the mandate to address and discuss all the health issues pertaining to indigenous people. Today, the importance of adopting a tribal specific approach is widely acknowledged when it comes to planning and assessing policies, programmes and health services. More and more frequently tribal health research results are presented this issue is giving rise to growing interest. Research on tribal health in India has been strengthened and given priority for funding in national research grants.

Despite increasing discussion on the need for strengthening TB control programme based on strong scientific evidences, little is concretely known about the burden of TB and operational feasibilities to implement in the tribal context. Particularly studies on TB research among tribal population are very limited. Though it is limited in number and its geographic coverage, it provides insight into the level of data and its use. The key challenge is to motivate policy makers to use the available evidence and priority setting tools to direct the limited available resources into the most effective interventions and also need to set priorities for TB research among different tribal population and different tribal areas.

5. Limitation of the study

We suggest cautious interpretation of these results in light of important limitation. We included 47 publications available in the MEDLINE database and this selection may have introduced some bias.

6. Conclusion

Control of TB in tribal areas continues to face major challenges. Efforts must continue to pursue high quality DOTS expansion. Addressing research on TB/HIV (Human Immunodeficiency Virus), Multi Drug Resistant Tuberculosis (MDR-TB), diabetics TB requires increasing effort and resources, as do other challenges facing other population and other areas such as migration, smoking, alcoholism and socio-economic conditions and high risk groups. Weak health system and scare human resources are constraints to programme implementation demands operation research to find out the feasibilities. As decision makers require competent, motivated researcher to provide quality technical analyses, they also need competent staff members to provide scientifically valid information and communicate the results as information for action. Operational research priorities identified by RNTCP[57] should be inclusive of tribal specific or need to include representative sample from tribes so that we can get answer for many research questions arised from tribal areas.

Conflicts of interest

The authors have none to declare.

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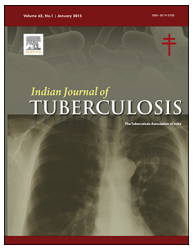

Review Article

Current Affairs, Future Perspectives of Tuberculosis and Antitubercular Agents

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ABSTRACT

Tuberculosis (TB) is the major threat for humans from past several decades. Even after advent of several antitubercular drugs, researchers are still struggling for the mycobacterial infections in humans are TB and leprosy. Chronic infections caused by *Mycobacterium tuberculosis* and *Mycobacterium leprae*. A particular problem with both of these organisms is that they can survive inside macrophages after phagocytosis, unless these cells are activated by cytokines produced by T-lymphocytes, because of this researchers are not yet succeeded in finding effective treatment on TB. In recent years TB has spread globally and became the major issue for world healthcare organizations. Some compounds like benzothiazinones shown promising activity against mycobacterium, few compounds are in pipeline which may exhibit improved pharmacological effect. Decaprenylphosphoryl-D-ribose 2′-epimerase (DprE1) is the vulnerable target for antitubercular drug discovery. DprE1 is a flavoprotein that along with decaprenylphosphoryl-2-keto-ribose reductase catalyses epimerization of decaprenylphosphoryl-D-ribose to decaprenylphosphoryl-D-arabinose through an intermediate formation of decaprenylphosphoryl-2-keto-ribose. This conversion makes DprE1 a potential drug target. Further research requires to tackle the biggest hurdles in Tuberculosis treatment, i.e. multi drug and extensively drug resistance.

1. Introduction

Tuberculosis (TB) is a disease of antiquity which is thought to have advanced at some point between the seventh and sixth centuries BC.[1] Current evaluations reveals that 33% of the total population is tainted bringing about somewhere in the range of 2 million passing for each year. The performance of the primary medications for TB treatment somewhere in the range of 50 years back – streptomycin, para-aminosalicylic acid, isoniazid – prompted good faith that the infection could be controlled if not destroyed.[2] These medicaments, combined and large expanding measures of medicinal services, brought on a fast decay of TB in numerous industrialized nations which delivered an atmosphere of lack of interest to the requirement for crisp medications. As a consequence of this apathy and the recognition by the pharmaceutical business that such operators would be probably not going to produce an appropriate

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rate of profitability, couple of new medications have been presented in the last 30 years.[3] However, since the 1980s the infection has been experiencing a recovery driven by an assortment of changes in social, restorative and financial components. In this manner, a sensational increment in immuno-suppressed people due mostly to Acquired Immune Deficiency Syndrome (AIDS) (additionally to tumor chemotherapy and organ-transplant hones), combined with expanding urbanization and destitution in creating nations, has traded off essential human services structures and prompted vast increments in TB rate. Accompanying with the resurgence of TB has been the event of multidrug-resistant disease which has uncovered the weaknesses of the present medication tool.[4]

In 1865 a French specialist, Jean-Antoine Villemin, demonstrated that TB was infectious, and in 1882 a German researcher named Robert Koch found the bacterium that causes TB. However, a large portion of a century went before medications were found that could treat TB. Until then, numerous individuals with TB were sent to sanatoriums, unique rest homes where they took after a recommended schedule each day. Nobody knows whether hospitals truly inhabited with TB; regardless of the possibility that they did, numerous individuals with TB could not stand to go to a hospital, and they passed on at home.[4,5]

A breakthrough came in 1943. An American researcher, Selman Waksman and one of his assistants, Albert Schatz, found a medication that could kill TB microscopic organisms. Somewhere around 1943 and 1952, two more medications were found. After these disclosures, numerous individuals with TB were dealt with, and the passing rate for TB in the United States dropped significantly. Every year, less and less individuals got TB.[6]

In 2012, one human life was smothered at regular intervals by TB. With these numbers, TB ties with Human Immunodeficiency Virus (HIV) (one life at regular intervals) and diabetes (one life at regular intervals). In spite of declining rates for occurrence and mortality interestingly inside 15 years of information gathering and progressing reconnaissance by the World Health Organization (WHO) during the most recent two years. In 2012, 8.6 million new cases happened and driving nations with the most noteworthy number of occurrence cases were India, China, South Africa, Indonesia, and Pakistan. 1.1 million recently contaminated TB patients were HIV positive. Other than a worldwide predominance of 12 million instances of dynamic TB in 2012, WHO evaluates the quantity of patients contaminated with the TB bacillus yet not yet having built up the dynamic illness at 2 billion.[7] TB is a bacterial disease, which influences the respiratory framework in around 90% of all cases.

2. *Mycobacterium tuberculosis*

Most mycobacteria species are saprophytic soil occupants, however a couple are critical pathogens, including the *Mycobacterium tuberculosis* complex, which can bring about TB in humans (*M. tuberculosis*, *M. africanum*, *M. caprae*, *M. bovis*, *M. canetti*, *M. microti*, *M. pinnipedii*) and *M. leprae* which causes disease. Atypical mycobacteria, which incorporate the *M. avium complex*, *M. kansaii*, *M. fortuitum*, and *M. chelonae*, opportunistic infections in immunologically compromised patients. The primary causative specialist of TB – *Mycobacterium tuberculosis* (Mtb) – was found and segregated by Robert Koch in 1882.[8] It is a pole molded bacillus of 1–4 μm length and 0.3–0.6 μm width. Cell division of Mtb happens each 12–24 h, which speaks to an ease back development rate contrasted with different microorganisms (15–60 min) and hampers antimicrobial treatment since most antibiotics interfere with cell division processes. The disease with the microorganism for the most part happens through bead/droplet infection.[9] Once Mtb has entered the host, the resistant framework will battle the disease by phagocytosis of Mtb into macrophages. By and large, microorganisms are absorbed inside macrophages by take-up into phagosomes, an intracellular compartment with low pH, a few compounds, and receptive oxygen species (ROS).

3. Cell wall of Mycobacterium

The uniqueness of all mycobacteria species is their cell envelope, which is especially rich in lipids and structures efficient and solid resistance shield to various ecological impacts, e.g. antibiotics and disinfectants. The cell divider is made out of two portions. The internal part contains a peptidoglycan (PG) layer, which is connected to the plasma film through the cell divider glycolipid phosphatidylinositol mannosides (PIMs). Covalently connected to the PG is a hydrophobic polysaccharide, the arabinogalactan (AG) with branched arabinose side chains.[10] The external fragment contains extractable lipids, e.g. trehalose monomycolate (TMM), trehalose dimycolate (TDM), sulfolipids, phenolic glycolipids, phthiocerol dimycocerosates, and complex polysaccharides and in addition little measures of proteins. Together with the mycolic corrosive chains, the free lipids frame a lopsided bilayer, which is likewise called the mycobacterial external membrane. The cell envelope is blended with complex free cell divider lipids, to be specific lipomannans (LM) and lipoarabinomannans. The respectability of the mycobacterial cell envelope is imperative for destructiveness and intracellular survival of Mtb.[11,12]

This excellent barrier exacerbates the antibiotic treatment of Tuberculosis because of its low porousness to drugs. Be that as it may, this one of a kind cell divider likewise involves a few one of a kind biosynthetic pathways, which incorporate a few compounds that are particular to mycobacteria and serve as focuses for the antimycobacterial chemotherapy. Therefore, as anyone might expect, numerous antitubercular drugs hinder biosynthetic pathways of cell divider segments.[13]

4. Tuberculosis – global burden

Tuberculosis remains a significant health issue globally, responsible for ill health among a large number of individuals every year. TB positions as the second driving reason for death from an irresistible malady around the world, after the human immunodeficiency infection (HIV). The most recent evaluations incorporated into this report are that there were 9.0 million new TB cases in 2013 and 1.5 million TB passings (1.1 million among HIV-pessimistic individuals and 0.4 million among HIV-constructive individuals). These aggregates are

higher than those incorporated into the 2013 worldwide TB report, essentially as a result of upward updates to evaluations of the quantity of TB cases and passings in Nigeria taking after the conclusion of results from the nation's first-historically speaking national TB commonness study. Given the extent of the populace and the high TB load in Nigeria, these updates have influenced worldwide appraisals. In spite of the fact that most TB cases and passings happen among men, the burden of illness among ladies is comparatively high.[14,15] In 2013, there were an expected 3.3 million cases and 510,000 TB passings among ladies, and additionally an expected 550,000 cases and 80,000 passings among children. TB mortality is unsatisfactorily high given that most passings are preventable if individuals can get to medicinal services for a determination and the right treatment is given. Short-course regimens of first-line tranquilizes that can cure around 90% of cases have been accessible for decades. TB is infectious and airborne. It positions close by HIV/AIDS as a main source of death around the world.[16]

5. Antitubercular agents and current research

Tuberculosis generally influences grown-ups in their most profitable years. Be that as it may, all age gatherings are at hazard. More than 95% of cases and passings are in creating nations. Individuals who are infected with HIV are 20–30 times more prone to create active TB. The danger of active TB is likewise more prominent in people experiencing different conditions that hinder the invulnerable framework. One million kids (0–14 years) fell sick with TB, and 140,000 youngsters passed on from the sickness in 2014. Tobacco utilize significantly expands the danger of TB sickness and demise. More than 20% of TB cases worldwide are owing to smoking.[17,18]

It will never be underscored enough that the full recognition of the treatment is most likely in any event as critical as the level of proficiency of the medications regulated for a probable cure. Much more terrible, the absence of treatment recognition is probably going to end up the primary driver of the event and spread of multi medication safe strains of *M. tuberculosis*. Two regimens rose up out of the numerous trials and include initial a two-month long treatment with four medications; either: streptomycin,[1] isoniazid,[3] rifampicin[7] and pyrazinamide[19] or: isoniazid[20], rifampicin[7], pyrazinamide[20] and ethambutol.[19] This is then trailed by four months of isoniazid[7] and rifampicin.[7] Symptoms, particularly hepatotoxicity, are an issue which now and again constrains an inconvenient treatment end. Patients taking after this regimen get to be non-irresistible after the initial couple of weeks however the rest of the months are crucial to overwhelm a moderate developing part of the bacilli furthermore to permit time for the host safe framework activity to accomplish a clinical cure. A considerable lot of them are analogs or prodrugs of the principle line drugs specified above and others dropped out of utilization.[15]

6. MDR and XDR resistance

Multi-Drug Resistance tuberculosis (MDR-TB) is connected with expanded mortality and longer treatment at much higher cost. The treatment results for Extensively Drug Resistance Tuberculosis (XDR-TB) are much poorer, especially among HIV-contaminated people in asset constrained settings. A few, however not all, strains of MDR-TB may have decreased bacterial wellness.[21–23] The term XDR (additionally referred to as outrageous medication resistance) TB was utilized interestingly as a part of November 2005 and temporarily characterized as TB cases in people harboring Mtb strains safe in vitro to in any event Isoniazid (INH) and Rifampicin (RMP) among first-line tranquilizes,[23] and to no less than three or a greater amount of the six fundamental classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and p-aminosalicyclic corrosive). Resulting reports proposed distinctive definitions for XDR TB.[24–27]

7. Emerging newer antituberculosis drugs

7.1. *Delamanid (OPC-67683)*

Delamanid (OPC67683; Fig. 1) is a nitro-dihydro-imidazooxazole derivative that inhibit mycolic corrosive amalgamation with potent in vitro and in vivo movement against medication safe strains of *Mycobacterium tuberculosis*.[28] Some random placebo studies have proved that delamanid could upgrade treatment alternatives for patients with MDR-TB.[22]

7.2. *Bedaquiline (TMC207)*

Bedaquiline (TMC207; Fig. 2) is a diarylquinoline that objectives the proton pump of adenosine triphosphate (ATP) synthase, prompting lacking union of ATP. Presently, bedaquiline appears to be a promising new anti-TB drug especially for the treatment of MDR-TB.[29]

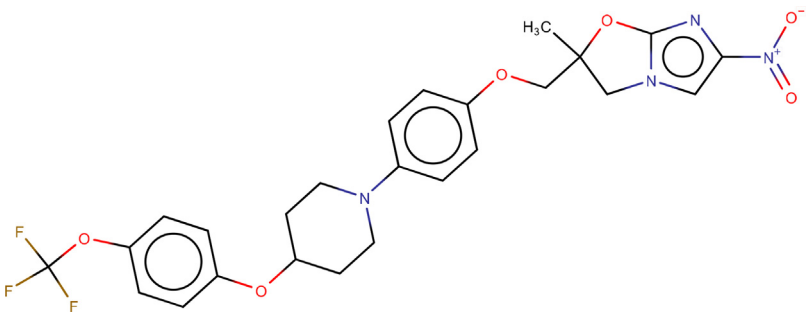

Fig. 1 – Structure of delamanid.

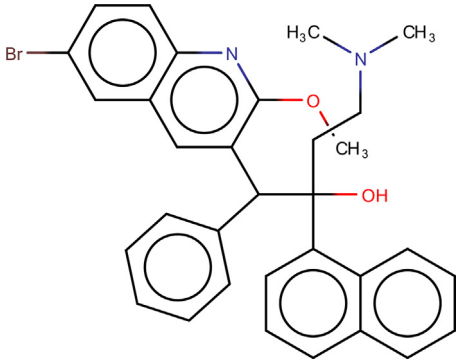

Fig. 2 – Structure of bedaquiline.

7.3. *Pretomanid (PA-824)*

The nitroimidazole-oxazine (PA-824; Fig. 3) is a derivative of metronidazole. It is assumed that PA-824 acts by repressing the combination of ketomycolates that are fundamental segments of the mycobacterial cell; and by giving nitric oxide amid enzymatic nitro decrease inside *Mycobacterium tuberculosis*, consequently harming the respiratory mechanical assembly. A randomized Early Bactericidal Activity (EBA) study in patients with medication delicate, spread positive pneumonic TB patients ($n = 69$) assessing oral PA-824 at 200, 600, 1000 or 1200 mg dosages for each day for 14 days uncovered that all dosage were very much tolerated, and had displayed equal movement.[30,31]

PA-824-moxifloxacin-pyrazinamide is possibly reasonable for treating drug-sensitive MDR-TB.[23]

7.4. *Oxazolidinones*

The oxazolidinones (Fig. 4) demonstration by means of aggressive restraint of the compound that ties the approaching exchange ribonucleic corrosive (RNA) with the complementary codon on the messenger RNA and hindering translation. Cycloserine (4-amino-1,2-oxazolidin-3-one) was the principle oxazolidinone that was utilized as a hostile to TB sedate.

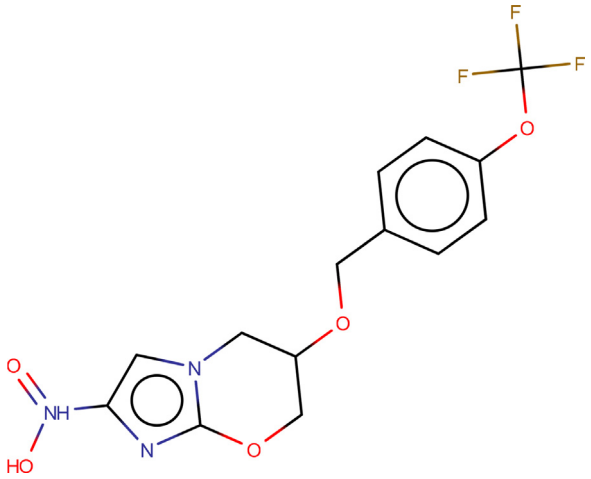

Fig. 3 – Structure of protomanid.

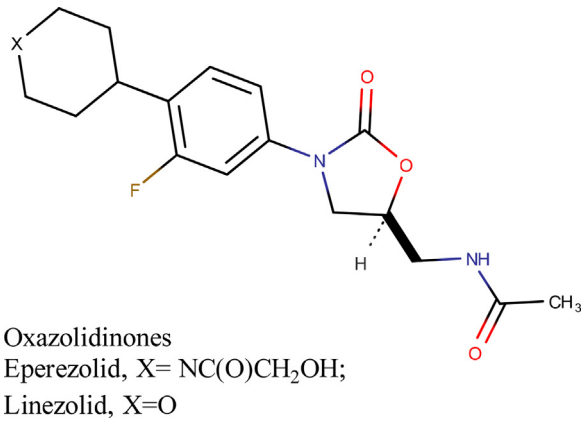

Fig. 4 – Structure of oxazolidinones.

Another oxazolidinedione, linezolid, has been utilized off-label as a part of the treatment of MDR-TB.[32]

7.5. *Substituted ethylenediamines*

SQ109 (Fig. 5) is a 1,2-ethylenediamine ethamubutol simple. It acts by focusing on the cell divider arrangement yet by hindrance of trehalose monophosphate transferase. It displays no cross-resistance with ethambutol. At present, SQ109 is experiencing safety and efficacy studies in people.[33,34]

7.6. *Benzothiazinones and Dinitrobenzamides*

Benzothiazinones and dinitrobenzamides act by focusing on the compounds in charge of the development of arabinans that are crucial parts of the cell. In perspective of their novel instrument of activity, these medications seem promising as hostile to TB drugs. 1,3-Benzothiazin-4-ones (benzothiazinone, BTZ; Fig. 6) are TB tranquilize applicants with high movement against *Mycobacterium tuberculosis* in vitro and in vivo.[35] A negligible inhibitory fixation (Minimum Inhibitory Concentration (MIC)) of 1 ng/mL against M. tuberculosis H37Rv was measured for the most encouraging BTZ, BTZ043; this MIC is an element of 20 beneath that of the cutting edge TB sedate, isoniazid. Hereditary and biochemical investigation uncovered that the objective of BTZs is the compound decaprenylphosphoryl-D-ribose 2′-epimerase (DprE1, Rv3790). DprE1, together with DprE2 (Rv3791), catalyzes the epimerization of decaprenylphosphoryl-D-ribose (DPR) to decaprenylphosphoryl-D-arabinose (DPA), which is an important precursor for the combination of cell-divider arabinans. Due to troubles in confining dynamic DprE1, subtle elements of the

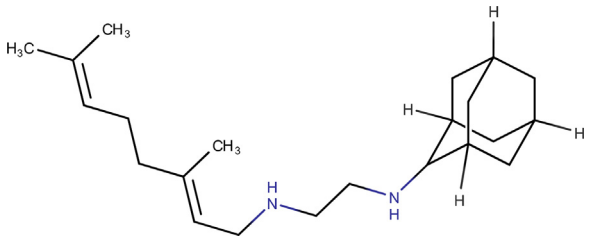

Fig. 5 – Structure of ethylenediamines.

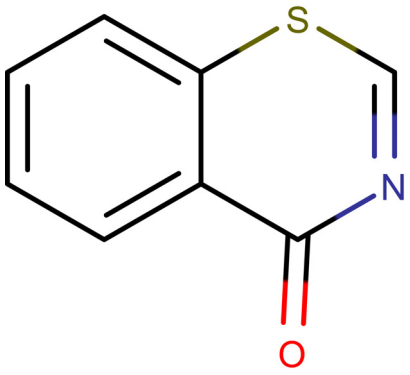

Fig. 6 – Benzothiazinone (BTZ).

component of activity of BTZs are rare. It is realized that a solitary point change in DprE1 (Cys387Ser or Cys387Gly) brings about a more than 250-overlap expanded MIC. Moreover, the nitro gathering of BTZs is crucial for action; its diminishment to either the amine or the hydroxylamine expanded the MIC no less than 500-fold. Following the disclosure of BTZs, another class of aggravates that objective DprE1 was identified: dinitrobenzamide DNB (Fig. 7) and its subsidiaries.[36]

8. Need for search of novel antitubercular agents

It is normal that advancement of the new viable hostile to TB treatment will bring us different results, for example, shortening the aggregate span treatment, change of the treatment culmination proportion, aversion and treatment for TB (MDR-TB) and decreasing the aggregate therapeutic consumption. Other TB sedate necessities to demonstrate the well pharmacokinetic dissemination and pervasion into lung tissue and cells. Besides, it is likewise fancied that the novel competitor shows the powerful bactericidal movement both against exponential and stable period of *M. tuberculosis* in vivo. For around 40 years, it was alarmingly quiet in the field of antitubercular medication improvement.[37]

The last first-line medication was presented in the 1960s, trailed by new blends and adaptions of the treatment regimens. Be that as it may, expanding death rates in the

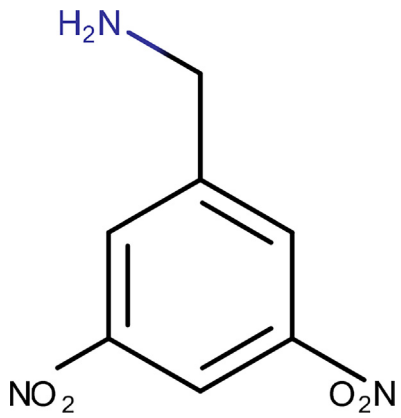

Fig. 7 – Dinitrobenzamide (DNB).

subpopulation of HIV infected patients and the rise of MDR/XDR TB has prompted a revaluating WHO began observation and control programs, organizations started TB medicate improvement projects, and open private associations (PPPs) were started to start the medication advancement pipeline and interface analysts from the scholarly world and industry (e.g. TB Alliance, Stop TB Partnership). Aside from the re-assessment and repurposing of existing antibiotics for the treatment of TB, a little number of new medication substances has since entered the pipeline. In spite of inside and out research and financing endeavors, the TB sedate pipeline still is exasperatingly void. To the gathering of re-purposed drugs have a place the fluoroquinolones moxifloxacin and gatifloxacin. The DNA gyrase inhibitors have been utilized off-mark to treat MDR TB and could supplant INH or EMB in first-line regimens by 2015, which is right now, assessed in stage III clinical trials.[38]

Nitroimidazoles OPC-67683 (delamanid) and PA-824 are prodrugs that require decrease by the deazaflavin subordinate nitroreductase (Ddn) to the relating dynamic des-nitro metabolites. Dad 824, right now in stage II, was appeared to be dynamic against recreating and non-imitating bacilli by means of intracellular NO discharge. Restraint of mycolic corrosive biosynthesis is likewise discussed. Delamanid hinders biosynthesis of methoxy mycolic and keto mycolic acids,[39] however the entire instrument of activity is still under study. Delamanid has as of late entered stage III clinical trials, and an after a negative assessment prior in 2013 a restrictive promoting approval in light of stage II information was suggested by the European Medicines Agency (EMA). Originally created as an ethambutol simple, the ethylenediamine subsidiary SQ109 indicates action against EMB-safe strains and focuses on an as of late found film transporter (MmpL3) and consequently, impairs the right gathering of the mycobacterial cell wall. SQ109 is as of now in stage II clinical trials. Oxazolidinones linezolid, its thiomorpholine simple PNU-100480 (sutezolid), and AZD5847, which restrain protein biosynthesis through official to the 23S rRNA of the 50S ribosome subunit, are in stage II as well. Capuramycines repress translocase-1 (TL-1), another objective in the PG biosynthesis. Further points of interest on current TB pipeline medications are examined in far reaching surveys.[40] Fig. 8 compresses focuses of current antitubercular operators (purple) and additionally those of current pipeline drugs (red).

9. Why DprE1 is potential target for antitubercular drug development

TB is an irresistible disease that spreads through air, for the most part influencing youthful grown-ups in their gainful years. Around 95% TB passings are in the creating universe of which 38% happens in India and China. Cell wall is a practical and defensive interface amongst outside and inward environment for each living cell. Interruption or restraint in its blend keeps the development and increase of the life form. Focusing on the cell wall amalgamation has been a fruitful approach in medication improvement. The causative microorganism *Mycobacterium tuberculosis* (*M. tuberculosis*) has a unique cell wall course of action, with layers of external lipids, mycolic corrosive, polysaccharides (AG), PG, plasma film,

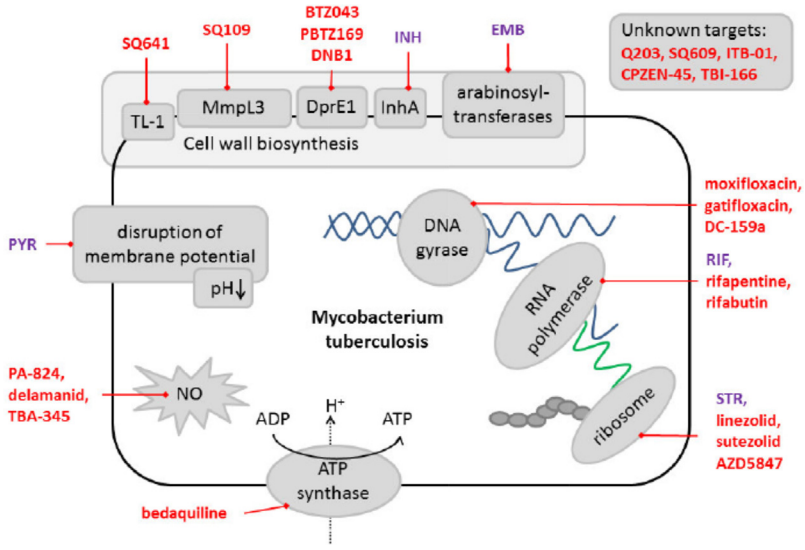

Fig. 8 – Current antitubercular agents.[17]

lipoarabinomannan (LAM), and PIM. The polysaccharides (AGs) are important precursor for bacterial cell wall union, decaprenylphosphoryl-b-D-ribose 20-epimerase (DprE1) is an oxidase required in the biosynthesis of DPA. It goes about as contributor of D-arabinofuranosyl deposits for the combination of arabinogalyctan. DprE1 is a flavoprotein that alongside decaprenylphosphoryl-2-keto-ribose reductase (DprE2) catalyzes epimerization of DPR to DPA through a transitional arrangement of decaprenylphosphoryl-2-keto-ribose (DPX). This NADP subordinate enzymatic response makes DprE1 a key part for cell development and survival, in this manner making it a potential medication target.[38,41]

The hereditary examinations of *M. tuberculosis*, *M. bovis* BCG, and *M. smegmatis* safe mutants uncovered that DprE1 is the objective of BTZs. DprE1 is a DPR oxidase, required in the biosynthesis of DPA, a key segment of the mycobacterial cell wall. DPA is the main known contributor of D-arabinofuranosyl deposits for the union of AG, an essential precursor for the mycobacterial cell wall center.[32,36] DprE1 is a flavoprotein that working together with decaprenylphosphoryl 2-ketoribose reductase (DprE2) catalyzes the epimerization of DPR to DPA, by means of the arrangement of the moderate DPX. Specifically, DprE1 catalyzes the oxidation of DPR to DPX, which is diminished to DPA by the NADH-subordinate DprE2 protein. In this specific circumstance, DprE1 was additionally appeared to be vital for cell development and survival. Two BTZ derivatives, depicted by the nitro gather supplanted by either an amino or a hydroxylamine gathering, are a great deal less dynamic.[42,43]

10. Future of antitubercular agents

The extensive length and complex nature of current TB treatment and the subsequent rise of MDR-TB and XDR-TB, and the inconsistency of key hostile to tuberculous medications with antiretroviral treatment (ART), all bolster the need to create novel, better medications and regimens for the treatment of TB.

TB is significantly less complex than the underlying TB treatment regimens, having been abbreviated from two years to six months; nonetheless they are still a long way from ideal. The advancement of an a few month regimen with once week by week dosing of three to four medications would bring about diminishing the term of treatment from the at present prescribed 28 weeks to eight to twelve weeks, and from roughly 130 dosages of a blend regimen to 10. Such a change must have a huge positive effect on control of the disease by enhancing quiet adherence, and on restraining improvement of medication resistance by enhancing treatment finish rates.[42] Be that as it may, achieving this goal of a two months regimen will probably require a significantly new helpful way. As already expressed, one of the key difficulties in the field of TB medication advancement is that the remedial unit is a mix regimen, not a solitary medication. It has been assessed that the clinical testing of a TB mix regimen containing a solitary new compound requires at least six years.[40]

11. Research priorities

Additionally, compressing the accessible proof, the surveys attempted for this overhaul uncovered various holes in current information about basic zones of the treatment for RR-/MDR-TB. Where proof was accessible it was normally allotted a low quality rating. This was one of the principle reasons why every one of the suggestions made in this rules amendment are restrictive.[44]

The WHO guidelines development group talked about the exploration needs and highlighted various needs. They recognized some issue territories which had as of now been singled out by before endeavors to characterize scrutinize needs for MDR-TB treatment, for example, preventive treatment for MDR-TB and enhancing proof on diminishment of regimen duration.[28]

Conflicts of interest

The authors have none to declare.

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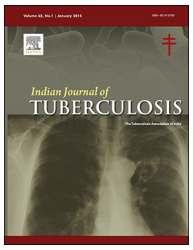

Original Article

Role of laparoscopy in diagnosing genital tuberculosis in suspected women: A cross-sectional study from a tertiary care hospital in Northern India

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ABSTRACT

This study was included 60 women with suspected genital tuberculosis, attending outpatient department of a tertiary care hospital. The aim was to evaluate the role and accuracy of laparoscopy in the diagnosis of genital tuberculosis. The patients were investigated for tuberculosis with Erythrocyte Sedimentation Rate, Montoux, chest X-ray, serum ELISA, CA125, ultrasonography, endometrial biopsy and laparoscopic biopsy. Culture or histopathology was taken as a gold standard for confirming the cases of genital tuberculosis. 30 patients were confirmed as positive. Comparison was made between the various diagnostic modalities. Baseline investigations like complete blood count, differential leukocyte count, ESR, Montoux, and some special tests like CA125 and serum ELISA were helpful in supporting the diagnosis in only some patients. The sensitivity, specificity, positive and negative predictive value of endometrial biopsy in diagnosing GT was 6.6%, 100%, 100% and 51.7% respectively. Laparoscopic gross visualization alone, staining, culture and histology were able to detect 86.6%, 33.3%, 50% and 63.3% of cases respectively. Many patients would have been missed if laparoscopy was not performed. It helps in macroscopic visualization of pelvic cavity and obtaining biopsies for ZN staining, culture and histopathology. This increases the pickup rate of positive cases and helps in confirmation of the diagnosis.

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1. Introduction

Tuberculosis is a major global health problem; particularly in many of the developing countries including India.[1] It is an infectious disease, afflicting 14 million people in India, mostly in the reproductive age group (15–45 years). Genital tuberculosis (GT) is a well recognized entity in the etiology of infertility in developing countries like India. It has been reported to range as high as 10–19% amongst Indian women to less than 1% in developed countries.[2] Female genital tuberculosis is often a silent disease sparing no age group but majority of patients are in the reproductive age. The actual incidence of genital tuberculosis may be under reported due to asymtomatic presentation and paucity of investigations.

GT is a chronic disease and often has low grade symtomatology with very few specific complaints. It is estimated 11% of patients lack symptoms.[3] The fallopian tubes are the initial site of involvement being affected in 100% cases of genital tuberculosis, followed by endometrium (79%), cervix (24%), ovaries (11%), vulva and vagina (0.07%). Most cases of confirmed genital tuberculosis will have a perfectly normal clinical examination, while a quarter of cases will present with an adnexal mass.[4]

Diagnosis of GT in early stage is very difficult. Early diagnosis may be associated with a favorable result before extensive genital damage occurs. Common presenting symptoms are nonspecific, hence diagnosis is difficult and elusive as affected patients have normal serological tests like haemogram including TLC and DLC, ESR, ELISA, Mantoux, normal chest x-ray but elevated CA125. Imaging by abdominal and pelvic ultrasonography or abdominal and pelvic CT scan is often very nonspecific. Findings on imaging have been suggested to be helpful but final diagnosis is revealed by culture and histology.

Diagnostic laparoscopy may aid in early diagnosis and safe management of genital tuberculosis, preventing unnecessary laparotomies. The accuracy of this modality in diagnosing genital tuberculosis is not proven though. This study was planned, therefore, to evaluate the accuracy of laparoscopy in the diagnosis of genital tuberculosis.

2. Methodology

This study was conducted in the department of gynecology in collaboration with the department of microbiology and pathology at a tertiary care hospital in Northern India. It was conducted from September 2010 to February 2012 after the approval of the Institutional Review Board. Ethical approval was obtained from institutional ethical committee.

This is a cross sectional study including 30 patients in the age group of 16–40 years who underwent diagnostic laparoscopy. All suspected cases of genital tuberculosis presenting with abdominal lump, adnexal mass, chronic pelvic pain, menstrual disorder or infertility were included in the study. Known cases of malignancy, pregnancy and endometriosis were excluded from the study. Informed consent was taken followed by detailed history, general physical and gynecological examination. Baseline investigations like complete blood count, differential blood count, Erythrocyte Sedimentation Rate, Mantoux, serum ELISA (IgG and IgM), HIV testing, ultrasonography and chest x-ray were done for all patients. Special investigation like CA-125 was also done in all the patients. Endometrial biopsy was done in 27 patients and the tissue obtained was sent for Zeil Neelson (ZN) smear staining, Lowenstien-Jensen culture and BACTEC culture and histopathological examination. In three patients, endometrial biopsy was not taken as patients were unmarried. Menstrual blood was collected and sent for ZN smear staining, LJ and BACTEC culture and histopathological examination in these patients.

All patients underwent diagnostic laparoscopy under general anesthesia using three port; a central 10 mm intraumbilical and two 5 mm lateral ports. A 0° Karl Storz telescope was used for visualization. Pneumoperitoneum was created by insufflations with carbon dioxide. The findings i.e. presence of tubercles, adhesions, tubo-ovarian mass and presence of free fluid were noted. Pouch of Douglas was inspected for presence of fluid which was aspirated if present, otherwise peritoneal washings were taken. Biopsies were taken from suspicious areas of omentum, peritoneum and fallopian tubes using biopsy forceps. The tissue sample was divided in two parts and one was kept in 5 ml distilled water or normal saline and another in the formalin. The fluid aspirated and the tissue samples kept in normal saline were sent for ZN staining, conventional and radiometric culture method (BACTEC 460 TB System). For histopathological study, the tissue biopsy sample from the lesions was fixed in 10% formalin. Polymerase chain reaction (PCR) of the tissue obtained by laproscopic biopsy was done in selected patients who could afford it from private laboratory as the facility of this investigation is not available in our hospital. The average procedure time was 20–30 min. Patients were discharged on the next day after operation.

Diagnosis of tuberculosis was based on the following criteria:

- Presence of acid fast bacilli (Ziehl-Nielson staining).
- Presence of tubercular bacilli on culture (Lowenstien-Jensen culture or BACTEC).
- Presence of a proliferative granulomatous lesion with central caseation necrosis surrounded by concentric layers of epithelial and giant cells with peripheral lymphocytes, monocytes and fibroblasts.

Culture (Lowentein Jensen or BACTEC) was taken as the gold standard in this study and it was used to compare with other diagnostic parameters. Patients who were diagnosed to have tuberculosis on the basis of above-mentioned criteria were started on category 1 antitubercular therapy.

Primary outcome measures were diagnostic yield of histopathology and acid fast bacilli staining (AFB) of laparoscopic biopsy specimen. Secondary outcome measures are diagnostic yield of other investigative modalities like Montoux, ELISA, ESR, ultrasound findings, gross laparoscopy findings and endometrial biopsy in diagnosing genital tuberculosis.

The data was analyzed with SPSS-Pe version. The data was expressed as percentage, mean with standard deviation (SD).

Sensitivity, specificity, positive predictive value and negative predictive values were calculated by Wolfram Alfa computational knowledge engine. The correlation between two categorical variables was determined using Chi-square tests. Agreement between two methods was observed by Kappa statistics. p value < 0.05 was considered statistically significant.

3. Results

The results of various diagnostic modalities like serology, laparoscopy, bacteriology and histopathology were compared. Laparoscopy was used as the final diagnostic modality for macroscopic visualization, and the final confirmatory diagnosis however was made on the basis of positive bacteriological result by culture of laparoscopic specimen. In the present study 15 patients were found to be culture positive and hence confirmed to have genital tuberculosis. All diagnosed cases were started on anti-tubercular therapy.

Table 1 depicts the demographic details and symptomatology. The study included patients of the age group 16–40 years with the mean age being 30 ± 5.3years. The majority of the positive cases of genital tuberculosis were in the age group of 31–35 years and ≤25 years both being 33.3%. The study showed that prevalence of genital tuberculosis was more in nulliparous (53.3%) compared to multiparous (46.5%) women. Majority of the patients of genital tuberculosis presented with primary infertility (33.3%), chronic pelvic pain (26.6%) and abdominal lump (26.6%). Constitutional symptoms in form of fever, weight loss, malaise, anorexia and night sweats were present in 4 patients and all 4 were diagnosed to be positive. Five out of nine patients of primary infertility were diagnosed to be positive for genital tuberculosis. Almost 73.3% of patients of genital tuberculosis had normal menstrual cycles. Amongst the positive cases, one patient gave history of secondary amenorrhea, two patients had metrorrhagia and one had dysmenorrhea.

Table 1 – Demographic and symptomatology profile of the study group.

Parameters	No. of patients (%)	No. of confirmed Positive cases [a](%)
Age (years)		
≤25	8/30 (26.6)	5/15 (33.3)
26–30	7/30 (23.3)	4/15 (26.6)
31–35	10/30 (33.3)	5/15 (33.3)
36–40	5/30 (16.6)	1/15 (6.6)
Marital status		
Married	27/30 (90)	12/15 (80)
Unmarried	3/30 (10)	3/15 (20)
Parity		
0	12/30 (40)	8/15 (53.3)
1	4/30 (13.3)	2/15 (13.3)
2–4	13/30 (43.3)	4/15 (26.6)
≥5	1/30 (3.3)	1/15 (6.6)
Constitutional symptoms	4/30 (13.3)	4/15 (26.6)
Infertility		
Primary	9/30 (30)	5/15 (33.3)
Secondary	1/30 (3.3)	1/15 (6.6)
Menstrual disorders		
Normal	26/30 (86.6)	11/15 (73.3)
Amenorrhea		
Primary	0	0
Secondary	1/30 (3.3)	1/15 (6.6)
Metrorrhagia	2/30 (6.6)	1/15 (6.6)
Dysmenorrhea	1/30 (3.3)	1/15 (6.6)
Chronic pelvic pain	14/30 (46.6)	4/15 (26.6)
Abdominal lump	4/30 (13.3)	4/15 (26.6)

[a] Includes all cases confirmed positive by culture (Lowenstein-Jensen and BACTEC 460 culture media used).
[b]Some patients had more than one symptom.

Table 2 depicts baseline tests, which were not, particularly found helpful in confirming the diagnosis of genital tuberculosis as only 8 out of 15 confirmed cases had leucocytosis and 6 had lymphocytosis. However ESR was raised and Mantoux were positive in all 15 confirmed positive cases, but are very non-specific tests. CA125 was raised in the range of 66–104 in 5 positive cases of GT. None of the patients in our study were found to be HIV positive. Imaging studies in form of radiography and ultrasonography helped in supporting the diagnosis of genital tuberculosis but confirmation needed further tests. Ultrasonography showed tubo-ovarian mass in 12 out of 15, enlarged mesenteric lymph nodes in three and ascites in one patient. All of these patients were confirmed to have genital tuberculosis. Ultrasound, especially trans-vaginal sonography can play important role in diagnosing genital tuberculosis. The findings in genital tuberculosis patients were ascites, mesenteric lymph node and tubo-ovarian masses of varying degrees. Five out of nine patients of primary infertility were diagnosed to be positive for genital tuberculosis. Out of five, two patients had grossly normal laparoscopic findings but the peritoneal fluid culture was positive and tissue histopathology showed granulomatous changes. Laparoscopic findings suggestive of genital tuberculosis were seen in 22 patients. In two patients with frozen pelvis there were dense adhesions and pelvic structures could not be visualized and there was straw colored free fluid in the pelvic cavity. Out of 22 patients 13 were confirmed to have genital tuberculosis by culture, either with BACTEC or LJ media. By laparoscopic visualization we can only make a provisional diagnosis however a definitive diagnosis of GT is possible only by the recovery of mycobacterium from tissue or fluid. The study showed that out of 22 patients with laparoscopic findings suggestive of genital tuberculosis, 19 were found to have granuloma in histology (63.3%) and 13 were culture positive (43.3%) of the patients and 10 were positive on staining (33.3%).

Culture of either tissue specimen or the fluid or peritoneal washings has helped in making the conclusive diagnosis more as compared to ZN staining as seen in Table 3. Laparoscopic findings and endometrial biopsy (taking presence of epitheloid granuloma in histology as positive case for genital tuberculosis) were not significantly correlated with culture findings as shown in Table 4. In none of the positive cases, endometrial biopsy showed histopathological findings of tuberculosis.

When the diagnostic yield of various diagnostic modalities were compared, as depicted in Table 5, Montoux and histopathology were found to be most sensitive (100%). Maximum specificity was for serum ELISA, ultrasonography and staining (100%). Maximum PPV was of serum ELISA, ultrasonography and staining (100%). Maximum NPV is of Mantoux and histopathology (100%).

Table 2 – Investigations in study population and confirmed positive cases.

Parameters	Total positive result (%)	Positive result among confirmed cases (%)
Mantoux	18/30 (60)	15/15 (100)
Leucocytosis	8/30 (26.6)	8/15 (53.3)
Lymphocytosis	6/30 (20)	6/15 (40)
ESR	17/30 (56.6)	15/15 (100)
Serum ELISA	10/30 (33.3)	10/15 (66.6)
CA125	5/30 (16.6)	5/15 (33.3)
HIV	0	0
Chest radiograph		
Normal	29/30 (96.6)	14/15 (93.3)
Abnormal	1/30 (3.3)	1/15 (6.6)
Ultrasound		
Ascites	1/30 (3.3)	1/15 (6.6)
Mesentric lymph nodes	3/30 (10)	3/15(20)
Tubo-ovarian mass	12/30 (40)	12/15 (80)
Laparoscopic findings		
Normal	8/30 (26.6)	2/15 (13.3)
Abnormal	22/30 (73.3)	15/22 (68.1)
Adhesions	12/30 (40)	12/15 (80)
Tubercles	7/30 (23.3)	7/15 (46.6)
Free fluid	12/30 (40)	12/15 (80)
Abnormalities in tubes	17/30 (70)	15/15 (100)
•Dilatation	5/30 (16.6)	5/15 (33.3)
•Kinking	3/30 (10)	3/15 (20)
•Pyosalpinx	1/30 (3.3)	1/15 (6.6)
•Hydrosalpinx	4/30 (13.3)	4/15 (26.6)
•Beaded tubes	6/30 (20)	6/15 (40)
Frozen pelvis	2/30 (6.6)	2/15 (13.3)
Laparoscopic guided biopsies		
Omental	2/30 (6.66)	0
Peritoneal	7/30 (3.17)	5/7 (71)
Tubal	21/30 (70)	14/21 (66.6)

Mantoux test with a range 11–40 mm; Leucocytosis ≥11,000; Lymphocytosis ≥40; ESR ≥20; CA125 ≥35 were considered positive.

4. Discussion

GT has become a challenging disease both from diagnostic and therapeutic point of view as it has few characteristic symptoms. The diagnosis of genital tuberculosis is seldom suggested from the history or physical examinations. The diagnostic dilemma arises because of the varied clinical presentation of the disease confounded by diverse results on imaging, laparoscopy, histopathology and a mixed bag of bacteriological and serological tests, each of which has its limitation in diagnostic sensitivity and specificity. If patients are adequately treated before their tubes are irreversibly damaged, the chances of successful pregnancy are reasonably good.[5]

Table 3 – Yield of various diagnostic modalities.

Parameters	No. of patients	Percentage (%)
Tissue biopsy by laparoscopy	30/30	100
Positive on ZN staining	8/30	26.6
Positive on culture	15/30	50.0
Positive on histology[a]	19/30	63.3
Peritoneal fluid	30/30	100
Positive on ZN staining	10/30	33.3
Positive on culture	13/30	43.3
Endometrial biopsy	27/30	90
Positive on ZN staining	1/27	3.7
Positive on culture	1/27	3.7
Positive on histology	0	0
Menstrual fluid	3/30	10
Positive on ZN staining	0	0
Positive on culture	3/3	100

[a] Presence of epitheloid granuloma is taken as positive and other causes of granuloma has been excluded in these patients.

In current study, 55.6% patients of infertility were diagnosed to have GT. Another Indian study revealed 3% incidence of GT among infertile women and the incidence was as high as 41% in women with tubal factor infertility.[6] Another study reported incidence of infertility in GT in Cuttack, India as 58%.[7]

As reported in this study, leucocytosis was present in 53.3%, lymphocytosis in 40%, serum ELISA was positive in 66.6% of the cases and ESR was raised in all positive cases. But these tests are non-specific. In a study comprising of 27 infertile women with a positive PPD skin test, only 11 had clear laparoscopic findings suggestive of genital tuberculosis.[8] Serological methods have been tried, but no single antigen has been found to be uniformly specific. However, the advantages of simplicity, cost effectiveness and scope of speedy automation make serology an attractive adjunct in diagnosis. Serological diagnosis should be done with caution in endemic countries. The serological tests may not be able to differentiate between infection and disease.

Table 4 – Comparison of laparoscopy findings, endometrial biopsy and serum ELISA with culture results.

	Culture			*p*-Value
	Total	Positive	Negative	
Gross laparoscopy findings				
Normal	8	2	6	
Abnormal	22	13	9	0.063
Total	30	15	15	
Serum ELISA				
Positive	10	0	10	
Negative	5	10	5	0.0022
Total	15	15	30	
Endometrial biopsy histopathology[a] (*n* = 27)				
Positive	0	0	0	
Negative	27	27	27	0.17
Total	27	27	27	

[a] Menstrual blood histology was not done as tissue present in the sample were inadequate for reporting.

Table 5 – Comparison of diagnostic yield of various tests.

Tests	Senstivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Mantoux	100	80	83.3	100
Serum ELISA	66.6	100	100	75
Ultrasonography	73.3	100	100	78.9
Gross laparoscopy findings	86.6	42.8	61.9	75
AFB staining of tissue from laparoscopic biopsy	66.6	100	100	68.7
Histopathology from laparoscopic biopsy	100	73.3	78.9	100

Ultrasonographic examination of the abdomen, pelvis and computerized tomography or magnetic resonance imaging may aid in diagnosis by identifying suspected cases but the final diagnosis can be made by histology or bacterial cultures. Endometrial biopsy is a part of infertility work-up. In our study, out of 27 endometrial biopsies performed only one patient was found to be culture and ZN stain positive; however, histology was normal. In this study, the sensitivity, specificity, PPV and NPV of endometrial biopsy in diagnosing genital tuberculosis is 6.6%, 100%, 100% and 51.7%, respectively. Menstrual blood was collected in 3 patients and staining and culture was positive in all of them; however, tissue was inadequate for histological examination. Paucibacillary nature of genital tuberculosis and unguided method of sampling may be the reason for low sensitivity of endometrial biopsy.

Laparoscopy has the dual advantage of pelvic organ visualization and sample collection from inaccessible sites for laboratory diagnosis. Biopsy can be taken with direct vision, often adding to the diagnostic accuracy of the procedure.[9,10] In our study, sensitivity, specificity, positive and negative predictive value of laparoscopy in diagnosis of GT was 86.6%, 42.8%, 61.9% and 75% respectively. The risk of complications due to laparoscopy has been known to be low, ranging from 2.6 to 6.5%.[11] However in our study none of the patients had any complications either during or after the procedure. Yang et al. conducted a large study comprising 1120 patients with infertility to conclude that laparoscopic examination is a very valuable tool for etiological diagnosis of tubal factor infertility.[12] In our setting, laparoscopy with directed biopsy proved to be an excellent tool for diagnosing GT.

The gross appearance of peritoneal tuberculosis may resemble that of a disseminated ovarian carcinoma. A frozen-section analysis should always be considered during the laparoscopy. If no carcinoma is detected, and histopathological examination is consistent with diagnosis of tuberculosis, unnecessary extensive surgery is avoided. Volpi et al. emphasized the importance of laparascopy for the differential diagnosis of tuberculosis in a gynecologic cancer center.[13] In another series of 135 patients with tuberculous peritonitis, 97% of cases were diagnosed on the basis of biopsy specimens taken during laparoscopy.[14]

Conventional bacteriology for isolation and identification of Mycobacteria has its specific advantages of being a conclusive diagnostic test. AFB positivity in smears depends on the bacillary load of the specimen and the type of the material. The sensitivity, specificity, PPV and NPV of ZN staining in this study was 66.6%, 100%, 100, 68.7% respectively. Different studies have reported a wide range of AFB positivity ranging from as low as 0% to as high as 75%.[15,16] Culturing of bacilli has a specificity approaching 100% and is considered as the gold standard.[17] Although microscopy is rapid, cheap and highly specific, its sensitivity has been shown to be as low for extrapulmonary TB. Biswas et al. detected 4.8% of suspected genital tuberculosis cases by culture and none by ZN stain.[18]

Nowadays, a series of automatic liquid culture systems are available contributing to faster diagnosis. Liquid mycobacterial cultures are useful, usually leading to isolation within days. The broth based BACTEC 460 system, introduced in 1980, has considerably improved the detection time of mycobacteria and it was a milestone in the advancement in mycobacteriology.

Liquid culture systems, such as BACTEC 460TB improves the diagnostic yield in abdominal TB by 64% over the traditional LJ medium.[19] Although studies of BACTEC in female genital tuberculosis are scarce, it is reported that it has double the sensitivity than that of culture on LJ medium. Malik et al. reported the sensitivity of BACTEC culture to be about 17.6% in the diagnosis of female genital tuberculosis.[20] However, there are concerns over use of BACTEC due to radioactive isotopes used, leading to health hazards. In the current study, AFB culture was positive in 50% by either BACTEC or conventional culture.

There are studies reporting that besides bacteriology, histopathology is a complimentary diagnostic tool for detection of TB granuloma in tissues.[15] Histopathology is often not satisfactory as inadequate specimens contribute to false negative results. Other reasons for false negative results include non-representative tissue samples, technical failure in processing biopsy, period of specimen collection in relation to disease stage and effect of HIV coinfection.[21] According to Indian literature the incidence of endometrial tuberculosis, as assessed by histopathology, varies from 1.53 to 6.4%.[22] Histopathology in current study was able to detect 63.3% of the cases of GT. Nogales-Ortiz et al. found abundant lymphoid follicles without granuloma in the endometrium in patients who have had both fallopian tubes involved.[23] Demonstration of granulomas in the histopathological tissue sample obtained can help in making the diagnosis of GT. Non-tuberculous causes of granulomas (sarcoidosis, brucellosis, and foreign body reaction) can usually be ruled out easily.

In this study laparoscopy by gross visualization alone was able to detect 86.6% of the cases of GT, 33.3% by ZN staining, 50% by culture (LJ + BACTEC) and 63.3% by histology. The gold standard for diagnosis of female GT is the isolation of M. tuberculosis from appropriate specimens, usually biopsy taken from offending pelvic genital organs laparoscopically and materials from the endometrium.[24] Laparoscopy can reveal presence of miliary granulomas, whitish yellow or opaque plaques surrounded by hyperemic areas over the fallopian tubes and uterus in acute stages. In chronic stages, the tubes show nodular salpingitis, patchy salpingitis, hydrosalpinx, pyosalpinx, or adhesions, inflammatory adenexal masses as was observed in the present study. When visual and histologic diagnoses are combined, laparoscopy also showed high sensitivity and specificity in diagnosing GT. In our study, histopathology of the tissue obtained by laparoscopy has showed granulomatous changes in 15 out of 15 culture positive patients. However in 4 culture negative patients, histopathology showed granulomatous changes.

Therefore it can be said that laparoscopic visualization and its assisted sampling for histological and bacteriological confirmation are essential. In this aspect histology and bacteriology are complimentary to each other. However, amongst the 15 confirmed cases of GT only one was picked up by endometrial biopsy. So if laparoscopic biopsy and fluid aspiration would not have been done we would have missed 14 cases of GT.

Prolonged hospital stay, along with the extensive and inconclusive investigations, adds to the costs of the management of patients with GT. Hence, in areas where advanced tests are not available, we advocate early referral for diagnostic laparoscopy. This approach can shorten the hospital stay, avoid unnecessary investigations, allow timely initiation of anti-TB therapy, reduce morbidities and reserve fertility.

5. Conclusion

Laparoscopy aids in speedy diagnosis and therefore, early implementation of treatment and thereby preventing massive and sometimes irreparable damage to vital reproductive structures. Diagnostic laparoscopy is recommended in all clinically suspicious cases of GT.

Conflicts of interest

The authors have none to declare.

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Original Article

Role of serum adenosine deaminase in pulmonary tuberculosis

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ABSTRACT

Background: Definitive laboratory diagnosis and confirmation of tuberculosis remains a major challenge because of lack of specificity and sensitivity of diagnostic methods especially in sputum smear negative tuberculosis. Many studies have proved the role of ADA in diagnosis of tuberculosis in effusion fluids and a decrease in ADA activity after treatment. This study was aimed to investigate the role of serum ADA level as an early diagnostic and prognostic marker for pulmonary tuberculosis (PTB).

Material and methods: This was a cohort study done on patients visiting the OPD Clinics of the department of Pulmonary Medicine at GMCH, Chandigarh. 50 sputum positive and 50 sputum negative tuberculosis patients and 100 controls were recruited. Serum ADA levels were measured at the start of treatment and again after two months of treatment. Its correlation with severity of disease was seen.

Results: Mean serum ADA (IU/L) was found to be 35.293 ± 30.941 in PTB patients and 11.819 ± 8.023 in control groups and the difference was found to be highly significant ($P < 0.00$). Mean ADA was 31.107 ± 29.32 in sputum positive patients, 39.478 ± 32.22 in sputum negative and 11.819 ± 8.0235 in control groups. No statistically significant difference was observed amongst sputum positive and sputum negative patients. The levels decreased significantly after intensive phase of treatment. At the cut off values of 14.6 IU/L, serum ADA had 78% sensitivity and 76% specificity (AUC = 0.801, P value < 0.00) to differentiate between PTB from healthy controls.

Conclusion: Serum ADA levels may be used as a biomarker for diagnosis of PTB and to evaluate the response to treatment at follow up.

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1. Introduction

Tuberculosis is one of the leading causes of morbidity and mortality, amongst infectious diseases.[1] India is a high burden country for Tuberculosis.[2] Caused by *Mycobacterium tuberculosis*, it can present as pulmonary tuberculosis (PTB) or extrapulmonary tuberculosis (EPTB). Sputum smear microscopy is routinely used for diagnosis of PTB. Despite best of the efforts, a clinician may have to face difficulties in smear negative patients, and sometimes, it becomes almost impossible to diagnose this entity. The presence of co-morbidities like diabetes mellitus, HIV and other immune compromised conditions further complicate the picture as they lead to atypical clinical and radiological presentations.[3,4] This delay in diagnosis and subsequent treatment leads to increased disease transmission and chances of drug resistance.[5] Hence, in the recent years, there has been a great demand for finding a rapid diagnostic method for the same.

Adenosine deaminase (ADA) is one such biomarker which is now a days being studied as a diagnostic tool in tuberculosis.[6–8] Literature is available on its role in effusion fluids.[9] However, limited literature is available regarding the use of serum ADA in active disease, and whether the levels fall with the recovery of the patients from infection.[6–8,10–12]

This study was planned to investigate the diagnostic value of serum ADA (as an alternative method) for diagnosis of active PTB, and find its diagnostic ability to differentiate sputum positive and sputum negative PTB patients. The levels of serum ADA in smear positive PTB patients were correlated with grading of sputum positivity. The effects of treatment on levels of serum ADA at the end of intensive phase (IP) were also studied to find its role as a prognostic marker.

2. Material and methods

The study was conducted in the Department of Pulmonary Medicine, in collaboration with the Department of Biochemistry, Government Medical College and Hospital, Sector 32, Chandigarh after taking approval of the Institute's Ethical Committee. This was a cohort study done on patients visiting the out patient clinics of the Department of Pulmonary Medicine. Taking the anticipated prevalence of 230/lac population/year of tuberculosis in India, 100 patients in the age group of 18–60 years were recruited. 100 subjects were taken as a control group for comparison.

The patients and controls were grouped as follows:

Group A: 50 age and gender matched sputum smear positive PTB patients.
Group B: 50 age and gender matched newly diagnosed sputum smear negative PTB patients diagnosed on radiological grounds or sputum culture being positive for M. Tuberculosis.
Group C: 100 age and gender matched apparently healthy individuals, selected out of the persons accompanying patients attending the Department of Pulmonary Medicine or volunteers from staff, doctors, etc.

EPTB, old treated cases of PTB, patients < 18 years of age and patients with other respiratory diseases were excluded.

All subjects undergoing the study were given necessary information and informed consent was taken on a standard proforma. Detailed history was taken from each subject and a thorough clinical examination was done. Investigations as per the requirement, varying from case to case, were carried out for the diagnosis of tuberculosis. In addition blood sample was collected from a vein in the ante-cubital fossa after the surface was sterilized with spirit. 5 ml of blood was collected in a disposable syringe under strict aseptic conditions. The blood thus collected was centrifuged immediately and serum separated. ADA levels were measured (Diazyme ADA assay kit; by enzymatic photometric method) on Hitachi 902, Random Access Chemistry Analyser on the same day, or else the serum samples were stored at $-20\ ^{\circ}C$. Serum ADA levels were measured at baseline in all the 3 groups, and repeated in patients of PTB (sputum positive and sputum negative) at the end of IP of treatment.

Levels of serum ADA in sputum positive PTB patients were correlated with severity of disease. Severity of disease was determined on the basis of acid fast bacilli (AFB) grading on sputum microscopy and was categorized as scanty, 1+, 2+ or 3+.

The patients were also evaluated for any underlying co-morbidities like hypertension (HTN), diabetes mellitus (DM) and human immunodeficiency virus infection (HIV).

3. Statistical analysis

All collected data was entered in the Microsoft excel spread sheet. Data was expressed as mean $\pm$ SD or median and inter-quartile range as appropriate for the parametric data and means were compared using paired *T*-test. *P* value of ≤ 0.05 was taken as statistically significant. All analysis was done in SPSS trial version 17 (statistical package for Social Sciences). Kolmogorov–Smirnov tests of normality were used to know normality of quantitative data. For skewed data, Mann–Whitney *U* test and Kruskal–Wallis test were used to assess differences between nonparametric data and Post Hoc test was done for pair wise comparison. Wilcoxon Signed Rank test was used to compare data at different points of time. Correlation analysis was done using Spearman's Rank test for skewed data and Pearson Chi-square test for normally distributed data.

4. Results

The 3 groups were matched for age and gender. The mean age of the patients was found to be 37.20 ± 15.29, 36.08 and 32.48 ± 9.381 years in the groups A, B and C respectively.

There were 33 males and 17 females in the sputum positive group (Group A) whereas sputum negative (Group B) had 30 males and 20 females. Amongst control group (Group C), there were 59 males and 41 females.

The co-morbidities in Group A and Group B are shown in Table 1.

Table 1 – Comorbidities in Group A and Group B.

	Group A	Group B
Diabetes mellitus	7	8
Hypertension	9	11
HIV	2	1

Mean serum ADA was found to be 35.293 ± 30.941 IU/L in PTB patients and 11.819 ± 8.023 IU/L in control groups. The difference in the mean values was found to be highly significant statistically ($P < 0.00$).

Mean serum ADA was found to be 31.107 ± 29.32 IU/L in sputum positive PTB, 39.478 ± 32.22 IU/L in sputum negative PTB and 11.819 ± 8.0235 IU/L in control groups (Fig. 1).

The difference in the mean values of serum ADA in sputum positive PTB and controls was found to be highly significant ($P < 0.00$). The difference in the mean values of serum ADA in sputum negative PTB and controls was also found to be highly significant ($P < 0.00$). However, no statistically significant difference was observed when mean serum ADA values of sputum positive and sputum negative patients were compared.

Serum ADA levels were measured at the start of treatment and again after completion of IP in PTB patients. The fall in the levels of serum ADA was found to be statistically significant in both smear positive and smear negative patients at the end of follow up at 2 months ($P < 0.00$ and $P < 0.008$ respectively) (Fig. 2).

The mean serum ADA levels were compared in cases showing different bacterial load shown in terms of sputum positivity. The results are shown in Table 2. The mean values of serum ADA did not show any statistically significant differences in relation to the bacterial load ($P = 0.722$).

Sensitivity and specificity was calculated by the help of ROC curves. Area under curve was found to be 0.801. ROC analysis showed that the cut off values for serum ADA to differentiate between PTB from healthy controls were found to be 14.6 IU/L with sensitivity of 78% and specificity of 76% (Fig. 3).

When the cut off values of 14.6 IU/L were applied to our study population, 75.8% of patients of pulmonary tuberculosis were found to be above the cut off value.

5. Discussion

Tuberculosis is a major health problem in India, and out of all its forms PTB is the commonest.[2] A definite diagnosis of PTB can be made with the presence of acid fast bacilli on sputum smear examination of a patient. Chest radiograph provides only a probable diagnosis and culture for tubercle bacilli is a sophisticated and time consuming process. To overcome this difficulty various biochemical tests have been tried from time to time which may help confirm the diagnosis of pulmonary tuberculosis.

Serum ADA has shown to provide promising results. The study was planned to find the diagnostic and prognostic role of serum ADA in patients with PTB. The cases and controls were age and sex matched.

In the present study, mean levels of serum ADA in patients of tuberculosis were found to be 35.29 ± 30.94 IU/L, as

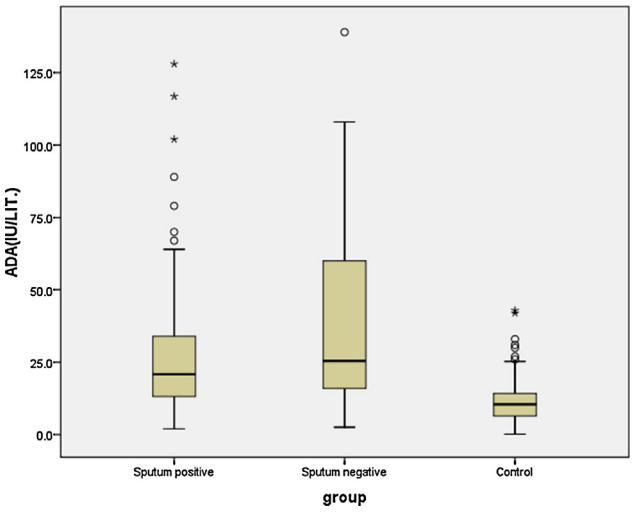

Fig. 2 – Serum ADA levels in sputum positive and sputum negative pulmonary tuberculosis at the end of intensive phase.

	N (50)	Mean ADA (IU/L)
SCANTY	13	29.46
1+	9	22.61
2+	16	25.31
3+	12	23.63

Fig. 1 – Base line serum ADA levels in cases and controls.

Table 2 – Mean ADA levels in relation to the bacteriological load.

	N (50)	Mean ADA (IU/L)
SCANTY	13	29.46
1+	9	22.61
2+	16	25.31
3+	12	23.63

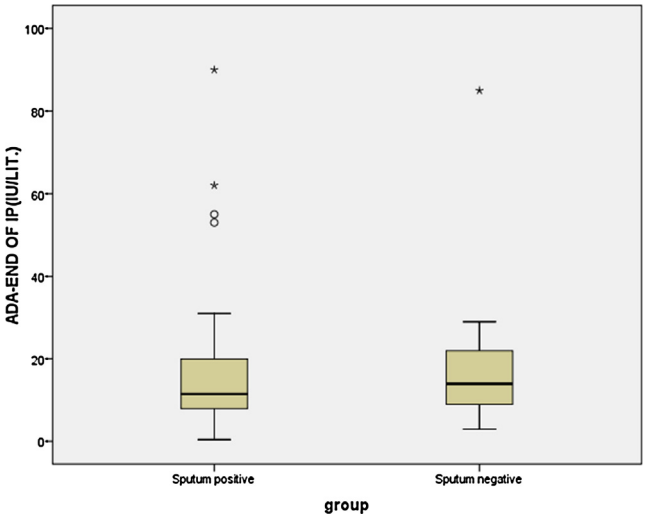

Fig. 3 – ROC analysis of serum ADA levels in cases and healthy controls.

compared to 11.81 ± 8.02 IU/L in healthy controls. The difference between the ADA levels in the two groups was found to be statistically significant ($P = 0.00$). Diagnostic value of serum ADA in patients of pulmonary tuberculosis has been estimated only in a few studies and similar results have been found.[12,13]

Previous numerous studies have reported an increase in ADA levels in pleural effusion pericardial effusion, peritoneum and CNS fluids.[14–16] However, the literature on the levels of ADA in serum is limited.

The main reason for the increased ADA levels in pleural effusion is the movement of T lymphocytes towards this area. Increase in ADA levels is the result of inflammatory reaction caused by monocytes and macrophages.[13,14] When alveolar macrophages are infected by mycobacterium, this enzyme could be found in serum during active pulmonary disease, which was the main reason for planning out this study. In concordance with other studies,[12,13] the encouraging results so obtained can help us use it as a supplementary diagnostic tool in 'difficult to diagnose' PTB patients.

The patients of pulmonary tuberculosis were further divided into two groups, sputum positive and sputum negative PTB. There was no statistically significant difference in the mean serum values of ADA when sputum positive and sputum negative cases of pulmonary tuberculosis were compared. Isolating AFB in the sputum smear of the patients depends on several factors like quantity and quality of the sputum, bacterial load, age and sex of the patient etc. Since no statistically significant differences between the sputum smear negative and positive patients were found when serum ADA was measured, it can be derived that irrespective of the sputum smear status ADA can help determine the patients who can be suffering from PTB, but difficult to diagnose on the basis of sputum smear examinations.

In the present study serum ADA levels were estimated twice in the patients of pulmonary tuberculosis, once at the time of enrolment and next at the end of IP of treatment. A significant decrease in the mean value of ADA was found after treatment in the patients of pulmonary tuberculosis and was consistent with other studies.[12,13] This gives an indication that serum ADA can act as a prognostic marker. A serial follow up of ADA levels can help in particularly those patients where sputum microscopy is not of much reliability because of poor patient related factors. Also, they can prove helpful in sputum smear negative patients, as in these patients it is very difficult to decide whether they need to be shifted from intensive to continuation phase, or IP needs to be extended.

In the patients of sputum positive PTB, severity of disease was categorized according to the bacterial load as scanty, 1+, 2 + and 3+. Of the 50 patients of sputum positive PTB 26% were scanty+, 18% were 1+, 32% were 2+ and 24% were 3+. When the mean ADA levels in patient of sputum positive PTB categorized on the basis of bacterial load were compared, no statistically significant differences were found, indicating that measurement of serum ADA activity has no role in determining the bacteriological load of the disease. Similar results were seen when patients of smear negative and positive PTB were compared. No significant differences in ADA levels as per bacterial load thus again reinforces the fact that the infection process in itself leads to an increase in the ADA levels in the serum. It does not thus correlate with the bacillary load or the extent of lung involvement. Serum ADA levels can thus elucidate the true picture and be a true reflection of the disease.

In the present study, the optimum cut off point for serum ADA levels for diagnosis of pulmonary tuberculosis was found to be 14.6 IU/L, using the ROC curve with sensitivity of 75% and specificity of 76%, a positive predictive value of 75.8% and a negative predictive value of 75.2%. The results of present study are in line with the available literature.[17–19] They suggest that serum values of ADA can be used as an aid to diagnosis of PTB but cannot replace the gold standard sputum smear microscopy.

6. Conclusion

Mean serum ADA was found to be significantly elevated in PTB patients as compared to healthy controls, though there were no statistically significant differences in the mean serum values of ADA in relation to the bacillary load or when sputum positive and sputum negative cases of PTB were compared. It was also found that the mean ADA levels decreased significantly with treatment.

Though, the study was conducted in narrow limitations, it has provided one small effort to arrive to the conclusion that serum ADA levels may be used as a supplementary aid for diagnosis and prognosis of PTB.

Conflicts of interest

The authors have none to declare.

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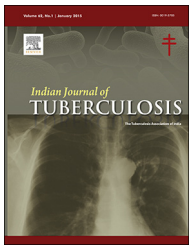

Original Article

Plasma levels of Rifampicin and Pyrazinamide with pre and post meal administration in tuberculosis patients

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ABSTRACT

Context: Various factors affect plasma concentrations of antitubercular drugs in different populations so dosing schedule should be adjusted after therapeutic drug monitoring.
Aims: To study variability in plasma concentrations of Rifampicin and Pyrazinamide with pre and post-meal administration of drugs in tuberculosis patients.
Methods and material: 52 patients of pulmonary tuberculosis, divided in to two groups, pre and post-meal through systemic randomization. After taking pre-dose sample, drugs were administered according to the group. Samples were withdrawn at 2, 4, 6, and 10 h after drug administration. Analysis of samples was done using HPLC.
Results: Mean ± 1SD of C_{max} of Rifampicin was 7.75 ± 2.82 μg/ml, mean ± 1SD of AUC_{0-10} was 42.17 ± 17.25 μg h/ml, adjusted T_{max} was 4.25 h. In pre-meal samples, the corresponding values were 7.75 ± 2.88 μg/ml, 42.83 ± 18.47 μg h/ml, 3.76 h and in post-meal samples 8.03 ± 2.30 μg/ml, 41.56 ± 16.46 μg h/ml and 4.75 h.

Mean ± 1SD of C_{max} levels of Pyrazinamide was 54.49 ± 21.86 μg/ml, mean ± 1SD of AUC_{0-10} was 337.94 ± 124.28 μg h/ml and adjusted T_{max} was 3.49 h. In pre-meal samples the corresponding values were 52.00 ± 19.13 μg/ml, 329.96 ± 112.11 μg h/ml, 3.23 h, and in post-meal samples 57.43 ± 23.61 μg/ml, 345.58 ± 136.99 μg h/ml, 3.54 h.
Conclusion: There is huge variability in the plasma levels of Rifampicin and Pyrazinamide in population of this sub-himalayan region.

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1. Introduction

Drug resistant tuberculosis has emerged as a serious health problem in 21st century and is on a rise because of the mistakes committed in the past by improper implementation of programmes and insufficient knowledge leading to exposure of the organism to variable and subtherapeutic concentrations of antitubercular drugs. Such exposure leads to acquired resistance which subsequently leads to increased primary drug resistance. It is known that various individual, environmental, administrative and other factors affect the plasma concentrations of these drugs in different populations at different times so it should be assured that this concentration remains within therapeutic range throughout the duration of treatment by finding out local factors and adjusting the doses accordingly.[1–3]

Considering these facts, the present study is planned to look for the variability in the plasma concentrations of two, first line antitubercular drugs, to find out whether the simultaneous ingestion of anti-tubercular drugs is appropriate and to find out whether the pre or post-meal administration of drugs changes the plasma levels significantly in the population of Kangra valley region of Himachal Pradesh or a reconsideration of dosing schedule is required.

2. Objectives

To study the variability in plasma concentrations of Rifampicin and Pyrazinamide with pre and post-meal simultaneous administration of drugs in the population of Kangra valley of Himachal Pradesh in India.

3. Design

The study was conducted in Dr. Rajendra Prasad Government Medical College and Hospital Tanda, District Kangra (HP) India. The protocol was approved by Institutional Ethical Committee before the recruitment of subjects started.

3.1. Material

Standard drugs were purchased from Merck Pharmaceutical. HPLC grade reagents methanol, acetonitrile and water were purchased of JT Baker's from the local supplier. 0.2 μm and 0.45 μm Millipore filters of Sigma–Aldrich were also purchased from the suppliers.

3.2. Instruments

LC 20 AD Shimadzu HPLC system with dual pumps, SPD 20AUV detector, Enable C18G guard column and Spincotech 250 × 4.6 mm column with manual injector was used in the study. LabSolutions software was used for analysis of samples. Centrifuge of Remi Elektro Technik, model Remi 8-C with maximum speed 5000 rpm was used.

3.3. Sample size calculation

It was calculated that to detect a plasma concentration variability of 10%, considering alpha error to be 0.05 a sample size of 62 will generate a power of 99%. Hospital records revealed that in the Pulmonary medicine department of Dr. R.P. Govt. Med. College, on an average 16 patients of new smear positive tuberculosis are admitted every month. So it was decided that all the patients of either sex aged between 18 to 50 years with New Sputum Positive Pulmonary (NSP) Tuberculosis reporting within the period of 6 months will be included in the study after explaining them the study protocol in their local language and taking their informed consent (time sample). All the patients included were given free treatment.

3.4. Diagnosis of patients

Diagnosis was made based on history, clinical examination, chest X-ray and sputum microscopy. After inclusion of the patients in the study, history of drug and nutritional supplement intake in the previous one week was taken. Detailed physical examination, screening for peripheral neuropathy and laboratory investigations (LFT, RFT, Routine Haematology, Blood glucose and HIV) were done. Pregnant women, nursing mothers, subjects with liver dysfunction and renal insufficiency, HIV positive subjects, subjects with any surgical or medical condition (present or history) which could significantly alter the absorption, distribution, metabolism or excretion of the study drug were excluded from the study.

3.5. Method

All patients were divided in to two groups 1 and 2 for Pre and post-meal administration of drugs. Patients were allotted different groups by systemic randomization. Participants were admitted night before drug administration and were given only tap water from 21:00 h.

In the morning (approximately 6:00 h) a predose pharmacokinetic sample was taken, antitubercular drugs (600 mg of isoniazid, 450 mg of Rifampicin, 1200 mg of Ethambutol and 1500 mg of Pyrazinamide) supplied through RNTCP were administered at 7:00 h with 200 ml of water and swallowed in sitting posture without chewing the drug. In group 1 dosing was done empty stomach and in group 2 dosing was done just after a meal comprising of pulses, vegetable, rice and chapati. Subjects were not allowed to exert physically. Post dose samples were withdrawn at 2, 4, 6, and 10 h after drug administration. Date and time of the sample collection were recorded and food was given 5 and 12 h after dosing.

3.6. Sample processing

Collected serum samples were kept in liquid nitrogen. On the day of processing, serum samples were taken out from nitrogen container and thawed at room temperature. One ml of serum was mixed with one ml of acetonitrile to allow denaturation of proteins. The sample was centrifuged at 3000 rpm for 10 min. Equal volume of supernatant was mixed with equal volume of acetonitrile. The sample was centrifuged

Table 1 – Plasma levels of Rifampicin in tuberculosis patients.

N	Time post dosing	Mean ± 1SD in μg/ml	Range in μg/ml	Median in μg/ml	N (undetected levels)
51	2 h	3.74 ± 3.67	0–12.34	2.90	13
49	4 h	5.59 ± 3.33	0–14.90	5.64	6
51	6 h	5.79 ± 2.84	0–11.08	5.67	4
47	10 h	3.31 ± 2.43	0–11.15	3.12	5

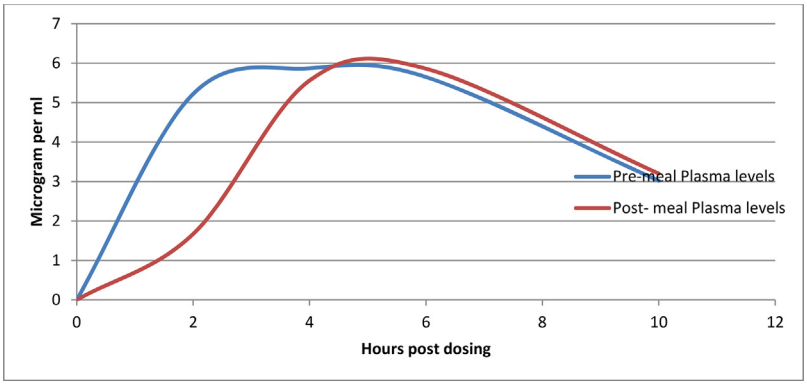

Fig. 1 – Median pre- and post-meal plasma levels of Rifampicin in tuberculosis patients.

again for 20 min for precipitation of any remaining protein. 20 μL of this supernatant was injected in HPLC system manually.

3.7. *Analysis*

Analysis of Pyrazinamide and Rifampicin was done simultaneously using external control. Mobile phase was acetonitrile and phosphate buffer 50:50 (v/v). pH of phosphate buffer was 6.8 and flow rate was 1 ml/minute. Run time was 12 min. Peaks of Pyrazinamide and Rifampicin were obtained at 2.9 and 6.2 min respectively.

3.8. *Calculation of pharmacokinetic parameters*

The non-compartmental pharmacokinetic analysis method was employed to determine the pharmacokinetic parameter of the drugs. C_{max} and T_{max} were obtained directly from the observed data. $AUC_{0–10}$ was calculated by the linear trapezoidal method.

3.9. *Statistical evaluation*

Students 't' test for comparison of means and ANOVA was used at appropriate places to compare the values of C_{max}, T_{max} and $AUC_{0–10}$ of various samples.

4. Results

A total of 52 patients with new smear positive tuberculosis were enrolled in the study. 37 (71.15%) patients were males and 15 (28.85%) were females with mean age ± SD being 44.8 ± 15.53 years. Mean weight ± SD of the patients was 45.52 ± 8.57 and mean body mass index (BMI) ± SD was 17.69 ± 3.41.

4.1. *Plasma levels*

A total of 250 blood samples were collected from 52 study subjects. 52 were control samples, 51 were 2 hour post dosing, 49 were 4 hour post dosing 51 were 6 hours post dosing and 47 blood samples were taken at 10 h after administration of antitubercular drugs.

4.2. *Rifampicin*

In blood samples mean ± 1SD of C_{max} of Rifampicin was 7.75 ± 2.82 μg/ml, range of C_{max} was 0–14.90 μg/ml and median of C_{max} was 7.85 μg/ml. Mean ± 1SD of $AUC_{0–10}$ was 42.17 ± 17.25 μg h/ml, range was 0–74.15 μg h/ml and median was 42.14 μg h/ml ($N = 44$). Adjusted T_{max} was 4.25 h (Table 1, Fig. 1).

4.3. *Pre-meal*

In 25 pre-meal samples, mean ± 1SD of C_{max} levels of Rifampicin were 7.75 ± 2.88 μg/ml; range of C_{max} was 2.19–14.90 μg/ml and median of C_{max} was 7.33 μg/ml ($N = 25$). Mean ± 1SD of $AUC_{0–10}$ was 42.83 ± 18.47 μg h/ml; range of $AUC_{0–10}$ was 0–69.46 μg h/ml and median $AUC_{0–10}$ 42.87 μg h/ml ($N = 21$). Adjusted T_{max} was 3.76 h.

4.4. *Post-meal*

In post-meal samples mean ± 1SD of C_{max} of Rifampicin was 8.03 ± 2.30 μg/ml; range was 3.40–11.48 μg/ml and median C_{max} was 8.09 μg/ml ($N = 24$). Mean ± 1SD $AUC_{0–10}$ was

Table 2 – Plasma levels of Pyrazinamide in tuberculosis patients.

N	Time post dosing	Mean ± 1SD in μg/ml	Range in μg/ml	Median in μg/ml	N (undetectable levels)
51	2 h	44.38 ± 23.64	0–100.27	42.09	2
49	4 h	41.70 ± 20.22	0–84.92	40.77	3
51	6 h	39.63 ± 18.83	0–95.13	38.65	1
46	10 h	24.86 ± 17.32	0–108.77	21.36	1

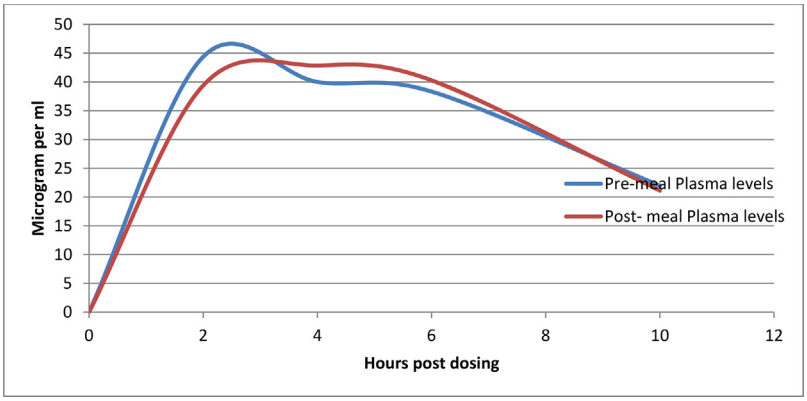

Fig. 2 – Median pre- and post-meal plasma levels of Pyrazinamide in tuberculosis patients.

41.56 ± 16.46 μg h/ml; range being 0–74.15 μg h/ml and median 41.84 μg h/ml ($N = 23$). Adjusted T_{max} was 4.75 h.

The *P* value of difference between AUC of pre and post meal samples of Rifampicin was 0.8108 and of difference between C_{max} of pre and post meal samples was 0.936.

4.5. *Pyrazinamide*

In the samples mean ± 1SD of C_{max} levels of Pyrazinamide was 54.49 ± 21.86 μg/ml; range of C_{max} levels was 17.46–108.77 μg/ml and median was 50.70 μg/ml ($N = 51$). Mean ± 1SD of $AUC_{0–10}$ was 337.94 ± 124.28 μg h/ml; range was 123.44–618.76 μg h/ml and median was 319.63 μg h/ml ($N = 45$). Adjusted T_{max} was 3.49 h (Table 2, Fig. 2).

4.6. *Pre-meal*

In pre-meal samples mean ± 1SD C_{max} of Pyrazinamide was 52.00 ± 19.13 μg/ml; range was 17.46–90.35 μg/ml and median of levels was 47.69 μg/ml ($N = 26$). Mean ± 1SD of $AUC_{0–10}$ of Pyrazinamide was 329.96 ± 112.11 μg h/ml; range was 123.44–508.94 μg h/ml and median was 313.63 μg h/ml ($N = 22$). Adjusted T_{max} was 3.23 h.

4.7. *Post-meal*

In post-meal samples mean ± 1SD of C_{max} levels of Pyrazinamide was 57.43 ± 23.61 μg/ml; range was 23.11–108.77 μg/ml and median was 51.74 μg/ml ($N = 26$). Mean ± 1SD $AUC_{0–10}$ of Pyrazinamide levels was 345.58 ± 136.99 μg h/ml; range was 141.27–618.76 μg h/ml and median was 353.68 μg h/ml ($N = 23$). Adjusted T_{max} was 3.54 h.

The *P* value of difference between C_{max} of pre and post meal samples of Pyrazinamide was 0.3671 and of difference between AUC of pre and post meal samples was 0.677.

4.8. *Association of various variables in the study*

No association was found between Rifampicin (mg/kg) and C_{max}; Rifampicin (mg/kg) and $AUC_{0–10}$; Pyrazinamide (mg/kg) and C_{max}; Pyrazinamide (mg/kg) and $AUC_{0–10}$ with correlation coefficient values of 0.118, 0.0508, 0.2742 and 0.3548.

When the patients were divided into three groups with C_{max} of Rifampicin on 1st day of treatment <6 μg/ml, 6- <9 μg/ml and 9 μg/ml or more, an association was found in the means of C_{max} of Rifampicin and Pyrazinamide of these three groups. The value of correlation coefficient of means of C_{max} of Rifampicin and Pyrazinamide of these three groups was 0.992 (Fig. 3). When the patients were divided into three groups with $AUC_{0–10}$ of Rifampicin on 1st day of treatment <30 μg h/ml, 30 μg h/ml- <50 μg h/ml and 50 μg h/ml or more, an association was found in the means of $AUC_{0–10}$ of Rifampicin and Pyrazinamide of these three groups. The value of correlation coefficient of means of $AUC_{0–10}$ of Rifampicin and Pyrazinamide of these three groups was 0.889 (Fig. 4).

5. Discussion

Therapeutic drug monitoring of antitubercular drugs is not done in India. Clinical markers of response of these drugs like loss of fever, stable or increasing weight, loss of night sweats, diminished cough etc. are used to judge the early success of regimen and finally the success is decided at the end of two

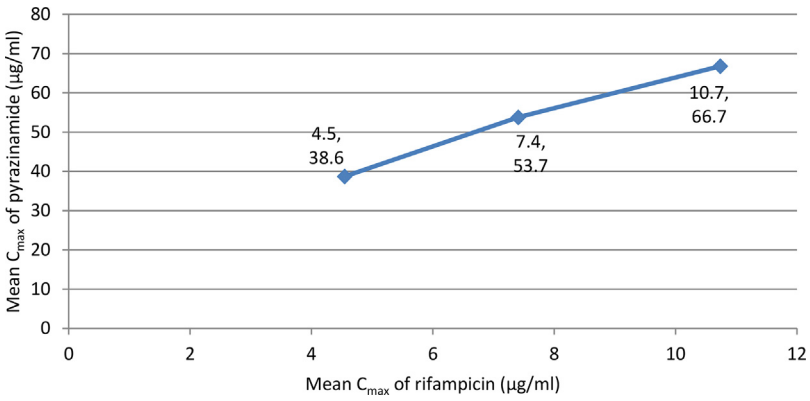

Fig. 3 – Association of C_{max} of Rifampicin and Pyrazinamide in tuberculosis patients.

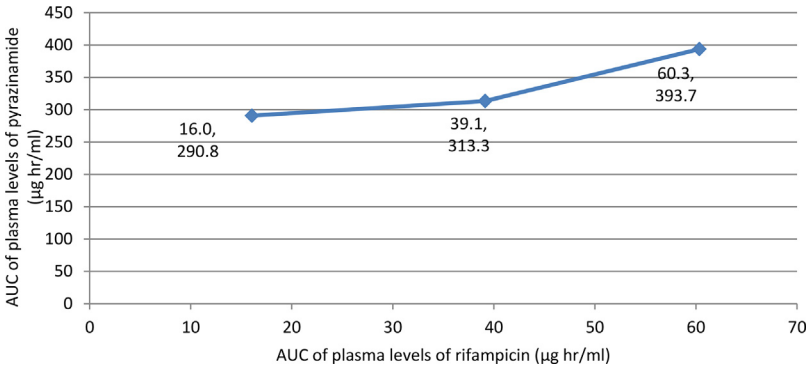

Fig. 4 – Association of AUC of Rifampicin and Pyrazinamide in tuberculosis patients.

months of therapy with the help of sputum microscopy. Even at this stage if the person is sputum positive, the intensive phase is extended for one more month without any correction in doses.[4] So, the problem with antitubercular regimen is the fact that by the time we assess the patient with these clinical and laboratory markers and decide whether the organism is responding to treatment regime or not, the organism is already exposed to sub-therapeutic concentrations for 8–12 weeks and has become resistant in many cases.

In our study the peaks of Pyrazinamide and Rifampicin were very clearly resolved without any disturbance in the surrounding region. In most of the samples it was a clear run after the elution of Rifampicin but we kept the running time fixed at 12 min and did not decrease it. We used only one HPLC column for processing all the samples. As the study progressed the RT of Rifampicin shifted from 6 min to 5.6 min. The RT of Pyrazinamide was constant throughout the study at 2.93 min.

Various studies have found some association between plasma levels of Pyrazinamide and Rifampicin and outcome of therapy. AUC has been found a more reliable variable than plasma levels by researchers. So this wide variability in average plasma levels, C_{max} and AUC of both Pyrazinamide and Rifampicin in the patients in our area is an area of concern.[5–10]

The plasma levels of Rifampicin reported in healthy volunteers are 8–24 µg/ml with T_{max} at 2 h with 600 mg daily regime.[11,12] In patients of this study, the levels reported range from 1.3 to 24.99 µg/ml. On dose/kg body weight basis, the doses of Rifampicin ranged from 6.92 to 17.31 mg/kg. Although the therapeutic relationship between Rifampicin concentrations and treatment response has not been defined in human studies, higher doses are associated with improved early bactericidal activity and better treatment results.[13–15] It is suggested that the drug's activity is concentration dependent thus the antibacterial activity will be more if the C_{max}/MIC is more.[16]

The plasma levels of Pyrazinamide described in healthy volunteers with a dose of 25 mg/kg daily regime are 20–50 µg/ml and with 50 mg/kg biweekly regime 40–100 µg/ml with T_{max} at 1–2 h.[11] In patients various studies report 29.9–84.4 mcg/ml.[6,17,18] The levels of Pyrazinamide in our study vary widely from 0 to 108.77 µg/ml. 50% of the patients showing therapeutic or more than therapeutic levels but in 50% of the patients the levels are sub-therapeutic. There was a wide variation in doses/kg body weight of Pyrazinamide also and they ranged from 23.08 to 57.69 mg/kg. One of the reasons for this wide variability is difference in body weight that generated a wide variability of drug dose per kg body weight.

5.1. *Pre and post-meal variability in plasma levels*

Food is considered an important factor affecting the bioavailability of antitubercular drugs. It is advised that antitubercular

drugs should preferably be administered empty stomach. The variability is reported more with Rifampicin than other drugs.[11] In our study, there was little difference in the mean, median and AUC_{0-10} levels of Rifampicin in pre and post meal samples but T_{max} was delayed by one hour. The levels of Pyrazinamide were more in post meal samples, difference in post and pre-meal mean, median and AUC values of Pyrazinamide was 5.43 μg/ml, 4.05 μg/ml and 15.62 μg h/ml respectively, though the T_{max} was almost 19 min more in post-meal samples.

It was observed that if people are divided into groups of high plasma levels moderate plasma levels and low plasma levels for Rifampicin the pattern remains same for Pyrazinamide. This shows that it is appropriate to know the plasma level status of a person for one drug and decide the dosing schedule. This information assumes importance because by knowing serum drug levels of a patient at the initiation of therapy, patients with low serum levels can be segregated for dose modification to achieve target therapeutic range thus helping in decreasing problem of resistance due to sub therapeutic levels.

6. Conclusion

It is evident from our study that there is a wide variability in plasma levels of Rifampicin and Pyrazinamide in the population of Kangra valley region. It was found that group of patients that had low levels of Rifampicin also had low levels of Pyrazinamide and vice versa. It is recommend that in this population serum drug level of all the patients for first line antitubercular drugs should be done at the initiation of therapy. By doing so, patients with low serum levels can be segregated for dose modification to achieve target therapeutic range thus helping in decreasing problem of resistance due to sub therapeutic levels. At the same time earlier screening of patients who generate very high plasma levels of these drugs and dose modifications in them can help in decreasing incidence of side effects of these drugs and also help in decreasing dropouts in the therapy. Dropouts are important contributors to drug resistance in the society. Many patients want to continue therapy but multiple side effects deter them from restarting it.

Conflicts of interest

The authors have none to declare.

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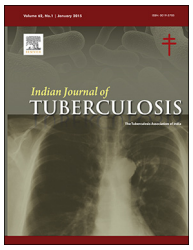

Original Article

Social support a key factor for adherence to multidrug-resistant tuberculosis treatment

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is emerging as a major public health problem globally. Treatment success rates in MDR-TB across the globe are not encouraging as completing MDR-TB treatment successfully is challenging due to high proportion of lost to follow up.

Methods: Using qualitative methods and grounded theory approach, in-depth interviews were conducted with MDR-TB patients and treatment providers. The social cognitive framework was explored as a way to guide understanding of the factors affecting treatment adherence among MDR-TB patients.

Results: Multiple factors influenced patient's decision to adhere to MDR-TB treatment. Self-motivation, awareness about disease and treatment, counselling support, family support, nutritional support and social support were important drivers for successful treatment. Providers related that motivational counselling, nutritional support, family support and social support encouraged treatment adherence.

Conclusion: To improve MDR-TB treatment adherence, a patient-centric approach should be considered at the programmatic level. There is a need to formulate strategy that includes motivational counselling, nutritional supplementation and social support mobilisation for treatment adherence. Participants suggested a Patient Support Group led treatment care model for better adherence and treatment success rates in MDR-TB treatment.

1. Background

Multidrug-resistant tuberculosis (MDR-TB) is emerging as a major public health problem globally and is deterring the achievements of TB control in many low and middle-income countries of World. India accounts for 64,000 MDR-TB cases out of 300,000 cases estimated globally.[1] Treatment success rates in MDR-TB are not encouraging. Globally, The treatment success rates in 2015 among those who were started on MDR-

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TB treatment was only 50%, mostly due to high death rates and loss to follow-up Similar to the global trends, the treatment success rate in India for 12,125 reported was 48% with 22% death rate and 19% lost to follow up (LFU).[2] To achieve the Global Plan to end TB target of 90%,[3] programs need to enhance the treatment adherence. Completing MDR-TB treatment successfully is challenging considering the long duration of treatment, number of pills and toxicity of drugs. Studies of predictors of poor treatment outcomes and risk factors associated with LFU have been conducted previously.[4–9] Studies conducted earlier from India[10,11] had highlighted factors affecting adherence to tuberculosis treatment, but there is limited knowledge about factors influencing treatment adherence in MDR-TB patients which is more complicated as compared to the first line anti-TB treatment. A qualitative study was conducted to understand patient and provider related factors to MDR-TB treatment adherence and successful treatment completion.

2. Methods

2.1. Study setting

This qualitative study occurred in seven districts of Maharashtra linked to Drug-resistant TB Centre (DRTBC) in Nagpur. This site was among the first pilot sites in country for implementing MDR-TB services. Case definitions[12,13] used are as shown in Table 1.

2.1.1. Study design and sampling

In-depth interviews were conducted during August 2012–February 2013 among a purposeful sample of 20 MDR-TB patients who were initiated on treatment from the DRTBC in Nagpur. Patients were selected from the treatment register for those who completed treatment successfully from September 2007 to March 2012. In-depth interviews were conducted with 10 treatment providers which included public, private providers and community volunteers.

A maximum variation sample strategy was used to ensure a diversified sample of patients. Participants were recruited purposefully to ensure heterogeneity in relation to age, sex, occupation, and residing in rural and urban areas.

2.2. Data collection

In-depth interviews were conducted in regional language. The patient interview guide included open-ended questions related to the diagnosis, treatment experiences, factors influencing treatment adherence, etc. Questions on training, experience of treating MDR-TB patients, challenges of providing treatment, reasons for successfully completion of treatment and their recommendations to improve adherence were included for providers. The interview guides for patients and providers were pilot tested and questions were readapted during concurrent analysis in accordance with a grounded theory methodological approach.

2.3. Theoretical framework

The social cognitive framework as conceptualised by Bandura was explored as a way to guide our understanding of the multiple factors affecting treatment adherence among MDR-TB patients. It incorporates personal factors, environmental factors, and behavioural factors.[14–16] It suggests, a multifaceted causal structure is linked with human motivation, action and well-being and offers both predictors of adherence and recommendations for its promotion.[14]

2.4. Data analysis

Audio-recorded data from interviews of patients and providers was transcribed verbatim and transcribed into English. Codes and themes were developed concurrently with data collection. Direct quotes that illustrated important themes were extracted and presented in this manuscript.

2.5. Ethical considerations

The study protocol was approved by the ethics committee of India's National Tuberculosis Institute for ethical clearance.

3. Results

We found that there were several factors interplaying for patient adherence of MDR-TB treatment. The factors emerged

Table 1 – Standard case definitions used under Programmatic Management of Drug resistant TB Guidelines in India.[16,17].

S. No.		Definitions
1.	MDR-TB case	MDR-TB is defined as M. tuberculosis resistant to isoniazid and rifampicin with or without resistance to other drugs.
2.	Adherence[16]	The extent, to which the patient follows medical instructions, a diet, and or fulfilling lifestyle changes, relates with agreed recommendations from a health care provider.
3.	Treatment success (includes cure and treatment completed)	**Cure**: A patient who has completed treatment and has been consistently culture negative (with at least 5 consecutive negative results in the last 12–15 months). If one follow-up positive culture is reported during the last three quarters, patient will still be considered cured provided this positive culture is followed by at least 3 consecutive negative cultures, taken at least 30 days apart, provided that there is clinical evidence of improvement. **Treatment completed:** A patient who has completed treatment according to guidelines but does not meet the definition for cure or treatment failure due to lack of bacteriological results.

during in-depth interviews with patients and providers that influenced positively treatment adherence and treatment success were mainly self-motivation, awareness about the disease, motivational counselling, family, nutritional and social support.

3.1. *Self-motivation*

During the interviews the strong motivating factor for adherence and overcoming barriers to complete the treatment was self-motivation and hopes and aspirations of good quality of life.

> ''I want to get out of this as early as I can... I want to be healthy again. I will go back to my village after getting cured...I have a shop there and my parents are staying there'' -Male/48yrs, Patient.

Concern for the family members and living for their loved ones motivated patients to complete the treatment in some cases.

> ''I had to tolerate a lot for my mother sake. I thought I am only son of my mother... There is nobody else in my family. I wanted to live for her, if something happens to me who am going to look after her.'' -Male/23yrs, Patient.

> ''To take medications it was really difficult. But for the sake of my children I had to bear it to any extent'' -Female/29yrs, Patient.

Having seen life threatening situation and fear of death motivated some patients to complete the treatment.

> ''I knew the disease is dangerous. I had seen one-two patient died in ward because of this disease when I was admitted, so I was scared about this. I didn't miss any dose.''
>
> -Male/25yrs, Patient.

3.2. *Awareness about the disease and treatment*

Correct information about the disease and treatment also influenced the patient's adherence.

> ''I knew taking medicine regularly is only solution ... I heard radio talk which is at 3pm once a week, some doctors talk...the doctors told in talk that TB is curable if someone (patients) take medicine regularly''-Male/48 yrs., Patient.

3.3. *Counselling support*

Provider counselling support was a critical factor which influenced patients to adhere to treatment and complete MDR-TB treatment.

> ''Initially, I had vomiting's and felt like head is rotating...I thought of quitting medicine but doctor explained me properly and gave some drugs. Because of him I am able to walk, otherwise I was not able to stand also''-F/35yrs, Patient.

> ''Patient sometimes as they are so depressed ...they lose interest in life. They need intense counselling. Some patients are chronic alcoholic; they are left alone by their family members. We have to make them understand the importance of taking medicines regularly and quitting alcohol.'' -Female/46yrs Private Practitioner DOT Provider.

> ''We have to talk to patients were politely, we can't shout at them. he [patient] faces lot of other challenges. If we don't counsel him properly he may quit at any time''
>
> -Male/30yrs, Government DOT provider.

3.4. *Nutritional support*

Some of the providers and patients emphasised that proper nutrition is needed to tolerate MDR-TB treatment.

> ''Nutrition affects a lot. Most of the patients are malnourished, and most are from the outskirts and from the lower strata. Economically they are in bad condition.''-Female/46 yrs., Private Practitioner DOT Provider.

> ''Patient who is on treatment needs good nutrition; if patient is from well to do family he can afford that, but if patient is from poor family he takes food whatever is available...because of this his body doesn't respond to medicines''-Female/36yrs, DOT Provider ASHA Worker.

A cured patient who later became a DOT provider noted the strong linkages between food, finances, and adherence to MDR-TB treatment.

> ''This disease needs proper food. The important thing is financial, if he (patient) doesn't have anything he will have to go for work...after taking these many medicines he cannot work, so he has to leave medications to work and earn.'' -Male/32 yrs., Patient.

3.5. *Family and community support*

Support by family and community members including peers, was an important factor influencing treatment adherence positively. When it came to family support for patients, mothers were mentioned as the most supportive caretaker by a majority of the patients.

> ''I used to sleep for 2–3 hrs during day after taking medicines. my mother in law and husband used to do all work at home. My family members supported me very much. They used to care for me a lot. Somebody else would have left his wife, my husband is very nice, and he cared for me very much.'' -Female/29 yrs., Patient.

> ''My mother took care of me during this painful treatment, like a mother takes care of her baby when he is in womb ''she suffered financial problems but never let me know that''. She always told you only take medicine, don't bother about anything else'' still now she buys half litre milk for me daily'' -Male/29yrs, Patient.

Some married female patients lacked support from their husbands who left them alone to be cared for by their (maternal) mothers.

"My husband and in laws did not bother to take me to doctor when I was ill. My husband didn't give me a single rupee for medicine. He did not work and used to drink excessive alcohol. I was ill; still I was working for them. So I called my mother, she took me to hospital." -Female/32 yrs., Patient.

Patient related peer support right from getting diagnosed at Lab to getting admitted at DR TB centre, being with the patient during the treatment, in some cases taking them to DOT centre and advice given by fellow patients as strong motivational factor to complete the treatment.

"I met one patient, he was about to complete the treatment. He had told me "there is no need to worry, don't be afraid"-Male/40yrs, Patient.

"Main thing I was motivated by a handicapped friend of mine, I thought he is living without both legs why can't I. he told me "you will be all right, take medicine regularly" -Male/25yrs, Patient.

3.6. *Social support*

Overwhelmingly patients and providers related that Social support was key factor for MDR-TB treatment adherence. employment support, nutritional support and at times financial support motivated the patients to complete treatment.

Some patients who are employed were supported by employer which motivated them to complete the treatment.

"I had left the job [worker at a shop] since I was ill. He [employer] called me back to work. . .he always enquired if I am taking medicines regularly. He changed duty timings for me. . .he has helped me a lot" -Male 40/yrs., Patient.

Patients on this long duration treatment struggle with finances and need support to meet the daily expenses including nutrition especially if the patient is breadwinner for the other family members.

"Sometimes when I saw my mother struggling with finances, I felt like I should go and do some work but I was not able to do anything as I felt drowsy after taking medicines. . . at times my friends & relatives gave me some money and grains to take care of daily needs"-Male/48 y, Patient.

"My neighbour's used to enquire. Have you taken medicine today? Have you taken food? If I had nothing to prepare food. . .they used to provide food" -Female/44yrs, Patient.

A government urban DOT provider who mobilised social support by coordination with local leaders, neighbours, medical staff and social workers in area for a poor female patient mentioned the importance of social support and suggested "Patient Support Group" (PSG) as shown in Fig. 1.

"Patient is with us for short time at the time he takes treatment. after that he returns back to society and has many challenges. We need a support group for patient" -Male/26yrs, DOT provider.

"Economically they are very backward, there is only one earning member in family and there are 4–5 members in family . . . If the earning member is suffering from disease, it

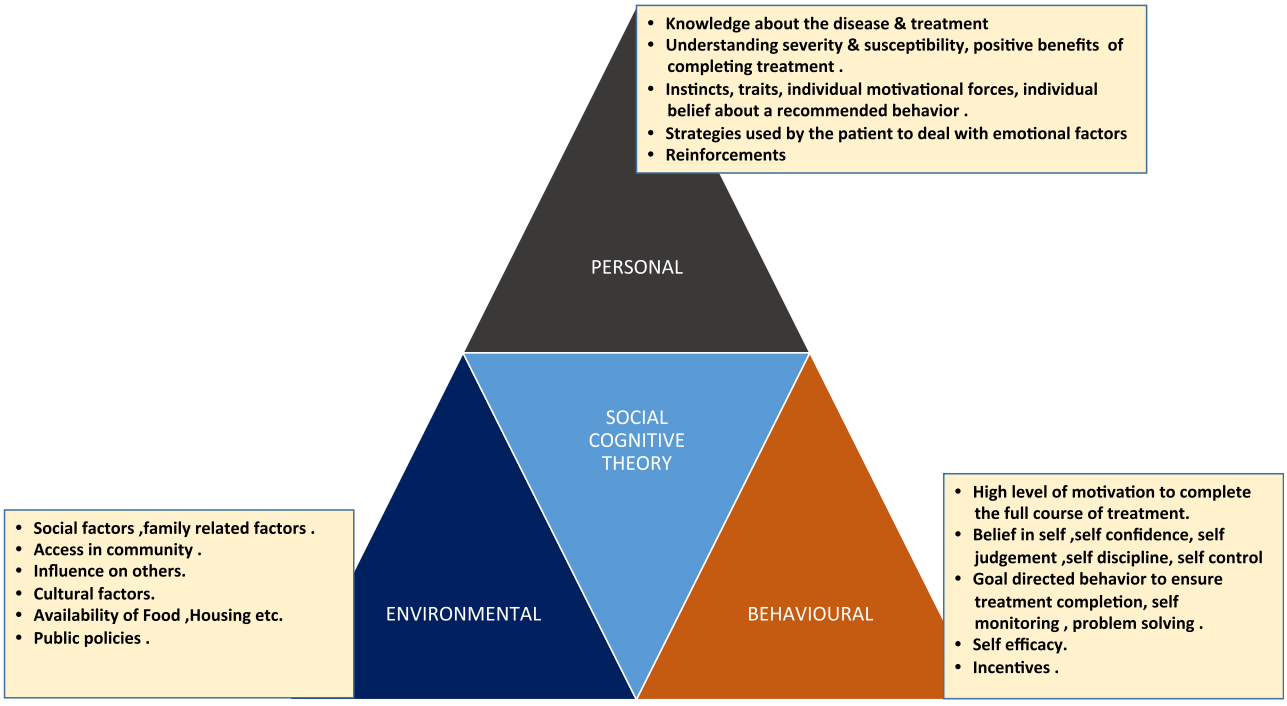

Fig. 1 – Structural adaptation of the social-cognitive theory among successfully treated MDR-TB patients.

affects the economy of the home. . .They need family support and social support also.'' -Female/32 yrs., DOT Provider.

4. Discussion

Completing MDR-TB treatment is a challenging task both for patients and providers. Long treatment duration, side effects of treatment; socioeconomic factors related to this disease, personal behavioural factors etc. make it more complex. Our study explored factors that positively contributed to successful treatment of MDR-TB, which has not been done previously as a qualitative study for MDR-TB treatment in India although there are several studies which are done for tuberculosis which is thrice weekly regimen in India and of 6–8 months duration treatment only.[10,17,18]

In this study we have identified there are multiple factors which influence the treatment adherence and treatment success. From the patients and providers perspective counselling was considered as important motivating factor which contributed to successful treatment. Studies conducted earlier emphasise counselling as important supplementary activity which will improve treatment adherence and reduce knowledge gaps.[19–21] Concerns were raised by providers regarding alcohol addiction in some of the MDR-TB patients as barrier to effective counselling and treatment adherence. Health care providers suggested that linkages to drug and alcohol de-addiction centres run by government will provide sufficient support system to the patients. This has also been highlighted in Russia study for tuberculosis patients.[22,23] Subjects shared that care for their immediate family members especially children or mother were strong drivers for completing MDR-TB treatment successfully in many patients. Fear motivation due to death associated with MDR-TB and patients having seen other patient died due to MDR-TB during their admission for pre-treatment evaluation in MDR-TB wards turned out to be an important factor that led to adherence and subsequently contributed to successful treatment in some patients. Although nutritional support for MDR-TB patients has been debated since long at several forums, it was considered as a supplement by patients and providers in the study. It is believed by many of the study participants that during MDR-TB treatment, patients need proper nutrition; the lack of proper nutrition would result in a decreased ability to tolerate the drugs. Some studies have underlined this fact.[24,25] Social support and family support have been found to be key motivating factors for the completion of the treatment. TB and Human Immunodeficiency Virus programs have developed linkages to various social welfare schemes in countries like Russia, India.[26,27] Care and support by government, peers, community volunteers, neighbours, society members, health staff, and family members was noted by a majority of the cured patients and DOT providers as the most important motivating factor to complete treatment. Similar findings were also found in studies in India.[11,28,29]

Study participants suggested a patient centric model of care which includes appropriate treatment and a support group for MDR-TB patient to ensure treatment completion successfully. Fig. 2 shows the PSG composition recommended by study participants (Fig. 2).

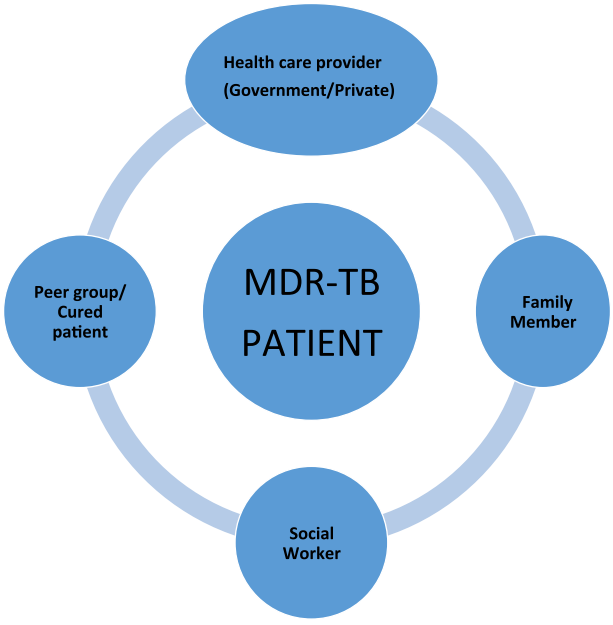

Fig. 2 – ''Patient Support Group'' (PSG) led model of care for MDR-TB treatment adherence.

5. Limitations

The study site was among the first site in India to report outcomes of MDR-TB patients and as the number of patients registered for treatment was limited, the sample used in the study was limited. Although the results of the study cannot be generalised, this study gives the viewpoint of patients and providers with regards to completing MDR-TB treatment successfully. This can help program managers and service providers to understand the motivators, design strategies to improve success rates.

6. Conclusion

Study highlights the factors according to patients and providers perspective which resulted in successful MDR-TB treatment. Self-motivation, awareness regarding disease and treatment, counselling support, family and social support positively influenced the treatment completion in MDR-TB patients. The support system recommended by participants based on their experience is the feedback to program managers and policy makers to devise strategies to improve adherence and success rates in this most challenging treatment. The ''PSG'' model of care for MDR-TB patients is the lesson learnt from the implementing site which can be replicated at much broader scale to assess the feasibility.

Author contributions

DRD, DJ contributed to the concept, design, Interviews, literature search, synthesis of information identified in the search, writing and editing of the manuscript, and data

collection and analysis. All others contributed in the literature search, writing, and review of the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors have none to declare.

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Original Article

Prevalence of chronic respiratory diseases from a rural area in Kerala, southern India

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ABSTRACT

Background: Chronic lung diseases are one of the leading causes of morbidity in developing countries. A community based survey was undertaken with an objective to estimate the prevalence of chronic respiratory diseases and to describe the profile of people with CRDs in the rural area Nilamel health block in Kollam district, Kerala, southern India.

Methods: A household information sheet and a translated respiratory symptom questionnaire based on International Union against Tuberculosis and Lung Disease (IUATLD) bronchial symptoms questionnaire was administered to 12,556 people above 15 years, selected randomly from Nilamel health block.

Results: Prevalence of self reported asthma was 2.82% (95% CI 2.52–3.12) and that of chronic bronchitis was 6.19% (95% CI 5.76–6.62) while other CRDs which did not fit to either constitute 1.89%. Prevalence of asthma among males was 2.44% (95% CI 2.05–2.85) while that of females was 3.14% (95% CI 2.71–3.57). Chronic bronchitis prevalence was 6.73% and 5.67% among males and females respectively.

Conclusion: Although India has devised a programme to combat cancer, diabetes, cardio vascular disease and stroke, none have been devised for chronic respiratory illness till date. Considering high prevalence and its contributions to morbidity and mortality, a comprehensive programme to tackle chronic respiratory diseases is needed.

1. Background

Lung diseases are one of the leading causes of death in developing countries. Chronic obstructive pulmonary disease (COPD) was the third leading cause of death worldwide.[1] Chronic respiratory diseases (CRDs) as a group accounted for 4.7% of global Disability Adjusted Life Years (DALY).[2] CRDs, if not diagnosed, treated and managed correctly can adversely affect individuals and health systems. But chronic respiratory disorders (CRDs), particularly asthma and COPD, have attracted very little special attention in low- and middle-income countries.

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A systematic review reports that the prevalence of chronic bronchitis in rural areas in India is somewhere between 6.5% and 7.7%.[3] The review also points to the limited number of community based studies estimating the prevalence of chronic respiratory diseases in India.[3] A large multi-centric study done in India (INSEARCH) to estimate the prevalence of CRDs reported wide variations in the prevalence of bronchial asthma among different cities ranging from 0.37% in Secunderabad to 4.45% in Thiruvananthapuram (Kerala) and that of chronic bronchitis from 0.61% in Guwahati to 13.54% in Thiruvananthapuram. The prevalence of chronic respiratory diseases, as reported by the INSEARCH study was very high in Thiruvananthapuram city as compared to all other 11 centres in India.[4]

Kerala, the southern State in India, has a reasonably strong primary health care system with a good infrastructure of primary health centres.[5] Government of Kerala had implemented a pilot project of the World Health Organisation (WHO) recommended Practical Approach to Lung health (PAL) strategy, with an intention to further strengthen the health system and to improve the quality of diagnosis, treatment and management of common chronic respiratory illnesses in primary healthcare settings.[6] PAL has been piloted in three health blocks with a population of approximately 550,000 in Kollam district and these areas reflects the typical state scenario in terms of geography and health care delivery. Estimating true burden of chronic respiratory diseases in the community was the first step in the implementation, as it was essential to further plan the logistics. Hence a community based survey was undertaken with an objective to estimate the prevalence of chronic respiratory diseases and to describe the profile of people with CRDs in the rural area Nilamel health block in Kollam district.

2. Methods

2.1. Study setting

Nilamel health block, situated in South East part of Kollam district, consists of four grama panchayats divided into 69 wards (lowest division of Local self Government) with a total population of around 125,034. Scheduled tribe constitutes 0.4% of the population. The area is bounded by mountains and forest on Eastern side. Sex ratio is 1113 females for 1000 males. Literacy rate for females is 92%. In 2014, 276 people with chest symptoms per 100,000 population were tested for TB and 35 new smear positive TB cases per 100,000 population were notified from this area.

2.2. Study population

Adult permanent residents of the area were eligible to be included. Children and adolescents less than 15 years were excluded, as PAL services in the state intended to include only people above 15 years, in initial phase.

2.3. Study tool

A household information sheet and a respiratory symptom questionnaire were designed based on International Union against Tuberculosis and Lung Disease (IUATLD) bronchial symptoms questionnaire and adopted from the Malayalam (regional language) translated and validated version of questionnaire used in INSEARCH study sponsored by Indian Council of Medical Research.[4,7] The questionnaire was pilot tested before initiation of the study.

2.4. Sampling and sample size

To detect a minimum prevalence of 5% with a relative precision of 20% and a design effect of 6.08 (intra cluster correlation from pilot study was 0.017, around 300 persons from a cluster) the sample size was estimated to be 11,557. Hundred consecutive houses were selected from each 40 randomly selected wards, with the first house selected randomly.

2.5. Data collection

160 community volunteers were trained over a day regarding data collection process. Training included study protocol, structure of study questionnaire, standardisation of asking questions, exercise on interviewing two households in the field and verification of the same. 40 multipurpose health workers (MPW) were trained separately for supervision of data collection and study protocol. 20% of the houses were re-visited by the MPW and 2% by doctors as part of quality control. The survey was carried out during December 26th, 2014 to January 3rd 2015. In case a house was locked, the data collectors would return at a later date, to at least three attempts.

2.6. Analysis

Asthma was diagnosed if the person answers ''yes'' to any of the questions a or b AND ''yes'' to any of the questions c, d or e.

(a) Have you ever experienced wheezing (without cold) or whistling sound from the chest during last 12 months?
(b) During last 12 months, have you ever woken up in the morning with a feeling of tight chest or breathlessness?
(c) Have your doctor ever told you that you are suffering from asthma?
(d) Have you ever had an attack of asthma in last 12 months?
(e) Have you ever taken any inhaler, rota haler or nebulisation or oral pills for breathlessness?

Chronic bronchitis was diagnosed by presence of cough with expectoration for three or more months in a year for two or more years assessed by asking

(a) Do you usually cough first thing in the morning?
(b) Do you usually bring up phlegm from your chest first thing in the morning?
(c) Did you had any of the above said problems for most of the morning for at least three consecutive months during last year?
(d) For how many years have you been experiencing the above said problem?

These definitions of bronchial asthma and COPD were validated in the field using physician diagnosis as gold standard in a previous multi-centric study done in India.[4,8] Double data

Table 1 – Age and gender specific prevalence of chronic respiratory diseases (N = 12,556).

Characteristics	Categories	Total number of people in the subgroup	Prevalence of asthma N (%)	Prevalence of chronic bronchitis N (%)	Prevalence of any CRDs N (%)
Age group	15–24 years	2604	44 (1.69%)	15 (0.58%)	116 (4.45%)
	25–34 years	2494	57 (2.28%)	19 (0.76%)	112 (4.49%)
	35–44 years	2459	61 (2.48%)	115 (4.67%)	219(8.90%)
	45–54 years	2085	62 (2.97%)	167 (8.01%)	297 (14.24%)
	55–64 years	1521	66 (4.33%)	224 (14.72%)	321 (21.01%)
	65–74 years	876	43 (4.91%)	154 (17.57%)	203 (23.17%)
	>75 years	517	21 (4.06%)	83 (16.05%)	108 (20.88%)
Gender	Male	6024	147 (2.44%)	406 (6.73%)	678 (11.25%)
	Female	6532	205 (3.14%)	371 (5.67%)	698 (10.68%)

entry was done by data entry operators in Epi Info software and was analysed using SPSS version 15 for Microsoft Windows. Descriptive statistics was done with frequencies and percentages and confidence intervals were calculated.

3. Result

A total of 17,205 people were covered during the survey which included 12,556 people above 15 years. Prevalence of any chronic respiratory disease in adults above 15 years was 10.9% (95% CI 10.35–11.45). Prevalence of self reported asthma was 2.82% (95% CI 2.52–3.12) and that of chronic bronchitis was 6.19% (95% CI 5.76–6.62) while other CRDs which did not fit to either constitute 1.89%. Prevalence of asthma among males was 2.44% (95% CI 2.05–2.85) while that of females was 3.14% (95% CI 2.71–3.57). Chronic bronchitis prevalence was 6.73% and 5.67% among males and females respectively (Table 1).

Among those diagnosed with CRD, 69.9% had received some medical treatment for the same in last year. 20% of those diagnosed to have chronic respiratory diseases reported that they had at least an inpatient admission in the past 12 months. When asked about whether the respiratory symptoms affects their day to day life, 36% of those with CRD strongly agrees, 35.7% agree, 13.1% were not sure and 15.2% disagree. 24.3% of those with CRD reported that they had smoked at least 100 cigarettes/bidis in their life time (48.7% of males and 0.43% of females) and 22.3% among them were current smokers (at least one cigarette/bidi in last month). Among the study participants, 12.9% (5.64% of males and 20.09% of females) reported that they were exposed to passive smoking from somebody else in the house. In the study, 42.29% of those with CRDs (30.34% of males and 53.18% of females) reported that they were usually exposed to the smoke from cooking inside the house using fire wood. 2.25% of the people with CRDs had a past history of TB.

4. Discussion

We used the interviewer administered questionnaire methods for estimating prevalence of chronic respiratory diseases, as is done in many other similar studies. The questionnaire method of assessing prevalence could under or over estimate the disease condition depending on the sensitivity and specificity of the tool and definitions. The questionnaire used for this study was tested for validity and reliability by previous researchers. Cluster of five questions in IUATLD respiratory symptoms questionnaire for differential diagnosis of asthma and COPD had registered a sensitivity of 85.6% and specificity of 91.4%.[9] Reporting and interviewer's bias could affect the estimates. However we have ensured standardisation of interview technique through systematic training. There will be considerable overlap of symptoms of bronchial asthma and COPD and the differentiation would be difficult in many cases even by physicians. For logistic reasons, we could not use spirometer or physician diagnosis. The strengths of our study were its large sample size, good study design, and good sampling strategy in identifying a representative population in the rural area of Kerala state.

Prevalence of self reported asthma was 2.82% and that of chronic bronchitis was 6.19% in the current study. Several estimates of current asthma point to rates ranging from1.2% in Belgium to as high as 25.5% in Australia.[10] The prevalence of asthma was 4.45% and that of chronic bronchitis among those with age more than 35 years was 13.5% in Trivandrum as reported by INSEARCH study.[4] INSEARCH study had estimated a relatively higher prevalence of asthma and COPD at Trivandrum as compared to other 11 centres in India.

With increasing age, due to decline in lung functions and the cumulative increase in exposure to environmental and other risk factors, the burden of chronic respiratory diseases are expected to increase. With a life expectancy of more than 71 years and with a fertility rate of 1.6, Kerala state has an expanding older age group and a shrinking younger age group. According to the 2011 Census, more than 12 per cent of Kerala's population comprises the elderly.[11]

The most important long-term COPD burden-reducing strategy is the control of tobacco smoke exposure. The recent efforts to combat smoking habits by implementing various anti-tobacco legislations and campaigns by Government of India are praiseworthy. The issue of smoking and second-hand smoking still exists and need to be resolved.

There was not much marked difference in the prevalence of CRDs among males and females. This is in contrast to many other studies which reported marked differences in the proportion of prevalence of CRDs among males and females.[12–15] The reasons could be explained by available epidemiologic data which demonstrate that domestic fuel smoke and indoor air pollution as important causes of CRDs in

never smokers. Although LPG is the predominant fuel used for cooking, the practice of using wood for some purposes like boiling water inside the houses is widely prevalent in the area.

A good proportion of individuals with CRD in the community had never smoked. Higher rates of external risk factors, including secondary smoking and indoor air pollution from the burning of biomass fuels could be the reason for CRDs among people without smoking history. Indoor air pollution from domestic fuel combustion could be a significant factor for high prevalence of chronic respiratory diseases. Similarly high prevalence in females highlights the importance to address risk factors other than smoking.

Although India has devised a programme to combat cancer, diabetes, cardio vascular disease and stroke, none have been devised for chronic respiratory illness till date. Considering high prevalence and its contributions to morbidity and mortality, a comprehensive programme to tackle CRDs is needed.

Conflicts of interest

The authors have none to declare.

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Original Article

Isoniazid and rifampicin heteroresistant *Mycobacterium tuberculosis* isolated from tuberculous meningitis patients in India

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ABSTRACT

Background: Heteroresistant *Mycobacterium tuberculosis* (mixture of susceptible and resistant subpopulations) is thought to be a preliminary stage to full resistance and timely detection, initiation of correct treatment is vital for successful anti tubercular therapy. The aim of this study was to detect multi drug resistant (MDR) and heteroresistant *M. tuberculosis* with the associated gene mutations from patients of tuberculous meningitis.

Methods: A total of 197 *M. tuberculosis* isolates from 478 patients of TBM were isolated from July 2012 to July 2015 and subjected to drug susceptibility testing (DST) by BACTEC MGIT and Genotype MTBDR line probe assay (LPA). Heteroresistance was defined as presence of both WT and mutant genes in LPA.

Results: Of 197 *M. tuberculosis* isolates, 11 (5.6%) were MDR, 23 (11.6%), 1 (0.5%) were mono resistant to isoniazid (INH) and rifampicin (RMP) respectively. Heteroresistance was detected in 8 (4%), 2 (1%) isolates to INH and RMP respectively. INH heteroresistant strains had WT bands with mutation band S315T1 whereas RMP heteroresistant strains had WT bands with mutation band S531L.

Conclusion: The prevalence of MDR *M. tuberculosis* was 5.6% in TBM patients with the most common mutation being ΔWT band with S315T1 for INH and ΔWT band with S531T for RMP. MGIT DST was found to be more sensitive for detecting overall resistance in *M. tuberculosis* but inclusion of LPA not only reduced time for early initiation of appropriate treatment but

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also enabled detection of heteroresistance in 8 (4%), 2 (1%) isolates for INH and RMP respectively.

1. Introduction

Tuberculous meningitis (TBM) is a devastating complication of tuberculosis and timely diagnosis and treatment with effective drugs are the key factors for successful management of these patients. Existence of multi drug-resistant (MDR) *Mycobacterium tuberculosis* is well recognized from both pulmonary and extrapulmonary tuberculosis and is posing a great threat to tuberculosis control programs worldwide.[1,2] Besides existence of MDR *M. tuberculosis*, patients with tuberculosis may also harbor both drug susceptible and resistant *M. tuberculosis* subpopulations together, representing a phenomenon called heteroresistance.[3] Heteroresistant *M. tuberculosis* is considered a preliminary stage to full resistance and failure to detect these bacteria may lead to treatment failure or spread of drug resistant bacteria to other patients.[3,4]

Heteroresistance can occur due to two mechanisms, one being acquisition of both susceptible and resistant bacterial populations from a partially treated patient or slow evolution of resistant clones from susceptible clones.[5,6] The prevalence of heteroresistance is largely unknown with few reports of heteroresistance to isoniazid (INH), rifampicin (RMP), ethambutol (EMB), streptomycin (STM) and flouroquinolones from pulmonary isolates.[3–10] This heteroresistance is dependent upon local epidemiology and is particularly common in regions with high rates of tuberculosis, especially drug resistant tuberculosis.[4] India accounts for more than 25% of the world's incident cases of tuberculosis with an estimated 35,400 MDR TB patients among incident TB cases in India.[2]

Numerous methods are available for drug susceptibility testing of *M. tuberculosis* isolates but detection of heteroresistant *M. tuberculosis* is in itself problematic as both the phenotypic and genotypic methods have their own limitations for detecting these strains.[3,5,8–10] A number of genotypic methods have become available for early detection of drug resistance but little is known if they can also detect heteroresistance. Recently, Genotype MTBDR line probe assay (LPA) has been proposed to be capable of detecting the presence of heteroresistance since the strips contain both WT and mutant probes.[3,10] This study was conducted to determine the prevalence of multi drug resistant and heteroresistance *M. tuberculosis* isolated from patients of TBM using Phenotypic Mycobacterial growth indicator tubes (MGIT) in BACTEC 960 and LPA.

2. Materials and methods

2.1. Study design

This prospective multi centric study was conducted from July 2012 to July 2015. CSF sample collected from consecutive patients suspected of tuberculous meningitis (treatment naïve) from four tertiary care institutes of Delhi viz. Department of Neurology, IHBAS, GB Pant Hospital, Guru Teg Bhahdur Hospital, and Chacha Nehru Bal Chikitsalaya, Delhi were subjected to Microbiological processing in Dept of Microbiology at Institute of Human Behaviour and Allied Sciences (IHBAS), Delhi.

Ethical approval for the study was obtained from the respective Institutional ethics committee. Informed written consent was obtained from all patients involved in the study.

2.2. Microbiological diagnosis

A total of 478 CSF samples collected from patients of suspected TBM (fulfilling Marias et al. criterion) were subjected to microscopy and culture in BACTEC MGIT 960 (MGIT 960, Becton Dickinson Systems, sparks, MD) as per standard methods.[12,13] *M. tuberculosis* was isolated from 197 patients and all these isolates were subjected to drug susceptibility testing (DST) by MGIT and Genotype® MTBDR assay (HAIN Life Sciences, Germany).[14,15]

2.3. Drug susceptibility testing (DST)

Phenotypic DST in BACTEC MGIT 960: DST was done by BACTEC MGIT 960 on all the culture isolates using standard critical concentration for INH (0.1 μg/ml), RMP (1 μg/ml), STM (1 μg/ml) and ETB (5 μg/ml) along with a 1/100 diluted control grown in drug free medium as per FDA-approved method.[14]

GenoType MTBDR*plus* assay: LPA was done as per manufacturer's instructions. Susceptibility to anti TB drugs was defined as presence of all wild type (WT) probes and no mutant probes. The absence of any of the wild-type bands and/or presence of any mutation band implies resistance to the respective drug. Heteroresistance was defined as presence of both WT and mutant gene probe.

Quality assurance: Five percent of the isolates from the study were sent to the National Tuberculosis Research Institute (National reference laboratory) for phenotypic drug susceptibility testing by BACTEC MGIT 960 for external quality assurance. For line probe assay positive (H37RV), negative and master mix controls were run in every assay.

Data analysis: Drug susceptibility test results of BACTEC MGIT and LPA were entered into spread sheet and compared manually for concordance, discordance. All the discordant results were repeat tested to rule out any technical error. Descriptive statistics were used to study the patterns of INH and RIF in *M. tuberculosis* isolates.

3. Results

Anti tubercular drug resistance: A total of 197 *M. tuberculosis* strains were isolated from 478 patients suspected of TBM in

Table 1 – Results of drug susceptibility testing by BACTEC MGIT 960 and LPA (n = 197).

Resistance	BACTEC MGIT 960		LPA	
	No	%	No	%
INH + RMP	7	(3.5)	8	(4.0)
Only INH	23	(11.6)	22	(11.1)
Only RIF	1	(0.5)	–	–
Only STM	10	(5.1)	ND	ND
Only ETH	3	(1.5)	ND	ND
INH + STM	2	(1.1)	ND	ND
INH + RMP + STM	1	(0.5)	ND	ND
INH + RMP + ETH	2	(1.1)	ND	ND
INH + RMP + STM + ETH	1	(0.5)	ND	ND

INH: isoniazid; RIF: rifampicin; STM: streptomycin; ETH: ethambutol.

the study. Of these 197 isolates, 11 (5.5%) were MDR TB, 23 isolates had mono resistance to INH, 2 isolates had resistance to INH and STM and only 1 isolate was mono resistant to RMP by BACTEC MGIT 960. By LPA, only 8 (4%) strains were found to be MDR TB (Resistant to INH and RMP) while 22 isolates (11.1%) revealed mono resistance to INH and there was no isolate with mono resistance to RMP. Table 1 summarizes the anti tubercular resistance pattern of all the isolates.

Eight isolates were detected heteroresistant to INH with 2 isolates hetero resistant to RMP. Of the 8 INH heteroresistant isolates 6 were detected resistant by BACTEC MGIT assay whereas 2 were identified as sensitive by MGIT. Both the rifampicin heteroresistant isolates were also detected resistant by BACTEC MGIT 960.

3.1. *Comparison of BACTEC MGIT 960 and LPA*

Out of 36 INH-resistant isolates by MGIT, 26 isolates were detected resistant to INH by LPA whereas 10 were detected as sensitive. Additionally 4 isolates resistant to INH by LPA were found sensitive by MGIT 960. Of the 12 RMP resistant isolates by BACTEC MGIT, 8 were found to be resistant by LPA but 4 were found to be sensitive. Thus out of 18 discordant results, discordance in INH susceptibility pattern was seen in 14 and discordance in RMP susceptibility pattern was seen in 4 isolates. One of these isolate had discrepant results for both INH and RMP (thus discrepancy in 17 *M. tuberculosis* isolates). Table 2 depicts the isolates with discrepant results.

3.2. *INH and RMP associated gene mutations by LPA*

Among 26 INH resistant isolates by both phenotypic and genotypic method, 17 (65.3%) were due to failing of katG WT gene with hybridization of katG MUT probe (s315T1 substitution), 6 were heteroresistant (Presence of WT bands with mutation band S315T1), 2 were due to failing of inhA WT1 gene with mutation band for C15T substitution. There was only one strain which has deletion of kat G WT gene. Out of 4 phenotypic sensitive and genotypic resistant isolates, 2 isolates had heteroresistance (Presence of WT bands with mutation band S315T1) whereas 2 depicted low level resistance to inh A (Table 3).

Table 2 – Discordant results in anti-tubercular drug resistance pattern with FDA approved method (BACTEC MGIT 960) and line probe assay.

DRUG	BACTEC	LPA	Mutation band in LPA
INH			
Strain no.			
1832	**S**	R	C15T
1922	**S**	R	ΔWT, S315T1
1913	R	S	–
2160	R	S	–
2472	R	S	–
2656	R	S	–
2948	S	R	WT, S315T1
3478	S	R	WT, S315T1
3608	R	S	–
3697	R	S	–
3892	R	S	–
3870	R	S	–
3941	R	S	–
3947	R	S	–
RMP			
Strain no.			
3478	R	S	–
3842	R	S	–
3844	R	S	–
3947	R	S	–

R: resistant; S: sensitive; WT: wild type present; ΔWT: wild type absent.

Out of 8 RIF resistant isolates by both phenotypic and genotypic method, three strains were due to failing of only WT8 band in 81 bp region of rpo gene, 2 isolates had deletion WT8 band with mutation in rpoB S531L (MUT 3 band), 1 isolate had deletion of WT8 and WT6 band and two strains were hetero resistance (presence of WT bands with mutation band rpoB S531L).

4. Discussion

The prevalence of primary MDR (resistance to INH and RMP) in *M. tuberculosis* isolates from patients of TBM was found to be 5.6% with additional INH resistance of the order of 18% considering phenotypic method as gold standard for detection of anti tubercular drug resistance. In India, the drug resistance pattern of *M. tuberculosis* isolated from tuberculous meningitis is not very well documented.[16–18] A study published in 2008 from National Institute of Mental Health and Neurosciences has shown prevalence of MDR in tuberculous meningitis patients to be 2.4% with mono resistance to INH of 12.5%. Baweja et al. in 2008 showed prevalence of MDR in tuberculous meningitis patients to be 18%.[15,16]

There was 91.3% concordance for detection of drug susceptibility/resistance in *M. tuberculosis* culture by the automated liquid culture-based system and LPA, suggesting that that most of the mutations conferring INH/RIF resistance in *M. tuberculosis* central nervous system isolates are incorporated in LPA.[18,19] Of the 14 discordant results for INH resistance, 10 isolates were phenotypic resistant but genotypic sensitive indicating some unidentified mutations in some other genomic regions (like ahpc, kasA, furA) not targeted by

Table 3 – Frequency and pattern of rpoB, katG, and inhA mutations in drug resistant *Mycobacterium tuberculosis* isolated from tuberculous meningitis patients by LPA.

Rifampicin				Isoniazid			
ΔWT	Rpob gene mutation	Codon	No of isolates	ΔWT probe	INH gene mutations KatG/inhA	Codon	No of isolates
WT 8	Nil	530–533	3	WT (katG)	MUT1 (katG)	315 S315T1	17
WT 8	MUT 3	S531L	2	–	MUT1 (katG)	S315T1	8
–	MUT 3	S531L	2	WT1 (inhA)	MUT1 (inhA)	15 C15T	4
WT8 WT 6	Nil	518–525 530–533	1	WT (katG)	Nil	315	1

ΔWT: deletion wild type.

this assay.[19,20] Four isolates were phenotypic sensitive but resistant by line probe assay. The reasons for discordance in these 4 isolates may be (1) low level resistance by detected LPA ($n = 1$) which could have been missed in phenotypic assay because of the use of higher concentrations of INH in phenotypic assay inhibiting the growth of *M. tuberculosis*, (2) presence of hetero resistance ($n = 2$) and failure of phenotypic assay to detect lesser number of resistant bacteria and (3) adaptation of the strain preventing phenotypic expression of Kat G mutation ($n = 1$). For rifampicin, 4 isolates were found resistant by BACTEC MGIT but sensitive by LPA and which could be due to some other mutations outside the 81 bp region of the *rpoB* gene not targeted by this assay.

To the best of our knowledge this the first study to report problem of heteroresistance in *M. tuberculosis* strains isolated from patients of TBM in India. Hetero resistance was detected in 8 (4%), 2 (1%) isolates for INH and RMP respectively. The prevalence of heteroresistance has been reported to vary from 9 to 20% in *M. tuberculosis* isolated from clinical samples.[7,21] In India, heteroresistant to INH and RMP has been reported in *M. tuberculosis* isolated from pulmonary specimens varying from 9.8% to 28.8%.[10,11] Heteroresistance most often results due to slow evolution of bacteria from a sensitive to resistant profile during treatment. This possibility is quite remote in the present setting as our patients were drug naïve and *M. tuberculosis* was isolated before initiation of anti tubercular treatment. This heteroresistance could have arisen due to transmission of both susceptible and resistant bacterial population from patients harboring mixed population during treatment or due to super infection in a chronically infected patient which is quite a possibility in TB endemic countries like India.

Out of 8 INH hetero resistance strains, 6 were detected resistant by both MGIT and LPA whereas 2 isolates were detected sensitive by BACTEC MGIT but resistant by LPA. The reason for discordance in the two phenotypic sensitive but genotypic resistant isolates could be masked expression of phenotypic resistance in presence of predominant sensitive bacterial population However there were 10, 4 isolates which were detected resistant to INH and RMP respectively by phenotypic assay but sensitive by LPA. These phenotypic resistant but genotypic sensitive isolates could have some other mutation outside the regions targeted by line probe assay but the presence of heteroresistance not detectable by LPA cannot be definitely ruled out as some of the reports have also shown that molecular tests cannot detect resistant bacteria if they are less than 5% in mixed population of susceptible and resistant population.[3] Thus both phenotypic and genotypic methods are needed to detect any type of resistance prevalent in *M. tuberculosis* isolates for treatment decisions.

5. Conclusion

The prevalence of primary MDR (resistance to INH and RIF) in *M. tuberculosis* isolates from patients of TBM was found to be 5.6% with heteroresistance to INH and rifampicin of 4%, 1% respectively. MGIT DST was found to be more sensitive for detecting overall resistance in *M. tuberculosis* but inclusion of Genotype MTBDR assay not only reduced time for early initiation of appropriate treatment but also enabled detection of hetero resistance.

Conflicts of interest

The authors have none to declare.

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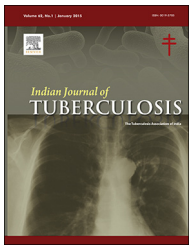

Original article

Amplification of Hsp 65 gene and usage of restriction endonuclease for identification of non tuberculous rapid grower mycobacterium

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ABSTRACT

Background: The rapid grower mycobacteria have emerged as significant group of human pathogen amongst the Runyon group IV organisms that are capable of causing infection in both the healthy and immunocompromised hosts. Study aimed to identification of species amongst rapid grower non tuberculous mycobacterial isolates by polymerase chain reaction – restriction enzyme analysis (PRA). Analysis and comparison of results with standard biochemical tests.
Methods: Rapid grower non tuberculous mycobacteria had been collected from liquid culture section during the study period. All isolates were identified by conventional biochemical tests. A 441 bp fragment of hsp65 genes was amplified and digested by two restriction enzymes, BstEII and HaeIII. Digested products were analyzed using polyacrilamid gel electrophoresis (PAGE).
Results: During study, 121 rapid grower mycobacterial isolates were subjected for species identification. Isolates were obtained from pulmonary samples (72) and extrapulmonary samples (49). In the PRA test 8 different types of rapid grower mycobacteria were identified after analyzing the fragments generated through restriction enzymes. *Mycobacterium chelonae* (57/121) was the most common isolate in pulmonary and extrapulmonary samples. *Mycobacterium fortuitum* (42), *Mycobacterium abscessus* (11), *Mycobacterium immunogen* (06), *Mycobacterium peregrinum* (02), *Mycobacterium smegmatis* (01), *Mycobacterium wolinskyi* (01), *Mycobacterium goodii* (01) were identified as other species of rapid grower non tuberculous mycobacteria.
Conclusion: PRA is a rapid and accurate system for the identification of species of non tuberculous mycobacteria. Results of PRA and biochemical tests are concordant up to 98%.

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1. Introduction

India is an agriculture based economy, where majority of rural people are exposed to soil, water, plants and livestock constantly. These are potential source of nontuberculous mycobacterium infection. The importance of several mycobacterial species other than the mycobacterium tuberculosis complex has increased in the recent past. It is now well recognized that nontuberculous mycobacteria (NTM) too cause pulmonary infection, lymph node infection, brain, bone, kidney, genital tract and skin/soft tissue infections in human beings. The guidelines for diagnosis, treatment and doses have been laid down by American Thoracic Society (ATS) and Infectious Disease Society of America (IDSA). The antibiotic susceptibility patterns and drug treatment are different from mycobacterial tubercular infections.[1,2] Therefore, there is a growing need to identify mycobacteria to the species level especially in tertiary care TB & chest referral centres. Analysis of PCR products has been the recent focus of considerable interest for separation of mycobacteria. Practice of molecular methods, polymerase chain reaction–restriction enzyme analysis (PRA) test has been considered as promising method for species identification of mycobacterium. Use of restriction enzyme Bst II and Hae III and analysis of the enzymatically digested amplified 65-kDa heat shock protein-encoding gene (hsp65) has been successfully applied by several researchers for the identification of mycobacterial species.[3–5] This method is simple, rapid, sensitive, time and labor saving than traditional biochemical methods.[6,7]

Rapid identification of each species is vital for prescribing the specific treatment regimen for each species of Mycobacteria.[8,9]

The aim of the present study was to evaluate the PRA method and to extend it to other mycobacterial species, especially rapidly growing nonchromogenic mycobacteria, often found in tap water and laboratory environment. Their multiple drug resistance nature is significant clinically.

2. Materials and methods

The study was conducted during October 2013–September 2014. Twice NTM positive samples were collected from the liquid culture section. The mucolytic N-acetyl L cysteine, sodium hydroxide (NALC-NaOH) treated samples were inoculated in BBL 7 ml MGIT media and placed in MGIT-960 system for automatic reading and hourly recording. The system indicts positive culture tubes, on reading >100 growth units. These were checked for acid fast bacilli by Ziehl Neelsen smear examination and further identification was done by immunochromatographic assay and PNB test. In case, two samples of same patient were found positive for NTM, they were further subjected for species identification. The identified NTM were divided into two groups on the basis of growth rate on solid Lowenstein Jensen media and examining the media daily for one week. The mycobacterium which grew within a week were grouped as 'rapid grower' and those which grew in more than a week were grouped as 'slow grower' mycobacterium. Species amongst the rapid grower mycobacterium was further identified by molecular method; PRA of a segment of the genes encoding 439-bp portion of the mycobacterial 65-kDa heat shock protein.

2.1. Sample preparation

A loopful of growth from the solid media transferred into 500 μl of Tris–EDTA (TE) buffer and centrifuge for 5 min at 14,000 rpm. Supernatant had been removed and was again centrifuged after adding 250 μl of Tris–EDTA buffer for 5 min at 14,000 rpm. The pellet was used for DNA extraction.[5] When growth was in liquid culture 500 μl of well vortexed sample was centrifuged for 5 min at 14,000 rpm. The pellet was re-suspended in 100 μl TE buffer.

2.2. DNA extraction

DNA was extracted by adding 250 μl of 5% of Chelex®-100 reagent in the concentrated bacterial suspension. Tubes were incubated in the 60 °C water bath for 10 min. Vortex the tubes for 15–20 s and then incubated it at 100 °C waterbath for 15 min. Again it was vortexed for 10–15 s and centrifuged for 3 min at 14,000 rpm supernatant was discarded and pellets were dissolved in 250–300 μl TE buffer and was used for PCR amplification.[5]

2.3. PCR amplification

A 50 μL PCR was set up by mixing 1× assay buffer, dNTP mix, primers TB11 Forward: 5′-ACCAACGATGGTGTGTCCAT-3′ and TB12 (Reverse: 5′-CTTGTCGAACCGCATACCCT-3′) and extracted DNA to each reaction tube. The composition of the PCR mixture (50 μL) was 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 10% glycerol, 200 μM (each) deoxynucleoside triphosphate, 0.5 μM (each) primer, and 1.25 U of Taq polymerase (Genex Bioscience, India). The reaction was subjected to 45 cycles of amplification in a thermocycler by using following program; initial denaturation for 1 min at 94 °C, annealing at 1 min at 60 °C and extension for 1 min at 72 °C this was followed by 10 min of extension at 72 °C.[5]

2.4. Contamination precautions

In order to avoid contamination, different steps like sample processing, reagent preparation, amplification and detection were done in a separate room.

2.5. Restriction analysis

For BstEII digestion, 10 μl of PCR product was added directly to a mixture containing 0.5 μl of 5 U of enzyme and 2.5 μl of restriction buffer. Mixture was incubated for 60 min at 60 °C. For Hae III digestion, 10 μl of PCR product was added directly to a mixture containing 0.5 μl of 5 U of enzyme and 2.5 μl of restriction buffer. Mixture was incubated for 60 min at 37 °C.

2.6. Evaluation of restriction patterns

After digestion, 4 μl of gel loading buffer (0.25% bromophenol blue) was added, and 10 μl of PCR product was loaded onto a 3%

agarose gel (Sigma Bioscience). Fragments were visualized by ethidium bromide staining and UV light. For interpretation of PRA patterns we designed a computerized work. The image was analyzed using a gel documentation system and compared with the standard pattern to identify the mycobacterium species as described by Telenti et al.[5] For quality control of the experiment, each batch was tested with a positive control (pure mycobacterial DNA of H37Rv) and a negative control (deionized water). Fragment band size was estimated using 100 base pair ladder (Sigma). Restriction fragment smaller than 50 bp was ignored in the results to avoid confusion with primers band. The species were identified by matching the band patterns as described by Telnati et al. and PRASITE Web site http://app.chuv.ch/prasite/index.html.chuv. The results were analyzed and compared with standard biochemical test.[5,6]

3. Results

In this study, total 121 species of rapidly growing mycobacteria were analyzed (Table 1). These were isolated from different clinical samples amongst which sputum (72) was most common, followed by lymph node aspirate (18), pleural fluid (11), skin (5), tracheal aspirate (3), ascitic fluid (3), CVP tip (4) and pus (5) (Table 2). DNA from the isolates was amplified using primers specific for hsp65 gene (Fig. 1). All the species showed distinctive band patterns for Bst II and Hae III enzymes except *Mycobacterium goodii* and *Mycobacterium wolinskyi*. The band size with Bst digestion ranged between 80 and 325 bp. Similarly, in Hae digestion band size ranged between 55 and 210 bp. By using 100 base pair ladder, the base pair were calculated by its proximity to neighboring base pair size. In total 12 different band patterns were seen following the Bst EII enzyme digestion and 14 different band sizes were seen with Hae III restriction enzyme (Fig. 2). With the help of 26 different band patterns, the rapid growers were divided into 8 species (Table 3). The most common species in restriction enzyme analysis was *Mycobacterium chelonae* (57) (Table 2). All the chelonae strains showed same restriction pattern of 325,140 and 125 bp with Bst enzyme and only one band size of 210 bp with Hae restriction (Table 1). The growth characteristic, growth rate and other biochemical reactions were not confirmed as *Mycobacterium abscessus* as the colonies were small pinpoint and visible growth was observed on 9th day of incubation. But in restriction enzyme analysis, band pattern clearly indicated the growth as *M. abscess* showing band 235,210 bp with Bst digestion and 145,706,055 bp after Hae digestion. Similarly, *M. goodii* was not confirmed in biochemical identification was identified as *M. goodii* in restriction enzyme analysis. During the study, *M. chelonae* species was identified as the most common isolate in pulmonary as well as extrapulmonary samples.

Table 1 – Laboratory phenotypic features of the clinically important species of rapidly growing mycobacterium.

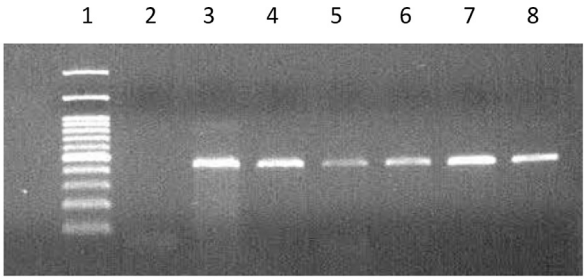
Sl. no.	Mycobacterial species	Pigment	Arylsul-3 days	MAC with no CV	Nitrate	Iron uptake	5% NaCl	Growth on		
								Sod citrate	Mannitol	Inositol
1	*M. chelona*	–	+	+	–	–	–	–	–	–
2	*M. fortuitum*	–	+	+	+	+	+	–	–	–
3	*M. peregrinum*	–	+	+	+	+	+	–	+	–
4	*M. abscessus*	–	+	+	–	–	+	–	–	–
5	*M. immunogenicum*	–	+	–	–	–	–	–	–	–
6	*M. smegmatis*	+	–	+	+	+	+	+	+	+
7	*M. wolenskii*	–	–	+	+	+	+	+	+	+
8	*M. goodii*	–	–	+	+	+	+	+	+	+

M. chelonae; *Mycobacterium chelonae*, *M. fortuitum*; *Mycobacterium fortuitum*, *M. peregrinum*; *Mycobacterium peregrinum*, *M. abscessus*; *Mycobacterium abscessus*, *M. immunogenicum*; *Mycobacterium immunogenicum*, *M. smegmatis*; *Mycobacterium smegmatis*, *M. wolenskii*; *Mycobacterium wolenskii*, *M. goodii*; *Mycobacterium goodii*, MAC; MacConkey, CV; Crystal Violet, Sod citrate; sodium citrate, Arylsul; aylsulfatase, NaCl; sodium chloride.

Table 2 – Species identification and isolation sources of 121 rapidly growing mycobacteria.

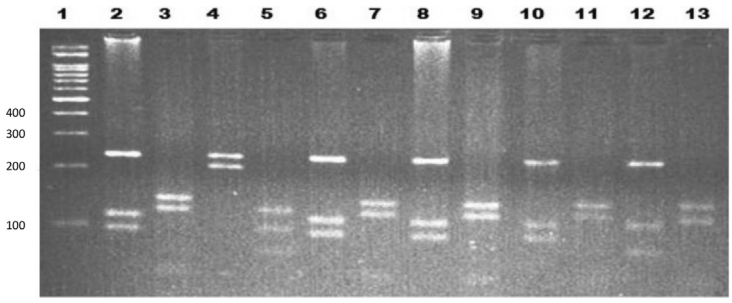
Sl. no.	Species	Sources							
		Sputum	Lymph node	Pleural fluid	Skin	Tracheal aspirate	Ascetic fluid	CVP tip	Pus
1	*M. chelonae*(57)	35	11	06	01	01	01	01	01
2	*M. fortuitum*(42)	28	04	03	02	02	01	01	01
3	*M. abscessus*(11)	05	02	02	01	–	–	–	01
4	*M. immunogenum* (06)	03	01	–	–	–	01	–	01
5	*M. peregrinum* (02)	01	–	–	01	–	–	–	–
6	*M. smegmatis* (01)	–	–	–	–	–	–	01	–
7	*M. wolinskyi* (01)	–	–	–	–	–	–	01	–
8	*M. goodii* (01)	–	–	–	–	–	–	–	01

M. chelonae; *Mycobacterium chelonae*, *M. fortuitum*; *Mycobacterium fortuitum*, *M. peregrinum*; *Mycobacterium peregrinum*, *M. abscessus*; *Mycobacterium abscessus*, *M. immunogenicum*; *Mycobacterium immunogenicum*, *M. smegmatis*; *Mycobacterium smegmatis*, *M. wolenskii*; *Mycobacterium wolenskii*, *M. goodii*; *Mycobacterium goodie*.
CVP; central venous pressure.



Lane 1 molecular marker; lane 2; negative control; lane 3 to 8 amplified product of Hsp65 gene of 439 bp

Fig. 1 – Amplification product of *Mycobacterium* species of Hsp-65 gene of rapidly growing mycobacteria.



Lane 1 ; DNA marker
Lane2,3; Bst: 80, 125,245, Hae; 135, 155 M.fortuitum
Lane 4,5; Bst: 220, 245, Hae 60,160 M.chelonae
Lane 6,7 ; Bst: 80,125, Hae; 135,150 M. fortuitum variant
Lane 8,9; Bst: 110, 125, 245 Hae; 120,140 M.gordonae
Lane 10,11; Bst: 85,125 Hae; 135,150 M.fortuitum variant
Lane 12,13; Bst: 85,245 Hae; 135,150 M.abscessus

Fig. 2 – Restriction enzyme analysis of rapidly growing mycobacteria.

Table 3 – Restriction enzyme analysis for identification of nontuberculous mycobacterium.

Sl. no	Molecular sizes (bp)		Organisms identified	Total no (%)
	BstE11	Hae111		
1	325,140,125	210	*M. chelonae*	57 (47)
2	245,220	155,145,95	*M. fortuitum*	42 (34.7)
10	235,210	145,70,60	*M. abscessus*	11 (9.1)
4	320,130	145,70,60,55	*M. immunogen*	06 (5)
5	230,120,80	140,120,50	*M. peregrinum* (02)	02 (1.7)
6	245,140,85	160,130	*M. smegmatis*	01 (0.8)
7	235,130,85	140,125,60	*M. wolinskyi*	01 (0.8)
8	235,130,85	145,125,60	*M. goodii*	01 (0.8)
Total				121

M. chelonae; *Mycobacterium chelonae*, *M. fortuitum*; *Mycobacterium fortuitum*, *M. peregrinum*; *Mycobacterium peregrinum*, *M. abscessus*; *Mycobacterium abscessus*, *M. immunogenicum*; *Mycobacterium immunogenicum*, *M. smegmatis*; *Mycobacterium smegmatis*, *M. wolenskii*; *Mycobacterium wolenskii*, *M. goodii*; *Mycobacterium goodie*.

4. Discussion

In this study, the highly conserved hsp-65 gene was targeted for amplifications. It has a high discriminating power to differentiate species of Mycobacteria as described earlier by Buchanan et al.[10] The restriction enzyme analysis of a 65-kDa heat shock protein (Hsp gene-439-bp) fragment is highly effective for differentiating mycobacteria to the species level. The results of amplification of hsp65 gene followed by restriction enzyme analysis of rapid grower mycobacterium

helped in discriminating 8 different types of rapid grower mycobacterium which were well correlated with biochemical identification results similar to Ringuet et al.[11] Differentiation between *M. chelonae* and *M. abscessus* by biochemical method is a difficult task, hence PRA test was used for rapid identification. Steingrube et al. has also discussed that Bst II, Hae III along with other enzymes AciI and CfoI gave the best separation of rapidly growing mycobacteria.[12] Their study showed that 60% of all RGM taxa studied were differentiated by HaeIII digests alone. All species studied by PRA test were readily discriminated from each other. *Mycobacterium fortuitum* and *Mycobacterium smegmatis* showing highest degree of similarity, were also identified by this test.

In our study, 8 different types of RGM species were identified through the similar way as discussed by Telenti et al.[5] and Plikaytis et al.[13] The study indicates that application of PCR-based methodology is useful at program level in order to differentiate the NTM within short span of time. With the introduction of liquid culture system for diagnosis of tuberculosis at national level, rapid growing mycobacterium are common isolates in clinical samples in many laboratories. PRA test is particularly useful in identifying mycobacteria when biochemical identification methods fail to differentiate the closely related species. The restriction enzyme digestion patterns obtained in the present study provides species separation similar to those described by Telenti et al.[5] with the exception of few band size measurements. Also, the routine recommendations to prevent PCR-linked contamination help to avoid false identification. We used separate manipulation rooms for reagent preparation, specimen preparation, amplification and detection. Even specimen centrifugations were performed in a separate aerosol-free area (distinct from the above three areas).

Among other rapid diagnostic methods; HPLC analysis of mycolic acids differentiates most of the *Mycobactenium* species; however, it is somewhat limited by the requirements of considerable expertise to interpret the chromatographs, and $>10^6$ bacteria are required to generate a reliable pattern and need expensive equipment which may not be available in a basic clinical microbiology laboratory.[14] The kit based tests, such as Accu Probe assay (Gen-Probe), INNO-LiPA line probe assay (Innogenetics, Ghent, Belgium) or GenoType assay (Hain Lifescience, Nehren, Germany) are costly and not readily available.[15] DNA sequencing is robust and accurate, though time-consuming and laborious.[16,17]

5. Conclusion

The study concluded that restriction enzyme analysis for identification of rapid grower mycobacterium is simple, sensitive, rapid and labor-saving, easy to do daily. It should not be hard to implement this system in reference laboratories, who would be enabled to identify species of clinical isolates of RGM within a short span of 1 or 2 working days. *M. chelonae* & *M. fortuitum* constitute 87% of total rapid growers and M. chelonae is the most common isolate in pulmonary as well as extrapulmonary specimen. Focus on the members of the rapidly growing mycobacteria will help in tackling the emerging importance of mycobacterial species in cases of sporadic infection or outbreaks. During the study, it was observed that the test lacked in analyzing and reporting the result with 5–10 bp band difference.

Conflicts of interest

The authors have none to declare.

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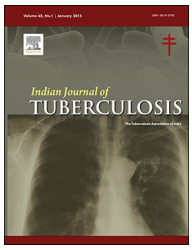

Original Article

Initial airflow obstruction in new cases of pulmonary tuberculosis: Complication, comorbidity or missed?

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ABSTRACT

Tuberculosis (TB) may have a similar spirometry findings as a chronic obstructive pulmonary disease but the prevalence of TB-induced airflow obstruction (AO) is still unknown.
Objectives: To measure frequency of AO in new TB cases at the beginning of treatment and to evaluate factors associated with obstructive abnormalities following TB diagnosis.
Materials and Methods: 317 patients that have no history of prior AO were recruited into the study with a median age of 39.0 years (IQR, 30.0–49.0). AO was defined using the $FEV_1/F(VC) <$ LLN.
Results: AO was detected in 29.97% (95/317) new TB cases. These patients had a more severe clinical manifestation of TB with a greater likelihood of cough, OR = 5.47 (95%CI 1.90–15.70) and wheezing, OR = 10.51 (95%CI 5.72–19.27), $p < 0.001$. The frequency of AO was positively associated with bronchoscopic evidence of narrowing of the main airways. Furthermore, from multiple logistic regression analysis we would assume that higher FEV_1 value in TB patients with AO was related to greater BMI and inversely associated with older age, female sex and radiographic extent ($p < 0.05$).
Conclusions: Obstructive pattern on spirometry frequently occurs in new TB cases without previously detected AO. This category of patients should be targeted for detailed follow-up, particularly, in high TB burden countries.

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1. Introduction

Tuberculosis (TB) is a growing problem in Ukraine because existing military conflict adds to the pre-existing challenges, such as a high rate of drug resistance and human immunodeficiency virus (HIV) co-infection.[1] However, TB case detection based on annual chest radiology rather than sputum smear microscopy (in 2015 there were only 2.6% TB cases identified by finding acid-fast bacilli (AFB) in primary health care)[27] leads to a delay in diagnosis with extensive lung lesions. Unfortunately, pulmonary dysfunction is a significant obstacle in achieving a desirable treatment outcome among TB patients.[12–14]

Airflow obstruction (AO) associated with active TB is often missed in routine practice.[3,7,33] AO may prolong sputum conversion time and delay healing of lung cavities,[9,14,35] despite effective TB treatment usually minimizes a restrictive ventilatory defect.[31] The prevalence of an obstructive abnormality (heterogeneous definitions) varies between 12.5 and 88.2% among different categories of TB patients.[2,35] Some authors considered airflow limitation as a ''red flag'' diagnostic tool for chronic obstructive pulmonary disease (COPD),[9,19] although others highlight active TB as an independent etiology of this phenomenon.[10,20,22,35] Nevertheless, discrepancies in study design and characteristics of selected participants, including sequelae of previous treatment[16] as well as coexistence of other diseases (HIV,[33] bronchial asthma (BA),[12] bronchiectasis[18] etc.), complicate estimates of the rate of AO among newly diagnosed TB patients.

Thus, the aim of the present study was to determine the frequency of initial AO among patients with new cases of pulmonary TB and to evaluate factors associated with obstructive abnormalities following TB diagnosis.

2. Study population and methods

2.1. Study design and participants

The present prospective cross-sectional study was carried out at the Regional Tuberculosis Dispensary in Vinnytsia from August 2007 to March 2012. Out of 2226 consecutively admitted patients aged 18 years or older with new cases of pulmonary tuberculosis, 352 (15.8%) were randomly selected and invited to participate in this study.

Inclusion criteria: 1) patients above 17 years of age with confirmed (culture positive) new case of pulmonary tuberculosis (a case never having previously received drug treatment for active TB or having received anti-TB drugs for less than one month); 2) at the time of spirometry test all participants could take anti-tuberculosis treatment, but not longer than one week.

Patients with any of the following conditions were *excluded*: 1) ever diagnosed with COPD, BA, bronchiectasis; 2) non-consenting patients; 3) ongoing treatment with β-blockers or corticosteroids; 4) pregnancy; 5) radiological evidence of lung pathology other than TB; 6) lack of cooperation; 7) technical difficulties; 8) mental or physical inability to perform the pulmonary function testing; 9) experience of smoking ≥10 pack/years; 10) intense/prolonged occupational exposure to noxious particles or gases; 11) exacerbation of allergic diseases; 12) HIV-positive patients.

Post-randomization exclusion of non-eligible patients ($n = 35$) was performed due to the following reasons: study personnel errors, $n = 4$; COPD, $n = 10$; bronchiectasis, $n = 1$; BA, $n = 3$; lung cancer/metastases, $n = 2$; ongoing treatment with corticosteroids, $n = 1$; allergy, $n = 2$; poor efforts during spirometry, $n = 4$; informed refusal patients, $n = 5$; HIV-positive individuals, $n = 3$.

The median age of the subjects ($n = 317$) was 39.0 years (IQR, 30.0–49.0). Comparative analysis of demographic characteristics between participants and adult population with new cases of pulmonary TB is shown in Table 1. Population data from 2010 was preferred for comparison as a midpoint of our study duration (2007–2013).

This study was approved by the Bioethics Committee at the National Pirogov Memorial Medical University of Vinnytsia and all participants gave written informed consent.

2.2. Methods

All patients underwent a standard evaluation that included complains, history, physical examination, chest radiography (CXR), laboratory investigations and lung function study.

2.3. Pulmonary function tests (PFTs)

Spirometry was performed and interpreted according to American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force on pulmonary function standards.[26,30] Measurements of forced expiratory volume in one second (FEV_1), vital capacity (VC), forced vital capacity (FVC) and forced expiratory flow between 25% and 75% of the FVC ($FEF_{25–75\%}$) were made using a portable Microlab Spiro (version 1.32, Rochester, UK) following the valid reference values of the European Community for Steel and Coal (ECCS). Pulmonary function tests (PFTs) were done in sitting position by qualified technologist under the direct supervision of the principal investigator.

Airflow obstruction was defined using the FEV_1/F(VC) ratio of less than the lower limit of normal (LLN) for relevant healthy population. The baseline VC or FVC has been chosen as a preferred parameter for diagnostic ratio calculating whichever was larger.[23] We analyzed flow-volume loop configurations to suspect predominant occurrence of the airway obstruction. Post-bronchodilator testing was performed if baseline spirometry showed an obstructive pattern. Significant reversibility was determined if after inhalation of 400 mcg salbutamol (four separate doses with 30-s intervals) and 15 min re-measurement - per cent/absolute changes in FEV_1 and/or FVC ≥12% and 200 ml compared with baseline values.[26]

Mouthpiece and transducer were cleaned and disinfected between patients to prevent the transmission of infection via direct contact with biological fluids.

2.4. Flexible fiberoptic bronchoscopy

Flexible fiberoptic bronchoscopy (FB) was performed in the procedure room via the oral route (Olympus; BF-PE2 or BF-TE2; Japan). There were standard indications: cough or

Table 1 – Characteristics of participants (n = 317) and adult population with new cases of pulmonary tuberculosis* (n = 30,314) in Ukraine.

Characteristics	Participants (n = 317)	Population (n = 30,314)	p-value
Male, n (%)	236 (74.4)	21,039 (69.4)	
Female, n (%)	81 (25.6)	9275 (30.6)	0.0545**
Rural residence, n (%)	123 (38.8)	10,358 (34.2)#	0.086**
Current smokers, n (%)	110 (34.7)	not available	
Ex-smokers, n (%)	19 (6.0)	not available	
Age distribution yrs., n (%)			
18–24	34 (10.7)	3290 (10.9)	
25–34	72 (22.7)	8161 (26.9)	
35–44	87 (27.4)	7402 (24.4)	
45–54	69 (21.8)	5868 (19.4)	
55–64	36 (11.4)	3181 (10.5)	
≥65	19 (6.0)	2412 (8.0)	0. 93,624##

* Ministry of Health report.[30]
\# Available data from mixed (adults + children) population with new TB cases.
** 2-sample z-test;
\## Mann–Whitney U test.

breathlessness unexplained due to the radiologic abnormalities (clinical suspicion of bronchial involvement), diffuse lung process on the CXR, recurrent hemoptysis, unexplained hoarseness, smear-negative cases (bacteriological confirmation of diagnosis), abrupt changes in the amount of sputum etc. More than half of the study participants have refused the FB through a fear of the discomfort during this procedure either they found the FB unnecessary or intolerable.

2.5. *Statistical analysis*

Data were analyzed with the use of statistical software SPSS V.20 and GraphPad Prism V.6 for Windows. We assessed the normality of the distribution by histogram and Shapiro-Wilks W test. Mean with 95% confidence interval (CI) and median with 25th–75th percentile (inter-quartile range (IQR)) presented normally and non-normally distributed variables, as appropriate. Multivariable logistic regression model was used to evaluate the independent predictors of airflow obstruction on spirometry. We rejected ''the null hypothesis'' if p-value was less than the threshold (0.05).

3. Results

Airflow obstruction has been detected in 29.97% (95/317) hospitalised patients with new pulmonary TB. The frequency of complaints and auscultatory findings accompanying with AO (FEV_1/F(VC) ratio below LLN) are given in Table 2. We also analyzed the differences between the probability of clinical sings happening among patients with AO and subjects without an obstructive pattern on spirometry (Fig. 1). Thus, odds ratio

Table 2 – Percentages of clinical signs and auscultatory findings combined with AO.

Findings	% of subjects, n = 95
Cough	95.8 (91)
Dyspnea	84.2 (80)
Wheezes	83.2 (79)
Fever	71.6 (68)
Loss of appetite	50.5 (48)
Weakness	46.3 (44)

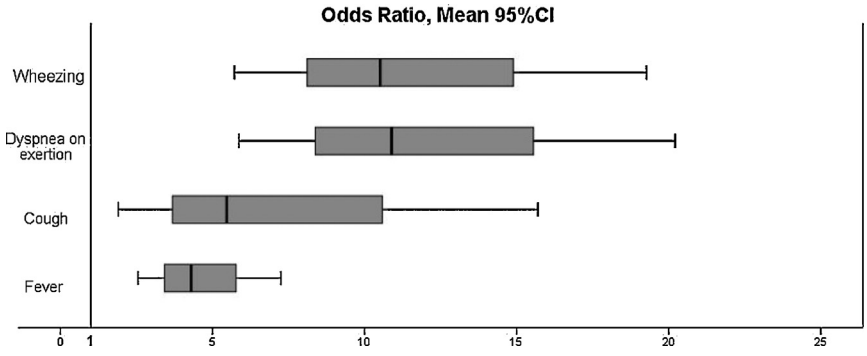

Fig. 1 – Association between airflow obstruction and probability of clinical signs in new TB cases. Legend: Pearson Chi-Square test, $p < 0.001$ for all cases

(OR) was calculated for cough OR = 5.47 (95% CI 1.90–15.70); dyspnea on exertion OR = 10.89 (95%CI 5.87–20.21); wheezing 10.51 (95%CI 5.72–19.27) and fever OR = 4.30 (95%CI 2.55–7.25), $p < 0.001$ for all cases. Of note, among underweight (BMI < 18.5) TB patients with airflow limitation, BMI value did not significantly correlate with FEV_1 (L) ($r = 0.35$, $p = 0.24$).

Radiographic manifestation of pulmonary TB were directly proportional to the frequency of obstructive abnormality on spirometry in subjects ($r = 1$, $p = 0.01$) – Fig. 2. Nevertheless, there were weak correlations with FEV_1 (L) ($r = -0.24$, $p = 0.018$) and respiratory impairment severity ($r = 0.32$, $p = 0.002$), classified according to ERS/ATS Task Force [33].

Significant post-bronchodilator reversibility was obtained in 53.5% (51/95) TB patients with AO. Meanwhile, only 37.9% (36/95) new TB cases with AO had post-bronchodilator FEV_1/F (VC) ratio less than LLN. Overall, flow-volume loop configurations revealed that majority of TB patients with AO had lower airway obstruction 37.9% (36/95) and dynamic central or intrathoracic upper airway obstruction 30.5% (29/95) (Fig. 3). To evaluate differences in endoscopic tracheobronchial pathology between TB patients with AO and without obstructive pattern on PFTs, we prospectively investigated 104 patients by FB. Table 3 summarizes the distribution of endobronchial findings in the target groups. Thus, any endobronchial pathology in new cases of pulmonary TB increased chances of obstructive abnormality on spirometry OR = 4.90 (95%CI 2.37–10.13) of what it would have been a normal endoscopic picture.

A binomial logistic regression was performed to evaluate the effects of age, gender, CXR pattern, smear microscopy, lung destruction, smoking, BMI and biomass/coal exposure on the likelihood that subjects have airflow obstruction, $\chi^2(9) = 17.67$, $p = 0.039$. The model explained 7.7% (Nagelkerke R^2) of the variance in AO and correctly classified 69.7% of cases. Only increasing age was associated with slightly greater likelihood of presence obstructive abnormality on PFTs - adjusted OR 1.02 (95% CI 1.00–1.04), $p = 0.02$.

Table 4 summarizes the stepwise multiple regression analysis. Unsurprisingly, BMI demonstrated the greatest positive impact on FEV_1 value whilst age, gender, domestic fuel and radiographic extent were associated with the biggest negative linear relation to operating margin.

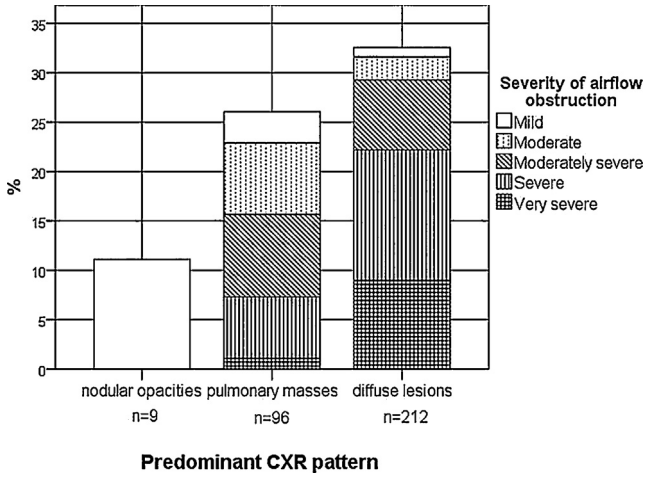

Fig. 2 – The relationship between pulmonary involvement due to TB and the frequency of airflow obstruction stratified by severity grading.

4. Discussion

The present study provides evidence that almost a third of hospitalised new cases with pulmonary TB (culture-confirmed) had AO (FEV_1/F(VC) < LLN) in Ukraine. We suggest AO might act as a surrogate marker of the severity of the clinical presentation and the extent of radiographic abnormality in newly diagnosed TB patients. However, the frequency of AO at first presentation with TB was greater than that noted by *Plit et al.* (11%), although his population was younger (median 35 versus 39 years in this study) and found by new inpatients rather than active annual CXR screening.[31]

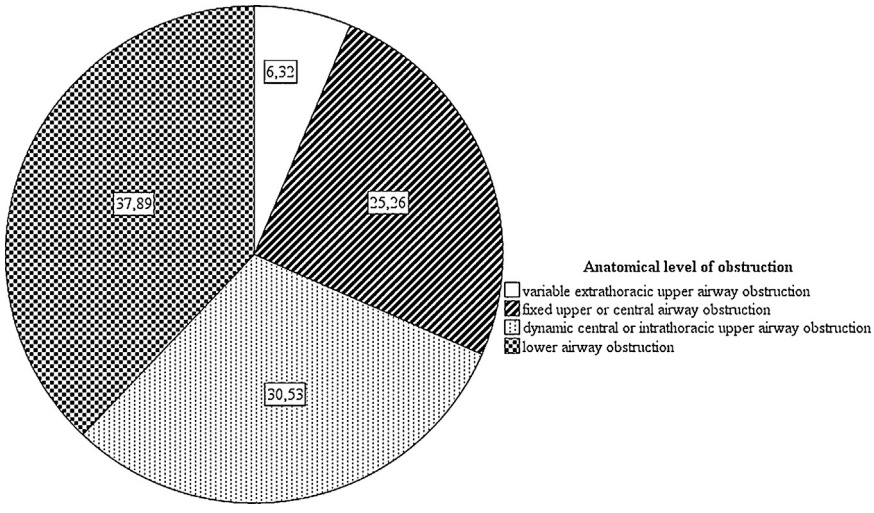

Fig. 3 – The frequency of airflow obstruction originating from different anatomical level.

Table 3 – Bronchoscopic findings and results of spirometry in new TB cases.

Endoscopic changes	FEV1/F(VC) < LLN (n = 58)	FEV1/F(VC)≥LLN (n = 46)
Normal endoscopic appearance, n (%)	3 (56.9)	36 (78.3)
Nonspecific inflammation, n (%)	19 (32.8)	7 (15.2)
Tuberculous endobronchitis, n (%)	6 (10.3)	None
Malignancy, n (%)	None	3 (6.5)*

TB, tuberculosis; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; LLN, less than the lower limit of normal for relevant healthy population.
* Mann–Whitney U test, $p = 0.4593$.

Table 4 – Results of multiple regression analysis with FEV_1(L) as dependent variable among TB patients with airflow obstruction.

Dependend variable	Predictors	Correlation coefficient	p-value	Standardized β coefficient	p-value
FEV_1, L	BMI	0.14*	0.173	0.22	0.013
	Domestic fuel	−0.20**	0.057	−0.20	0.024
	Sex	−0.32**	0.002	−0.34	<0.001
	Radiographic extent	−0.20#	0.058	−0.22	0.014
	Age	−0.40*	<0.001	−0.34	<0.001

Model summary: $F = 9.91$, $R^2 = 0.36$, $p < 0.001$. FEV_1, forced expiratory volume in one second; TB, tuberculosis.
* Pearson's Correlation Coefficient.
** Chi-squared test.
# Spearman's Coefficient of Rank Correlation.

Overdiagnosis of COPD or BA in patients with TB-induced airway narrowing can occur as a result of endobronchial lesions,[16] compression by enlarged mediastinal lymph nodes,[5,13] paravertebral[28] or retropharyngeal abscess[6] and even cellular bronchiolitis.[7]

Bronchospasm or bronchial hyperresponsiveness may play a key role in the development of AO in TB patients.[29,35] Proinflammatory cytokines by airway epithelial cells, contamination of cavities by *Aspergillus* and by nontuberculous mycobacteria may also contribute to hypersensitivity disorders (including AO).[8,11,24]

In accordance with previous findings,[2,9] the clinical significance of AO depends on its severity and cause. AO can be considered as a self-limiting disorder under standard chemotherapy either effectively cured by taking broncholitics/corticosteroids[31,35] or may remain as a progressive, irreversible abnormality (defined as COPD) in the post-treatment period.[2,25] Post-bronchodilator reversibility was detected in 15.0% of new TB cases with positive smear microscopy and fibrocavitary lesions[32] and 6.3% of patients with severe dyspnea and post-tuberculous lung destruction.[34] Unlike previous data, we found reversibility in half (52.82%) of new TB patients, but it does not rule out positive clinical response to bronchodilators in another half of the subjects.[30] Nevertheless, we determined that the post-bronchodilator ratio FEV_1/F (VC) was <LLN in 11.4% new active TB cases, while in population-based cross-sectional study carried out in Latin America[25] the prevalence of post-bronchodilator AO was 30.7% among individuals with a history of TB. Therefore, development of adjuvant interventions to prevent or to suspend further deterioration of lung function in individuals with TB could be useful tool for vast majority of patients.

Our results were consistent with several studies noting the important relationships between AO and chest radiographic pattern of TB patients.[3,15,17] Although we calculated no significant correlation between FEV_1(L) and CXR changes ($r = -0.20$, $p = 0.058$) in comparison to earlier published literature ($r = -0.41$, $p < 0.001$)[31] because only patients with airflow limitation were taken into account. The logistic regression has determined only increasing age as an important predictor of initial AO among new TB patients ($p = 0.02$), whereas *Radovic et al.* were focused on pulmonary TB cases with "extensive" lesions and normal PFTs at the beginning of treatment.[32] Therefore, this approach seems to need exclusion of the vast majority of such TB patients that might have restrictive, mixed or obstructive abnormalities.[3]

Multiple regression analysis in our study revealed strong evidence about negative associations between FEV_1 (L) and female sex. In Ukraine women traditionally are more exposed to fuel by heating with coal or wood. Positive impact of BMI on FEV_1 (L) among TB patients could be explained by less proportion of malnourished or cachectic patients with severe clinical presentation and skeletal muscle wasting. Interestingly, the frequency of AO in patients with prior TB was irrespective to more hard smoking history, as confirmed earlier.[19]

The main strengths of our study were prospective design, using strong criteria for participants selection (culture-confirmed TB cases, low limit of normal value on spirometry with post-bronchodilator testing), relatively large sample and avoidance of self-reported measurements.

We would like to note some limitations of this study. First, cross-sectional design cannot prove causality. Second, we did not estimate the effect of passive smoking in our sample. Nonetheless, Ukraine has one of the highest smoking rates in the world and AO might have an inverse relationship with second hand smoking. Third, we had no opportunity to perform methacholine challenge test and chest computed

tomography in our clinic. Therefore, concomitant BA and bronchiectasis cannot be fully excluded even without typical clinical presentation and no prior history of allergy.[4] The present analysis has not focused on family income, dietary intake and living in correctional settings, although these factors may increase risk of AO.[21]

The main difficulty was to distinguish restrictive defect from mixed dysfunction (restrictive and obstructive). However, alternative methods of lung volumes measurement, e.g. the body plethysmography and nitrogen washout have also limited application in active TB patients due to potential harm of contamination.[23,30] In this context non-contact lung function assessment is a perspective option.

5. Conclusions

We found that new cases of pulmonary TB were frequently accompanied by initial AO. This category of patients was older and had more severe clinical manifestation of TB, as well as more often endobronchial pathology. We encourage further investigations to establish the clinical significance of AO associated with TB and consensus in treatment strategy: who should be treated, how long and which drugs are preferred.

Conflicts of interest

The authors have none to declare.

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Original article

Efficacy of alternate day Directly Observed Treatment Short-course (DOTS) in skeletal tuberculosis – A retrospective study

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ABSTRACT

Objective: To assess the efficacy of alternate day (thrice a week) Directly Observed Treatment Short-course (DOTS) regimen spanning six to nine months in providing sustained cure for skeletal tuberculosis (TB) under programmatic conditions.
Design: Retrospective cohort study.
Setting: An urban district tuberculosis centre in India under the Revised National Tuberculosis Programme.
Participants: A cohort of 218 patients treated with alternate day DOTS regimen for skeletal TB between 2007 and 2012.
Methods: All patients with the diagnosis of skeletal TB registered between 2007 and 2012 who successfully completed treatment were followed up for evidence of disease recurrence or relapse using structured interviews conducted between August 2013 and October 2015 after ensuring a minimum follow up of two years.
Results: Of the 200 patients eligible for follow up in this study, 117 (58.5%) had a minimum follow up of two years. The remaining 83 cases could not be traced. 105 (89.7%) of these 117 patients were symptom free for two years or more after the completion of treatment. There were four cases who had a relapse of the disease within two years of completion of treatment. Eight cases were administered further ATT soon after the completion of treatment under DOTS.
Conclusions: This study confirms the efficacy of the alternate day DOTS regimen in successfully treating all forms of skeletal TB, including spinal TB, with a success rate of 89.7%.

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1. Introduction

South East Asia Region (SEAR) accounts for nearly 40% of the global morbidity and mortality due to tuberculosis (TB) with 4.5 million prevalent cases and 440,000 deaths reported from the region in 2013.[1] India, with a population of 1.25 billion, in turn contributed to 24% of the estimated global incidence of TB cases and about 20% of global TB-related deaths for the same year.[1]

India launched its Revised National Tuberculosis Control Programme (RNTCP) in 1997 to tackle the burgeoning TB menace and expanded it to cover the entire country by 2006.[2] Under this programme more than 650 TB suspects per 100,000 population were being examined and over 1.5 million TB patients started on the internationally recommended Directly Observed Treatment Short-course (DOTS) annually.[3]

The DOTS strategy was launched by WHO in 1995 for TB control and more than 180 countries around the world are currently implementing this programme.[2] Under DOTS, TB mortality in India has been reduced from 39 per 100,000 population in 1990 to 23 per 100,000 population in 2010 and the prevalence of TB brought down to 256 per 100,000 population in 2010 from 456 per 100,000 population in 1999.[3]

Skeletal TB accounts for approximately 2% of all TB cases and 11% of all extrapulmonary TB (EPTB) cases.[4] Traditionally RNTCP has been asserting that "in the absence of neurological complications, skeletal TB can be effectively treated with 6 months of Short Course Chemotherapy (SCC). There is no role for surgery on a routine basis".[4] A landmark 10 year follow up study by the Tuberculosis Research Centre (TRC) and Chennai Medical College established the efficacy of Short Course Chemotherapy (SCC, 6 months) in the treatment of spinal TB.[5] Several other studies suggest that 6–9 months of chemotherapy should be adequate in treating most cases of spinal TB.[6,7] Valsalan et al. recently reported that DOTS was comparable to other standard regimens in treatment of spinal TB with fewer side effects.[8]

However, many practicing orthopaedicians continue to employ longer (12 months or more) regimens especially in cases of spinal TB, as also for other forms of skeletal TB, and have remained sceptical about the alternate day DOTS regime of RNTCP.[9,10] This has lead to poor referrals to RNTCP, suboptimal utilization of DOTS services and prolonged treatment duration.

Given the aforesaid background, this study was planned to retrospectively review the efficacy of alternate-day DOTS in skeletal TB under programmatic conditions as per RNTCP guidelines.

2. Objective

To retrospectively assess the efficacy of alternate day (thrice a week) DOTS regimen spanning 6–9 months in providing sustained cure for skeletal TB (not requiring any further anti-tuberculous therapy for at least two years) under programmatic conditions at an urban district tuberculosis centre.

3. Methods

In this study we examined the treatment data of all the patients of skeletal TB treated by the DOTS regimen between 2007 and 2012 at Ramakrishna Mission Free TB Clinic, Karol Bagh, New Delhi, India. This Clinic has served as a District Tuberculosis Centre (DTC) right from the inception of RNTCP in 1998. Starting with a service population of approximately 500,000 in 1998, it was catering to a 700,000 population base through 18 DOTS centres and seven Designated Microscopy Centres (DMCs) in 2012. It is equipped to conduct basic diagnostic investigations (sputum microscopy and X-ray studies, besides FNAC at its attached Medical Centre). All residents of the area served by this Clinic wishing to be treated under RNTCP are registered at this DTC. The skeletal TB cases were either diagnosed at the Clinic by visiting orthopaedic surgeons or referred for treatment from other centres (both government and private) and were treated with alternate day DOTS as per standard RNTCP protocols (see Annexure I).

The electronic TB Programme Management System (e-TBPMS) maintained at the centre provided a list of all patients diagnosed with "Skeletal TB" between 2007 and 2012. The computerized registry as well as the manual record cards of these patients were reviewed for data on demographic profile, site of lesion(s), method of diagnosis, date and duration of treatment, category of DOTS therapy and treatment outcomes.

All patients with skeletal TB registered between 2007 and 2012 who successfully completed treatment were included for follow up in this study. Follow up interviews were conducted between August 2013 and October 2015 after ensuring that a minimum of two years had elapsed between the date of completion of treatment and the date of the follow up interview. The e-TBPMS co-ordinator attempted to contact all patients included in the study, first over phone and then by personal visits to patients at the addresses they provided while registering for treatment.

The interview focussed on the following questions:

(1) Was the patient free from symptoms at the end of treatment?
(2) Did the patient have to continue taking anti-TB therapy (ATT) after completion of DOTS?
(3) Did the patient suffer a relapse of TB at the same or other site after completion of treatment as noted in their record at the centre? If yes, did they receive ATT?
(4) Is the patient currently free of symptoms?

The data obtained from the interviews was correlated with the information available in the treatment records of the patients.

4. Results

During the six-year period under review (2007–2012), 11,274 patients received DOTS at the DOT centres under the DTC. This included 3086 patients (27.4%) treated for EPTB and 218 patients (1.93%) treated for skeletal TB. Of the 218 patients with skeletal TB considered for this study, 116 (53.2%) were

Table 1 – Distribution of skeletal TB lesions by site.

Skeletal site	Frequency of skeletal site involvement (%)
Spine	137 (62.8)
Joints	66 (30.3)
Long bones	14 (6.4)
Bone marrow	1 (0.5)
Site not mentioned	4 (1.8)
Total	222[a] (218 patients)

[a] 4 patients had multifocal skeletal TB.

female and 102 male. The majority of these patients (78.4%) were in the 11–40 years age group. The spine was the commonest site of skeletal involvement (62.8%). 31 patients (14.2%) had skeletal lesions as part of multi-focal TB; four of these had TB involving two different skeletal sites.

Out of the 218 patients who took ATT for skeletal TB, 172 (78.9%) were new cases. 163 of these received the Category I regimen and six were put on the Category III regimen; three patients received a non-DOTS regimen. Forty-six (21.1%) patients with a previous history of ATT were put on the Category II treatment. Histopathological evidence of the disease was available in 16 patients and 12 patients had a microbiological confirmation of TB (were AFB positive). Eighty patients had MRI evidence of the disease and seven had their lesions investigated by CT scans. The rest were initiated on ATT on clinical and radiological grounds (Table 1).

Of the 218 patients treated for skeletal TB, 200 (92.2%) completed treatment successfully and were considered for follow up in this study. Among the 18 patients excluded from follow up analysis, eight did not complete treatment, four died while on treatment, four received a non-DOTS regimen, and two patients were transferred to other districts for treatment. Out of the 200 patients who were treated successfully, 179 cases (89.5%) had completed treatment by 9 months. The majority of these patients had spinal TB and that explains the longer duration of treatment (9 months). 49 cases (24.5%) had 6 months of therapy, the standard treatment duration for uncomplicated bone TB cases (Table 2).

Of the 200 patients included for follow up (at least two years after completion of treatment) in this study, 83 (41.5%) could not be traced for follow up. For rest of the cases follow up results are detailed in Table 3.

Table 2 – Duration of treatment of skeletal TB patients successfully treated between January 2007 and December 2012.

Treatment duration	Number of patients (%)
6 months (±15 days)	48 (24.0%)
7 months (±15 days)	5 (2.5%)
8 months (±15 days)	50 (25.0%)
9 months (±15 days)	76 (38.0%)
10 months (±15 days)	9 (4.5%)
11 months (±15 days)	7 (3.5%)
12 months (±15 days)	5 (2.5%)
Total	200

5. Discussion

Skeletal TB comprises 2% of all TB cases and 9–11% of all cases of EPTB in India. Though these figures do not translate into very large numbers, they constitute an important subset of TB patients as spinal involvement in more than 50% of these cases poses a high risk for the development of disabilities, besides occupational and socio-economic problems. Though RNTCP traditionally recommended 6 months of alternate day DOTS for uncomplicated bone TB and 9 months of alternate day DOTS for TB spine,[11] there are few published studies documenting the validity of these recommendations under programmatic conditions and orthopaedicians have remained skeptical of both short-course and alternate day regimens.[12] This study validates the efficacy of the alternate day DOTS regimen as prescribed by RNTCP in the treatment of all forms of skeletal TB under operational conditions.

However, an expert group has recently suggested that all cases of bone and joint TB should be treated with an extended course of 12–18 months of anti-tubercular drugs, even though the group did not undertake a systematic review of literature. The expert group also noted the lack of consensus on what constitutes the healed status of skeletal TB.[13]

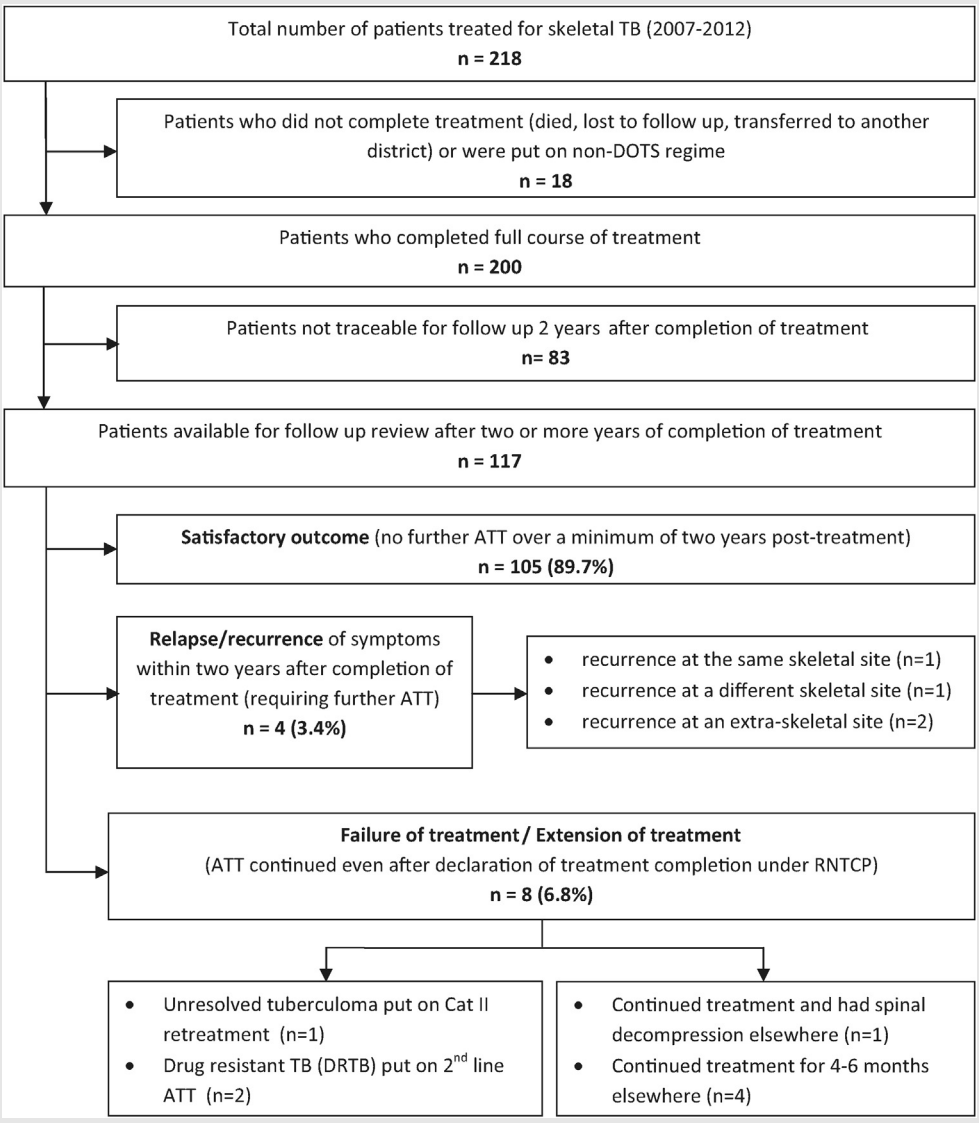
India's RNTCP does not categorically spell out the case definition and treatment success criteria for skeletal TB under operational conditions. Given the endemicity of TB, Indian orthopaedicians diagnose TB largely on clinical and radiological grounds[14] before referring patients for DOTS. Histopathological and microbiological confirmation of the disease is established only in a limited number of cases. Clinico-radiological and haematological evidence of cessation of disease activity with no evidence of recurrence up to two years post completion of treatment is taken to signify healed status of disease.[12] Our study used the 'no evidence of recurrence up to two years post completion of treatment' criterion for validating cure.

Of the 200 patients eligible for follow up in this study, 117 (58.5%) had a minimum follow up of two years. The remaining 83 cases could not be traced. 105 (89.7%) of these 117 patients were symptom free for two years or more after the completion of treatment. This treatment success rate of 89.7% compares very favourably with the overall treatment success rates of 88% and 70% for new and retreatment cases of sputum positive pulmonary TB that RNTCP achieved in 2013.[15]

There were four cases who had a relapse of the disease within two years of completion of treatment; only one case had a relapse at the same site, the rest had relapse at different sites. The relapse rate of 5.1% in our study is far lower than the nearly 10% relapse rate among newly treated smear positive cases of pulmonary TB reported in various studies from India.[16] This possibly reflects the paucibacillary nature of skeletal TB.

Of the eight cases who were administered further ATT soon after the completion of treatment under DOTS, two cases required second line drugs for drug resistant TB (DRTB), underscoring the need for drug susceptibility testing in all cases where failure of standard regimen is suspected. The five other patients who continued taking ATT for 6 months after

Table 3 – Two-year follow up status of skeletal TB patients treated between January 2007 and December 2012.



completion of treatment at our clinic may actually be reflecting the bias of many orthopaedicians in favour of longer treatment regimens for skeletal TB, a bias that needs to be addressed.

In our follow up cohort, 83 patients (41.5%) were not traceable two years after completion of treatment. This occurred primarily because the service area of the DTC where the study was conducted is a commercial area and is home to many daily wage workers and petty traders residing in temporary tenements for short periods of time. As considerable time had elapsed since these people had shifted residence they could neither be traced over the phone nor by personal visits to the addresses provided by them at the start of treatment. Additionally, there were patients who had apparently moved to Delhi with the aim of receiving appropriate treatment. Several of this group were no longer traceable. These patients however can be classified as 'missing at random' and their exclusion from analysis does not affect the validity of our results.[17,18]

The data reviewed in this study is the complete data of a DTC for the period of study. It is projected to have captured 70% of incident cases of TB in the DTC's area of service and is a reliable representation of the epidemiological features of skeletal TB in the community served.[3] Further, as the DOTS services at the DTC where the study was undertaken were offered to all patients referred for anti-tubercular treatment, irrespective of the status of disease or the presence of co-morbidities, the study cohort was free from any exclusion bias. The 89.7% success rate two years post completion of treatment should allay fears of clinicians about the efficacy of alternate day DOTS regimen. Even though RNTCP is currently recommending daily DOTS and extended treatment for skeletal TB, our study highlights the efficacy of shorter duration of ATT.

6. Conclusion

This study confirms the efficacy of the alternate day DOTS regimen in successfully treating all forms of skeletal TB, including spinal TB, with a success rate of 89.7%.

Conflicts of interest

The authors have none to declare.

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Annexure I. Treatment regimens for DOTS during the period of study

Treatment groups	Type of patient	Regimen[a]		Total duration
		Intensive phase (IP)	Continuation phase (CP)	
Category I (New)[d]	Sputum smear-positive Sputum smear-negative Extra-pulmonary Others	$2H_3R_3Z_3E_3$ 8 weeks, 24 doses	$4H_3R_3$ 18 weeks, 54 doses	6 months, 78 doses
Category II (Previously treated)[e]	Smear-positive relapse Smear-positive failure Smear-positive treatment after default Others[b]	$2H_3R_3Z_3E_3S_3$ $/1H_3R_3Z_3E_3$ 12 weeks, 36 doses	$5H_3R_3E_3$ 22 weeks, 66 doses	8 months, 102 doses
Category III (New, not seriously ill)[f]	Not seriously ill sputum smear-negative or extra-pulmonary	$2H_3R_3Z_3$ 8 weeks, 24 doses	$4H_3R_3$ 18 weeks, 54 doses	6 months, 78 doses

[a] Adapted from Central TB Division, DGHS, GoI, Revised National Tuberculosis Control Programme (RNTCP), Training module for medical practitioners, 2007:4; 2010:29–30.

[b] The number before the letters refers to the number of months of treatment. The subscript after the letters refers to the number of doses per week. The dosage strengths are as follows: isoniazid (H) 600 mg, rifampicin (R) 450 mg, pyrazinamide (Z) 1500 mg, ethambutol (E) 1200 mg, streptomycin (S) 750 mg.

- Patients who weigh 60 kg or more receive additional rifampicin 150 mg.
- Patients who are more than 50 years old receive streptomycin 500 mg. Patients who weigh less than 30 kg, receive drugs as per paediatric weight band boxes according to body weight.

[c] In rare and exceptional cases, patients who are sputum smear-negative or who have extra-pulmonary disease can have recurrence or nonresponse. This diagnosis in all such cases should always be made by an MO and should be supported by culture or histological evidence of current, active TB. In these cases, the patient should be typed as 'Others' and given treatment regimen for previously treated.

[d] New includes former categories I and III.

[e] Previously treated is former category II.

[f] Category III regimen was discontinued in 2008.

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Original article

Evaluation of loop mediated isothermal amplification (LAMP) assay in the diagnosis of tubercular lymphadenitis: A pilot study

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ABSTRACT

Tubercular lymphadenitis (TBLA) contributes to 30–40% of extrapulmonary TB cases in the immunocompetent individuals and 40–50% in people with HIV. Current diagnostic methods for TBLA like Gene-Xpert or PCR are costly and conventional methods like fine needle aspiration cytology, histopathology lack sensitivity and specificity. Culture which is considered as gold standard require high turnaround time. Loop mediated isothermal amplification (LAMP) assay has been developed as a novel technique for nucleic acid amplification and has shown promising results in the diagnosis of pulmonary tuberculosis. Present study evaluated the Nu-LAMPTM TB Kit (RAS Life Sciences Pvt. Ltd, a bioMerieux group company) for diagnosis of TBLA comparing with conventional tests (cytology, ZN smear, culture). The sensitivity, specificity, PPV and NPV of LAMP assay was found to be 33.3%, 91.2%, 40% and 88.57% as compared to 100%, 76.5%, 42.9% and 100% of ZN staining and 100%, 73.5%, 40% and 100% of cytopathology. The low sensitivity of LAMP assay in the present study addresses the need for comparison and validation of the commercially available LAMP kits before used for patient diagnosis.

1. Background

Tubercular lymphadenitis (TBLA), the involvement of the lymphatic system by tuberculosis (TB) contributes to 30–40% of extrapulmonary TB cases in the immunocompetent individuals and 40–50% in people with HIV.[1] TBLA commonly presents as gradually increasing painless swelling and can mimic malignancy. Accurate and timely diagnosis permit cure and

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prevents development of potential complications ranging from cellulitis to carotid artery rupture etc.[2]

The conventional methods for diagnosis of TBLA include fine needle cytology (FNAC), Ziehl Neelsen (ZN) staining of smear, and culture. Histopathology and cytology have low sensitivity and specificity and culture has high turnaround time.[3] Nucleic acid detection tests provide rapid, sensitive and specific results for TBLA diagnosis. Semi-automated cartridge based nucleic acid detection tests like Gene-Xpert MTB/RIFassay (Cepheid diagnostics Pvt. Ltd.) which does not require advanced laboratory infrastructure as well as trained professionals are expensive in terms of initial capital investment as well as running cost.[4] Thus, there is a need for a rapid, sensitive and economical test for diagnosis of tubercular lymphadenitis. The loop-mediated isothermal amplification (LAMP) assay has been developed as a novel technique for nucleic acid amplification. Unlike PCR, it can be performed using a simple water bath and a positive LAMP reaction can be visualized with naked eyes using a UV illuminator. LAMP assay has shown promising results in the diagnosis of pulmonary tuberculosis and TB-LAMP (Eiken Chemicals Pvt. Ltd., Japan) has been endorsed by World Health Organization (WHO) recently for diagnosis of pulmonary tuberculosis.[5] However, there are only few studies assessing the utility of LAMP assay in the diagnosis of extrapulmonary tuberculosis. Thus a pilot study was conducted to evaluate the diagnostic accuracy of a commercial LAMP assay (Nu-LAMPTM TB Kit, RAS Life Sciences Pvt. Ltd, a bioMerieux group company) on FNAC samples from cases of suspected tuberculous cervical lymphadenitis by comparing it with conventional methods (cytology, ZN smear and culture on LJ Media).

2. Methods

The study was conducted between November 2016 and January 2017 after approval from the institute ethics committee (approval No.: T/EM-F/Micro/16/07). Fine needle aspirates were collected from all patients with suspected tubercular lymphadenitis attending the outpatient departments of All India Institute of Medical Sciences, Bhubaneswar during the study period were included in the study. Features of unilateral single or multiple, painless, slow growing mass or masses developing over weeks to months, non-responding to antibiotics were considered as inclusion criteria.[6] Patients who had received antitubercular therapy in past or have received ATT for a period of more than 4 weeks were excluded from the study. All samples were subjected to cytopathology, ZN staining for detection of acid fast bacilli (AFB), culture on LJ media and LAMP assay. The first three tests were done following the standard protocol. LAMP assay was done using NuLAMP^{TM} TB kit (RAS Life science Pvt Ltd, Telangana, India). Two hundred microliter of each sample was subjected to DNA extraction by using the RAS DNA extraction kit based on salting out method as per manufacturer's instructions. Extracted DNA was used to set the LAMP assay using NuLAMP^{TM} TB kit. Briefly, 5 μL of the extracted DNA along with positive control and negative control supplied with kit were then added to the RAS master mix (17.0 μL RAS TB reaction buffer, 1.5 μL fluorescent detection reagent, 1.5 μL Ras *Bst* enzyme) and placed in thermocycler at 65 °C for 1 min for 60 cycles. The final results were read using UV reader of low wave length (254 nm). A fluorescent green colour occurring in the reaction tubes were considered as positive and absence of fluorescence was considered as negative test. Reading was noted independently by two different observers and consensus reports by both were considered for result analysis. The results of each of the tests were compared with culture. The sensitivity, specificity, positive predictive value and negative predictive value were calculated as per standard statistical formula.

3. Results

A total of 40 fine needle aspirates were obtained from 40 patients with clinical suspicion of tubercular lymphadenitis. Of the 40 patients 23 (57.5%) and 17 (42.5%) were females and males respectively. The median age was 30 years (range 3–75 years) and majority of the patients belonged to the age group of 21–40 years.

Of the 40 samples tested, cytology showing epithelioid granuloma was found in 15 samples, whereas positivity for mycobacteria was maximum by ZN staining for acid fast bacilli (AFB) (14/40) followed by culture (6/40) and LAMP assay (5/40). More number of samples tested in cytology laboratory were positive for ZN stain (14/40) compared to samples examined in microbiology lab (8/40).

Of the five samples positive by LAMP assay, two were also positive by the other three assays (ZN staining, cytology and culture). Of the remaining 3 LAMP positive samples, one was positive by ZN staining and cytology but was negative on culture, and the remaining two LAMP positive samples were negative by all the three assays. LAMP assay had the highest specificity (91.2%) followed by ZN stain (76.5%) and cytology (73. 5%). The sensitivity of LAMP assay was found to be the lowest (33.3%) among the three assays, whereas the sensitivity of cytology and ZN staining was 100% each. All the three tests had a positive predictive value between 40% and 42.9%. The negative predictive value of LAMP assay was lower (88.57%) than that of both ZN stain and cytology (both 100%). The details of the sensitivity, specificity, positive predictive values, negative predictive values of the individual tests are given in Table 1.

4. Discussion

LAMP assay is an isothermal nucleic acid amplification technique which can be used with minimum infrastructure for rapid diagnosis of variety of infectious diseases. In 2016, World Health Organization (WHO) recommended that TB LAMP may be used as a replacement for sputum microscopy for diagnosis or as a follow-on test to smear microscopy in adults with suspected pulmonary tuberculosis based on the LAMP assay developed by Eiken Chemical Company Ltd.[7] Several LAMP based diagnostic assays are now available commercially for both pulmonary and extrapulmonary tuberculosis. Tubercular lymphadenitis (TBLA) is the commonest manifestation of extra-pulmonary TB and contributes to

Table 1 – Diagnostic accuracy of various tests compared to culture as reference standard.

Test	Culture		Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
	Positive	Negative				
Ziehl Neelsen Stain						
ZN stain Positive	6	8	100 (54.1–100%)	76.5 (58.8–89.2%)	42.9 (29–57.9%)	100
ZN stain Negative	0	26				
Cytology						
Cytology Positive	6	9	100 (54.1–100%)	73.5 (55.6–87.1%)	40 (27.6–53.9%)	100
Cytology Negative	0	25				
LAMP assay						
LAMP Positive	2	3	33.3 (4.3–77.7)	91.2 (76.3–98.1%)	40 (12.2–76.1%)	88.57 (81.3–93.2%)
LAMP Negative	4	31				

around 25% of all the cases of TB.[8] There have been a few studies on the utility of LAMP based assay for diagnosis of TBLA.[9] Hence, we evaluated a commercially available kit (Nu-LAMP TB Kit) for diagnosis of TBLA in a pilot study.

Our findings of low culture positivity (15%) compared to microscopy to detect acid fast bacilli (35%) are in agreement with study by Sharma et al. and Pahwa et al.[9,10] Such findings have been explained to be due to sample inadequacy, low bacterial load, and features peculiar to lymphadenitis like damage to tubercle bacilli from immunologically competent cells in the sample which result in reduced viability of mycobacteria which take up the acid-fast stain but fail to grow in culture. A low ZN positivity in the sample tested in microbiology lab as compared to pathology lab in our study has also been observed by Sharma et al.[9] The discrepancy was most likely due to the fact that, half of the sample was utilized for cytopathology study and the remaining was divided into three equal parts and used for ZN stain, culture and LAMP.[9] Thus, the sample available for ZN stain reporting at microbiology laboratory was 1/6 compared to 1/2 of sample at cytopathology laboratory.

The sensitivity of LAMP assay using different gene targets (*IS6110*, *mpb64*, *sda* etc.) for detection of culture proven extrapulmonary and tubercular lymphadenitis has been reported in the range of 84–95%.[9,11] The sensitivity of LAMP assay in this study (33.3%) was lower than that reported in literature which could be due to the gene targets or presence of inhibitors in the sample. Although LAMP assay has been demonstrated to have higher inhibitor resistance compared to PCR assay,[12] failure to amplify should nevertheless be confirmed by spiking experiments. This was not done and is one of the limitations of the study.

Commercial and In house LAMP based studies for the diagnosis of tuberculosis have used different DNA extraction methods [phenol chloroform isoamyl alcohol or column based DNA extraction method], salting out method, Procedure for Ultra Rapid Extraction (PURE) as well as different gene targets (MPB64, IS 6110, or multi gene target)[11] which can affect the sensitivity and specificity of the test. However, it is also to be noted that the present study is a pilot study conducted with less number of samples, hence large number of samples needs to be tested for further confirmation.

Three samples which were positive on LAMP assay were not positive on culture were assigned as false positives. Culture being less sensitive, other criteria like use of other WHO endorsed molecular assay like Gene-Xpert, Eiken TB-LAMP or use of composite reference standard which includes response to treatment, and radiology could have been used as gold standard for evaluation of this assay. LAMP assay has been projected for its field applicability. This is because it does not require thermocycler. In the present study though thermocycler has been used the test could have been done in dry/water bath also. Integration of DNA extraction along with the amplification test and visual reading with naked eye can be helpful in reality for field testing.

The present study is one of the few studies conducted on LAMP assay on extrapulmonary tuberculosis sample. However, the small sample size is its major limitation which needs further evaluation.

5. Conclusion

The present pilot study showed low sensitivity of the LAMP assay tested as compared to ZN stain and cytology. However, given the small sample size in the present study, the assay should be evaluated in large number of samples and in different laboratory settings before drawing concrete conclusion. The low sensitivity of the LAMP assay in the present study for detection of *Mycobacterium tuberculosis* DNA from lymph node aspirate addresses certain important issues regarding the implementation of commercially available LAMP assays for diagnosis in a clinical set up. These include the need of comparative evaluation of all the assays available

commercially and validation of each test with different clinical samples such as sputum, pus, aspirate, body fluids and C.S.F etc. before being recommended for diagnosis.

Conflict of interest

The authors have none to declare.

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Case report

Germ cell tumor causing pleural effusion: A diagnostic dilemma

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ABSTRACT

Straw colored pleural fluid with raised adenosine deaminase (ADA) levels in young healthy adults usually raises suspicion of tuberculosis, sometimes leading to laxity in carrying thorough physical examination and missing out some important clues with potential disastrous consequences. A 35-year-old male was diagnosed to have left pleural effusion and antitubercular treatment was started on the basis of straw colored, lymphocyte-predominant pleural fluid with significantly raised ADA levels. When there was no improvement after 1 month of treatment he was investigated further and found to have a mediastinal mass along with hydro-pneumothorax. Fine needle aspiration cytology (FNAC) of the mass was done twice at different centers with different reports followed by biopsy from the mass to settle the diagnosis. Histopathological examination revealed yolk sac tumor. Testicular ultrasound showed a mass with ill-defined hypoechoic areas and lobulated margins in left testis, which was missed on clinical examination. Serum lactate dehydrogenase (LDH) and alpha fetoprotein (AFP) levels were found to be elevated. Beta-human chorionic gonadotropin (β-hCG) was normal. The final diagnosis of nonseminomatous germ cell tumor with mediastinal metastasis was made. The present case underlines the importance of good clinical examination, an art which is diminishing with availability of sophisticated investigations and a thin line of difference between potentially curable and fatal diagnosis, especially in young population, where malignancy is overlooked as a differential diagnosis. Furthermore, despite all its advantages, too much reliance on FNAC may be responsible for misdiagnosis in certain cases.

1. Introduction

Straw colored pleural fluid with increased adenosine deaminase (ADA) levels in countries endemic for tuberculosis like India in otherwise healthy younger population is usually considered to be of tubercular origin and this may lead to missing out looking for leads pointing to certain uncommon causes for the same. Testicular cancers account for only 1% of all cancers in males.[1] However, these are the commonest solid

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tumors in males between 15 and 35 years of age.[2] Testicular cancers frequently metastasize to the mediastinum. Prompt diagnosis should be made in such cases as they have high cure rates.

2. Case report

We present a case of 35-year-old male who presented with chief complaints of fever for two months, shortness of breath for one month and dry cough for four days. Patient was investigated outside and diagnosed to have left sided pleural effusion (Fig. 1). Straw colored pleural fluid was aspirated which was lymphocyte-predominant with ADA levels of 75 IU/L, which was a significant increase. Diagnosis of left tubercular pleural effusion was made and anti-tubercular treatment (ATT) was started (HRZE) according to body weight. Patient presented to our center after one month of taking ATT with no clinical improvement. On examination there were decreased breath sounds on left side and trachea was shifted to right side. Pleural fluid aspiration was done which showed thick hemorrhagic fluid, cytological examination of which showed blood only. Intercostal chest tube drainage was done and hemorrhagic fluid was drained. CECT chest was subsequently done and it showed left loculated hydro-pneumothorax with left posterior mediastinal mass in contact with aorta (Fig. 2). FNAC done from the mass was suggestive of adenocarcinoma (Figs. 3 and 4). Tumor was staged as $TxNxM_1$. Patient was planned for chemotherapy. Meanwhile patient's condition deteriorated and he went to another higher center where repeat FNAC of mass was done which was suggestive of squamous cell carcinoma. Patient was accordingly started on carboplatin and etoposide. Due to variation in the two reports, biopsy was subsequently done from the mass and histopathological examination revealed yolk sac tumor (Fig. 5). Thereafter, testicular ultrasound was done which showed ill defined hypoechoic areas with lobulated margins in the left testis which was missed on clinical examination. Serum LDH and AFP were then ordered and found to be raised. Beta-hCG was normal (Table 1). Whole body scintigraphy was done which showed no evidence of bony metastasis. MRI brain was also normal. Hence, diagnosis of nonseminomatous germ cell

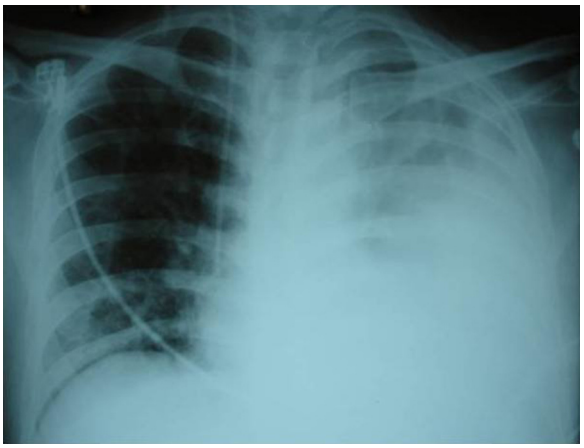

Fig. 1 – Chest radiograph showing left sided pleural effusion.

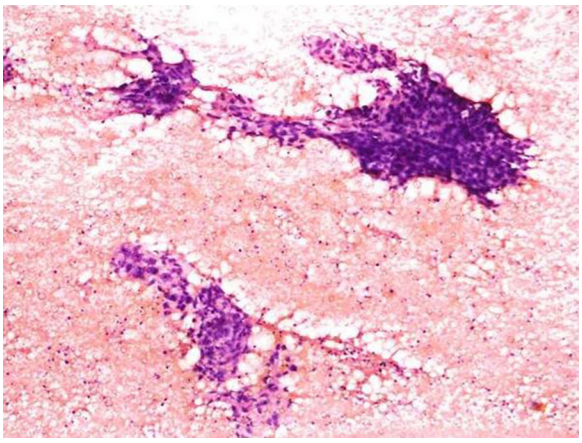

Fig. 3 – Aspirate smear showing tissue fragment and sheet of loosely cohesive tumor cells (H&E 100×).

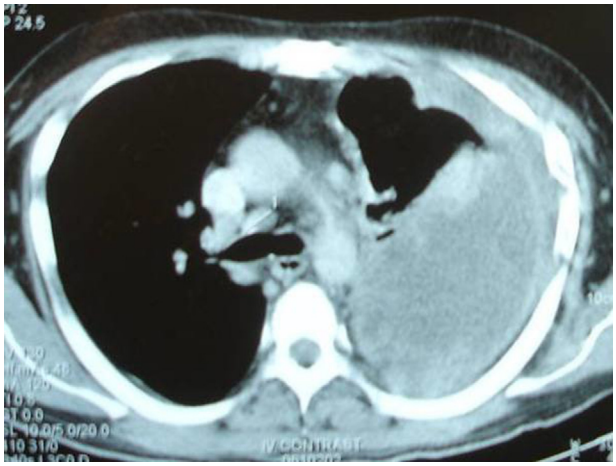

Fig. 2 – CECT Chest showing mass in the left hemithorax encasing the collapsed left lung.

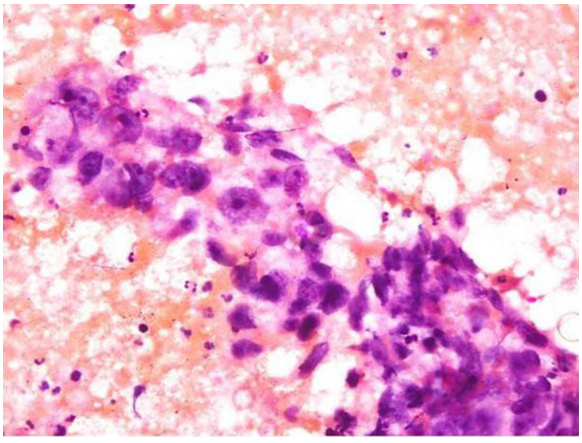

Fig. 4 – Photomicrograph showing moderately anaplastic tumor cells with conspicuous nucleoli and vacuolated cytoplasm (H&E 400×).

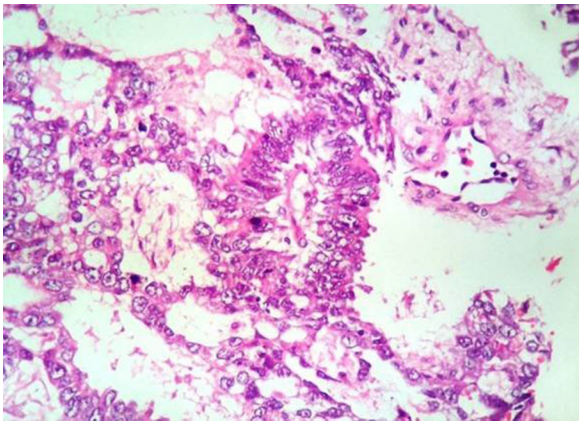

Fig. 5 – Histopathologic section showing Schiller–Düval body in yolk sac tumor (H &E 400×).

Table 1 – Serum tumor markers before and after treatment.

Tumor marker	Before treatment	After treatment	Normal value
Alpha Fetoprotein (ng/mL)	5790	20	<7.2
Beta-hCG (IU/L)	1.15	0.26	<6.5
Serum LDH (U/L)	2032	350	225–450

Beta-hCG: beta-human chorionic gonadotropin; serum LDH: serum lactate dehydrogenase.

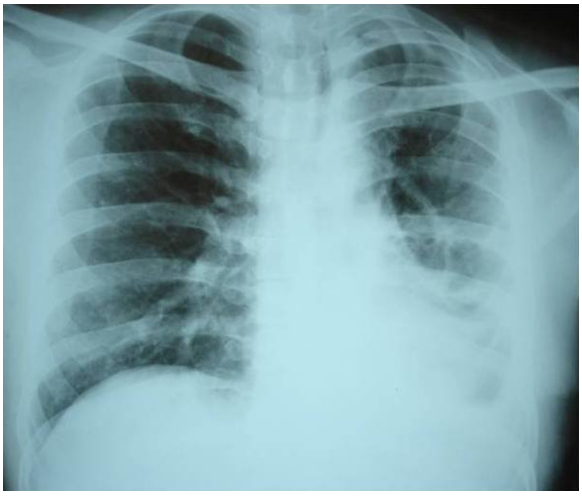

Fig. 6 – Chest radiograph after treatment.

tumor with mediastinal metastasis was made. Patient underwent orchidectomy whose histopathological examination further confirmed the diagnosis and he was treated with bleomycin, etoposide and cisplatin. Patient also underwent surgical removal of the mediastinal tumor after three cycles of chemotherapy. There was subsequent decrease in serum LDH and AFP levels. Patient subsequently recovered completely from the disease (Fig. 6). On follow up after three years, patient is healthy and alive with no evidence of recurrence of disease.

3. Discussion

Testicular cancer has three main types: germ cell tumors, non-germ cell tumors, and extragonadal tumors. Germ cell tumors, which are the most common, are classified as either seminoma or nonseminoma, based on histology. Of the three main types of testicular cancer, nonseminomatous germ cell tumors (NSGCTs) are second only to seminomas in terms of frequency.[3] Testicular cancer usually manifests as a painless swelling/enlargement of the testis and only about 10% of patients complain of new onset pain in the testicle. Nearly a quarter of patients with metastatic disease experience symptoms like low back pain caused by tumoral metastasis to the retroperitoneal lymph nodes.[4] The serum markers α-fetoprotein, β-human chorionic gonadotropin, and lactate dehydrogenase can be useful for diagnosis, treatment and surveillance.[4,5] Radical orchidectomy is the primary treatment for most patients presenting with a suspicious testicular mass. Orchidectomy is both diagnostic and therapeutic. Given that the testis can act as a sanctuary site for tumor cells from chemotherapeutic agents, orchidectomy should be performed even in the case of metastases suitable for biopsy.[4,6] Patients with metastatic NSGCTs are usually treated using a multimodal approach consisting of systemic chemotherapy followed by consideration of post chemotherapy retroperitoneal lymph node dissection.[4,7] As testicular cancers are curable even in the presence of metastatic disease, the correct diagnosis and staging is a critical component for therapeutic decision making and prognosis. Yolk sac tumor exhibits diverse cytologic patterns which include papillary, cohesive clusters, acinar formations and scattered cells with vacuolated cytoplasm and conspicuous nucleoli.[8] At times, coarse clumped chromatin and irregular nuclear membranes are observed.[9] Schiller-Düval bodies, a characteristic feature on histopathology is occasionally seen on FNAC.[10] Aforementioned cytologic features can lead to a mistaken diagnosis of adenocarcinoma/squamous cell carcinoma on FNAC as happened in this case. Therefore, high degree of suspicion is required in such cases for correct diagnosis. Anterior mediastinal involvement is seen in primary mediastinal germ cell tumors which is expected in view of their proposed thymic origin whereas posterior mediastinal involvement occurs in case of metastases from germ cell tumors,[11] as seen in the index case.

The current case underlines the importance of good clinical examination, an art which is diminishing with availability of sophisticated investigations. It also signifies that despite all its advantages, FNAC might not be the best tool in certain situations to clinch the correct diagnosis. The case highlights the thin line of difference between a potentially curable and a fatal diagnosis and the important role played by the pathologist in this regard. Testicular cancers are curable even in the presence of metastatic disease. Thus, high degree of suspicion is required in these cases. Malignancy should always be kept in differential diagnosis even in younger population with undiagnosed pleural effusion.

Conflicts of interest

The authors have none to declare.

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Case Report

Primary oral tuberculosis on the tongue mimicking squamous cell carcinoma

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ABSTRACT

Tuberculosis is chronic granulomatous disease with rare oral manifestations. But if so are overlooked by most of the health care professionals. Clinically, most of the times, a tuberculous ulcer may mimic an ulcer of malignant origin and may be misdiagnosed. So, keeping in mind the etiology and the nature of the ulcer, it should be differentially diagnosed and a histopathological examination should only confirm the final diagnosis of the ulcer.

1. Introduction

Tuberculosis is a chronic granulomatous disease that can affect any part of the body and seldom takes an oral form. Almost 30–60% of all cases occur in the developing countries and India alone accounts for nearly one-fifth of the global burden of tuberculosis and is one of the major cause of morbidity and mortality worldwide.[1,2] In 2007, the incidence of 139 per 100,000 active *Mycobacterium* TB infections has been reported and out of these patients, a significant proportion exists in whom the extrapulmonary site with active infection is manifested.

Oral tuberculous lesions may be either primary or secondary in occurrence. Primary lesions are uncommon, seen in younger patients and present as single painless ulcer with regional lymph node enlargement. The secondary lesions are common, often associated with pulmonary disease, usually present as single, indurated, irregular, painful ulcer covered by inflammatory exudates in patients of any age group but relatively more common in middle aged and elderly patients.[3]

After obtaining prior permission from the review board of the Institute as well as the patient, the present communication reports a rare case of primary infection of tuberculosis with manifestation pertaining to tongue which on clinical presentation mimicking an oral squamous cell carcinoma.

2. Case report

A 25-year-old female reported to the Department of Oral Medicine & Radiology with a complaint of ulcer on the tongue since 4 months. The ulcer was painful and enlarged gradually to its present size. The medical history of the patient revealed that she was diagnosed with primary tuberculosis 5 months back

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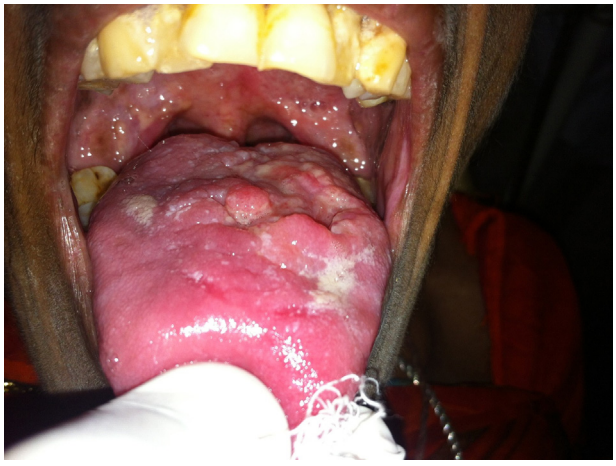

Fig. 1 – Clinical presentation of the ulcer over the dorsum of the tongue.

and since then she was on the WHO regimen – I by her physician but was not regular in taking medications as revealed by the patient. The reports of the patient revealed that there was pulmonary involvement. Personal history of patient revealed that she was a habitual smoker and took almost 6–7 cigarettes per day since 5 years. No significant findings were noticed on extraoral examination. Intraoral findings revealed a very poor oral hygiene. On intraoral examination (Fig. 1), an ulceroproliferative, rough, irregular-shaped ulcer was seen on the dorsum of the tongue measuring 4 cm × 2 cm in size extending anteriorly 1 cm away from the tip of the tongue, posteriorly up to the base of the tongue and laterally extending up to the margins of tongue. The base of the ulcer appeared to be granular. The margins of the ulcer were slightly elevated and indurated. The ulcer was surrounded by a small area of erythema, which was painful on touch. The histopathological analysis of a tissue from the ulcer revealed chronic granulation tissue with necrosis along with few scattered epitheloid cells (Fig. 2). Thus, a final diagnosis of oral tuberculosis was given.

3. Discussion

TB is caused by the bacterium mycobacterium tuberculosis, which is an aerobic, non-motile, non-capsulated, nonspore-forming, and rod-shaped organism.[4]

The fact that *M. tuberculosis* cannot invade the intact mucosa of oral cavity as the squamous epithelium is resistant to invasion to tubercle penetration has been attributed to the

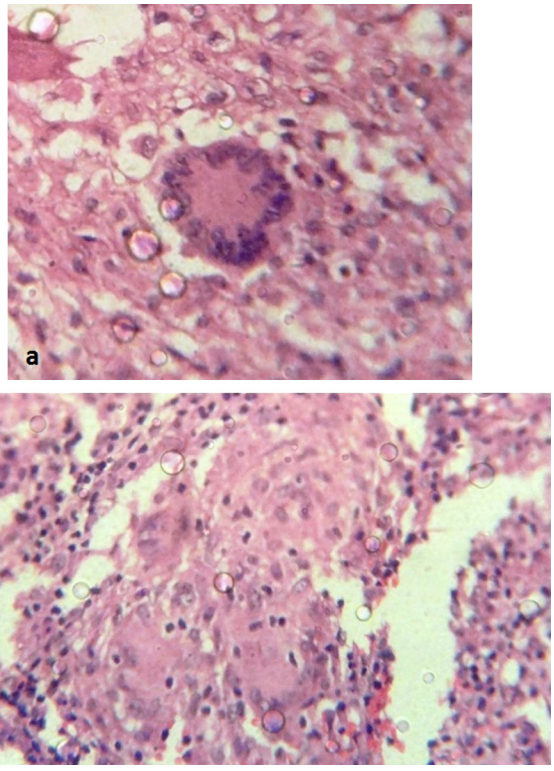

Fig. 2 – (a and b) Histopathological section showing chronic granulation tissue with necrosis along with few scattered epitheloid cells.

cleaning action of saliva, in the presence of salivary enzymes, tissue antibodies, and the thickness of the protective epithelial covering. Any break or loss of the natural barrier may be as a result of trauma, chronic irritation or inflammation, leukoplakia, tooth extraction, or poor oral hygiene and may provide a route of entry for the organism.[5] The ulcer is formed by the breakdown of tubercles and has undermined edges.[6]

But at times of atypical presentation, it may mimic a malignant ulcer.[7,8] More often, the tubercular ulcers are irregular and have punched out margins,[9] whereas in the present case, the clinical appearance of the ulcer was ulceroproliferative type and the margins were indurated.

The differential diagnosis of a tuberculous ulcer of the oral cavity includes aphthous ulcers, traumatic ulcers, syphilitic ulcers, actinomycosis, Wegener granuloma, and malignancy.[10]

The cases that have been reported by other authors where the most likely clinical presentation is of squamous cell carcinoma have been listed in Table 1.

Table 1 – Tubercular ulcer mimicking SCC as reported by other authors.

Author	Year	Presentation of ulcer
Mahajan S et al.[1]	2007	Reported a case of tubercular ulcer on the right retromolar area to the soft palate. The ulcer had an indurated base and everted margins.
Ram H et al.[8]	2012	Reported a case of tubercular ulcer on the left angle of mouth. The ulcer had irregular margins and the floor contained a yellowish granular tissue. It had indurated and rolled margins.
Nicoara et al.[10]	2013	Reported a case of tubercular ulcer on the buccal mucosa mimicking squamous cell carcinoma. The ulcer had an ulceroproliferative appearance, with irregular borders and granular base

4. Conclusion

To conclude, tuberculosis of oral cavity is rare and if present, it can mimic squamous cell carcinoma more than any other pathology of oral cavity. Whenever oral health professionals come across an oral ulcer with chronic non-healing history a differential diagnosis of tubercular ulcer should be considered. And if not done so, the chances of cross infections can increase on part of the health care professional and diagnosis may be inconclusive.

Conflicts of interest

The authors have none to declare.

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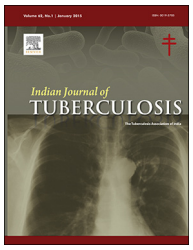

Case Report

An extremely rare association of Sweet's syndrome with active pulmonary tuberculosis

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ABSTRACT

Sweet's syndrome is a skin manifestation of various systemic infections, drugs, malignancies and autoimmune disorders. There are very few case reports describing the relationship between Sweet's syndrome and non-tubercular mycobacterium infection. Further development of Sweet's syndrome secondary to mycobacterium tuberculosis (active pulmonary tuberculosis) is extremely uncommon and this is the second well established case reported from India. Here we report a forty eight year old man who presented with multiple erythematous and tender plaques over neck, palms and sides of soles. He also had high grade fever, headache, myalgias, cough, chest pain and difficulty in breathing. With clinical possibilities of (1) Sweet's syndrome with pulmonary involvement and (2) Sweet's syndrome secondary to pulmonary infection, we send the skin biopsy for histopathological examination and also advised routine laboratory plus imaging investigations to find out the underlying cause. Clinical and lab parameters together with the biopsy report fulfilled the diagnostic criteria for Sweet's syndrome. Further chest X-ray findings, demonstration of acid fast bacilli of mycobacterium tuberculosis on sputum smear microscopy and MGIT report confirmed the diagnosis of pulmonary tuberculosis. Patient was put on colchicine and standard anti-tubercular drugs. Significant improvement was noticed in skin lesions within five days of treatment and no recurrence has been seen for the past six months.

1. Introduction

Sweet's syndrome is a multisystem inflammatory disorder characterized by sudden onset of high grade fever, typical skin lesions and dense dermal neutrophilic infiltrate on histopathology. Fever, arthritis and conjunctivitis are common systemic features of Sweet's syndrome but pulmonary involvement is quite rare.[1] In most (70%) cases, this syndrome is idiopathic but its frequent associations include infections, pregnancy, inflammatory disorders and malignancies. Amongst infections, occurrence of Sweet's syndrome with mycobacterium infection is uncommon, only few case reports of non-tubercular mycobacterium infection or extra-pulmonary tubercular infection are there.[2] Further the reporting of active pulmonary tuberculosis and Sweet's syndrome is extremely uncommon with only a single case reported from India.[3] If a patient of Sweet's syndrome present with

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pulmonary symptoms it is very important to differentiate whether lung involvement is due to underlying infective cause or due to pulmonary Sweet's itself. Usually systemic corticosteroids are treatment of choice for Sweet's syndrome but they are contra-indicated in active pulmonary tuberculosis. In developing countries like India where tuberculosis is endemic, active screening must be done for mycobacterium infection. Alternative drugs like colchicine, dapsone, clofazamine, potassium iodide and indomethacin should be preferred over steroids in such cases.

2. Case report

A 48-year-old man presented to us with fever, body-aches and multiple red raised painful skin lesions over neck, palms and soles for a period of three days. Previous records revealed that fifteen days earlier patient was treated for fever, cough and chest pain with antibiotics (cefpodoxime) and antipyretics. He had marked improvement in fever and chest pain after medication. But shortness of breath and cough was persistent, which further deteriorated after appearance of the skin lesions. He gave the history of intermittent productive cough for 5–6 months but he denied for associated haemoptysis or weight loss or loss of appetite. There was no history of preceding drug intake, alteration of bowel or urinary habits or any other systemic complaint. On examination patient was febrile, dyspnoeic, sick looking with pulse rate 112/min, respiratory rate 24/min and fever 38 °C. Cutaneous examination revealed multiple well defined erythematous and tender plaques over neck, palms and soles (Figs. 1 and 2). Pulmonary and other systems were normal on examination. With clinical possibilities of (1) Sweet's syndrome with pulmonary involvement and (2) Sweet's syndrome secondary to underlying pulmonary infection, skin biopsy was taken and simultaneously routine investigations were sent to find out the associated diseases. Laboratory investigations showed Hb 11 g/dl, WBCs 13,000/μl, neutrophils 76%, positive C reactive protein and elevation of ESR to 46 mm/h. Tests for HIV, hepatitis were non-reactive and further blood sugar level, urine microscopy, hepatic and renal profile were unremarkable. Although his montoux test was non-contributory but chest X-ray revealed cavities in right upper lobe along with bilateral patchy infiltrates (Fig. 3). Further reporting of acid fast bacilli on sputum microscopy and specific demonstration of mycobacterium tuberculosis bacilli on MGIT confirmed the diagnosis of pulmonary tuberculosis. Histological examination showed dense neutrophillic infiltrate within the edematous dermis with no evidence of leucocytoclastic vasculitis (Fig. 4). Thus the diagnosis of Sweet's syndrome secondary to sputum positive pulmonary tuberculosis was established and patient was put on standard anti-tubercular drugs along with colchicine 0.5 mg three times daily doses. Significant improvement was noticed in skin lesions within five days and patient recovered completely in a period of four weeks. We stopped colchicines in a period of three months after tapering its doses from thrice daily to once daily schedule.

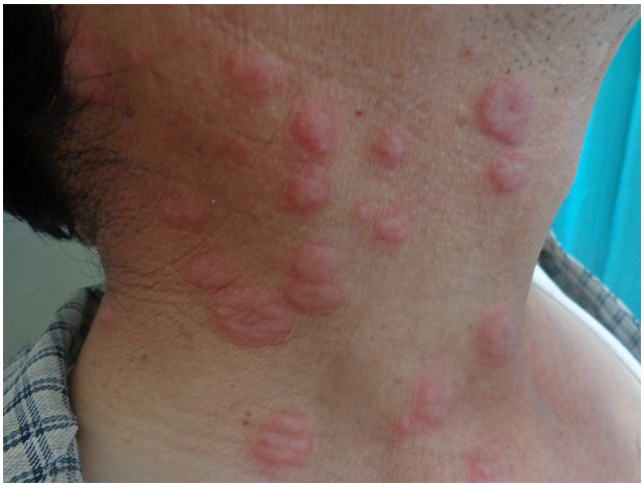

Fig. 1 – Multiple erythematous plaques with central vesiculation at places over neck.

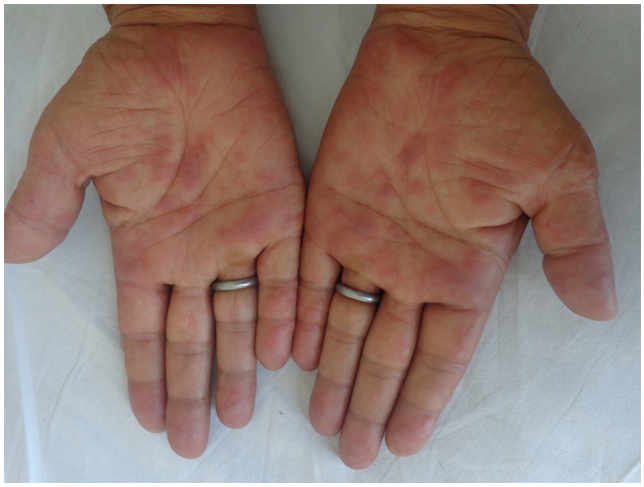

Fig. 2 – Multiple erythematous and tender plaques over palms in a patient of Sweet's syndrome.

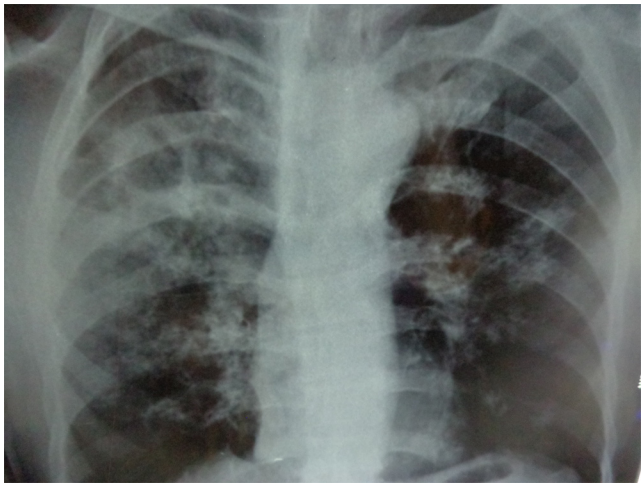

Fig. 3 – X-ray chest showing cavitary lesions in right upper lobe and bilateral patchy infiltrates.

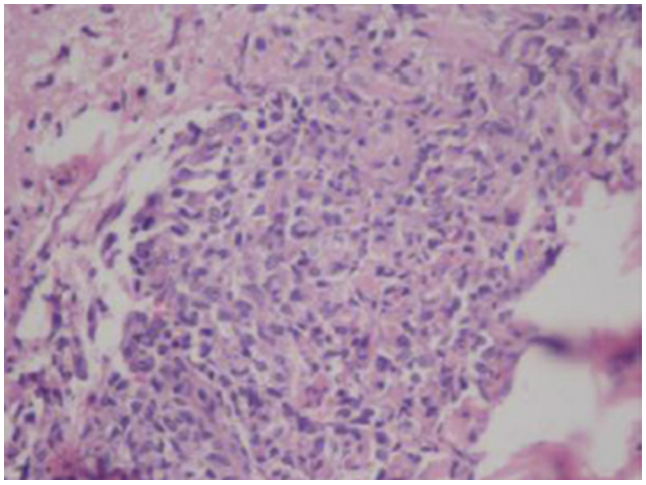

Fig. 4 – Histopathological examination revealing dense dermal neutrophilic infiltrate within the edematous dermis with no evidence of vasculitis.

3. Discussion

Sweet's syndrome was described in 1964 in British journal of dermatology as an 'acute febrile neutrophillic dermatoses by Dr. Robert Douglas Sweet. In 1986 Su and Liu proposed diagnostic criteria for classical Sweet's syndrome, which later on was modified by Von den Driesch in 1964.[3] Sweet's syndrome is classified as classical type, malignancy associated and drug induced. In about 75% of cases there is preceding viral or bacterial infections including cytomegalovirus, hepatitis, HIV, streptococcus, salmonella, yersinia and rarely mycobacterium tuberculosis.[4] Most of the case reports showing relationship between mycobacterial infection and Sweet's syndrome document that underlying disease is either extra-pulmonary tuberculosis or infection by atypical mycobacteria.[2,5,6] One author described Sweet's syndrome concomitant with tuberculosis and cervical cancer.[7] Another author reported Sweet's syndrome and pulmonary tuberculosis in Crohn's disease patient who was on treatment with anti-TNF-α.[8] The compounding factors like cervical cancer and Crohn's disease are independent risk factors to induce Sweet's syndrome and anti-TNF α can lead to tuberculosis due to immunosupression. Therefore from above case reports this is difficult to establish the primary relationship between Sweet's syndrome and pulmonary tuberculosis. Though the case reports by Singh[9] and Ledoult et al.[10] strengthen the primary association between mycobacterium tuberculosis infection and Sweet's syndrome. Further, to our knowledge this is the second well established case of Sweet's syndrome primarily associated with sputum positive pulmonary tuberculosis reported from India as first case was reported by Karmakar et al.[4]

Amongst inflammatory disorders, inflammatory bowel disease is most common association. Rheumatoid arthritis, Sjogren's syndrome and systemic lupus erythematosus are other auto-immune disorders reported with Sweet's syndrome. Malignancy associated Sweet's syndrome is seen nearly in 20% of patients and most of the malignancies are hematopoietic in origin.[4] Medications like granulocyte colony stimulating factors, furosemide, hydralazine, and sulphamethoxazole-trimethoprim can induce Sweet's syndrome with definitive history of preceding drug intake.[3] Almost all these associations were ruled out in our case by clinical and investigative workup. The exact pathogenesis of Sweet's syndrome is unknown but authors suggest that it is neutrophil mediated hypersensitivity reaction to an antigen and Interleukin-1, G-CSF play key role in maintenance of this hypersensitivity reaction. Classical presentation of Sweet's syndrome is fever, neutrophilia and erythematous skin lesions. Musculoskeletal (arthritis) and ocular symptoms (conjunctivitis, uveitis, and scleritis) are frequently observed but pulmonary, cardiac and neurological involvement is rare.[1] For the diagnosis of Sweet's syndrome major criteria are (1) sudden onset of tender erythematous skin lesions (papules, plaques or nodules) and (2) dense dermal neutrophilic infiltrate in the absence of vasculitis on histopathology. Further (1) fever more than 38 °C, (2) ESR>30 mm/h, neutrophill count >70%, positive C-reactive protein, (3) association with underlying infection, pregnancy, inflammatory disease or malignancy and (4) excellent response to systemic corticosteroids constitute the minor criteria.[3] To establish the diagnosis of Sweet's syndrome two major and two minor criteria are required while in our patient both major criteria and three out of four minor criteria were met. In a pulmonary symptomatic patient underlying pulmonary infection (bacterial, viral and specifically mycobacterium infections) triggering the Sweet's syndrome has to be differentiated from pulmonary involvement by Sweet's syndrome itself. Pulmonary Sweet's syndrome manifest with dry cough and dyspnoea. Chest X-ray reveals diffuse lung infiltrates or pleural effusion and CT scan usually confirms the lung involvement in Sweet's syndrome.[1] In our case X-ray findings, lab results and treatment response (to anti-tubercular drugs and colchicines) were consistent with pulmonary tuberculosis so we didn't feel need to go for CT scan of chest.

On one hand steroids are first line of treatment for pulmonary involvement in Sweet's syndrome while on other hand they are contraindicated in active pulmonary tuberculosis. So differentiation between two is utmost important and further it is emphasized that even in chest asymptomatic patients active search must be done to find out tubercular focus before starting oral steroids.

Oral corticosteroids relieve general symptoms within hours, skin lesions within 2–5 days and induce complete cure between 1 and 12 weeks. Despite excellent initial response, Kemmet and Hunter have reported recurrences in 30% cases.[11] Further long term oral corticosteroids therapy leads to reactivation of latent tuberculosis and dissemination of focal tuberculosis. In view of above, we put our patient on colchicine along with standard regimen of anti-tubercular drugs. Patient remitted well and the gastrointestinal side effects which generally limit the use of colchicine were not seen. Potassium iodide, dapsone, clofazamine, cyclosporine and infliximab are other drugs implicated for the treatment of Sweet's syndrome.

4. Conclusion

Sweet's syndrome is very common neutrophillic dermatosis often treated by systemic corticosteroids. In developing

countries like India active search must be done to find out underlying latent or undiagnosed tuberculosis. Corticosteroids should be used cautiously as they can manifest the latent tuberculosis or disseminate the focal tuberculosis. Colchicine is a safe and effective alternative drug to treat Sweet's syndrome concomitant with pulmonary tuberculosis.

Conflicts of interest

The author has none to declare.

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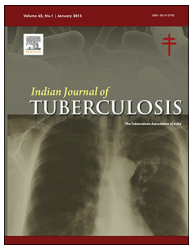

Case report

A case of hepatic tuberculosis: A tuberculoma

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ABSTRACT

Tuberculosis (TB) is a common cause of morbidity and mortality worldwide and its eradication in the United States has stalled for the first time in decades. Isolated hepatic TB is an extremely uncommon form of extrapulmonary TB. Here we present a case of a tuberculous liver abscess and suggest that TB should be considered in patients who fail to respond to antibiotics and prompt diagnostic intervention.

1. Introduction

Liver abscess is a rare disease, originally described by Hippocrates, which was previously uniformly fatal. Improvement in diagnostic imaging and antimicrobials has led to an improvement in outcome. It is a rare condition with a slight predominance in males and some association with diabetes mellitus, underlying hepatobiliary or pancreatic disease or occult gastrointestinal (GI) or intra-abdominal malignancy.[1] However, limited literature exists regarding isolated tuberculous liver abscesses in the absence of pulmonary disease.

2. Case report

A 40-year old obese Indian-American woman with no known medical history presented with 2 weeks of progressive right upper quadrant abdominal pain and low grade fevers. The pain was sharp and radiated to her back. She also described associated nausea, non-bloody vomitus and loose bowel movements. There was no history of weight loss, cough, sick contacts or recent travels. Admission blood counts demonstrated a white blood cell count of 7.8×10^9/L with 81% neutrophils and 14% bands and a platelet count of 89×10^9/L. Comprehensive metabolic panel was notable for a sodium of

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136 mmol/L, potassium 2.6 mmol/L, bicarbonate 17 mmol/L, total bilirubin 3.6 mg/dL, aspartate aminotransferase (AST) 471 units/L, alanine aminotransferase (ALT) 132 units/L and a normal serum creatinine. The INR was 2.07. The toxicology screen, acute hepatitis panel and human immunodeficiency virus (HIV) testing were all negative.

A computed tomography (CT) scan of the abdomen showed a complex right upper quadrant mass with low density cystic components centered on the porta hepatis with a conglomerate of necrotic lymphadenopathy (Fig. 1). A magnetic resonance imaging (MRI) was done due to persistence of symptoms, which demonstrated a complex cystic mass with involvement of the pancreatic head and segment 6 of the liver consistent with abscess. She was treated with typical antibiotics as well as Albendazole for a liver abscess. She continued to deteriorate clinically and blood cultures were persistently negative. Subsequently abscess drainage was performed and yielded thick, foul-smelling brown pus. The gram-stain demonstrated gram-positive rods that were strongly acid-fast positive. Acid-fast culture confirmed a *Mycobacterium tuberculosis* abscess.

3. Discussion

For the first time in two decades tuberculosis (TB) incidence in the United States has not decreased.[2] Infection with *Mycobacterium tuberculosis* is common, with nearly one-third of the world's population affected at any given time. *Mycobacterium tuberculosis* remains a major cause of death worldwide.[3] Extrapulmonary sites of infection can affect any organ with the most common affected sites being lymph nodes, pleura and osteoarticular areas. The diagnosis of extrapulmonary TB can be elusive and require a high index of suspicion. Antituberculous therapy can minimize mortality but may need to be initiated empirically.[4] Hepatobiliary and pancreatic TB are rare and often associated with military TB occurring in immunocompromised patients. The clinical presentation is non-specific but may include: anorexia, malaise, low grade fevers, weight loss, night sweats, melena, mass or abscess and obstructive jaundice. Diagnosis of TB at these obscure sites is difficult and often requires microbiological or histopathological examination.[5]

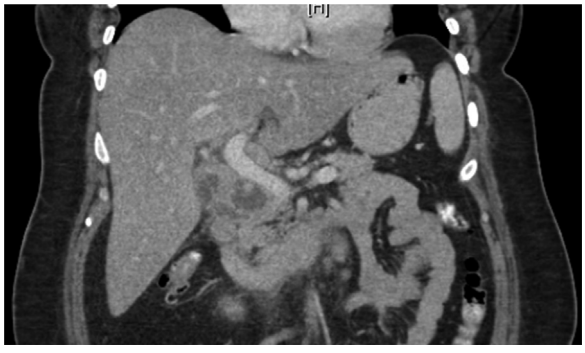

Fig. 1 – Complex right upper quadrant mass with low density cystic components centered on the porta hepatis as above. No resultant biliary or main pancreatic duct dilatation. A conglomerate of necrotic lymphadenopathy.

TB is most commonly isolated from the lungs and hepatic involvement is frequently seen in patients with disseminated TB. Miliary hepatic TB is rare and is often situated within lobules as the infection is thought to be carried from the lung via the hepatic artery and at times from the GI tract via the portal vein.[6] Three morphological types of hepatic TB have been described: (1) military hepatic TB associated with generalized military TB, (2) primary TB of the liver without involvement of other organs and (3) primary pyogenic lesions in the liver. The accepted criteria for diagnosis of hepatic TB include: (1) Acid Fast Bacilli (AFB) in liver tissue, (2) tubercle bacilli elsewhere plus hepatic granulomas with or without caseation, (3) typical macroscopic appearance on laparotomy or peritoneoscopy and (4) response to antituberculous therapy.[7]

Primary tuberculous hepatic abscesses, with no evidence of infection elsewhere are uncommon. The diagnosis is often difficult to make and often made post-mortem.[8] Primary involvement of the liver in TB is rare due to the low tissue oxygen level which makes liver inhospitable for the bacilli.[9] Imaging, although helpful in identifying liver abscess, is not helpful in differentiating tuberculous liver abscess from other pyogenic liver abscesses. CT findings include military, nodular or cystic lesions with or without ring enhancement, however, these radiologic findings have a low specificity and generally not helpful in making a definitive diagnosis.[10]

4. Conclusion

Pyogenic liver abscesses are a known cause of abdominal pain. Isolated tuberculous abscesses are a rare but deadly subset of liver abscesses. TB should be considered in patients who are from endemic areas or who do not respond to standard therapy. Liver abscesses, in patients who do not quickly respond to antibiotics should be pursued with percutaneous drainage to identify causative organisms. Percutaneous drainage is a safe and effective first-line management of liver abscesses.[11] Antituberculous therapy is recommended for 12 months in combination with percutaneous drainage.[12] The prognosis of liver abscess varies with the time of diagnosis: if diagnosed early and effective treatment administered the prognosis is good.

Conflicts of interest

The authors have none to declare.

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Letter to the Editor

IS6110 PCR for the early diagnosis of paediatric tuberculosis in India

Dear Editor,

India with 1.21 billion people is the second most populous country of the world after China. Children constitute about 39% of the total population of the country. Tuberculosis (TB) continues to be a major public health problem in India with 2.2 million new cases annually, making it the highest TB burden country in the world. Children get expose to TB from adult active patients and childhood TB may comprise about 10% total new TB cases in country.[1] Despite the high burden of TB and HIV/AIDS, little attention has been directed to the paediatric TB in the country.

I read with interest the paper ''PCR targeting IS6110 in diagnosing tuberculosis in children in comparison to MGIT culture'' by Dayal et al., published in the July 2016 issue of Indian Journal of Tuberculosis.[2] The results of this study[2] suggest that IS6110 PCR can be used for the early diagnosis of paediatric TB. However, limitations of the IS6110 PCR have not been highlighted by the authors in this paper.[2]

The IS6110 is a specific genetic marker for the identification of *Mycobacterium tuberculosis* complex and *M. tuberculosis* strains typically contain multiple copies (up to 25) of IS6110 sequence in their genome. However, several studies from different parts of the world have reported the presence of clinical *M. tuberculosis* strains with single or no copy of IS6110 in their genome.[3,4] It has been reported that 8–11% of *M. tuberculosis* strains in South-East Asia do not contain IS6110 sequence.[3] In India, it has been reported that 11% clinical isolates of *M. tuberculosis* lacked IS6110 element in their genome and prevalence of zero copy number isolates varies from 9 to 10% in all four regions (north, south, east and west) of country.[4] The studies from southern part of the country reported high frequency (19.2–62.5%) of *M. tuberculosis* isolates with zero copy number of the IS6110 element.[5] The absence of IS6110 element in clinical isolates of *M. tuberculosis* may leads to false negative results and undermines the utility of IS6110 PCR as diagnostic tool.

Previously, variable sensitivity (70–90%) and specificity (83.5–99.0%) of IS6110 PCR has been reported for the diagnosis of paediatric TB. It has been found that the sensitivity and specificity of IS6110 PCR mainly depends on the source of the clinical sample, localization of disease, co-existence of HIV infection and other technical parameters. Therefore, IS6110 PCR cannot be considered as a test of choice to rule-out *M. tuberculosis* infection in paediatric patients. The samples with IS6110 PCR negative results should be re-tested using other tests and/or PCR targeting other molecular targets to ensure accurate diagnosis of paediatric TB, especially in the population of South-East Asia including India.

Conflicts of interest

The author has none to declare.

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Letter to the Editor

Soft tissue tuberculosis – An unusual presentation of a common disease

Keywords:
Tuberculosis
Soft tissue
Acid fast bacilli

ABSTRACT

Tuberculosis (TB) has reached epidemic proportions in India with a myriad of clinical presentations. Extra pulmonary TB can present in a wide variety of clinical forms and its identification requires a high degree of clinical suspicion. Soft tissue infection by Mycobacteria is rare. The diagnosis is often not thought of owing to the rarity of this entity.

Dear Editor,

Extra pulmonary manifestations of tuberculosis (TB) are becoming very common in the present era and this parallels with the rise in incidence of HIV infection.[1]

In this context, we would like to report an interesting case of TB involving the soft tissue. A 55 year old female, farmer by occupation, presented to the surgical Out Patient Department (OPD) with the complaints of swelling in the left wrist and right calf for a duration of 6 months. She had history of intermittent low grade fever for the same duration. On examination, she was thin built, afebrile and had pallor. On local examination, the left wrist swelling was multilobulated, 6 cm × 5 cm in size occupying the dorsal and lateral aspect of the left wrist. The swelling was non-tender, tense, cystic and the skin over the swelling was stretched with dilated blood vessels. The swelling over the right calf was 15 cm × 12 cm, non-tender, tense and cystic. The differential diagnosis was lipoma/neurofibroma.

Blood investigations: Haemoglobin – 10 g%, total WBC count – 15,430/μl, differential count – neutrophils 88%, lymphocytes 12%, Erythrocyte Sedimentation Rate (ESR) – 52 mm, HIV 1 and 2 antibodies – negative.

Ultrasonogram findings: Multiple thick walled cysts in the left wrist insinuating between tendons, suggestive of ganglion/bursitis. A large complex cyst, posterior aspect of right leg, arising between the tendons, suggestive of popliteal cyst. Fine Needle Aspiration Cytology (FNAC) was done which yielded thick viscid whitish material which was blood tinged.

On microscopy, amorphous eosinophilic material with patchy necrosis and a mixed inflammatory infiltrate was seen. A Ziehl–Nielsen stain showed a heavy load of acid fast bacilli. Hence a diagnosis of Mycobacterial infection of the soft tissue was made. The patient was admitted and worked up for pulmonary TB. The chest X-ray revealed bilateral miliary patches. She was started on Anti Tubercular Therapy (ATT), showed clinical improvement and was discharged.

The WHO global TB report 2016 states that there is an estimated 10.4 million new TB cases of which India has the highest TB burden with approximately 2.84 million new cases reported in 2015.[2] TB as a disease entity has diversified in its clinical presentations with emergence of rarer and unusual forms of extra pulmonary disease. Extra pulmonary disease can involve any of the organ systems. However the commonest involved organs include lymph nodes, Central Nervous System (CNS), pleura, musculoskeletal system and genitourinary system (Fig. 1).[3]

TB of the soft tissue is a rare entity and occurrence of this in an immune competent person is still rarer.[4,5] Although disseminated disease involving the reticulo endothelial system, GI tract and musculoskeletal systems have been reported, multiple soft tissue swellings is a rare presentation.[1] Such a presentation rarely brings TB into the spectrum of

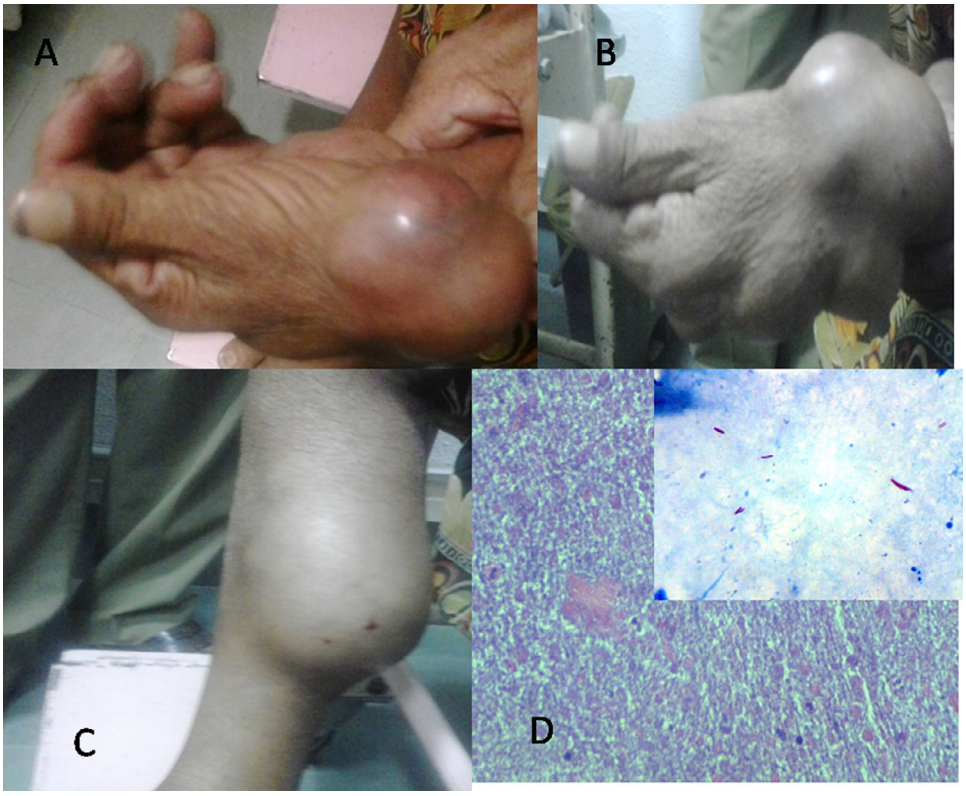

Fig. 1 – (A, B) Soft tissue swelling left wrist, (C) swelling in the right calf region, (D) FNAC (40×) areas of necrosis – inset (100×) – acid fast bacilli in groups.

differential diagnosis. This case emphasises the need to consider TB in the differential diagnosis of soft tissue swellings especially in India where the disease is rampant and remains one of the leading causes of morbidity and mortality. Timely diagnosis not only offers cure but also eliminates unnecessary surgical interventions.

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Abstracts

Drug resistance patterns among extra-pulmonary tuberculosis cases in a tertiary care centre in North India

Sharma SK, Chaubey J, Singh BK, Sharma R, Mittal A, Sharma A. *Int J Tuberc Lung Dis*. 2017;**21(10)**:1112–17. https://doi.org/10.5588/ijtld.16.0939

Background: Extra-pulmonary tuberculosis (EPTB) is a growing public health concern, and data on drug resistance are limited.
Material and methods: Specimens from 2468 clinically diagnosed EPTB patients received at the Intermediate Reference Laboratory (IRL) of a tertiary centre in India were subjected to Ziehl–Neelsen staining, Xpert® MTB/RIF testing, liquid culture and drug susceptibility testing (DST) using automated BACTEC MGIT™ 960™. Line-probe assay (LPA) was performed on all culture-positive isolates. Gene sequencing was performed on rifampicin-resistant/multidrug-resistant TB (RR/MDR-TB) and phenotypic/genotypic discrepant isolates.
Results: The culture positivity rate was 18.9% (483/2553). The sensitivity and specificity of Xpert in diagnosing EPTB were respectively 70.8% (95%CI 66.5–74.8) and 97.7% (95%CI 96.9–98.3), with liquid culture as the reference standard. Prevalence of RR/MDR-TB was 10.1% (49/483). Prevalence of pre-extensively drug-resistant TB (pre-XDR-TB) was 18.4% (09/49), whereas the prevalence of XDR-TB among MDR-TB patients was 2% (01/49). The sensitivity of genotypic DST for the detection of rifampicin resistance was 92.7% (95%CI 81.1–98.5) and specificity was 99.3% (95%CI 97.5–99.9), with 100% concordance between Xpert and LPA.
Conclusion: The burden of drug resistance, including M/XDR-TB, among EPTB patients is high. Novel molecular tests can help in early diagnosis and treatment to prevent disease progression and amplification of resistance.

Conflicts of interest

The authors have none to declare.

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Why are HIV-infected people not started on antiretroviral therapy? A mixed-methods study from Gujarat, India

Chawla S, Shringarpure K, Modi B, Sharma R, Rewari BB, Shah AN, Verma PB, Dongre AR, Kumar AMV. *Public Health Action*. 2017;**7(3)**:183–92. https://doi.org/10.5588/pha.16.0108

Setting: Five purposively selected antiretroviral therapy (ART) centres in Gujarat, India.
Objectives: To assess the proportion of ART-eligible people living with the human immunodeficiency virus (PLHIV) who were not initiated on ART within 2 months of being recorded as eligible, to identify factors associated with non-initiation and to explore reasons from the provider's perspective.
Design: We used a mixed-methods design (triangulation) of (1) a quantitative phase involving record reviews and cohort analysis (Poisson regression) of PLHIV registered during April 2014–March 2015, and (2) a qualitative phase involving one-to-one interviews with 25 providers.
Results: Of 2079 ART-eligible PLHIV, 339 (16%) were not started on ART within 2 months. PLHIV with CD4 counts of <350 cells/μl and patients who were labourers, hospitalised, bedridden or registered with certain ART centres were more likely not to be initiated on ART. Qualitative results were categorised into two broad themes: government health system- and patient-related challenges, which validated and complemented the quantitative findings.
Conclusion: Several patient subgroups at greater risk of ART non-initiation were identified, along with reasons for risk; this has important programme implications for achieving the UNAIDS 90–90–90 goal, and particularly the second 90 component of having 90% of diagnosed PLHIV start ART.

Conflicts of interest

The authors have none to declare.

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Acquaintance to artificial neural networks and use of artificial intelligence as a diagnostic tool for tuberculosis: A review

Dande P, Samant P. *Tuberculosis*. 2017;**106**. https://doi.org/10.1016/j.tube.2017.09.006

Tuberculosis [TB] has afflicted numerous nations in the world. As per a report by the World Health Organization [WHO], an estimated 1.4 million TB deaths in 2015 and an additional 0.4 million deaths resulting from TB disease among people living with HIV, were observed. Most of the TB deaths can be prevented if it is detected at an early stage. The existing processes of diagnosis like blood tests or sputum tests are not only tedious but also take a long time for analysis and cannot differentiate between different drug resistant stages of TB.

The need to find newer prompt methods for disease detection has been aided by the latest Artificial Intelligence [AI] tools. Artificial neural network [ANN] is one of the important tools that is being used widely in diagnosis and evaluation of medical conditions. This review aims at providing brief introduction to various AI tools that are used in TB detection and gives a detailed description about the utilization of ANN as an efficient diagnostic technique. The paper also provides a critical assessment of ANN and the existing techniques for their diagnosis of TB. Researchers and Practitioners in the field are looking forward to use ANN and other upcoming AI tools such as fuzzy-logic, genetic algorithms and artificial intelligence simulation as a promising current and future technology tools towards tackling the global menace of tuberculosis. Latest advancements in the diagnostic field include the combined use of ANN with various other AI tools like the fuzzy-logic, which has led to an increase in the efficacy and specificity of the diagnostic techniques.

Conflicts of interest

The authors have none to declare.

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Evaluation of a rapid molecular drug-susceptibility test for tuberculosis

Xie YL, Chakravorty S, Armstrong DT, Hall SL, Via LE, Song T, Yuan X, Mo X, Zhu H, Xu P, Gao Q, Lee M, Lee J, Smith LE, Chen RY, Joh JS, Cho YS, Liu X, Ruan X, Liang L, Dharan N, Cho S-N, Barry III CE, Ellner JJ, Dorman SE, Alland D. *N Engl J Med*. 2017;**377**:1043–54. https://doi.org/10.1056/NEJMoa1614915

Background: Fluoroquinolones and second-line injectable drugs are the backbone of treatment regimens for multidrug-resistant tuberculosis, and resistance to these drugs defines extensively drug-resistant tuberculosis. We assessed the accuracy of an automated, cartridge-based molecular assay for the detection, directly from sputum specimens, of *Mycobacterium tuberculosis* with resistance to fluoroquinolones, aminoglycosides, and isoniazid.

Methods: We conducted a prospective diagnostic accuracy study to compare the investigational assay against phenotypic drug-susceptibility testing and DNA sequencing among adults in China and South Korea who had symptoms of tuberculosis. The Xpert MTB/RIF assay and sputum culture were performed. *M. tuberculosis* isolates underwent phenotypic drug-susceptibility testing and DNA sequencing of the genes *katG*, *gyrA*, *gyrB*, and *rrs* and of the *eis* and *inhA* promoter regions.

Results: Among the 308 participants who were culture-positive for *M. tuberculosis*, when phenotypic drug-susceptibility testing was used as the reference standard, the sensitivities of the investigational assay for detecting resistance were 83.3% for isoniazid (95% confidence interval [CI], 77.1–88.5), 88.4% for ofloxacin (95% CI, 80.2–94.1), 87.6% for moxifloxacin at a critical concentration of 0.5 μg per milliliter (95% CI, 79.0–93.7), 96.2% for moxifloxacin at a critical concentration of 2.0 μg per milliliter (95% CI, 87.0–99.5), 71.4% for kanamycin (95% CI, 56.7–83.4), and 70.7% for amikacin (95% CI, 54.5–83.9). The specificity of the assay for the detection of phenotypic resistance was 94.3% or greater for all drugs except moxifloxacin at a critical concentration of 2.0 μg per milliliter (specificity, 84.0% [95% CI, 78.9–88.3]). When DNA sequencing was used as the reference standard, the sensitivities of the investigational assay for detecting mutations associated with resistance were 98.1% for isoniazid (95% CI, 94.4–99.6), 95.8% for fluoroquinolones (95% CI, 89.6–98.8), 92.7% for kanamycin (95% CI, 80.1–98.5), and 96.8% for amikacin (95% CI, 83.3–99.9), and the specificity for all drugs was 99.6% (95% CI, 97.9–100) or greater.

Conclusions: This investigational assay accurately detected *M. tuberculosis* mutations associated with resistance to isoniazid, fluoroquinolones, and aminoglycosides and holds promise as a rapid point-of-care test to guide therapeutic decisions for patients with tuberculosis. (Funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and the Ministry of Science and Technology of China; ClinicalTrials.gov number, NCT02251327.)

Conflicts of interest

The authors have none to declare.

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Community-based active case finding for tuberculosis in rural western China: A cross-sectional study

Chen C, Yang C-G, Gao X, Lu Z-Z, Tang F-X, Cheng J, Gao Q, Cárdenas V. *Int J Tuberc Lung Dis*. 2017;**21(11)**:1134–9. https://doi.org/10.5588/ijtld.17.0123

Setting: Current passive case finding strategies are not effective at identifying tuberculosis (TB) patients in rural China.

Objective: To evaluate a community-based, active case finding (ACF) scheme in identifying symptomatic individuals with TB.

Design: We conducted door-to-door household visits of all residents aged □15 years at two rural sites to screen for TB symptoms. Individuals with symptoms were enrolled and asked to provide three sputum samples. All participants underwent chest X-ray, and microbiologic detection of *Mycobacterium tuberculosis* from sputum samples using microscopy, solid culture and Xpert® MTB/RIF was performed.

Results: Among the 19,334 residents screened for TB symptoms, 865 (4.5%) reported having □1 symptom. A total of 52 TB cases were detected, 11 of whom had microbiologic confirmation. Xpert identified all five *M. tuberculosis* culture-positive cases and yielded an additional three diagnoses. Prevalence of newly detected TB at the two sites through ACF was respectively 475 and 196 per 100,000 population. These estimates are respectively four and eight times, on average, higher than those identified through passive surveillance during the previous 5-year period for the two sites.

Conclusion: Community-based symptom screening followed by laboratory tests was found to be feasible and effective in increasing TB case finding in rural China.

Conflicts of interest

The authors have none to declare.

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Revisiting acid-fast bacilli microscopy of concentrated sputum smears as an efficient tool for the diagnosis of tuberculosis: A study from a tertiary care centre in Southern India

Anto Jesuraj Uday Kumar J, Dhar C, Srinivasa H. *J Tuberc Res*. 2017. https://doi.org/10.4236/jtr.2017.52016

Background and objectives: With 2.2 million new cases every year, tuberculosis (TB) continues to be an epidemic of large

proportions in India. Conventional direct sputum smear microscopy, though limited in its sensitivity, is still the most common method of testing for TB. Newer techniques such as concentrated sputum microscopy, have shown some promise in improving this limited sensitivity. We have compared the efficacy of concentrated sputum versus the direct smear technique in 1000 sputum samples of patients suspected to be suffering from TB.
Methods: A total of 1000 sputum specimens were collected for direct acid-fast bacilli (AFB) smear, concentrated AFB smear and culture from St. John's Medical College and Hospital. 39 contaminated samples were (3.9%) omitted during the final analysis. Mycobacterial culture was used as the reference standard method for the detection of TB.
Results: 184 and 198 of the 961 samples were found to AFB positive by direct smear microscopy and concentrated smear technique respectively. The measured sensitivity and specificity of direct smear microscopy were 69.86% and 95.82%, while that of concentrated smear microscopy was 76.71% and 95.96% respectively. 33 samples found to be negative by the direct smear method turned out to be positive by the concentrated smear technique.
Conclusions: Though our study suggests no significant statistical difference between the two techniques of detecting pulmonary tuberculosis, we recommend the use of the concentrated technique in centres such as ours, where facilities are already in place. In this way, the number of cases of TB that remain untreated may significantly come down.

Conflicts of interest

The authors have none to declare.

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Incidence of active tuberculosis in individuals with latent tuberculosis infection in rural China: Follow-up results of a population-based, multicentre, prospective cohort study

Gao L, Li X, Liu J, Wang X, Lu W, Bai L, Xin H, Zhang H, Li H, Zhang Z, Ma Y, Li M, Feng B, Du J, Sui H, Zhao R, Su H, Pan S, Guan L, Shen F, He J, Yang S, Si H, Cheng X, Xu Z, Tan Y, Chen T, Xu W, Peng H, Wang Z, Zhu T, Chen X, Zhou X, Guan X, Jin Q for the LATENTTB-NSTM Study Team. *Lancet Infect Dis*. 2017;**17 (10)**:1053–61. https://doi.org/10.1016/S1473-3099(17)30402-4
Background: The management of latent *Mycobacterium tuberculosis* infection is a new priority action for the WHO End Tuberculosis (TB) Strategy. However, national guidelines on latent tuberculosis infection testing and treatment have not yet been developed in China. Here, we present the results from the 2-year follow-up of a study that aimed to track the development of active disease in individuals with latent tuberculosis infection, identify priority populations for latent infection management, and explore the most suitable latent infection diagnostic approach.
Methods: A population-based multicentre prospective study was done in four sites in rural China, between 2013 and 2015. The baseline survey in 2013 measured the prevalence of latent tuberculosis infection using QuantiFERON-TB Gold In-Tube (QFT) and tuberculin skin test (TST) in eligible participants. During the follow-up phase between 2014 and 2015, we assessed individuals who had tuberculosis infection at baseline (QFT-positivity or TST tuberculin reaction size [induration] of ≥10 mm) for the development of active disease through active case finding. Eligible participants included in follow-up survey had a birth date before June 1, 2008 (5 years or older in 2013), and continuous residence at the study site for 6 months or longer in the past year. Participants with current active tuberculosis at baseline survey were excluded.
Findings: Between September 1, 2013, and August 31, 2015, 7505 eligible participants (aged 5 years or older) were included in tuberculosis infection test positive cohorts (4455 were QFT positive, 6404 had TST induration ≥10 mm, and 3354 were positive for both tests) after baseline examination. During the 2-year follow-up period, 84 incident cases of active tuberculosis were diagnosed. Of participants who developed active tuberculosis, 75 were diagnosed with latent infection by QFT, 62 were diagnosed by TST, and 53 were diagnosed by both tests. An incidence rate of 0.87 (95% CI 0.68–1.07) per 100 person-years was observed for individuals who tested positive with QFT, 0.50 (0.38–0.63) per 100 person-years for those who tested positive with TST ($p < 0.0001$), and 0.82 (0.60–1.04) per 100 person-years for those who tested positive with both tests. Male sex and a history of tuberculosis were significantly associated with increased risk of disease development with adjusted hazard ratios of 2.36 (95% CI 1.30–4.30) for male sex and 5.40 (3.34–8.71) for a history of tuberculosis.
Interpretation: Our results suggest that high-risk populations in communities in rural China, such as individuals at a high risk of disease reactivation from previous tuberculosis, should be targeted for latent infection screening and treatment with an interferon-γ releasing assay rather than a TST.
Funding: National Science and Technology Major Project of China, Program for Changjiang Scholars and Innovative Research Team in University of China, CAMS Innovation Fund for Medical Sciences, and Sanming Project of Medicine in Shenzhen.

Conflicts of interest

The authors have none to declare.

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A label-free biosensor based on localized surface plasmon resonance for diagnosis of tuberculosis

Sun W, Yuan S, Huang H, Liu N, Tan Y. *J Microbiol Methods*. 2017;**142**:41–5. https://doi.org/10.1016/j.mimet.2017.09.007
A biosensor based on localized surface plasmon resonance (LSPR) was developed to detect the antibody of *Mycobacterium tuberculosis* using the fusion protein CFP10-ESAT6 as an antigen. To explore the diagnostic potential of the biosensor for tuberculosis (TB), the fusion protein CFP10-ESAT6 was immobilized on gold nanorods (Au NRs) by chemical modification, and the functionalized Au NRs were subsequently incubated with serums collected from TB patients, non-tuberculous pulmonary disease patients or healthy individuals. The change in the LSPR properties of Au NRs from the specific interaction between the antigen and antibody was monitored, and detection of the target antibody was completed based on the proposed biosensor. Serum analysis showed that the sensitivity of the biosensor was 79% and the specificity was 92%. Therefore, the LSPR biosensor is a valuable tool for serodiagnosis of TB.

Conflicts of interest

The authors have none to declare.

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Defining the research agenda to measure and reduce tuberculosis stigmas

Macintyre K, Bakker MI, Bergson S, Bhavaraju R, Bond V, Chikovore J, Colvin C, Craig GM, Cremers AL, Daftary A, Engel N, Ferris France N, Jaramillo E, Kimerling M, Kipp A, Krishnaratne S, Mergenthaler C, Ngicho M, Redwood L, Rood EJJ, Sommerland N, Stangl A, van Rie A, van Brakel W, Wouters E, Zwerling A, Mitchell EMH. *Int J Tuberc Lung Dis*. 2017;**21(suppl 1)**: S87–S96. https://doi.org/10.5588/ijtld.17.0151

Crucial to finding and treating the 4 million tuberculosis (TB) patients currently missed by national TB programmes, TB stigma is receiving well-deserved and long-delayed attention at the global level. However, the ability to measure and evaluate the success of TB stigma-reduction efforts is limited by the need for additional tools. At a 2016 TB stigma-measurement meeting held in The Hague, The Netherlands, stigma experts discussed and proposed a research agenda around four themes: (1) drivers: what are the main drivers and domains of TB stigma(s)?; (2) consequences: how consequential are TB stigmas and how are negative impacts most felt?; (3) burden: what is the global prevalence and distribution of TB stigma(s) and what explains any variation? (4): intervention: what can be done to reduce the extent and impact of TB stigma (s)? Each theme was further subdivided into research topics to be addressed to move the agenda forward. These include greater clarity on what causes TB stigmas to emerge and thrive, the difficulty of measuring the complexity of stigma, and the improbability of a universal stigma 'cure'. Nevertheless, these challenges should not hinder investments in the measurement and reduction of TB stigma. We believe it is time to focus on how, and not whether, the global community should measure and reduce TB stigma.

Conflicts of interest

The authors have none to declare.

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