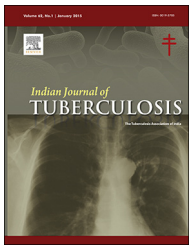
INDIAN JOURNAL OF TUBERCULOSIS

EDITORIAL BOARD

Available online at www.sciencedirect.com

ScienceDirect

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



Editorial

Informative, large cohorts of meningeal tuberculosis

While numerically of minor importance among all tuberculosis cases, tuberculous meningitis is nevertheless of major importance in clinical practice. It is difficult to diagnose, and the longer the delay to treatment initiation, the more disastrous the outcome after appropriate chemotherapy. Once a patient is presenting with Medical Research Council Grade III tuberculous meningitis (a Glasgow Coma Scale below 10/15), the chance of disability-free survival is less than 50%.[1] Because the disease is relatively and absolutely rare, comprehensive studies providing statistically meaningful data are difficult to come by. Earlier this year, two very large cohorts of tuberculosis meningitis among adults were published from Indonesia[2] and Viet Nam[3] respectively, accompanied by a thoughtful editorial comment.[4] The Indonesian study reported on 608 patients, the Vietnamese comprised 764. These are unusually large sample sizes for such a rare condition. Coupled with the thorough investigations, they thus provide solid information on pathogenetic aspects of tuberculous meningitis that not only help a better understanding and insight into the interplay between clinical presentation and outcome and relevant components of the patient's genetic profile, but they also have diagnostic and quite possible therapeutic implications.

One of the key foci in both studies was leukotriene A4 hydrolase, an enzyme co-determining intracerebral inflammatory response. The activity of the hydrolase may lead to either a hyperinflammatory or hypoinflammatory response in meningeal tuberculosis, and either can have a detrimental effect on survival. Mutations that occur in the gene encoding leukotriene A4 hydrolase influence the genotype of the hydrolase, the type and extent of inflammatory response and thus the clinical presentation and prognosis.[4]

In both studies, the impact of the genotypic polymorphism of the enzyme was studied. The results were actually perplexing and the discordances much more complex to grasp than one had anticipated. In short, the studies did not demonstrate equivalent findings. In Viet Nam, patients with a heterozygous genotype causing hypoinflammation had a higher risk of death than those with a homozygous type (causing hyperinflammation). In Indonesia, no such association could be found. The two studies thus differed in the role of the inflammatory response genotype on the risk of death.[4] This is puzzling and suggests that unidentified effect modifiers and/or confounding factors play additional roles that could not be pinpointed in these two studies although a multitude of factors were examined and controlled for.

There were also substantial differences in fatality in the two studies that cannot be fully explained (among not HIV infected 41% in Indonesia versus 19% in Viet Nam). Other relevant clinical parameters were identified that influenced fatality. In Indonesia, a strong neutrophil response and fever increased the risk of death.[2] As expected, HIV infection increased the risk of a fatal outcome in both studies.

While knowledge continues accumulating about the pathogenesis of tuberculosis in general and meningeal tuberculosis in particular, these simultaneously published studies also hold an important lesson about research in a wider sense. It is decidedly unusual to have the opportunity of getting insight into the natural history of such a rare condition as meningeal tuberculosis from a cohort exceeding 600 cases. The rarity is topped by the fact that there were two such studies published simultaneously in the same Journal both investigating a very similar question. Had we had the opportunity of getting the results from only one of these two studies, many of us might be inclined to taking its main finding at face value. The opportunity to get insight into two of such studies teaches us how science moves at her best. There are discordances to resolve, and the best studies such as these challenge us to deepen our research endeavors even more to understand the natural history even better and thus ultimately improving our diagnosis and the patient's treatment outcome. This might be particularly important in such a rare condition as tuberculous meningitis that all too often results in a disastrous outcome if we are unable to diagnose it in a timely fashion and provide a treatment that is not only an issue of the right drug combination but also and perhaps often even more importantly, addressing increasingly correctly the type of inflammatory response that causes the most deleterious damage to the patient's brain.

REFERENCES

1. Thwaites G. Tuberculous meningitis. *Medicine (Baltimore)*. 2013;41:683–685.

2. van Laarhoven A, Dian S, Ruesen C, et al. Clinical parameters, routine inflammatory markers, and LTA4H genotype as predictors of mortality among 608 patients with tuberculous meningitis in Indonesia. *J Infect Dis*. 2017; 215:1029–1039.
3. Thuong NTT, Heemskerk D, Tram TTB, et al. Leukotriene A4 hydrolase genotype and HIV infection influence intracerebral inflammation and survival from tuberculous meningitis. *J Infect Dis*. 2017;215:1020–1028.
4. Fava V, Schurr E. Evaluating the impact of LTA4H genotype and immune status on survival from tuberculous meningitis. *J Infect Dis*. 2017;215:1011–1013 [Editorial Commentary].

Hans L. Rieder MD, MPH
Tuberculosis Consultant Services and University of Zurich, Switzerland

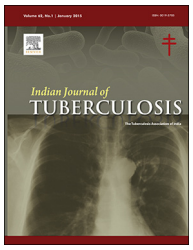
Received 30 June 2017

http://dx.doi.org/10.1016/j.ijtb.2017.07.001
0019-5707/

Available online at www.sciencedirect.com

ScienceDirect

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



Short communication

Sarcoidosis vs tuberculosis: Diagnostic mystery still unresolved

Rashi Jain, Anant Mohan *, *Randeep Guleria*

All India Institute of Medical Sciences, India

ARTICLE INFO

Keywords:
Sarcoidosis
Tuberculosis
Diagnosis
Differentiation

ABSTRACT

Sarcoidosis and tuberculosis are chronic, multisystemic, granulomatous disease of alike clinical, radiological and histopathological manifestations. Idiopathic nature of the disease and a strong clinical similarity with tuberculosis make the effectiveness of various clinical examinations for the diagnosis of sarcoidosis difficult in a tuberculosis endemic area. Presently confirmation of a diagnosis of sarcoidosis in most cases requires a biopsy which is often not confirmatory. A variety of novel medical approaches is under research to replace invasive diagnostic procedures for a simple non-invasive investigation for the identification of sarcoidosis. Here we discussed the studies focussing on the features that can be useful for distinguishing sarcoidosis from tuberculosis. Multiple studies have found molecular, cellular, immunological and clinical biomarkers efficient to lead the way of clinicians for the exact diagnosis of sarcoidosis.

1. Introduction

Sarcoidosis is a chronic, multisystemic, enigmatic granulomatous disorder of undetermined aetiology characterised by tissue infiltration by mononuclear phagocytes and lymphocytes. The close clinicoradiological and histopathological similarities between sarcoidosis and tuberculosis make the confident confirmation of sarcoidosis challenging. The presence of bilateral hilar lymphadenopathy, while considered a radiological hallmark of sarcoidosis, is by no means pathognomic and may be encountered in tuberculosis, lymphomas, other infections, including fungal, and certain occupational diseases such as asbestosis.[1] To further complicate the issue, symptoms of pulmonary sarcoidosis are very non-specific and often mimic a host of other respiratory disorders. On the other hand, features considered typical of pulmonary tuberculosis, such as a miliary distribution of lesions, can also occur in sarcoidosis.[2]

The pathological hallmark of sarcoidosis is the presence of noncaseating, compact, 'naked' granulomas while inflammation in tuberculosis is more intense with plenty of 'caseous' necrosis in the centre of granulomas.[3] However, necrotizing granulomas do occur in sarcoidosis[4] and conversely, one may demonstrate noncaseating granulomas in tuberculosis.[5] This clearly indicates again that histopathological features alone are not specific enough to discriminate between these two disorders.

Amidst all these puzzles, a variety of novel medical approaches is being utilised for accurate differentiation between sarcoidosis and tuberculosis. Multidisciplinary approaches are indeed required in India where the prevalence

* *Corresponding author.*
E-mail address: anantmohan@yahoo.com (A. Mohan).

http://dx.doi.org/10.1016/j.ijtb.2017.09.001

of tuberculosis is extremely high. Since the therapeutic approach for tuberculosis and sarcoidosis is radically different, i.e. anti-tubercular drugs and corticosteroids respectively, misdiagnosis may produce an adverse impact on patients' health.

2. Molecular diagnosis

The advent of molecular analysis has reinvigorated the study of disease pathology and helps distinguish diseases with similar clinical presentations. Active research is ongoing to unearth suitable and reliable differentiating features between sarcoidosis and tuberculosis as well. Bloom et al.[6] identified 144 differentially expressed transcripts including IFN-inducible genes between TB and active sarcoidosis. A detailed comparison between pulmonary tuberculosis and sarcoidosis including whole-blood gene expression profiling, microRNA expression, and multiplex serum analytes showed multiple common and differential features between two groups.[7] Four miRNA, 691 significantly regulated genes and 12 serum analytes were shown to acquire different direction between the patient groups. In another study, protein microarray, enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC) and receiver-operating characteristic (ROC) curve all together verified differential expression of intercellular adhesion molecule 1 (ICAM-1) and leptin between sarcoidosis and tuberculosis.[8] Koth et al.[9] measured the transcriptional signature of peripheral blood from patients with sarcoidosis and assessed overlap and differences in their gene expression with pulmonary tuberculosis. They used machine learning algorithms to build a classifier for distinguishing sarcoidosis from pulmonary tuberculosis. This analysis suggested GBP6 [guanylate-binding protein 6]; SEPT4 [Septin 4]; TIMM10 [translocase of inner mitochondrial membrane 10]; and NOG [Noggin] are downregulated in sarcoidosis but not in tuberculosis.

Overall molecular approaches are being aggressively explored for diagnosing sarcoidosis, but are limited by the non-uniformity in their results. Further robust studies might carve a pathway to diagnose sarcoidosis and tuberculosis with more specificity.

3. Cellular diagnosis

Cytological examination of patients with sarcoidosis may possibly prove the sole or supporting evidence for distinguishing from tuberculosis. Bronchoalveolar lavage (BAL) cellular analysis could be used for differentiating sarcoidosis from tuberculosis. BAL lymphocyte percentage, neutrophil count and CD4/CD8 ratio have good sensitivity and specificity for differentiating between the two conditions.[10] In majority of cases, the presence of high-grade lymphocytosis, low neutrophil percentage and high CD4/CD8 ratio in BAL act as a good predictor for sarcoidosis. Sarcoidosis patients exhibit the higher percentage of CD4+T lymphocytes and CD4/CD8 ratio while the lower count of CD8+T lymphocytes and mast cells.[12] Recently, a relatively lesser known inflammatory marker, neutrophil/lymphocyte ratio has attracted attention and is being explored as a promising tool for differentiating sarcoidosis from tuberculosis.[13]

4. Immunological diagnosis

Sarcoidosis and tuberculosis both are accompanied by a chronic inflammation resulting in mononuclear cell infiltrates and granuloma formation. Comparative assessment of cell and humoral mediated immune response may aid in discriminating the two diseases. As a pilot experiment, Levy et al.[14] utilised ELISA to test if serum anti-TB IgG levels could be a tool for such differentiation. The authors found that patients with sarcoidosis were highly different from patients with tuberculosis. Serum anti-TB IgG levels were significantly higher in tuberculosis patients than sarcoidosis ones ($p < 10^{-6}$). This study has been conducted three decades ago with no further reproduction; hence its reliability is not established. The analysis of cytokine network participating in the immunopathogenesis of sarcoidosis and tuberculosis may enhance our knowledge to make these diseases indistinguishable from each other. Cytokine profile in serum and BAL of tuberculosis and sarcoidosis patients were evaluated to get any difference between them.[15] Cytokine profile in BAL of pulmonary tuberculosis and pulmonary sarcoidosis was found indistinguishable and provided no diagnostic value. In blood, the only Interleukin-4 (IL-4) level was comparable between tuberculosis and sarcoidosis. The author utilised all other differences in serum markers to create a logistic regression model to classify a sample as tuberculosis or sarcoidosis. Barring some limitations, this study was a sincere effort for distinguishing the two diseases but failed to achieve the desired objective.

A patent was recently accepted in the United States for diagnosis of sarcoidosis based on a blood test using mycobacterial catalase-peroxidase (mKat G). It is a whole blood Interferon gamma (IFNγ) release assay. For a positive test of sarcoidosis, IFNγ concentration in mKatG condition minus the IFNγ concentration in background condition must be greater than 100 pg/ml and IFNγ concentration in mKatG condition must be greater than the IFNγ concentration in the PPD condition.[16] In Indian settings, use of this technique is questionable due to widespread exposure to tuberculosis.

5. Nucleic acid identification

Quantification of *Mycobacterium tuberculosis* (MTB) genome has greater diagnostic performance in discriminating patients with sarcoidosis from those with tuberculosis.[17] Applying ROC curves it was analysed that MTB genome copies number of 1.14×10^3 copies per ml should be preferred as quantitative cutoff value for the differentiation with the sensitivity 96.8% and specificity 98.1%.

6. Clinical and histopathological diagnosis

On clinical grounds, a histological diagnosis may prove reliable for differentiation between sarcoidosis and tuberculosis, two

diseases mimicking each other. Lymph nodes of patients with sarcoidosis and tuberculosis were biopsied through endobronchial ultrasonography-guided transbronchial needle aspiration (EBUS TBNA) and examined for their sonographic features.[18] Along with a positive tuberculin skin test (TST), heterogeneous echotexture or coagulation necrosis in the lymph nodes on EBUS were found having diagnostic value for tuberculosis over sarcoidosis. In a parallel study, the size of lymph nodes on endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was considered for comparison between the two disorders.[19] Tuberculosis nodes were significantly smaller than those in sarcoidosis. In a specific group of patients with enlarged hilar and paratracheal lymph nodes, labial biopsies were examined for having any discriminatory value.[20] Non-caseating granuloma in the labial biopsy in sarcoidosis vs normal minor salivary glands in tuberculosis might be considered for differentiating the two diseases.

In contrast to the above results, a study by Kaur et al.[20] did not find any morphological features of lymph node granulomas that could help distinguish tuberculosis from sarcoidosis.

7. Conclusion

The distinction between sarcoidosis and tuberculosis continues to challenge clinicians and researchers alike. No single parameter or feature has been able to reliably differentiate one from the other. Based on the results collected from cellular, molecular, immunological, histopathological and clinical studies, a decisive model could be developed to distinguish the two diseases. Though individual results are not accepted by research communities unanimously, collective data from various dimensions may make it possible to substitute invasive diagnostic procedures for a simple non invasive investigation for sarcoidosis.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Woodring JH, Vandiviere HM, Fried AM, Dillon ML, Williams TD, Melvin IG. Update: the radiographic features of pulmonary tuberculosis. *AJR Am J Roentgenol*. 1986;146 (3):497–506.
2. Gupta D, Kumar S, Jindal SK. Bilateral miliary mottling without hilar lymphadenopathy: a rare presentation of sarcoidosis. *Lung India*. 1996;14(2):87.
3. Rosen Y. Pathology of sarcoidosis. *Semin Respir Crit Care Med*. 2007;28(1):36–52.
4. Karkhanis V, Joshi JM. All that caseate is not TB. *Lung India*. 2007;24(3):100.
5. Mukhopadhyay S, Gal AA. Granulomatous lung disease: an approach to the differential diagnosis. *Arch Pathol Lab Med*. 2010;134(5):667–690.
6. Bloom CI, Graham CM, Berry MPR, et al. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. *PLOS ONE*. 2013;8(8).
7. Maertzdorf J, Weiner J, Mollenkopf H-J, et al. Common patterns and disease-related signatures in tuberculosis and sarcoidosis. *Proc Natl Acad Sci USA*. 2012;109(20):7853–7858.
8. Du S-S, Zhao M-M, Zhang Y, et al. Screening for differentially expressed proteins relevant to the differential diagnosis of sarcoidosis and tuberculosis. *PLOS ONE*. 2015;10(9):e0132466.
9. Koth LL, Solberg OD, Peng JC, Bhakta NR, Nguyen CP, Woodruff PG. Sarcoidosis blood transcriptome reflects lung inflammation and overlaps with tuberculosis. *Am J Respir Crit Care Med*. 2011;184(10):1153–1163.
10. Greco S, Marruchella A, Massari M, Saltini C. Predictive value of BAL cellular analysis in differentiating pulmonary tuberculosis and sarcoidosis. *Eur Respir J*. 2005;26(2):360–362.
12. Iliaz S, Iliaz R, Ortakoylu G, Bahadir A, Bagci B, Caglar E. Value of neutrophil/lymphocyte ratio in the differential diagnosis of sarcoidosis and tuberculosis. *Ann Thorac Med*. 2014;9(4):232.
13. Levy H, Feldman C, Wadee AA, Rabson AR. Differentiation of sarcoidosis from tuberculosis using an enzyme-linked immunosorbent assay for the detection of antibodies against *Mycobacterium tuberculosis*. *Chest*. 1988;94(6):1254–1255.
14. Thillai M, Eberhardt C, Lewin AM, et al. Sarcoidosis and tuberculosis cytokine profiles: indistinguishable in bronchoalveolar lavage but different in blood. *PLoS ONE*. 2012;7(7):e38083.
15. Moller DR. *Diagnostic Blood Test for Sarcoidosis*. 2015. 20150192592. Available from: http://www.freepatentsonline.com/y2015/0192592.html.
16. Zhou Y, Li HP, Li QH, et al. Differentiation of sarcoidosis from tuberculosis using real-time PCR assay for the detection and quantification of Mycobacterium tuberculosis. *Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG World Assoc Sarcoidosis Granulomatous Disord*. 2008;25(2):93–99.
17. Dhooria S, Agarwal R, Aggarwal AN, Bal A, Gupta N, Gupta D. Differentiating tuberculosis from sarcoidosis by sonographic characteristics of lymph nodes on endobronchial ultrasonography: a study of 165 patients. *J Thorac Cardiovasc Surg*. 2014;148(2):662–667.
18. Fritscher-Ravens A, Ghanbari A, Topalidis T, et al. Granulomatous mediastinal adenopathy: can endoscopic ultrasound-guided fine-needle aspiration differentiate between tuberculosis and sarcoidosis? *Endoscopy*. 2011;43 (11):955–961.
19. Tabak L, Ağirbaş E, Yilmazbayhan D, Tanyeri H, Güç U. The value of labial biopsy in the differentiation of sarcoidosis from tuberculosis. *Sarcoidosis Vasc Diffuse Lung Dis Off J WASOG*. 2001;18(2):191–195.
20. Kaur G, Dhamija A, Augustine J, Bakshi P, Verma K. Can cytomorphology of granulomas distinguish sarcoidosis from tuberculosis? Retrospective study of endobronchial ultrasound guided transbronchial needle aspirate of 49 granulomatous lymph nodes. *CytoJournal*. 2013;10(1):19.

FURTHER READING

11. Drent M, Wagenaar SS, Mulder PHG, Van Velzen-Blad H, Diamant M, Van den Bosch JMM. Bronchoalveolar lavage fluid profiles in sarcoidosis, tuberculosis, and non-Hodgkin's and Hodgkin's disease: an evaluation of differences. *Chest*. 1994;105(2):514–519.

Available online at www.sciencedirect.com

ScienceDirect

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

Review Article

Role of essential trace elements in tuberculosis infection: A review article

Aliyeh Sargazi [a], *Roghayeh Afsar Gharebagh* [b], *Alireza Sargazi* [a], *Halimeh Aali* [c], *Hamid Owaysee Oskoee* [d], *Zahra Sepehri* [e,*]

[a] *Students Research Committee, Zabol University of Medical Sciences, Zabol, Iran*
[b] *Assistant Professor of Cardiology, Department of Cardiology, Urmia University of Medical Sciences, Urmia, Iran*
[c] *Internist, Department of Internal Medicine, University of Medical Sciences, Zabol, Iran*
[d] *Department of Infectious Diseases, Tabriz University of Medical Sciences, Tabriz, Iran*
[e] *Research and Technology Department, Zabol University of Medical Sciences, Zabol, Iran*

ARTICLE INFO

Article history:
Received 8 October 2016
Accepted 17 March 2017
Available online 11 April 2017

Keywords:
Tuberculosis
Copper
Calcium
Iron
Zinc
Selenium

ABSTRACT

Malnutrition is one of the risk factors in tuberculosis (TB) infection. Mineral levels perturbation is seen in patients with TB. Moreover there are some strategies to starve pathogens of essential metals. Here we decided to conclude association between some essential elements and TB. Copper, calcium and iron are essential for hosts' immune system although calcium and iron are necessary for Mycobacterium tuberculosis vitality. Changing these elements alongside with anti-TB therapy is suggested for better treatment outcomes.

1. Introduction

Tuberculosis (TB) stays a serious health treat in the world. Malnutrition is seen particularly in TB infected patients in low income countries. Smoking, alcohol usage, gender, age, body mass index and socioeconomic status are some of the main risk factors correlate with TB.[1] It is assumed that poor nutrition reduces immune response and decreases body ability in combat with microbes. Mineral levels are detected in imbalanced levels in patients with TB.[2] Mostly in TB patients with insufficient dietary regimen the level of essential elements is low although higher dose of elements such as iron are seen in some patients.[3] There are many evidences about correlation between iron, zinc, copper, calcium, selenium and Mycobacterium tuberculosis (MTB) virulence. It has been well established that the balance of iron, copper, and zinc influences the success of MTB infections. Other studies demonstrated correlation between the levels of iron, copper, selenium, calcium and zinc in patients with TB.[2] Copper, selenium and

* *Corresponding author at*: Internist, Research and Technology Department, Zabol University of Medical Sciences, Zabol, Iran. Tel.: +98 54 32230770; fax: +98 54 32230768.
E-mail address: fkiyani7@gmail.com (Z. Sepehri).

http://dx.doi.org/10.1016/j.ijtb.2017.03.003

zinc are critical components of many enzymes and their free forms is toxic to MTB.[4] Moreover, iron is an essential nutrient for MTB growth.[5] Animal hosts take advantage of 'nutritional immunity' which is a strategy to starve pathogens of essential metals.[6,7] In this study we have tried to conclude the relation between some essential elements and TB.

2. Physiological functions

Copper: Copper presents in various physiological functions protein and carries by ceruloplasmin in human body. It is involved in the structure of dopamine-β-monooxygenase in nervous system or lysyl oxidase in connective tissues.[8] It is also a component of oxidant metalloenzymes such as Cytochrome-c-oxidase, Cu–Zn-superoxide dismutase.[9] The free copper toxicity with corporation of hydrogen peroxide is used against mycobacterium by our immune defense.[10,11]

Calcium: Calcium is one of the main metals in human body structure. Its significant role in musculoskeletal and nervous systems is known to everyone. Also calcium plays a determinant role in the generation of proinflammatory responses.[12] Calcium and vitamin D modulate genes of immune and inflammatory pathways.[13] Calcium have critical role in actin organization and dynamics at the synaps and reveal calcium influx through CRAC channels which may modulate T cell receptors in immune system.[14] Furthermore calcium is a critical component in oxidative stress pathways.[15]

Iron: Iron is an essential element for development of almost all body systems. It is found in body mostly in the form of complex binding proteins, such as transferrin, lactoferrin, and ferritin.[16] More over iron is essential for both parasite and host cells vitality therefore pathogens are specialized by high affinity uptaking mechanisms.[17] Iron especially associate with cell mediated and neutrophil activities in innate immunity and T cell function, immunoglobulin secretion and IL-6 secretion in acquired immunity.[18]

Zinc: Zinc is a trace element which is essential for human growth and development particularly in neurologic, reproductive and immune systems. It is a component of many metalloenzymes such as superoxide dismutase and a part of promoter in transcription or expression of some genes such as IL-2.[19] Zinc deficiency alone or alongside with vitamin A deficiency influence innate and acquired immune systems via effect on epithelial, neutrophil, macrophage, nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) and CD4 T cells.[20]

Selenium: Selenium is necessary for musculoskeletal, cardiovascular, hormonal and reproduction systems maintenance and development. It is a component of iodothyronine deiodinases which attends in thyroid hormones regulation.[21] Selenium presents anti-toxicity activities against heavy metals such as cadmium, mercury, lead and silver.[22,23] Selenoproteins such as selenium dependent glutathione peroxidase (GPx) are involved in anti-oxidative stress and inflammation regulation responses.[21,24] Furthermore selenoprotein P as an anti-oxidative diminishes reactive nitrogen specie.[25,26] It also influence on serum antibody titer, lymphocyte B and T count and proliferation.[27]

3. Antibacterial and anti-mycobacterial activities

Copper: Cooper ions have tendency to be compound with other molecules and yield different properties such as antibacterial effects. It is seen that copper deficiency cause infection susceptibility. Hence, Cu is an essential nutrient for many bacteria, but it is also toxic because it provides hydroxyl radicals.[28] It has long been assumed that copper compounds are capable to inhibit fungi and bacteria growth.[29] The copper compounds showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Mycobacterium tuberculosis*.[30,31] MTB is destroyed in vitro by copper concentration lower than what is found in macrophage phagosomes.[32]

Calcium: Supplementing calcium results in increased oxidative burst and reduces bacterial loads. Hypercalcemia occurs in TB in contrast some studies showed low calcium level and vitamin D sensitivity in patients with TB.[33] Serum calcium perturbation is associated with vitamin D abnormalities.[34] Vitamin D deficiency is correlated with the high TB incidence, especially in extrapulmonary TB.[35]

Iron: Iron is an essential element for MTB development. It is shown that increased dietary iron has correlation with TB morbidity and mortality.[36] Furthermore excessive serum iron is observed in MDR-TB patients. Iron acquisition is necessary for *E. coli*, *Vibrio cholerae*, *Brucella melitensis* and both gram negative and gram positive bacteria development.[37]

Zinc: The most famous zinc compounds with antibacterial activities are zinc oxide and zinc acetate. Although zinc is a necessary element for bacterial growth in optimum concentration range but its bactericidal effect in high concentrations has been proved on *S. aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *E. coli*, *Mycobacterium tuberculosis* and other bacteria species.[38]

Selenium: Selenium has an important role in immune system maintenance and MTB clearance. It cooperates in antioxidant compounds to protect host from viral, bacterial and fungal infections. It especially have protective role against coxsackievirus, influenza virus, human immunodeficiency virus, *Escherichia coli*, *Staphylococcus aureus*, *Actinobacillus pleuropneumoniae*, *Mycobacterium tuberculosis*, *Candida utilis* and *allbicans*.[39]

4. Physiological mechanisms

Copper: The IFN-γ-mediated activation of macrophages is the key component of anti-TB immune response. It cooperates with ATP7A which is a copper-transporting ATPase, oxygen and nitrogen intermediates, hydrolytic enzymes and bactericidal peptides. It is a potent redox and is used in electron transferring system.[40] Cu(II) pyrophosphate complex acts against both drug sensitive and resistant MTB,[41] therefore MTB export copper metal out of intracellular spaces using P-type ATPases.[42] Almost 24% of all transportation genome in MTB is specialized for copper transportation.[43]

Calcium: Calcium dependent phagosome maturation involves mycobacterial inhibition of sphingosine kinase and reveals survival of MTB within human macrophages.[44] It

regulates immune response via voltage gated calcium channels (VGCC). The VGCC-blocked dendritic cells (DCs) stimulate T cell and macrophages activation against MTB.[45]

Iron: The success of *M. tuberculosis* is related to its ability to offload the toxic metal and thrive in the host. MTB secrete high affinity chellators and siderophores named mycobactins which are necessary for virulence.[46,47] It produces two forms of mycobactins. One is carboxymycobactin which is an amphiphilic molecule and the other is mycobactin which is a lipophilic molecule.[48] Iron is used as a prosthetic group for superoxide dismutase in order to convert superoxide which is a potent antibacterial agent into hydrogen peroxide which is a weaker one. In next step MTB converts hydrogen peroxide to water.[49] Some studies showed that success of MDR-TB is related to its ability to evade burst mechanism employed by phagocytes to kill ingested MTB.

Zinc: *Mycobacterium tuberculosis* use zinc metalloprotease-1 to inactivate host immune defense.[50] Besides an enzyme of MTB named Cu,Zn superoxide dismutase protects this bacteria from toxic effects of host macrophages oxidative burst products. It is assumed this enzyme is contributed with MTB resistance.[51] The zinc dependent mechanisms in MTB are contributed with ferric/zinc uptake regulators (Fur/Zur).[52] Although zinc is an essential element for MTB, it is toxic in higher concentrations. Zinc is an antioxidant and anti-inflammatory agent in body.[53] Zinc has a central role in immune defense from skin barrier stabilization till lymphocyte generation. It is necessary for proper action of neutrophils, macrophages, natural killer cells, B and T lymphocytes and their productions.[54] Particularly it is associated in high expression of zinc transporter in T helper 1 cells and IFN-γ secretion.[55] Moreover lower zinc level in patients with TB is associated with higher concentrations of C-reactive protein (CRP) which is an indicator of acute phase response.[56]

Selenium: This element is essential for T lymphocyte proliferation and differentiation.[57] Patients with CD4 cell count less that 200 per square millimeters show higher risk for TB infection. Selenium is associated in maintaining T cell function therefore it decreases TB risk.[57] It is necessary for both B and T cell development and enhances their function.[27] Selenoprotein P, W and GPx are the most famous compounds in which selenium is participated. The main role of these compounds as an antioxidant is to decrease oxidative stress by reducing peroxides and hydrogen peroxide.[58]

5. Treatment and drug interactions

Copper: Some studies showed copper level did not significantly change during anti-TB therapy.[59] Higher copper concentration in patients with TB and its role in mycobacterial virulence suggest its usage in infection treatment.[56,60] One of these treatment regimens is by complexing disulfiram and copper. It causes MTB to be more susceptible during medical treatment.[61] We have seen in nano-sized studies that incorporation of copper complexes with isoniazid had increased antimycobacterial activity and decreased cytotoxicity against the host cells.[62] Furthermore the copper derivative of isonicotinoyl-dithiocarbazic acid shows high minimum inhibitory concentration (MIC) against MTB. Moreover copper compounds with fluorinated isonicotinoylhydrazones and carboxamidrazones showed increased bactericidal ability.[63,64] It is shown that Casiopeínas copper-based compounds usage alone have considerable effects on resistant TB patients and its usage combined with ethambutol is effective on both resistant and susceptible cases.[65]

Calcium: Hypercalcemia is observed in patients with TB. It is shown that dietary Vit-D and hypercalcemia degree and duration are correlated.[66] It is discussed that rifampicin when combined with isoniazid has no significant effect on patients' calcium serum level although it causes clinically significant derangement of vitamin D metabolism.[67] In contrast some studies showed calcium-Vit D metabolism perturbation during rifampicin and isoniazid therapy.[68]

Iron: Reduced level of iron is observed in patients with TB before therapy initiation and active TB is associated with anemia.[69] It is seen that iron level has been increased four months after TB treatment initiation.[70] In a clinical trial association between dietary iron and TB was evaluated and increased risk of potent infection and mortality among HIV-TB infected patients was observed.[36] Also adverse reaction to ethambutol was observed in drug-induced haemolytic anemia.[71] There is not enough evidences encourage using iron supplement or iron chelator besides recommended anti-TB drug regimens.

Zinc: It is shown that implementation of zinc supplement with vitamin A has improved anti-TB treatment outcomes after two months. It also has converted sputum smear results in TB infected patients.[72] Zinc level is lower in pulmonary TB and multidrug resistant patients.[73] Zinc can be used for evaluating treatment outcomes. Zinc level increased and Zn/Cu ratio decreased significantly after two months therapy in TB infected patients.[59] However some studies contrasts with these results.[74] There are no sufficient data about using zinc supplement alongside with TB therapy but there are evidences demonstrated that using zinc will increase ethambutol cytotoxicity.[54]

Selenium: Selenium concentration in serum of TB infected patients is lower in comparison with healthy individuals.[56] Serum selenium low level is associated with pulmonary infection three time higher risk.[75] This also has capacity to predict TB risk in HIV infected drug users.[57] Using vitamin A and selenium supplementation alongside with standard TB chemotherapy decreased oxidative stresses, enhanced antioxidants and revealed better treatment outcome.[76]

6. Conclusion

TB infection is associated strongly with malnutrition. Perturbation in patients' serum essential elements will threaten host body cells vitality and sometimes provide better conditions for pathogen activities. On the other hand essential elements level changes in host body according to an immune strategy named 'nutritional immunity' during infection. It changes into very low concentrations to starve pathogens or very high and toxic concentrations to destroy them. Our results demonstrated association between copper, calcium, iron, zinc, selenium and TB. Higher concentrations of copper, selenium, zinc and calcium and lower concentrations of iron are treats for

MTB.[32,72] Therefore the monitored level of serum elements is advised to be considered during TB treatment.

Conflicts of interest

The authors have none to declare.

Acknowledgments

This study was enrolled in Zabol University of Medical Sciences Research department and Students Research Committee. We acknowledge all those who cooperated in this project.

REFERENCES

1. Kirenga BJ, Ssengooba W, Muwonge C, et al. Tuberculosis risk factors among tuberculosis patients in Kampala, Uganda: implications for tuberculosis control. *BMC Public Health*. 2015;15(1):1.
2. Riccardi G, Pasca MR, Buroni S. Mycobacterium tuberculosis: drug resistance and future perspectives. *Future Microbiol*. 2009;4(5):597–614.
3. Hoang T, Agger EM, Cassidy JP, Christensen JP, Andersen P. Protein energy malnutrition during vaccination has limited influence on vaccine efficacy but abolishes immunity if administered during Mycobacterium tuberculosis infection. *Infect Immun*. 2015;83(5):2118–2126.
4. Neyrolles O, Mintz E, Catty P. Zinc and copper toxicity in host defense against pathogens: Mycobacterium tuberculosis as a model example of an emerging paradigm. *Metal Economy Host–Microbe Interact*. 2015.
5. Wells RM, Jones CM, Xi Z, et al. Discovery of a siderophore export system essential for virulence of Mycobacterium tuberculosis. *PLoS Pathog*. 2013;9(1):e1003120.
6. Weinberg ED. Nutritional immunity: host's attempt to withhold iron from microbial invaders. *JAMA*. 1975;231(1):39–41.
7. Kehl-Fie TE, Skaar EP. Nutritional immunity beyond iron: a role for manganese and zinc. *Curr Opin Chem Biol*. 2010;14(2):218–224.
8. Al-Bayati MA, Jamil DA, Al-Aubaidy HA. Cardiovascular effects of copper deficiency on activity of superoxide dismutase in diabetic nephropathy. *N Am J Med Sci*. 2015;7(2):41.
9. Harris ZL, Gitlin JD. Genetic and molecular basis for copper toxicity. *Am J Clin Nutr*. 1996;63(5):836S–841S.
10. Lukasewycz O. Copper deficiency suppresses the immune response of mice. *Science*. 1981;213(4507):559–561.
11. Wagner D, Maser J, Lai B, et al. Elemental analysis of *Mycobacterium avium*-, *Mycobacterium tuberculosis*-, and *Mycobacterium smegmatis*-containing phagosomes indicates pathogen-induced microenvironments within the host cell's endosomal system. *J Immunol*. 2005;174(3):1491–1500.
12. Noble A, Truman JP, Vyas B, Vukmanovic-Stejic M, Hirst WJ, Kemeny DM. The balance of protein kinase C and calcium signaling directs T cell subset development. *J Immunol*. 2000;164(4):1807–1813.
13. Protiva P, Pendyala S, Nelson C, Augenlicht LH, Lipkin M, Holt PR. Calcium and 1, 25-dihydroxyvitamin D3 modulate genes of immune and inflammatory pathways in the human colon: a human crossover trial. *Am J Clin Nutr*. 2016;103(5):1224–1231.
14. Hartzell CA, Jankowska KI, Burkhardt JK, Lewis RS. Calcium influx through CRAC channels controls actin organization and dynamics at the immune synapse. *Elife*. 2016;5.
15. Ermak G, Davies KJ. Calcium and oxidative stress: from cell signaling to cell death. *Mol Immunol*. 2002;38(10):713–721.
16. Weinberg ED. Iron loading and disease surveillance. *Emerg Infect Dis*. 1999;5(3):346.
17. Litwin CM, Calderwood S. Role of iron in regulation of virulence genes. *Clin Microbiol Rev*. 1993;6(2):137–149.
18. Li J, He K, Liu P, Xu LX. Iron participated in breast cancer chemoresistance by reinforcing IL-6 paracrine loop. *Biochem Biophys Res Commun*. 2016;475(2):154–160.
19. MacDiarmid CW, Taggart J, Jeong J, Kerdsomboon K, Eide DJ. Activation of the yeast UBI4 polyubiquitin gene by Zap1 transcription factor via an intragenic promoter is critical for zinc-deficient growth. *J Biol Chem*. 2016;291(36):18880–18896.
20. Rice JM, Zweifach A, Lynes MA. Metallothionein regulates intracellular zinc signaling during CD4(+) T cell activation. *BMC Immunol*. 2016;17(1):13.
21. Navarro-Alarcon M, de la Serrana HL, Perez-Valero V, Lopez-Martinez C. Serum selenium levels as indicators of body status in cancer patients and their relationship with other nutritional and biochemical markers. *Sci Total Environ*. 1998;212(2–3):195–202.
22. Caurant F, Navarro M, Amiard JC. Mercury in pilot whales: possible limits to the detoxification process. *Sci Total Environ*. 1996;186(1–2):95–104.
23. Mousa SA, O'Connor L, Rossman TG, Block E. Pro-angiogenesis action of arsenic and its reversal by selenium-derived compounds. *Carcinogenesis*. 2007;28(5):962–967.
24. Van Cauwenbergh R, Robberecht H, Van Vlaslaer V, Deelstra H. Comparison of the serum selenium content of healthy adults living in the Antwerp region (Belgium) with recent literature data. *J Trace Elem Med Biol*. 2004;18(1):99–112.
25. Li N, Gao Z, Luo D, Tang X, Chen D, Hu Y. Selenium level in the environment and the population of Zhoukoudian area, Beijing, China. *Sci Total Environ*. 2007;381(1–3):105–111.
26. Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. *Sci Total Environ*. 2008;400(1):115–141.
27. Hawkes WC, Kelley DS, Taylor PC. The effects of dietary selenium on the immune system in healthy men. *Biol Trace Elem Res*. 2001;81(3):189–213.
28. Halliwell B, Gutteridge J. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J*. 1984;219(1):1.
29. Sorenson JR. 6 copper complexes offer a physiological approach to treatment of chronic diseases. *Prog Med Chem*. 1989;26:437–568.
30. Xu D, Shen Z, Shi Y, He Q, Xia Q. Synthesis, characterization, crystal structure, and biological activity of the copper complex. *Russ J Coord Chem*. 2010;36(6):458–462.
31. Wang X, Du Y, Fan L, Liu H, Hu Y. Chitosan-metal complexes as antimicrobial agent: synthesis, characterization and structure–activity study. *Polym Bull*. 2005;55(1–2):105–113.
32. Wolschendorf F, Ackart D, Shrestha TB, et al. Copper resistance is essential for virulence of Mycobacterium tuberculosis. *Proc Natl Acad Sci USA*. 2011;108(4):1621–1626.
33. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol*. 2009;6(10):621–630.
34. Rook GA. The role of vitamin D in tuberculosis. *Am Rev Respir Dis*. 1988;138(4):768–770.
35. Davies P. A possible link between vitamin D deficiency and impaired host defence to Mycobacterium tuberculosis. *Tubercle*. 1985;66(4):301–306.

36. Gangaidzo IT, Moyo VM, Mvundura E, et al. Association of pulmonary tuberculosis with increased dietary iron. *J Infect Dis*. 2001;184(7):936–939.
37. Hoekstra D, van der Laan JW, de Leij L, Witholt B. Release of outer membrane fragments from normally growing *Escherichia coli*. *Biochim Biophys Acta (BBA): Biomembr*. 1976;455 (3):889–899.
38. Xie Y, He Y, Irwin PL, Jin T, Shi X. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Appl Environ Microbiol*. 2011;77 (7):2325–2331.
39. Humann-Ziehank E, Menzel A, Roehrig P, Schwert B, Ganter M, Hennig-Pauka I. Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. *Metallomics*. 2014;6 (10):1869–1879.
40. Crichton R, Pierre J-L. Old iron, young copper: from Mars to Venus. *Biometals*. 2001;14(2):99–112.
41. Hoffman AE, DeStefano M, Shoen C, et al. Co(II) and Cu(II) pyrophosphate complexes have selectivity and potency against Mycobacteria including Mycobacterium tuberculosis. *Eur J Med Chem*. 2013;70:589–593.
42. Argüello JM, González-Guerrero M, Raimunda D. Bacterial transition metal P1B-ATPases: transport mechanism and roles in virulence. *Biochemistry*. 2011;50(46):9940–9949.
43. Agranoff D, Krishna S. Metal ion transport and regulation in Mycobacterium tuberculosis. *Front Biosci*. 2004;9:2996–3006.
44. Malik ZA, Thompson CR, Hashimi S, Porter B, Iyer SS, Kusner DJ. Cutting edge: Mycobacterium tuberculosis blocks Ca^{2+} signaling and phagosome maturation in human macrophages via specific inhibition of sphingosine kinase. *J Immunol*. 2003;170(6):2811–2815.
45. Gupta S, Salam N, Srivastava V, et al. Voltage gated calcium channels negatively regulate protective immunity to Mycobacterium tuberculosis. *PLoS ONE*. 2009;4(4):e5305.
46. De Voss JJ, Rutter K, Schroeder BG, Su H, Zhu Y, Barry CE. The salicylate-derived mycobactin siderophores of Mycobacterium tuberculosis are essential for growth in macrophages. *Proc Natl Acad Sci USA*. 2000;97(3):1252–1257.
47. Reddy PV, Puri RV, Chauhan P, et al. Disruption of mycobactin biosynthesis leads to attenuation of Mycobacterium tuberculosis for growth and virulence. *J Infect Dis*. 2013;jit250.
48. Ratledge C, Dover LG. Iron metabolism in pathogenic bacteria. *Annu Rev Microbiol*. 2000;54(1):881–941.
49. Yuniastuti A. The role and characteristic of antioxidant for redox homeostasis control system in Mycobacterium tuberculosis. *Int Res J Microbiol*. 2012;3:416–422.
50. Vemula MH, Medisetti R, Ganji R, et al. Mycobacterium tuberculosis zinc metalloprotease-1 assists mycobacterial dissemination in zebrafish. *Front Microbiol*. 2016;7:1347.
51. Piddington DL, Fang FC, Laessig T, Cooper AM, Orme IM, Buchmeier NA. Cu,Zn superoxide dismutase of Mycobacterium tuberculosis contributes to survival in activated macrophages that are generating an oxidative burst. *Infect Immun*. 2001;69(8):4980–4987.
52. Lucarelli D, Russo S, Garman E, Milano A, Meyer-Klaucke W, Pohl E. Crystal structure and function of the zinc uptake regulator FurB from Mycobacterium tuberculosis. *J Biol Chem*. 2007;282(13):9914–9922.
53. Prasad AS. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol*. 2014;28(4):364–371.
54. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr*. 1998;68(2 suppl):447s–463s.
55. Aydemir TB, Liuzzi JP, McClellan S, Cousins RJ. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. *J Leuk Biol*. 2009;86 (2):337–348.
56. Koyanagi A, Kuffo D, Gresely L, Shenkin A, Cuevas LE. Relationships between serum concentrations of C-reactive protein and micronutrients, in patients with tuberculosis. *Ann Trop Med Parasitol*. 2004;98(4):391–399.
57. Shor-Posner G, Miguez MJ, Pineda LM, et al. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. *J Acq Immune Defic Syndr*. 2002;29(2):169–173.
58. Guertin KA, Grant RK, Arnold KB, et al. Effect of long-term vitamin E and selenium supplementation on urine F2-isoprostanes, a biomarker of oxidative stress. *Free Radic Biol Med*. 2016;95:349–356.
59. Ciftci TU, Ciftci B, Yis Ö, Guney Y, Bilgihan A, Ogretensoy M. Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. *Biol Trace Elem Res*. 2003;95(1):65–71.
60. Festa RA, Jones MB, Butler-Wu S, et al. A novel copper-responsive regulon in Mycobacterium tuberculosis. *Mol Microbiol*. 2011;79(1):133–148.
61. Dalecki AG, Haeili M, Shah S, et al. Disulfiram and copper ions kill Mycobacterium tuberculosis in a synergistic manner. *Antimicrob Agents Chemother*. 2015;59(8):4835–4844.
62. Silva PBd, Souza PCd, Calixto GMF, et al. In vitro activity of copper(II) complexes, loaded or unloaded into a nanostructured lipid system, against Mycobacterium tuberculosis. *Int J Mol Sci*. 2016;17(5):745.
63. Sandbhor U, Padhye S, Billington D, et al. Metal complexes of carboxamidrazone analogs as antitubercular agents. 1. Synthesis, X-ray crystal-structures, spectroscopic properties and antimycobacterial activity against Mycobacterium tuberculosis H37Rv. *J Inorg Biochem*. 2002;90(3):127–136.
64. Maccari R, Ottanà R, Bottari B, Rotondo E, Vigorita MG. In vitro advanced antimycobacterial screening of cobalt(II) and copper(II) complexes of fluorinated isonicotinoylhydrazones. *Bioorg Med Chem Lett*. 2004;14 (23):5731–5733.
65. Barbosa A, Caleffi-Ferracioli K, Leite C, et al. Potential of Casiopeínas® copper complexes and antituberculosis drug combination against Mycobacterium tuberculosis. *Chemotherapy*. 2016;61(5):249–255.
66. Sharma SC. Serum calcium in pulmonary tuberculosis. *Postgrad Med J*. 1981;57(673):694–696.
67. Perry W, Erooga M, Brown J, Stamp T. Calcium metabolism during rifampicin and isoniazid therapy for tuberculosis. *J R Soc Med*. 1982;75(7):533–536.
68. Brodie M, Hillyard C. Calcium metabolism during rifampicin and isoniazid therapy for tuberculosis. *J R Soc Med*. 1982;75 (11):919.
69. Karyadi E, Schultink W, Nelwan RH, et al. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. *J Nutr*. 2000;130(12):2953–2958.
70. Edem V, Ige O, Arinola O. Plasma vitamins and essential trace elements in newly diagnosed pulmonary tuberculosis patients and at different durations of anti-tuberculosis chemotherapy. *Egypt J Chest Dis Tuberc*. 2015;64(3):675–679.
71. Nicolini A, Perazzo A, Gatto P, et al. A rare adverse reaction to ethambutol: drug-induced haemolytic anaemia. *Int J Tuberc Lung Dis*. 2016;20(5):704–705.
72. Karyadi E, West CE, Schultink W, et al. A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status. *Am J Clin Nutr*. 2002;75(4):720–727.
73. Ghulam H, Kadri SM, Manzoor A, et al. Status of zinc in pulmonary tuberculosis. *J Infect Dev Ctries*. 2009;3(5):365–368.

74. Visser ME, Grewal HM, Swart EC, et al. The effect of vitamin A and zinc supplementation on treatment outcomes in pulmonary tuberculosis: a randomized controlled trial. *Am J Clin Nutr*. 2011;93(1):93–100.
75. Rayman MP. The importance of selenium to human health. *Lancet*. 2000;356(9225):233–241.
76. Seyedrezazadeh E, Ostadrahimi A, Mahboob S, Assadi Y, Ghaemmagami J, Pourmogaddam M. Effect of vitamin E and selenium supplementation on oxidative stress status in pulmonary tuberculosis patients. *Respirology*. 2008;13(2): 294–298.

Available online at www.sciencedirect.com

ScienceDirect

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

Review Article

Drug development against tuberculosis: Past, present and future

Mahesh S. Vasava, Manoj N. Bhoi, Sanjay K. Rathwa, Mayuri A. Borad, Sneha G. Nair, Hitesh D. Patel *

Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, India

ARTICLE INFO

Article history:
Received 24 October 2016
Accepted 15 March 2017
Available online 14 April 2017

Keywords:
Tuberculosis
Drug development
Drug resistance
Antimicrobials
Anti-TB agents

ABSTRACT

Infection of *Mycobacterium tuberculosis* (MTB) was observed as early as 5000 years ago with evidence, which is a primeval enemy of the humanoid race. MTB is the pathogen which is responsible for causing the infectious disease tuberculosis; it remains a major cause of morbidity and mortality in poor low-income countries as well as in developing countries because of non-availability of reliable laboratory facilities. The current treatment for drug-resistant tuberculosis (TB) is lengthy, complex, and connected with severe harmful side effects and poor outcomes. The present cure against tuberculosis has substantial restrictions, in terms of their efficiency, side-effect outline, and complication of handling.

Furthermore, the emergence of multi-drug resistant tuberculosis (MDR-TB) outbreaks during the 1990s and additionally in recent times the vast deadly strains of extensively drug-resistant tuberculosis (XDR-TB) and totally drug resistance tuberculosis (TDR-TB) is hampering efforts to control and manage tuberculosis (TB). As a result, novel methodologies for the treatment of multi-drug-resistant and extensive drug-resistant tuberculosis (TB) are severely desired. A number of new potential anti-tuberculosis drug candidates with novel modes of action have been entered in clinical trials in recent years. These agents are most likely to be effective against resistant strains. The treatment landscape is beginning to shift, with the recent approvals by Food and Drug Administration to the new TB drugs bedaquiline and delamanid. Also, the pipeline of potential new treatments has been fulfilled with several compounds in clinical trials or preclinical development with promising activities against sensitive and resistant MTB bacteria. An additional new chemical entity is also under development. The already existing drugs with their suggested mode of treatment as well as new probable anti-tuberculosis drug moieties which are at present in the pipeline has been summarized in this review.

* *Corresponding author.*
E-mail addresses: maheshvasava@gujaratuniversity.ac.in (M.S. Vasava), drhiteshpatel1@gmail.com, hitesh13chem@rediffmail.co (H.D. Patel).

Abbreviations: TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; MDR-TB, multi-drug resistant tuberculosis; XDR-TB, extensively drug resistant tuberculosis; TDR-TB, totally drug resistant tuberculosis; INH, isoniazid; WHO, World Health Organization; RIF, rifampicin; PZA, pyrazinemide; EMB, ethambutol; ETH, ethionamide; CDC, Centers for Disease Control and Prevention; CBC, Complex Blood Count; GI, gastrointestinal; LFT, liver function test; PO, per os; *katG*, *catalase-peroxidase*; NADP, nicotinamide adenine dinucleotide phosphate; MRC, Medical Research Council; rRNA, ribosomal RNA; FQs, fluoroquinolones; FDA, Food and Drug Administration; MIC, minimum inhibitory concentration; CYP, cytochrome; GLP, Good Laboratory Practice.

http://dx.doi.org/10.1016/j.ijtb.2017.03.002
0019-5707/

1. Introduction

The human tuberculosis are established over 6000 years of age which is proposed from another DNA investigation of a tuberculosis genome reproduced in southern Peru, in 2014. Analysts conjecture that humans initially acquired tuberculosis in Africa around 5000 years ago.[1] It was concluded by Gutierrez and her colleagues that in East Africa an early progenitor of *Mycobacterium tuberculosis* (MTB) was present as early as 3 million years ago, and it was suggested by them that, at that time it may have infected early humanoids.[2] MTB, the bacteria that cause tuberculosis (TB) was discovered by Dr. Koch on March 21, 1882. In the United States and Europe, TB killed one out of every seven people during that time. Dr. Koch's discovery was the most important step taken toward the control and elimination of this deadly disease.[3] After a century of Dr. Koch's discovery, World Health Organization (WHO) and the International Union declared the first World TB Day in 1982.[3] The timeline history of tuberculosis is represented in Fig. 1.

In 1997, it was estimated that one-third of the human population (approximately 1.86 billion people) are infected with *M. tuberculosis* worldwide.[4] Tuberculosis, TB (tubercle

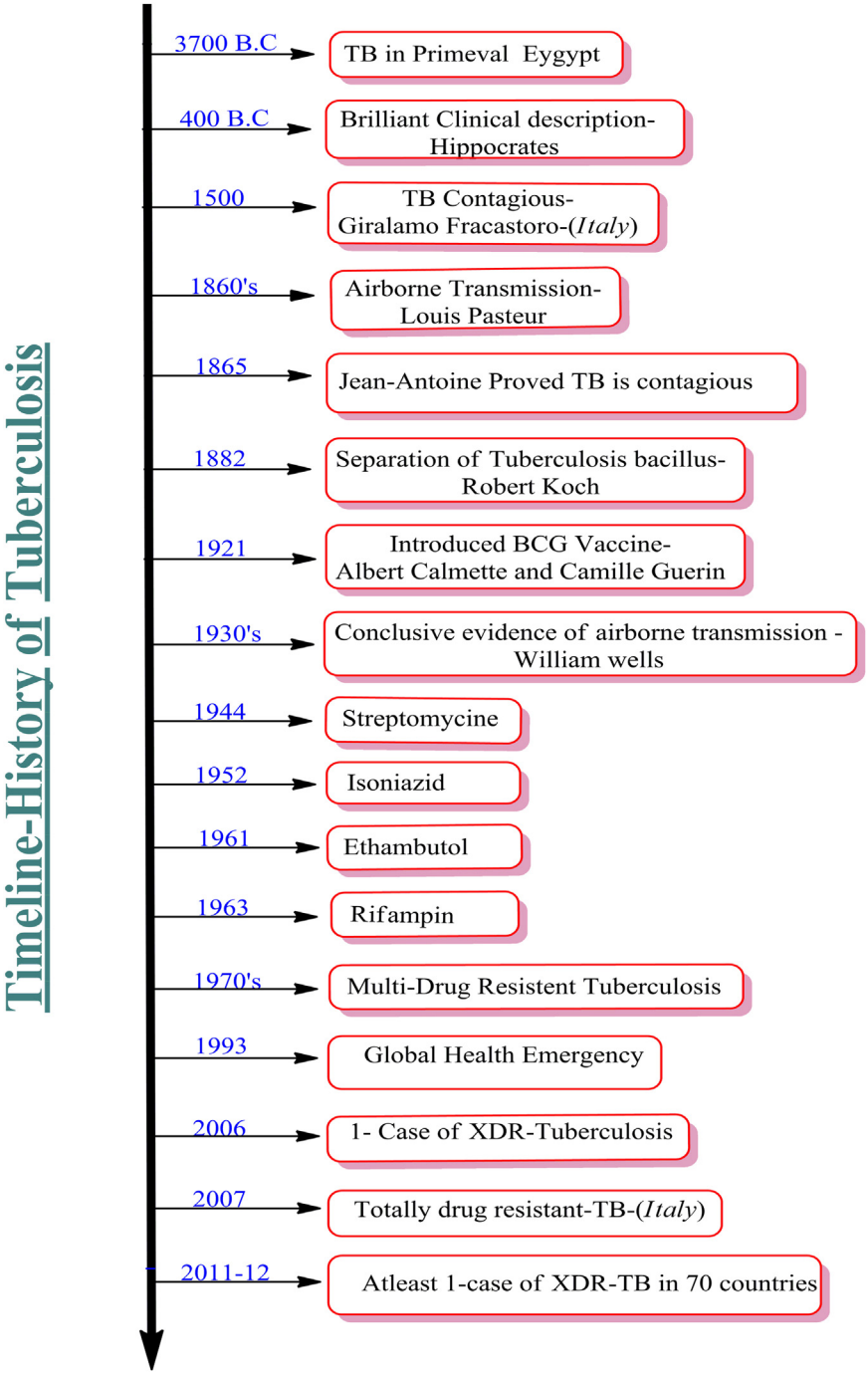


Fig. 1 – Timeline history of tuberculosis.

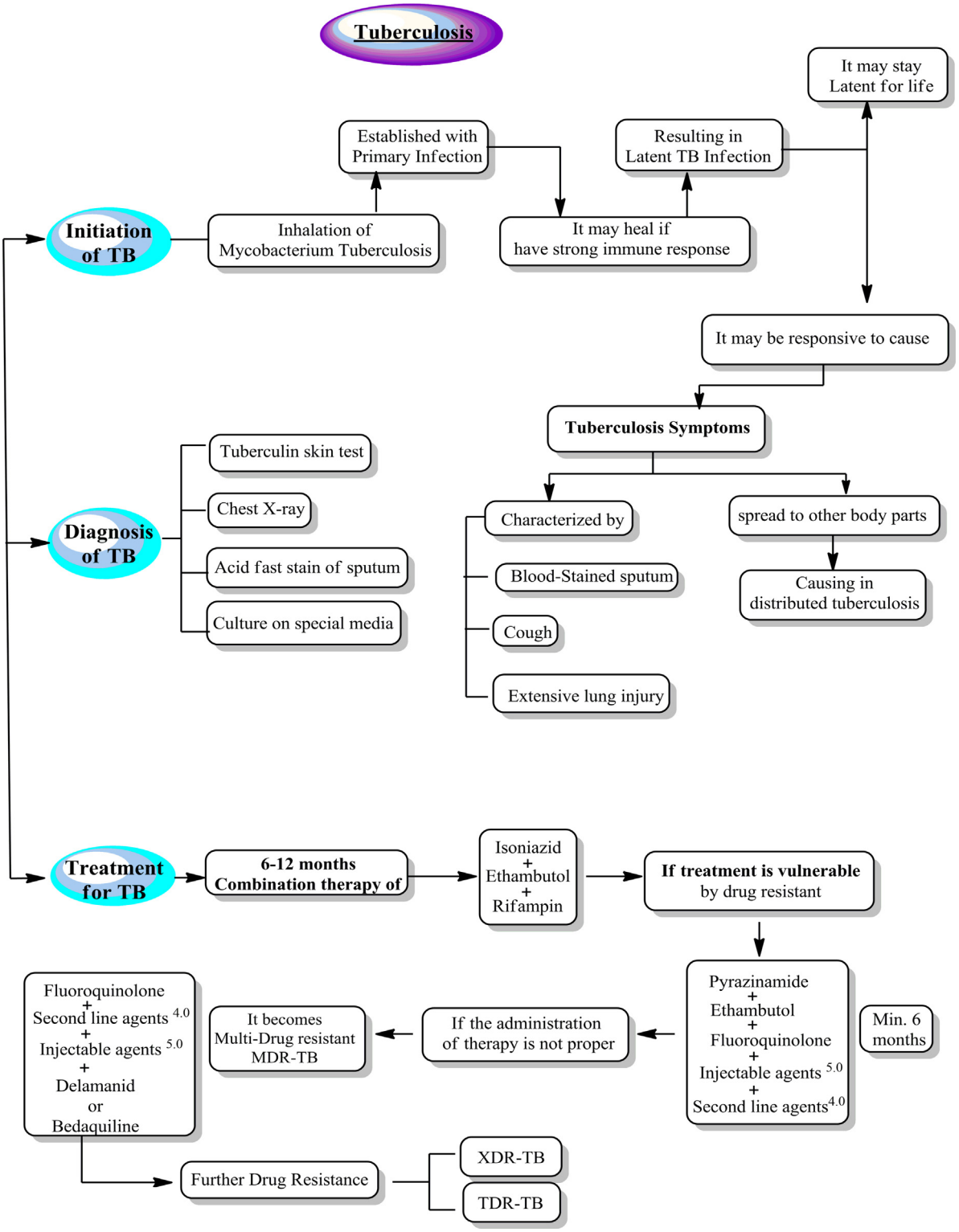


Fig. 2 – Graphical representation of tuberculosis – inhalation, diagnosis and treatment.

bacillus), or MTB (*Mycobacterium tuberculosis*), in the past also called phthisis, phthisis pulmonitis, or consumption, is a widespread, infectious disease caused by various strains of mycobacteria, usually *M. tuberculosis*.[5] TB is spread through inhalation of airborne *M. tuberculosis* cells, which multiply in macrophages and within the large cystic tubercles, they form liquified tissue surrounded by infected macrophages.[6] After the inhalation of TB bacteria, it establishes with primary infection and it may cure if human have a strong immune system. But if the immune system response is weaker than bacteria will spread and the infection becomes Latent TB, which may responsible for Tuberculosis disease (Fig. 2).

The cell wall of mycobacteria plays an essential role in growing the bacteria. The cell wall of *M. tuberculosis* is made up of two segments, upper and lower. The peptidoglycan of *M. tuberculosis* is covalently attached to arabinogalactan which in turn is attached to mycolic acid with their long mero mycolate and short alpha chains. This is the cell wall core of *M. tuberculosis* and is known as a mycolyarabinogalactan-peptidoglycan complex. The upper segment of the cell wall is made up of free lipids, some with longer fatty acids complementing the shorter alpha chain and vice versa. Cell wall proteins, phosphatidylinositol mannosidase, the phthiocerol containing lipids, lipomannan, and lipoarabinomannan also can be found at upper segment of the cell wall.[7] Furthermore, the cell envelope of *M. tuberculosis* also contains an additional layer beyond peptidoglycan that rich in unusual lipids, glycolipids, and polysaccharides.[8] *M. tuberculosis* is an intracellular pathogen. *M. tuberculosis* is able to parasite human mononuclear phagocytes.[9] *M. tuberculosis* will spend most of its life cycle in macrophages.[10] *M. tuberculosis* has the ability to multiply inside the macrophage phagosome. *M. tuberculosis* can remain dormant for a few years without the symptoms. When the immune system of the hosts is low, the dormant *M. tuberculosis* will become active and cause the infection. There are two types of tuberculosis that are childhood-type tuberculosis and adult-type tuberculosis.

However, all age groups are at risk of TB, but it mostly affects young adults, in their productive years. In developing countries, 95% cases end in death. Tuberculosis (TB) remains one of the world's deadliest communicable diseases. TB killed around 1.5 million people (out of which 1.1 million HIV-negative and 0.4 million HIV-positive), in 2014. About 9.6 million people are estimated to be falling ill with TB worldwide, in 2014 (5.4 million men, 3.2 million women, and 1.0 million children). Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive.[11] Approximately 1000 million people were infected, over which 150 million people get sick and 36 million died of TB between 2000 and 2015.[12]

According to the worldwide survey, patients suffering from TB as well as HIV ranks the top position in leading to an increased mortality rates. The mortality rate survey done in the year 2014 for HIV indicated that 0.4 million TB death among HIV-positive people occurred out of the total of 1.2 million.[13] 15% of AIDS patients globally die of TB and approximately 12 million individuals are co-infected, and roughly every year. Furthermore, the emergence of new infectious forms of TB such as multi-drug-resistant tuberculosis (MDR-TB) and extremely drug-resistant tuberculosis (XDR-TB) and its synergy with HIV has fueled its epidemic nature.[3]

Over the decades a grim statistic changed little, TB kills 300,000 Indians annually: one death every 2 min.[14] In Mumbai, India, 80% of previously untreated patients were infected with *M. tuberculosis* resistant to at least 1 drug, and 51% were infected with *M. tuberculosis* resistant to both isoniazid and rifampin.[15] MDR-TB increases treatment costs by a factor of 10–100-fold, and TB control programs may spend up to 30% of their budgets on the 3% of patients with TB who have with MDR-TB.[16] 300 million Indians infected by TB, which proves India is a highest TB burden country in the world, accounting for 21% global incidence.[17]

MDR-TB (multi-drug resistant) is emerging the worldwide threat.[18] MDR-TB is well-defined by the resistance to the two most potent first-line anti-TB drugs isoniazid and rifampicin, and have need of treatment with more expensive second-line drugs, which, in addition to being costly, cause more adverse events and show lower cure rates.[19] The term XDR-TB (extensively drug resistant) introduced the first time in March 2006 and it causes via MDR-TB with additional resistance to any fluoroquinolone and one of the second-line injectable drugs (kanamycin, amikacin, or capreomycin).[18,19] Recently, cases of unclearly defined TDR-TB were reported.[20] TDR-TB (totally drug resistant, sometimes called as XDR TB or extremely drug resistant TB), is Tuberculosis bacteria which are resisting against all the first-line and second-line anti-TB drugs.[21] Human TB is linking the growing list of bacterial diseases arriving the post-antibiotic era.

Existing economical and effective four-drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) were introduced before 50 years ago, and till date, no novel drug has been developed except delamanid and bedaquiline, which is used against MDR-TB. Most of the arsenal composed drugs were discovered in 1950 to 1960 for first-line TB treatment. For the treatment of TB, the first compound used in 1944, was streptomycin.[22] There is an urgent need to develop newer, potent and safer anti-tubercular agents which are less prone to resistance.[23] In this review, we highlight the short history of tuberculosis and the drug development against TB as well as the recent drug development. This further fueled our knowledge to improve the treatment and prevention of this deadly disease.

2. Therapy against TB

Tuberculosis (TB) treatment has become very difficult after the emergence of existing drug resistant against a bacterium named *M. tuberculosis* which is preventable and curable. In a human body, TB bacteria can attack the spine, kidney, lungs, and brain, but lungs have more risk of attack. All TB bacteria infected peoples do not get sick. There are two TB related exist conditions, one of which is latent TB infection and other is active TB disease (Fig. 2). In both Latent TB and Active TB case they are curable. When people have TB bacteria in the body, but they are not active and they do not get sick it's called as latent TB infection. Latent TB infection cannot spread in the human body and do not have TB symptoms. In latent TB infection, if TB bacteria become activated, it converts into TB disease and people become sick. Therefore, latent TB infected people, it is more superior to get preserved for not emerging it to TB disease. Nevertheless, if the immune system of human cannot stop the evolution of TB bacteria, they are multiplying in the body and it became active TB disease. In TB disease, TB bacteria are active and make people sick. People already diagnosed with TB disease have chances of spreading it to others.

The treatment duration for tuberculosis is minimum 6–12 months with the combination of first-line anti-TB agents. However, if the treatment is vulnerable by drug resistance of bacteria, it further treated with the combination therapy of second line agents, fluoroquinolones, and some injectable agents (Fig. 2).

Table 1 – Anti-tuberculosis agents for the treatment of drug-susceptible and drug-resistant tuberculosis.

Regimen	Recommended regimens for the treatment of drug-susceptible pulmonary tuberculosis			
	Initial-phase drugs	Interval/doses	Continuation-phase drugs	Interval/doses
1	INH,RIF,PZA,EMB	7 days/week for 56 doses or 5 days/week for 40 doses	INH,RIF	7 days/week for 126 doses or 5 days/week for 90 doses or twice weekly for 36 doses or once weekly for 18 doses
2	INH,RIF,PZA,EMB	7 days/week for 14 doses, then twice weekly for 12 doses or 5 days/week for 10 doses, then twice weekly for 12 doses	INH,RIF	Twice weekly for 26 doses or once weekly for 54 doses
3	INH,RIF,PZA,EMB	Twice weekly for 24 doses	INH,RIF	Twice weekly for 54 doses
4	INH,RIF,EMB	7 days/week for 56 doses or 5 days/week for 40 doses	INH,RIF	7 days/week for 217 doses or 5 days/week for 155 doses or twice weekly for 62 doses

Source: American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of tuberculosis. MMWR Recomm Rep. 2003; 52(RR-11):1–77.

For active TB who are presumed to have a drug-susceptible disease with newly diagnosed patients, the WHO recommended the standard drug regimen by includes an Initial phase of 2 months of a standard four-drug combination (INH, RIF, PZA, and EMB), followed by a continuation phase of 4 months of INH and RIF. The detail description of recommended regimens is given in Table 1 by American Thoracic Society, CDC, and Infectious Diseases Society of America. Drugs are administered by mouth and are listed in Table 2 with their recommended daily dose and notable adverse effects.

The current treatment for drug-susceptible TB requires four drugs used in combination for 2 months, followed by two drugs used in combination for 4 months.

3. The past

3.1. *Existing first line anti-TB agents*

3.1.1. *Rifampicin*

Rifampicin-1972 (1)

In 1957, the Lepetit Pharmaceuticals research lab in Italy was brought a soil sample for analysis from a pine forest on the French Riviera. There, a research group supervised by Piero Sensi and Maria Teresa Timbal discovered a new bacterium. This newly discovered bacterium appeared of interest since it was producing a new class of molecules with antibiotic activity. They decided to call these compounds ''rifamycins'' and the reason behind the name of this newly discovered compound because of the French crime story Rififi it is about a jewel heist and rival gangs which is particularly fond by Sensi, Timbal, and the researchers.[24] In 1959, after two years of attempts to find more stable semisynthetic products, and this newly produces molecule have a high efficacy and good tolerability and was named as ''rifampicin''. Rifampicin **(1)** was first sold in 1971.[25]

In 1972, as an anti-tubercular drug Rifampicin was first introduced and it was an extremely effective against *M. tuberculosis*. Rifampicin targets by binding with the mycobacterial DNA-dependent RNA polymerase and thereby attack the organism and kill by interfering in the transcription process.[26]

Rifampicin along with isoniazid forms the backbone of first line and short course chemotherapy by reason of its high bactericidal action. Because of the widespread application and results in the selection of mutants resistant to other components of short course chemotherapy is increasing resistance to rifampicin. Hence, resistance to rifampicin can

Table 2 – Existing first-line anti-tuberculosis drug candidate.

First-line anti-tuberculosis drug candidate with side effects and monitoring				
Drugs	Dosage	Route	Opposing effects	Observing
Isoniazid	5 mg/kg/day up to 300 mg/day	PO	Asymptomatic elevation of LFTs, clinical hepatitis, fatal hepatitis, peripheral neurotoxicity, central nervous system effect, lupus-like syndrome, hypersensitivity reactions, monoamine poisoning, Drug interaction	LFTs
Pyrazinemide	40–45 kg:1000 mg/day 56–75 kg:1500 mg/day 76–90 kg:2000 mg/day	PO	Hepatotoxicity, GI symptoms, gouty polyarthralgia, asymptomatic hyperuricemia, dermatitis acute gouty arthritis, transient morbilliform rash	LFTs, serum uric acid
Ethambutol	40–45 kg:800 mg/day 56–75 kg:1200 mg/day 76–90 kg:1600 mg/day	PO	Retrobulbar nueritis, peripheral nueritis, cutaneous reactions, fever	Vision tests
Rifampin	10 mg/kg/day up to 600 mg/day	PO	Hematologic toxicity, uveitis, GI reactions, polyarthralgia, hepatotoxicity, pseudojaundice, rash, flu-like syndrome, orange discoloration of bodily fluids, significant drug interactions	LFts, CBC
Rifabutin	5 mg/kg/day up to 300 mg/day	PO	Cutaneous reactions, GI reactions, Flu-like syndrome, hepatotoxicity, severe immunological reactions, leukopenia, thrombocytopenia, orange discoloration of bodily fluids, significant drug interactions	LFts, CBC
Rifapentine	10 mg/kg/day up to 600 mg/day	PO	Same as Rifampin	LFts, CBC

Source: American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of tuberculosis. MMWR Recomm. Rep. 2003; 52(RR-11):1–77.
CBC – complex blood count, GI – gastrointestinal, LFT – liver function test, PO – per os (by mouth).

be considered as a surrogate marker for multiple drug resistant tuberculosis (MDR-TB).[27] Spontaneous mutation, which occurs at a rate of 108 in *M. tuberculosis* is the root for Resistance to Rifampicin.[28] RNA polymerase has a composed of four different subunits α, β, β' and σ, encoded by *proA*, *rpoB*, *rpoC* and rpoD, respectively, is highly conserved among bacterial species complex oligomer. Characterization of the *rpoB* genetic factor in *Escherichia coli* confirmed that mutations within the *rpoB* locus conversed conformational changes resulting in defective binding of the drug and consequently resistance.[29]

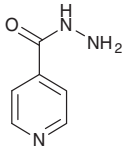
Consequently, the rpoB locus of *M. tuberculosis* was characterized and mutations conferring the resistant trait were identified in an 81-bp RIF Resistance Determining Region (RRDR) of the *rpoB* RNA component equivalent to codons 507–533.[29,30] The most commonly associated mutations with rifampicin resistance in the majority of studies is mutations in codons 516, 526 and 531.[28,31]

Alternatively, mono-resistance to rifampicin is quite rare and nearly all rifampicin resistant strains are also resistant to other drugs, exclusively to isoniazid. This is the object why rifampicin resistance is considered as a surrogate marker for MDR-TB.[32] Recently, the genome sequencing studies have discovered the achievement of compensatory mutations in *rpoA* and *rpoC*, encoding α and β' subunits of *RNA polymerase*, with mutations in *rpoB*, in rifampicin resistant strains.[33]

3.1.2. *Isoniazid*

In 1952, INH **(2)** has been the keystone in tuberculosis chemotherapy for almost half a century since its discovery as an effective anti-tuberculosis drug.[34] INH is a prodrug, and its anti-tuberculosis function requires in vivo activation by *katG*, an enzyme with the dual activities of catalase and peroxidase. In the early 1990s, when the primary mycobacterial *catalase-peroxidase* gene (*katG*) was cloned and sequenced until the association of this enzyme with isoniazid activation was not demonstrated.[35]

Resistance to INH is related with a variability of mutations disturbing one or more genes such as those encoding *catalase-peroxidase* (*katG*),[36] the enoyl-acyl carrier protein reductase involved in mycolic acid biosynthesis (*inhA*).[37]



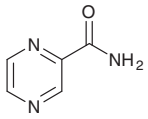
Isoniazid-1952 (2)

Currently, two intracellular targets for isoniazid the fatty-acid enoyl-acyl carrier protein reductase (*InhA*), and a complex of an acyl carrier protein (*AcpM*) and a β-ketoacyl-ACP synthase (*KasA*) investigated by Vilchèze et al. and Slayden et al.[38,39] Low-level resistance to isoniazid was exhibited via the clinical isolation of these enzymes, which are involved in the synthesis of mycolic acids, and mutations have been found in the promoter regions, or less commonly in the genes that encode these proteins (*inhA*, *acpM*, and *kasA*).

Mutations in *katG* (codon 135) or *inhA* were also found in cases of isoniazid resistance, the role of *kasA* mutations

in isoniazid resistance is currently unclear, because similar mutations were also found in isoniazid-susceptible isolates.[40] Mutations in *dfrA* could possibly play a role in resistance to isoniazid, which is one recent interesting finding showed that the 4R isomer of the isoniazid NADP adduct origins inhibition of the dihydrofolate reductase (*DfrA*) in *M. tuberculosis*.[41] Furthermore, 16 other proteins identified by an analysis of the proteome of isoniazid targets in *M. tuberculosis*, in addition to *InhA* and *DfrA*, that were bound by these adducts with high affinity, which could signal other not yet clearly defined actions of isoniazid on the bacteria.[42] Several studies have found single nucleotide polymorphisms in other genes in isoniazid-resistant clinical isolates of *M. tuberculosis*, including *kasA* and the oxy *RahpC* and fur *AKatG* intergenic regions.[43–45] A recent study has also found that a silent mutation in *mabA* conferred isoniazid resistance through up regulation of *inhA* in *M. tuberculosis*.[46]

3.1.3. *Pyrazinamide*

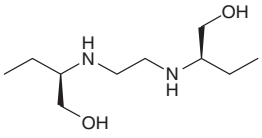


Pyrazinamide-1954 (3)

In 1936, a nicotinamide analog, pyrazinamide (PZA) **(3)**, it was first chemically synthesized.[47] In 1945, was found that a nicotinamide have tuberculostatic activity in Guinea-pigs and mice, and later, in 1947 there followed the investigation of various related drugs. Yeager et al. were found that an analog of nicotinamide-pyrazinamide to be more active in mice, and the first clinical trials of it were reported in 1952.[48] The nicotinamide had certain activity against mycobacteria in animal models and based on an unexpected observation of this pyrazinamide was discovered as a potent anti-TB drug.[49] TB treatment period is 9–12 months and pyrazinamide is a perilous frontline TB drug that shows an antique role in shortening it to 6 months.[49,50] The population of *M. tuberculosis* persistence that are not killed by other drugs, it is killed by pyrazinamide, because PZA has a powerful sterilizing activity .[51] The mechanism of action of PZA is well known, in *M. tuberculosis* pyrazinoic acid (POA), the active moiety of PZA, has been shown to prevent several functions at acidic pH.[52,53] The main cause for this is often that PZA is extraordinarily absolutely different from common antibiotics, that are primarily active against growing bacterium and do not have any or very little activity against non-growing persisters. Nevertheless, PZA is precisely the alternative to common antibiotics as a result of it's no or very little activity against growing tubercle bacilli and is primarily active against non-growing persisters.[54,55]

The pyrazinamide (*PZase*)/nicotinamidase, which is encoded by the *pncA* gene in *M. tuberculosis* and as a result PZA is a converted to the active form in POA.[56] The conversion to POA from PZA is also kind of like the nitrilase superfamily, The generates of a tetrahedral intermediate that collapses with the loss of ammonia and subsequent hydrolysis of the thioester bond by water due to the nucleophilic attack by the active site of cysteine[57,58] In 1967, McDermott's group found that the PZA resistance in *M. tuberculosis* is related to loss of nicotinamidase and *PZase*.[59,60] The major mechanism of PZA resistance is due to mutations in the *pncA* gene encoding *PZase*/nicotinamidase.[60,57] Although the extremely different and scattered distribution of *pncA* mutations, there is some degree of clustering at three regions of *PncA*: 3–17, 61–85, and 132–142.[61] Recently, how very diverse mutations can contribute to PZA resistance was solved by the crystal structure of *M. tuberculosis PncA*.[61] Additionally, it was shown that some PZA-resistant clinical isolates such as DHM444 without *pncA* mutations[62] and *Mycobacterium canettii* had mutations in the drug target *RpsA*.[61,63] A current study, has challenged the earlier model by suggesting that POA prevents trans translation, a route of ribosome spacing in *M. tuberculosis*. The study was accomplished in pyrazinamide resistant strains deficient mutations in *pncA* however, that had mutations in *rpsA* recognizing the ribosomal protein 1 (*RpsA*) because the planned target. POA was confirmed to be bound to *RpsA* and overexpression of *RpsA* conferred increased resistance to pyrazinamide.[64] PZA is a significant frontline drug which is a fight against TB. However, the growing emergence and epidemics of MDR, XDR-TB and TDR-TB call for the crucial development of new drugs which have the ability to kill the bacteria.[65] Developing new medicine that has an activity for persister TB bacterium is crucial for more shortening this TB medical aid.

3.1.4. *Ethambutol*



Ethambutol-1960 (4)

The new anti-tuberculosis agent, ethambutol (EMB) **(4)** was discovered by the Lederle Company in 1961,[66] which was additionally shown to be active in tuberculosis-infected guinea pigs.[67] The newly discovered compound N,N-diisopropylethylenediamin by group of lederel laboratories, was highly active against members of the genus mycobacterium, particularly against *M. tuberculosis* both in vitro and in vivo experimentally infected mice which is found during a screening program designed to rest selected compounds for potential anti-microbial activity,[68] their observation were both exciting and intriguing, since the chemical structure of this compound was completely unknown to any other known anti-tuberculosis drug. The lederle group started an autonomous program to define a structural characteristic of the diamine (organic compound) needed for its action on mycobacterium and to search out related compounds processing higher activity and laser animal toxicity.

Wilkinson et al. (1961, 1962)[69,70] was first described Synthesis of ethambutol in two reports and the first study of the anti-tuberculosis activity of ethambutol in experimental animals was first reported by Thomas et al. (1961)[67] and Wilkinson et al. (1962)[70] The drug is usually in salt form, dihydrochloride salt, water soluble and a white crystalline

Table 3 – Frist line Anti-TB agent with Mode of action and resistance.

Drugs	Target	Mode of action	Mutation
Rifampicin	*rpoB* (β-subunit of RNA polymerase)	Inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA dependent RNA-polymerase	81-Base pair Codons – 516, 526 and 531 [28,31] (Ser531Leu, His526Tyr, and Asp516Val)
Isoniazid	*katG* (catalase/peroxidase) *inhA* (enoyl reductase) *ahpC* (alkyl hydroperoxide reductase)	Block mycolic acid biosynthesis	Codon 315 in *katG* (Ser315Thr) [40] Codon – 138 and 328
Pyrazinamide	*pncA* *PZase*	Inhibiting the enzyme fatty acid synthesis	Mutations at amino acid residues 3–17, 61–85 and 132–142 [61]
Ethambutol	*embB* (arabinosyl transferase)	Blocking the formation of mycobacterium cell wall	Overproduction of *embCAB* gene cluster [74] (*embB* codon 306)

material that is heat stable. Ethambutol dihydro chloride inhibited *M. tuberculosis* or *M. smegmatis* in vitro at a concentration of 1–2 mg/ml and was highly effective in the treatment of mice infected with *M. tuberculosis* which is showed by Wilkinson et al. (1962).[70]

EMB has an established place as a first line anti-tuberculosis agent since more than 50 years to till date, it has a valued for the protection that it offers companion drugs against the development and consequences of drug resistance. EMB is shows potent activity against resistant strains l31 of isoniazid- and streptomycin and organisms of the genus *M. tuberculosis*. Thomas et al. was reported that EMB was inactive with other bacteria, fungi, and viruses in both activity, in vitro and in vivo in tests.[67,68]

Takayama and Kilburn[71] showed a method that controlled the growth of trehalose mono- and dimycolates within the medium, the transfer of arabinogalactan into the semipermeable membrane (cell wall) of *Mycobacterium smegmatis*, which is inhibited by EMB. In addition, it had been shown to inhibit the transfer of [D-14C] glucose into the D-arabinose residue of arabinogalactan.[72] Although the proof implicating arabinosyltransferases as the target of EMB, only in recent times has insight into the molecular genetics of arabinan biosynthesis been achieved.[73] A two-gene locus (*embAB*) in *Mycobacterium avium* that encodes arabinosyltransferases mediating polymerization of arabinose into arabinogalactan is identified by Belanger et al. Further, newly, three genes encoding a putative EMB target in *M. smegmatis* were cloned, sequenced, and characterized by molecular genetic strategies. These genes are organized as an operon, and because two of the three genes are homologous to *embAB* in *M. avium*, they were designated *embC*, *embA*, and *embB*.[74] Telenti et al. have now demonstrated that natural resistance to EMB results from an accumulation of genetic events determining overexproduction of the *embABC* proteins and structural mutations at codon 306 in *embB*.[74] The target(s) of EMB might presumably be arabinosyltransferases, which are included in the biosynthesis of AG and LAM and that have numerous degrees of sensitivity to EMB.[75] EMB in order inhibits arabinan biosynthesis in both AG and lipoarabinomannan (LAM).[74] The resistance of EMB was due to overproduction of the natural EMB target and not to an EMB resistance determinant. Lately, the *embCAB* gene cluster from *M. tuberculosis* and *M. smegmatis* was identified by Telenti et al.,[74] and showed that EMB resistance could result from overproduction of the *emb* protein(s), structural mutation of the *EmbB* protein, or both. On the other hand, the involvement of each gene from the *embCAB* operon to EMB resistance was not determined (Table 3).

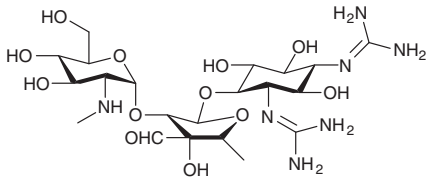
4. Existing second line anti-TB agents

4.1. *Streptomycin*

A huge antimicrobial spectrum was showcased by aminoglycosides-aminocyclitol antibiotics (hereafter named amino glycosides) which are nothing but water soluble, cationic molecule. Incorporation of the six-membered aminocyclitol ring has been depicted in the vast array of structural diverse compounds, moniker amino glycosides. Due to the poor oral adsorption, amino glycosides are administered intravenously or by injection in the treatment of bacterial infections caused by both gram-positive and gram-negative organisms.[76] No matter a few troubles of toxicity and bacterial resistance, these antibiotics still continue to be a seriously vital factor of our modern antimicrobial arsenal.

Streptomycin **(5)** turned out to be first isolated, through Albert Schatz, a graduate student, in the laboratory of Selman Abraham Waksman at Rutgers university in a studies undertaking funded via Merck and Co.[74,77,78] by Selman Waksman (for which he become awarded the Nobel Prize in physiology and medicine in 1952) on October 19, 1943 this presaged the identification and characterization of a clinically beneficial aminoglycosides over the 50 years. This led to the discovery of aminoglycoside antibiotics, streptomycin in 1944 by Schatz et al. (1944).[79] The significance of those preliminary findings become at once obvious and its use in the therapy of tuberculosis became stated to within one year of the development of streptomycin,[80] and it stays as a key component of present anti-mycobacterial treatment.[81] The primary randomized trial of streptomycin toward pulmonary tuberculosis was carried in 1946–1947 by means of the MRC Tuberculosis Research Unit beneath the chairmanship of Sir Geoffrey Marshall (1887–1982). The trial became each

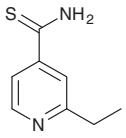
double-blind and placebo-controlled. It has been accepted widely in the primary randomized therapeutic trial.[82]



Streptomycin-1943 (5)

Translational errors and slowdown of translocation occurs due to the disturbance in several steps of protein synthesis by streptomycin.[83–85] Foot printing and mutation studies shows that streptomycin is a protein synthesis inhibitor which binds firmly to single site on 16S *rRNA*.[86–88] Foot printing research confirmed that streptomycin protects specific residues of 16S *rRNA* inside the 30S subunit[89] and may be connected to unique portions of 16S *rRNA*.[90] Moreover, a mutation in Euglena chloroplast 16S *rRNA* led to streptomycin resistance[91] and mutations in distinct regions of *E. coli* 16S rRNA changed the ribosomal response to streptomycin.[92–97] Streptomycin also interacts with ribosomal proteins in the 30S subunit[98,99] and mutations in S4, S5 and S12 ribosomal proteins are shown to influence its binding.[100] Speculation in this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the *mRNA* strand. This consequences in an unstable ribosomal-*mRNA* complex, leads to a frameshift mutation and defective protein synthesis; leading to cellular death.[101] Humans have ribosomes which are structurally different from the ones in bacteria, so the drug does no longer have this effect in human cells. At low concentrations, but, streptomycin best inhibits the increase of the bacteria with the aid of inducing prokaryotic ribosomes to misinterpret *mRNA*.[102] Streptomycin can bind to *E. coli 16S rRNA* in the absence of ribosomal proteins and might guard bases in the interpreting center from dimethyl sulfate (DMS) attack.[103]

4.2. *Ethionamide*



Ethionamide-1960 (6)

After its discovery in 1956, Ethionamide **(6)** was used to cure tuberculosis and MDR-TB. Ethionamide generally used in treatment of drug resistant TB falls in the class of antibiotic drug with thioamide groups. The treatment of MDR and XDR-TB Ethionamide is used as a part of regimens commonly concerning five drug treatments and it has been endorsed to use in aggregate with the fluoroquinolones.[104] In 2009 Wyeth pharmaceuticals become bought Ethionamide by means of Pfizer (is an American global pharmaceutical business enterprise established in NY metropolis) and it is bought underneath the brand call Trecator or Trecator SC with the aid of Wyeth prescription drugs.[105] Due to the structural similarity of ETH and PTH (prothionamide) to INH and it is clear that all of these drugs inhibit mycolic acid biosynthesis.[106,107]

The bacterial cellular wall has been an effective target for many drugs.[108] Many anti-tuberculosis agents are acknowledged to inhibit cell wall biosynthesis. INH, which is one of the maximum efficient and the most extensively used anti-tuberculosis drugs, has been the difficulty of intensive research at some stage in the beyond decade.[109–111] Both *M. tuberculosis* and *Mycobacterium bovis* BCG are extremely liable to INH (minimum inhibitory concentrations (MIC), 0.02–0.2 mg/ml). ETH, a structural analog of INH, is a useful second line anti-tuberculosis drug. The 2 drugs have nearly same results in that each strongly inhibit the synthesis of mycolic acids.[112,113] The cloning and characterization of the gene *Rv3855*, which we now coin *ethR*, that confers resistance to ETH, however now not to INH when it is overexpressed in either *M. smegmatis*, *M. Bovis* BCG or *M. tuberculosis* on a multicopy vector. Moreover, a transposon mutant of *ethR* results in ETH allergic reaction in *M. bovis* BCG. Further, genetic and biochemical proof suggests that *ethR* encodes a transcriptional regulator that isn't directly implicated in mycolic acid biosynthesis however performs a critical function within the law of a second open reading frame (ORF), which is responsible for the activation of ETH. Evaluation of the locus surrounding *ethR* discovered the presence of an adjacent gene now termed *ethA*, which encodes a putative monooxygenase, the expected activator of ETH. Overexpression of *ethA* brought about hypersensitive reaction to ETH in mycobacteria. Therefore, the data supplied are like minded with the belief that *ethR* represses *ethA*, which encodes the equal protein of *katG* implicated inside the activation of ETH.[114] It changed into proven that a single amino acid mutation of *inhA*, S94A, was enough to confer resistance to each ETH and INH in *M. tuberculosis*.[115]

4.3. *Fluoroquinolone*

For the effective second line medication of MDR-TB, fluoroquinolones use have currently increased. The synthetic derivatives of the parent nalidixic acid results in ciprofloxacin and ofloxacin which are nothing but obtained as by product of the antimalarial chloroquine.[116] Two critical enzymes for bacterial viability, topoisomerase II (DNA *gyrase*) and topoisomerase IV are inhibited by fluoroquinolones serves as the basis of mechanism of action. These proteins are encoded with the aid of the genes *gyrA, gyrB, parC*, and *parE*, respectively.[117] Primary mechanism of development of fluoroquinolone resistance in *M. tuberculosis* is through chromosomal mutations in the quinolone resistance-figure out region of *gyrA* or *gyrB*. The most common mutations located are at position 90 and 94 of *gyrA* however mutations at position 74, 88 and 91 have additionally been stated.[118,119] At present, 8-methoxy fluoroquinolones derivatives gatifloxacin, moxifloxacin and DC-159a, have showed extreme activities against MDR-TB bacteria and may be able to reduce the duration of currently available TB treatment. Thus, a new anti-TB agents from quinolones are needed to develop

Table 4 – List of currently available FDA-approved flouroquinolone antibacterial drugs for systemic use.

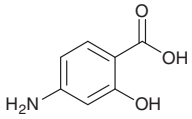
Brand name	Avelox	Cipro	Cipro extended-release[b]	Factive	Levaquin	Moxifloxacin injection	Ofloxacin[b]
Active Ingredient	Moxifloxacin[a]	Ciprofloxacin[a]	Ciprofloxacin extended-release[a]	Gemifloxacin[a]	Levofloxacin[a]	Moxifloxacin	Ofloxacin[a]

Source: http://www.fda.gov/Drugs/DrugSafety/ucm500143.htm.

[a] Available as generic.
[b] Available only as generic.

the treatment of tuberculosis with combination regimens (Table 4).

4.4. *Para-aminosalicylic acid*



Para-Aminosalicylic acid-1948 (7)

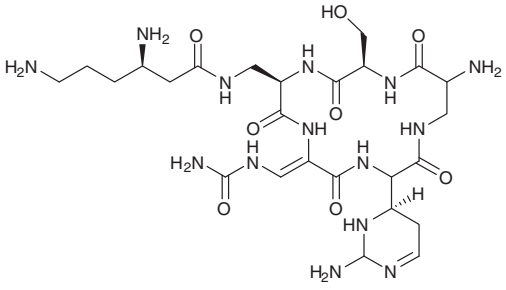
In combination with isoniazid and streptomycin, para-aminosalicylic acid or PAS **(7)** was initially used for the treatment of anti-tuberculosis, however now it is considered as a second line drug for the MDRTB treatment regimen. Till date its mode of action is not completely known. PAS has to compete with the dihydropteroate synthase, which is interfering in the process of folate synthesis as it is the analog of para amino benzoic acid. A study utilizing transposon mutagenesis recognized mutations as a part of the *thyA* gene connected with imperviousness to PAS that were likewise present in clinical isolates resistant to PAS.[120] In a board of 85 clinical MDR-TB detaches, transformations in folC were distinguished in five isolates resistant to PAS. In any case, only under 40% of PAS safe strains had transformations in *thyA* showing that still different components of resistance to the medication may exist.[121]

5. Exsisting injectable anti-TB agents

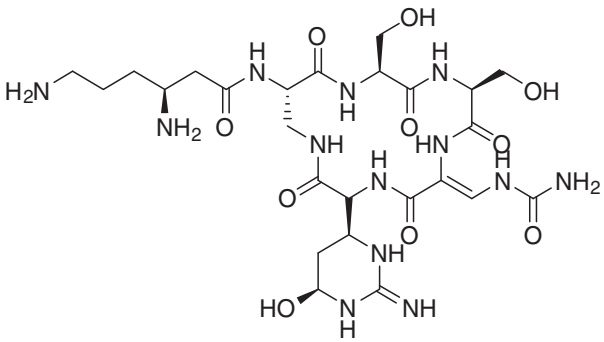
5.1. *Capreomycin and viomycin*

Capreomycin **(8)** is an important class of antibiotics which have a good mechanism of actions against multi-drug-resistant tuberculosis (MDR-TB), was discovered from Streptomyces Capreolus in 1960 and it belongs to the tuberactinomycins.[122,123] Capreolus a subspecies of saccharothrix metabolism is produce the capreomycin, a macrocyclic peptide antibiotic.[123] Capreomycin seems to inhibit the translation of mycobacteria, and with the help of mycobacterial ribosomes, it can inhibit phenylalanine synthesis in an in vitro translation assay. Furthermore, capreomycin does not interfere with *mRNA* binding to the ribosome which is signifying by Comparable inhibition was understood whether or not ribosomes were preincubated with *mRNA*.[124]

Capreomycin and viomycin **(9)** are cyclic peptide antibiotics and the structurally similar scaffold which are primarily active against mycobacteria. Both drugs show potent activity against *M. tuberculosis* by inhibition of bacterial growth via blocking protein synthesis on the ribosome.[125] Additionally, both drugs targeted bacterial protein synthesis by binding to the well-maintained intersubunit bridge B2a, made by interaction among helix 69 (H69) of the 23S *rRNA* and helix 44 (h44) of the 16S *rRNA*.[126]



Capreomycin-1963 (8)

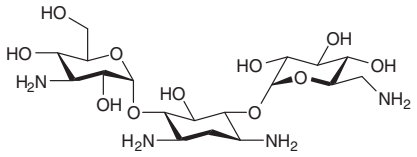


Viomycin (9)

After the miscarriage treatment with first-line drugs, capreomycin is used clinically against TB bacteria.[127] Capreomycin-resistant clinical isolates are normally resistant against various anti-tuberculosis agents which include kanamycin. Cross-resistance among kanamycin and capreomycin became previously observed in a variable fraction of kanamycin-resistant in *M. tuberculosis* strains[128] even though the molecular mechanism of this resistance was no longer known.

Inhibition of translocation at some point of peptide elongation is the principle mechanism of drug action facilitating the activity of antibacterial drugs[129,130] the loss of 2-O-methylation of C1920 (*rRNA* nucleotides are numbered according to those for *E. coli* throughout) in H69 and that of C1409 in h44 by *TlyA* reduces susceptibility to capreomycin .[131,132] *Thermus thermophiles TlyA* modifies most effective C1920 in H69 of 23S *rRNA*, but not C1409 in h44 of 16S *rRNA*. Inactivation of *TlyA* in *T. thermophiles* does not show effect on its sensitivity to capreomycin.[133] Maus at el. reported that the mutations in the 3 part of the 16S *rRNA* gene (*rrs*), particularly at positions 1401, 1402, and 1484, it is responsible for capreomycin resistance [134].

5.2. *Kanamycin*



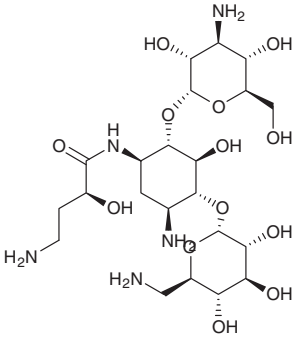
Kanamycin-1957 (10)

Umezawa et al. have first reported kanamycin **(10)** a group of aminoglycoside antibiotics in 1957 by isolation of *Streptomyces kanamyceticus* at the National Institute of Health of Tokyo, Japan. Kanamycin was first introduced as a clinical drug in 1958. Umezawa et al. were elucidate that the mechanism of inactivation of kanamycin due to a kanamycin-resistance organism in 1967, 10 years after the discovery of kanamycin.[135,136]

Furthermore, within a year after the discovery of Kanamycin, Kanamycin A has been commercially produced as the most common form of a drug, these antibiotics gained practically instant acceptance due to their wide range of activity, specifically against severe staphylococcal and mycobacterial infections.[137] Kanamycins interact with the 30S subunit of the prokaryotic ribosome, inducing vast quantities of mistranslation and indirectly inhibiting translocation at some stage in protein synthesis, resulting in death.[138]

5.3. *Amikacin*

Amikacin **(11)** a nephrotoxic, ototoxic and semi-synthetic aminoglycoside antibiotic, is a derivative of kanamycin A. Amikacin disrupts bacterial protein synthesis by binding to the 30S ribosome of inclined organisms, is similar to other aminoglycosides. Binding interferes with *mRNA* binding and *tRNA* acceptor websites main to the manufacturing of non-functional or toxic peptides.[139] The bactericidal effect of amikacin may confer the mechanism of action of is not completely known.



Amikacin-1976 (11)

Table 5 – Second-line anti-TB agent with mode of action and resistance.

Drugs	Target	Mode of action	Mutation
Streptomycin	*rpsL* (S12 ribosomal protein) *rrs* (16S rRNA) *gidB* (7-methylguanosine methyltransferase)	Binds to the 16S rRNA, interferes with translation proofreading, and thereby inhibits protein synthesis	Mutation in codon 43 and 88 from lysine to arginine in rpsL
Capreomycin	*rrs* (16S rRNA) *tylA* (rRNA methyltransferase)	Inhibit protein synthesis by binding to the 70S ribosomal unit	Mutations in the 16S rRNA gene
Ethionamide	*inhA* (enoyl reductase)	Inhibit the mycolic acid biosynthesis	C-15T mutation in the regulatory region S94A and I194T
p-Aminosalicylic acid	*thyA* (thymidylate synthase A)	Inhibit the folic acid synthesis	Mutation in thyA Thr202Ala
Fluoroquinolones	*gyrA/gyrB* (DNA gyrase)	Inhibiting the activity of both the DNA gyrase and the topoisomerase IV enzymes.	Mutations in quinolone resistance-determining region (QRDR) in gyrA
Kanamycin	*rrs* (16S rRNA) *eis* (aminoglycoside acetyltransferase)	Inhibit the protein synthesis by binding to the four nucleotides of 16S rRNA and a single amino acid of protein S12	Mutations in the codon 1401, 1402 and 1484 in the rrs (16S rRNA)
Amikacin	*rrs* (16S rRNA)	Inhibit the protein synthesis by binding to the 30S ribosomal subunit	Same as kanamycin

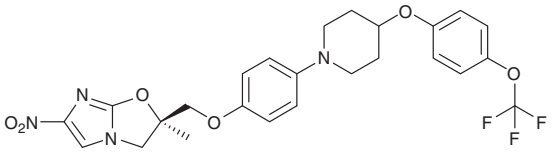
The mechanism of action of aminoglycosides by binding to the bacterial 30S ribosomal subunit, causing misreading of *t-RNA*, leaving the bacterium not able to synthesize proteins important to its increase. Aminoglycosides are beneficial in infections regarding aerobic, Gram-negative micro-organism, which includes pseudomonas, acinetobacter, and enterobacter. Further, a few mycobacteria, consisting of the micro-organism that causes tuberculosis, are susceptible to aminoglycosides. Aminoglycosides can also use against Gram-positive bacteria which is responsible for bacterial infections, however, different types of antibiotics are more potent and less destructive to the host. Especially in endocarditis, the aminoglycosides had been used along with penicillin-related antibiotics in streptococcal infections for their synergistic consequences, in the past. Aminoglycosides are mostly ineffective against the anaerobic micro-organism, viruses and fungi's. The aminoglycosides primarily act by binding to the aminoacyl site of 16S ribosomal RNA in the 30S ribosomal subunit, leading to a misreading of the genetic code and inhibition of translocation. The preliminary steps required for peptide synthesis are uninterrupted, such as binding of *mRNA* and the affiliation of the 50S ribosomal subunit, however, elongation fails to arise due to disruption of the mechanisms for making sure translational accuracy. The ensuing antimicrobial activity is usually bactericidal against susceptible aerobic gram-negative bacilli[140] (Table 5).

6. The present

6.1. *Recently approved by FDA*

6.1.1. *OPC-67683*

The new nitro-dihydroimidazooxazole delamanid (Deltyba™, known as OPC-67683) **(12)**, in adults having resistant forms of pulmonary TB is used extensively, which has further significantly broadened the inventory of the treatment options.[141]

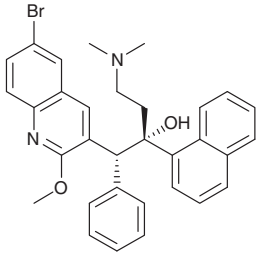


OPC-67683 (Delamanid)-2006 (12)

Despite the inclusion of the drug in the international guidance for the treatment of MDR-TB since April 2014[142] an easy access to countries with the greatest need till continues to be challenging. A score of less than ten patents outside the normal clinical setting could access the drug by the end of December 2014.[143] Otsuka (the drug's developer) announced in a recent "Fight-Back Initiative" held by WHO Global Laboratory Initiative Partners Forum in Geneva that a nitro-dihydro-imidazooxazole derivative Delamanid (OPC-67683) is a mycolic acid biosynthesis inhibitor which is devoid of mutagenicity with exceptionally high potent activity against TB including MDR-TB. This is supported by its low minimum inhibitory concentration (MIC) (range: 0.006–0.024 lg/ml) in vitro and highly effective therapeutic activity at low doses in vivo.[142,144]

The primary metabolites produced by the orally dosed nitro-dihydro-imidazooxazole derivative Delamanid kill tuberculous myobacteria by inhibiting the bacteria to create a building block that is important for their cell walls, which in turn leads to the blockage of the synthesis of mycolic acids.[141] Nitroimidazoles drugs realease nitric acid on metabolization which in turn lead to destroying TB bacteria.[142,145] Patients co-infected with TB/HIV could be effectively treated due to the long half-life of OPC-67683, the lack of metabolization by CYP enzymes and its efficacy in immunocompromised mice. At relatively lower concentration methoxy-mycolic and keto-mycolic acid synthesis (like INH) are inhibited due to the uniqueness in the structure of the cell wall of mycobacteria.[142] A synergistic interaction occurs when OPC-67683 combines with RIF or EMB in vitro with devoid of any antagonistic interaction with the first-line drugs RIF, INH, EMB and SM.[142] When a combination of OPC-67683 with RIF and PZA is taken for two months which is further continued for 2 months by taking a combination of RIF leads to an elimination of all lung bacterial load within 3 months and complete elimination occurs in mouse models after 4 months.[142] A reductive activation by *M. tuberculosis* is required by Delamanid to exert its activity. A mutation was found in the *Rv3547* gene in experimentally generated delamanid resistant mycobacteria, indicating its role in the commencement of the drug.[142]

6.1.2. *TMC-207*



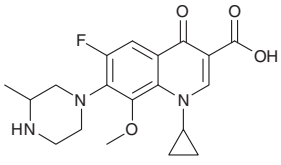
TMC-207 (Bedaquiline)-2005 (13)

TMC207 **(13)** is counted on to be the most potent of molecules through subsequent in vivo testing of activity against *M. tuberculosis*.[142] An excellent activity against susceptible drugs like MDR and XDR *M. tuberculosis* strains with no cross-resistance to current first-line drugs is exhibited by TMC207 MDR.[146] A greater potency is shown by TMC207 against mutated drug-resistant strains than susceptible isolates which indicates a unique mechanism of action. Despite the appearance of TMC207-resistant *M. tuberculosis* strains, full susceptibility occurs to other anti-TB drugs such as RIF, INH, SM and EMB. On co-administration of TMC207 with RIF decrease its levels significantly as TMC207 is readily metabolized by CYP3A4 and, and this leads in developing incompatible with antiretrovirals.[147] Very few similarities are there between mycobacterial and human protein encoded by *atpE* gene which codes only C subunit of ATP synthase, inhibition of mycobacterium membrane-bound ATP synthase by TMC207 is of greater potential.[148] Negative cultures in

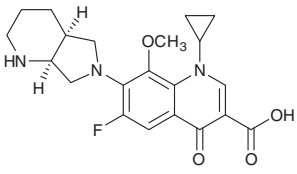
mouse models after two months is observed when TMC207 is combined with first-line drugs RIF, INH and PZA. While a combination of TMC207 and PZA for only two months leads in complete eradiation of lung *M. tuberculosis* proving it to be a synergistic effect. A successful complete eradication of lung and spleen infection within two months in drug sensitive mouse model occurs by using TMC207 with MDR-TB regimen (amikacin, ethionamide, moxifloxacin and PZA).[149] The results obtained by the phase II clinical trials led to an increased approval in using bedaquiline in treating MDR-TB which is available under the trade name Sirturo. However, due to unexplained deaths and QT interval prolongation a "black box" warning is also accompanied with the medicine. Recent reviews and evaluation of this new drug have been published.[150,151] A completely new target of action for an antimycobacterial drug was observed as bedaquiline inhibits ATP synthase of *M. tuberculosis*. This mode of action was revealed by analyzing *M. tuberculosis* and *M. smegmatis* mutants defiant to bedaquiline. The only mutation observed by sequencing the genome of the mutants which is further compared to susceptible strains is observed in the *atpE* gene, which encodes the c part of the F0 subunit of the ATP synthase.[152] A63P and I66M found in bedaquiline resistant mutants are the most prevalent mutation in the *atpE* gene. I66 due to modification by introduction, reduces the proper binding of bedaquiline to its target molecule.[146,153] It was observed in a study to assess the mechanism of resistance to bedaquiline in *M. tuberculosis* that only 15 out of 53 resistant mutants had mutations in *atpE*. While other 38 strains lacked mutations in *atpE* or even in the F0 or F1 operons, which indicates that other mechanisms of resistance are still possible.[154]

7. Currently evaluated for their anti-TB activity

7.1. *Gatifloxacin and moxifloxacin*



Gatifloxacin-1992 (13)



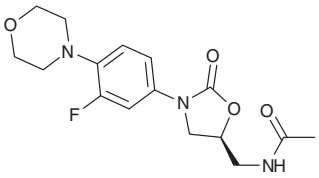
Moxifloxacin-1999 (14)

For an effective treatment of the respiratory tract infections, fluoroquinolones-gatifloxacin **(13)** and moxifloxacin were marketed in 1999. Both these fluoroquinolones molecules are at present in phase III clinical trials for the cure of TB.[155]

A 8-methoxyquinolone, moxifloxacin **(14)** manufactured by Bayer, Newbury, UK has been proved an important fluoroquinolone to have a wide range of activity against bacterial pathogens, including community-acquired pneumonia, and has a good safety record.[156,157] In clinical trials conducted for the execution of the respiratory infections use of this agent is readily made.

The treatment of patients intolerant of first-line anti-TB agents is done by making use of moxifloxacin which is recommended by the American Thoracic Society, Centers for Diseases Control and Prevention, and the Infectious Diseases Society of America.[158] In addition to this for the treatment of MDR-TB, moxifloxacin of quinolines base forms the central drug.

7.2. *Linezolid*



Linezolid-1998 (15)

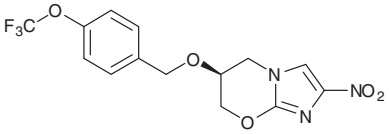
For drug-resistant, gram-positive bacterial infections linezolid **(15)** (Zyvox, Pfizer) belonging to new class of anti-microbial agents, oxazolidinones was accepted in 2000.[159] In vitro activity of *M. tuberculosis* with MIC90 from 0.5 to 2.0 mg/l is depicted by linezolid.[160,161] In 2003 linezolid interaction with humans having MDR-TB was reported.[162] Protein synthesis inhibitor, linezolid interacts only with domain V of the 23S *rRNA* portion of the 50S ribosomal subunit of bacteria.[163,164] The use of linezolid in prolonged anti-TB regimens is suppressed due to its toxicity. An approximate of 40–90% of patients suffer adverse events while 6% and 68% discontinue the use of linezolid due to the neuropathy (including optic neuritis) and myelosuppression according to the published reports.[165]

8. The future

8.1. *Under various phases of clinical and preclinical development*

New and novel compound discovery is still challenging. Despite a lot of work been done on whole-genome sequencing of *M. tuberculosis* genome-derived targeted approaches areas are yet to be explored to understand their complete potential .[166] A simple shifting from single-enzyme target to a phenotypic screening of whole bacterial cell leads to an impetus in the screening efficacy of the novel targets.[167] Furthermore, many novel anti-TB drug candidate with novel mechanism of actions, are in the preclinical-hit-to-lead optimization phase and also in pre-clinical developments, the pipe-line for the early clinical development phase is very small (Fig. 3). Two exciting new drugs (bedaquiline and delamanid) are currently approved by Food and Drug Administration (FDA) and two existing drug with combination dose moved beyond Phase-III study and have shown promising results in clinical trials in the past year.

8.1.1. *PA-824*



Pretomanid (PA-824) (16)

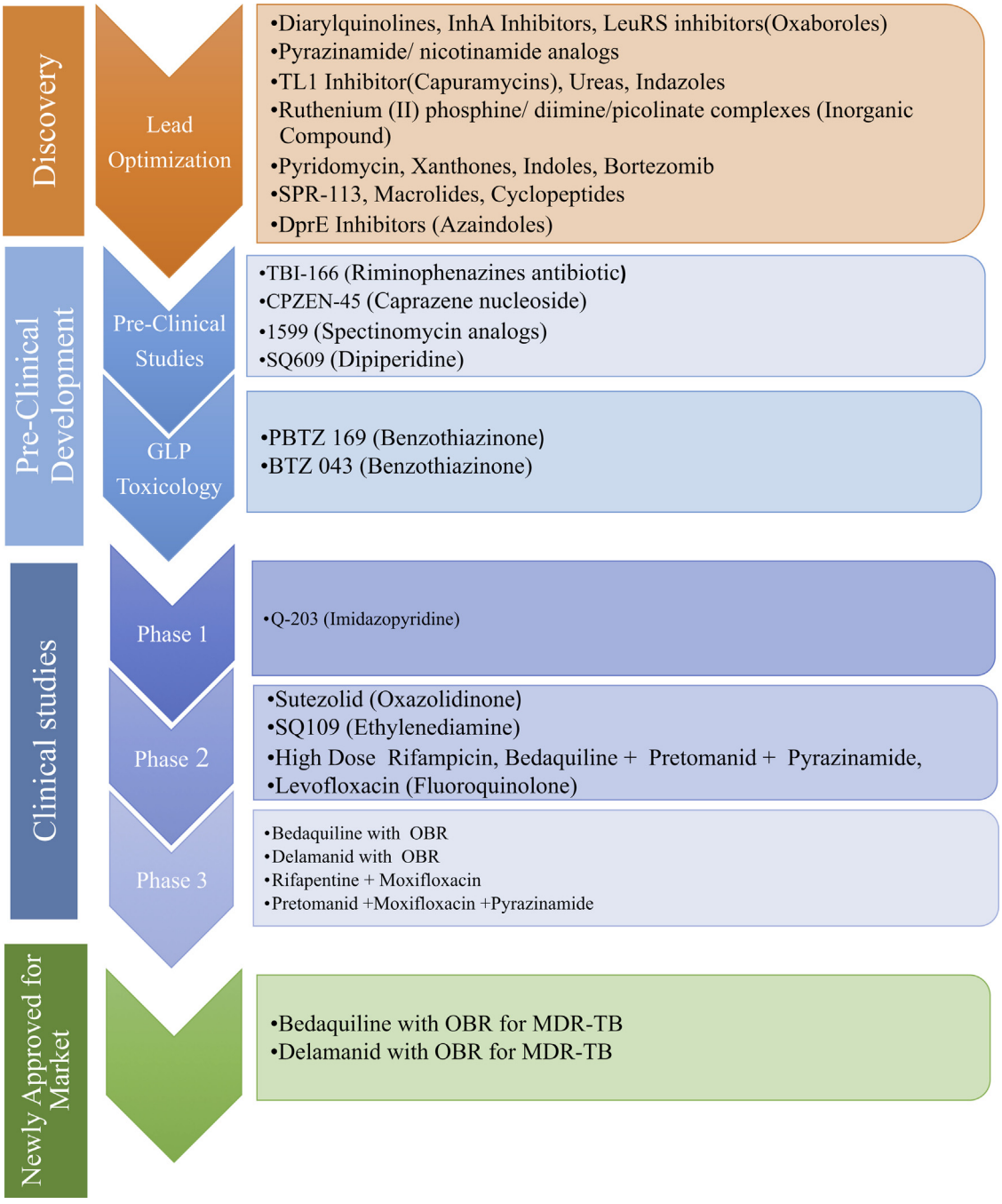
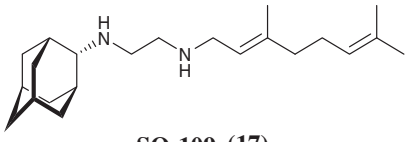


Fig. 3 – Global tuberculosis drug pipeline (GLP – Good Laboratory Practice).
***Source*: Working Group for New TB Drugs (www.newtbdrugs.org/pipeline).**

PA824 **(16)** is a bicyclic subsidiary of nitroimidazole that demonstrated particular activity against *M. tuberculosis*. PA824 should be initiated by a nitroreductase to apply its action and it hinders the amalgamation of protein and cell wall lipids.[168,169] The mechanism of resistance to PA824 has been appeared to be most ordinarily connected with the loss of a particular glucose6phosphate dehydrogenase (FGD1) or the dezaflavin cofactor F420. As of late, a nitroimidazooxazine specific protein creating minor basic changes in the medication has additionally been identified.[170]

8.1.2. SQ109



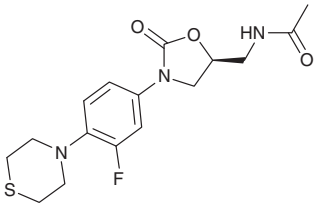
SQ-109 (17)

SQ109 **(17)** was develop by a collaboration of Sequella and NIH in the library of 63,000 diamine scaffold which is undergo broad studies in rats, dogs, and monkeys. More than 15 years was spent on research to develop SQ109 and applied its scientific expertise in tuberculosis (TB).[171] The SQ109 scaffold is a synthetic analog of ethambutol that has appeared in vitro and in vivo activates against drug susceptible and drug resistant *M. tuberculosis*.[172] Combinations of SQ109 with standard anti-TB drugs demonstrate both better efficacy and shorter time to achieve the same reduction in *M. tuberculosis* as standard therapy with ethambutol which is validated by numerous in vivo studies in the chronic mouse model of TB.[173]

SQ109 works by interfering with mycolic acid assembly in the core of bacterial cell wall which results in trehalose monomycolate (a precursor of the trehalose dimycolate) accumulation. Transcriptional studies have demonstrated that like other cell wall inhibitors, for example, isoniazid and ethambutol, SQ109 impels the interpretation of the *iniBAC* operon required for efflux pump functioning.[173] Moreover, by creating spontaneously produced safe mutants to SQ109 analogs and performing entire genome sequencing, transformations in the *mmpL3* quality were distinguished, recommending *mmpL3* as the target of SQ109 and signaling *mmpL3* as a transporter of trehalose mono mycolate.[174]

8.1.3. *PNO-100480*

The oxazolidinones (sutezolid) **(18)** discovered by E.I. Du Pont Nemours & Company in the 1980s, and later developed at Pharmacia and Upjohn (now part of Pfizer), has promising activity against drug-susceptible and drug-resistant TB. This ultimately led to linezolid, 1, and an analog, eperezolid.[175] A morpholinyl analog of linezolid sutezolid (PNU-100480 [U-480]) in the hollow-fiber, mouse, and whole-blood models exhibited advanced efficacy against *M. tuberculosis*.[176,177] Morpholinyl oxazolidinone, sutezolid (PNU-100480, PF-02341272) and AZD5847 (also known as AZD2563) have completed phase I clinical trials. Pfizer and AstraZeneca are functioning on these two identified anti-tubercular compounds.[178]



Sutezolid (PNO-100480) (18)

The oxazolidinones contain another class of protein combination inhibitors that block interpretation through a novel component by keeping the arrangement of the initial complex. The anti-tuberculosis movement of PNU-100480 (PNU) was initially reported in 1996.[179] When both drugs were administered at 100 mg/kg of body weight, it was observed that with subsequent experiments with a murine model indicated that PNU proved to be more active than LZD, but clinical relevance of LZD was not established and no clear difference was observed when their activities were compared for lower doses.[180]

Cynamon and associates initially reported the counter TB action of PNU in a murine model after intravenous contamination of outbred CD-1 mice, the start of treatment at somewhere around 1 and 7 days after disease, and treatment for 4 weeks.[181] Sutezolid (PNU100480) is a morpholinyl analog of linezolid with preparatory proof for prevalent adequacy against *M. tuberculosis*. In the mouse model, sutezolid abbreviates standard treatment by 1 month, though linezolid does not[182] in the entire blood society display, the maximal bactericidal action of sutezolid (−0.42 log/day) is more than twice that of linezolid (−0.16 log/day, $P < 0.001$).[179] Time-subordinate killing has been accounted for in entire blood and empty strands.[183]

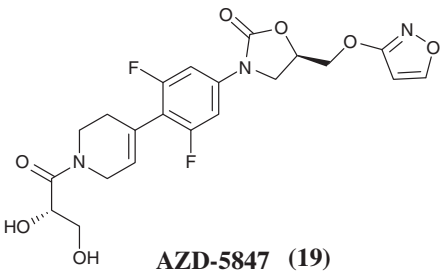
Bactericidal movement against intracellular mycobacteria is primarily because of the guardian (PNU100480), while a sulfoxide metabolite (PNU101603) Contributes altogether to action against extracellular mycobacteria. Phase 1 studies reveals no irregular hematologic or biochemical discoveries, nor did cases of peripheral or ophthalmic neuropathy, in healthy volunteers who were direct sutezolid 600 mg twice every day for 28 days.[184] Demonstrating a safety profile better than correspondingly dosed linezolid.

This is the principal investigation of sutezolid in patients with aspiratory tuberculosis. The principle discoveries were that measurements of 600 mg BID and 1200 mg QD given for 14 days were for the most safe, all around endured, and brought about promptly perceivable bactericidal movement in both sputum and blood. Its effect in sputum was managed all through the full time of treatment. These discoveries support further improvement of sutezolid as a part of a new tuberculosis regimen.[185]

Sutezolid got an Orphan Drug assignment in both U.S. and E.U. is at present in clinical improvement for the treatment of adult pumonary TB brought about by drug sensitive or drug-safe strains of *M. tuberculosis* (IND #104806). Sutezolid was safe and very much tolerated at dosages up to 1200 mg per day for up to 14 days, or 600 mg twice every day for up to 28 days.[183]

8.1.4. *AZD5847*

AZD5847 **(19)** [(5R)-3-(4-{1-[(2S)-2,3-dihydroxypropanoyl]-1,2,3,6-tetrahydropyridin-4-yl}-3,5-difluorophenyl)-5-[(isoxazol-3-yloxy)methyl]-1,3-oxazolan-2-one] belongs to the oxazolidinone drug class and has a molecular formula of $C_{21}H_{21}F_2N_3O_7$ and a relative molecular mass of 465.4 Da.[184] The agent has 2 chiral centers with no evidence of isomerization. AZD5847 has no measurable pK_a over the physiological pH range, and its melting point is 153 °C.

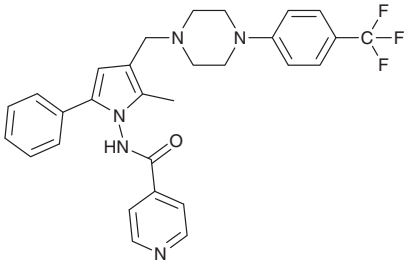


AZD-5847 (19)

AZD2563 a novel class of oxazolidinone was discovered by AstraZeneca and it initially intended for Gram-positive

bacteria's.[185] The medication was originally named AZD2563 and considered as a prodrug (AZD2563 disodium phosphate [DSP] for intravenous imbuement) in stage 1 clinical trial; we have reprofiled AZD2563 for its hostile to TB action. In these studies, AZD2563 and AZD2563 DSP have been renamed AZD5847 and AZD5847 DSP, individually, and information on its in vitro antimicrobial action against a board of medication defenseless and medication safe clinical disconnects of *M. tuberculosis*, its bactericidal movement against *M. tuberculosis* $H_{37}Rv$ in broth or in human macrophages, and portrayal of resistant mutants were distributed recently.[186]

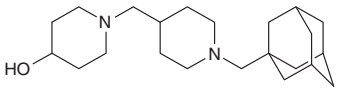
8.1.5. *LL3858*



LL-3858 (20)

Extremely restricted data on the advancement of pyrroles as anti-mycobacterial agents is right now accessible. Pyrroles derivatives were observed to be dynamic against standard and drug-sensitive *M. tuberculosis* strains in vitro[187] that demonstrated higher bactericidal action than isoniazid when administered as monotherapy to infected mice. In mouse models, a 12 weeks treatment with LL-3858 **(20)** or more isoniazid and rifampicin, or LL-3858 or more isoniazid-rifampicin-pyrazinamide, disinfected the lungs of all contaminated mice. Tests directed in mice and dogs demonstrated that the compound is all around retained, with levels in serum over the MIC and preferable half-life and Cmax over those appeared by isoniazid. No data is accessible concerning the atomic components that intervene LL-3858's bactericidal action.[188]

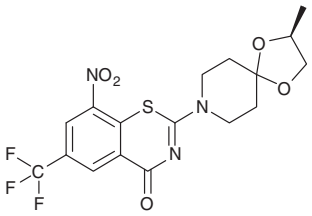
8.1.5.1. *SQ609*.



SQ-609 (21)

SQ609 **(21)** was discovered by Sequella and it is a new diamine scaffold and currently, SQ609 is under IND-directed preclinical studies and evaluation in clinical trials. It has a different mechanism of action from existing TB drugs and potent and specific activity against both drug-sensitive and drug-resistant forms of *M. tuberculosis*, low toxicity, activity in in vivo models of *M. tuberculosis* infection, and a favorable safety and pharmacology profile.[189] A number of new dipiperidine analogs were designed and they demonstrated activity against *M. tuberculosis* after the clinical development of SQ109.[190] A concentration of 4 mg/mL of SQ609 was able to restrain 90% of bacterial growth in *M. tuberculosis* infected macrophages in vitro with no toxic effect. While in vivo evaluation of SQ609 was done in *M. tuberculosis* $H_{37}Rv$ infected mice. SQ609 at 10 mg/kg for 2 weeks was used once daily for the treatment of C3H/He mice intravenously infected. SQ609 was appeared to avoid the weight reduction of the animals and could prolong therapeutic 2 weeks after the end of the treatment.[190] SQ609 is at present being assessed in preclinical studies.

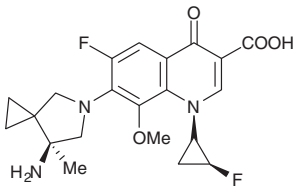
8.1.5.2. *BTZ043*. BTZ043 **(22)** is discovered by Medical Centre of the University of Munich (LMU), Hans-Knöll-Institut (HKI) and German Center for Infection Research (DZIF) and it is currently in a preclinical development.[191] BTZ043 was described as 1,3-benzothiazin-4-one or benzothiazinone (BTZ), is a new class of TB drug candidate with novel and high antimycobacterial activity.[192]



BTZ043 (22)

Furthermore, the lead compound, (S)-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BTZ043) was demonstrate that have in vitro, ex vivo and in vivo activity against *M. tuberculosis* and also found to be active against drug susceptible and MDR clinical isolates of *M. tuberculosis*.[193] The mechanism of action of BTZ043 was initially spotted at the cell wall biogenesis level by transcriptome analysis. Moreover, the target of the drug was identified at the level of the gene *rv3790* via genetic analysis, using in vitro generated mutants, which together with *rv3791* encode proteins that catalyze the epimerization of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA), a precursor for arabinan synthesis needed for the bacterial cell wall.[191] *DprE1* and *DprE2* were proposed as names for these two key enzymes.[190] More recent, studies have characterized more precisely the mechanism of action of BTZ043 by showing that the drug is activated in the bacteria through reduction of an essential nitro group to a nitroso derivative, which can react with a cysteine residue in *DprE1*.[194]

8.1.5.3. *DC159a*.

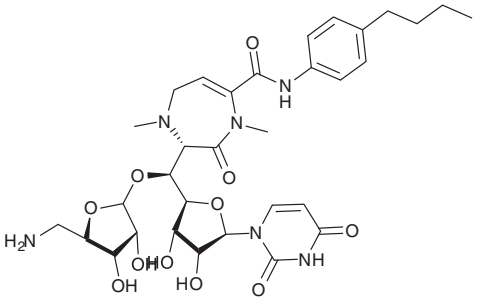


DC-159a (23)

DC-159a **(23)** is a new class of compound for the treatment of MDR-DB which is derived from a new generation of fluoroquinolones. DC-159a illustrate potent activity against various respiratory pathogens, as well as *GyrA* activity, which shows very significant roles in DNA replication, same as other quinolone derivatives. But the mechanism of action of DC-159a is still under investigation.[195,196]

Some other anti-TB fluoroquinolones get resist against quinolone resistant multi-drug-resistant tuberculosis strains (QR-MDR-TB) and they become inactive, but DC-159a showed good in vitro and in vivo activities against MDR-TB. However, DC-159a-resistant mutants shown patterns of mutations in *GyrA* diverse than the ones observed in quinolones-resistant strains.[197] As a result, it has been suggested that DC-159a may possibly be a replacement of new drug candidate for the cases of QR-MDR-TB treatment. DC-159a showed MIC90 of 0.06 mg/mL against drug-susceptible strains (n ¼ 21) and 0.5 mg/mL against QR-MDR strains (n ¼ 11).[198] In *M. tuberculosis* $H_{37}Rv$ infected mice, during the initial phase of treatment (2 months), the activity of DC-159a alone (25 mg/kg) was superior to moxifloxacin at 25 mg/kg and equivalent to moxifloxacin at 50 mg/kg.[199] Additional preclinical studies are currently in progress.

8.1.5.4. CPZEN-45. CPZEN-45 **(24)** was derived from the caprazamycins, which are natural products isolated from *Streptomyces* sp. MK730-62F2. Caprazene (CPZEN), a core structure of the caprazamycins, proved to be a good precursor of anti-TB antibiotics. CPZEN-45 was the most promising new tuberculosis drug candidate based on the study of structure activity relationships on a range of CPZEN derivatives showed excellent activity against *M. tuberculosis*.[200] Due to the poor absorption from the gastrointestinal (GI) tract of the compound, it shows a solubility of around 10 mg/ml in water and has low oral bioavailability. CPZEN-45 shows excellent activity against MTB strains in vitro and as a result, it appears to be a promising candidate for the treatment of TB. The minimum inhibitory concentration (MIC) of CPZEN-45 is 1.56 g/ml for *M. tuberculosis* ($H_{37}Rv$) and 6.25 g/ml for MDR-TB.[201]

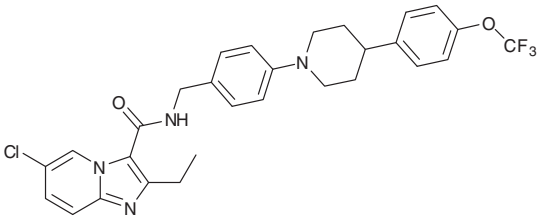


CPZEN-45, (Caprazene-45) (24)

CPZEN-45 can be a very good candidate for the treatment of MDR-TB and XDR-TB because it has in no way been utilized in treatment; therefore, no bacterial strain resistance to this compound is estimated. No matter its appealing capabilities, development of an oral formulation for CPZEN-45 won't be possible because of its meager solubility and potentially low bioavailability. The parenteral preparation of this compound may be possible, however, the everyday injections for TB treatment are undesirable and would reduce the enthusiasm for its use by patients and clinicians.

Consequently, unconventional drug transport strategies have to be explored for this new drug. The efficacy of TB treatment with this new TB drug candidate can hypothetically increase due to a preparation of CPZEN-45 as a powder for inhalation. Hence, the overall goal is to illustrate that when administered to the lungs as a respirable powder that CPZEN-45 is powerful in reducing TB infection. For the purpose of identification of the major objective of CPZEN-45, the effect of caprazamycin and CPZEN-45 on the incorporation of radio labeled precursors into cellular macromolecules was estimated in *B. subtilis* 168. Furthermore, CPZEN-45 inhibited glycerol incorporation dominantly in *B. subtilis*.[202,203]

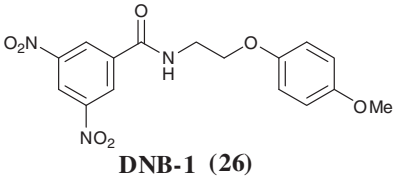
8.1.5.5. Q-203. Q203 **(25)** is a new class of TB drug candidate which is derived from imidazopyrimdines,[204] it blocks the respiratory cytochrome bc1 complex which is very important to preserve the proton gradient and ATP synthesis and as a result the growth of *M. tuberculosis* is prevented.



Q-203 (25)

Despite the fact that the drug has a comparable target as bedaquiline, it inhibits ATP synthesis more potently in both aerobic and hypoxic environments. Furthermore, Q203 is shown potent activity against multi-drug-resistant (MDR-TB) and extensively drug resistant (XDR-TB) bacteria of *M. tuberculosis* from humanoid, and data from mice models show a 100–1000-fold reduction of colony-forming units and a blocking of granuloma formation.[205]

8.1.5.6. DNB-1.

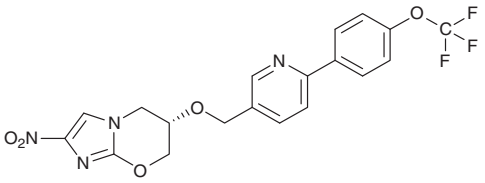


DNB-1 (26)

Dinitrobenzamide analogs as DNB1 **(26)**, these derivatives were also shown to inhibit decaprenylphospho-arabinose synthesis by targeting decaprenylphosphoribose 20 epimerase *DprE1* and are currently under development.

8.1.5.7. TBA-354. The researchers at the Auckland Cancer Society Research Centre (ACSRC) and Maurice Wilkins Centre for Molecular Bio discovery was designed new in the compound, TBA-354 **(27)** in collaboration with the University of Illinois at Chicago and the TB Alliance group. Preclinical

studies showed that TBA-354 has been more potent than another compound in its class, however, PA-824 was already shown ability in clinical trials. Approximately after 50 years, this is the first novel class of drugs to be developed for TB and it is newly designed to work against the persistent form of the TB .[206] According to TB Alliance, TBA is currently under phase-1 clinical trial. This research program arose from a rather speculative offer to the GATB (Global Alliance for Tuberculosis Drug Development) from the ACSRC (Auckland Cancer Society Research Centre) to help with their PA-824 second generation development program due to their specialty in the chemistry of nitroimidazole.



TBA-354 (27)

Since the introduction of metronidazole, the nitroimidazoles were extensively used to treat anaerobic bacterial and protozoal infections.[207] Under specific environmental situation or selectively bio-reduced by an enzyme specific to the target pathogen the compounds of this group are safe. Various nitroimidazole derivatives have shown potent activity against members of the *M. tuberculosis* bacteria.[208] More than one thousand nitroimidazole analogs have been pursued by the Global Alliance for the development of TB drugs.[209–211] Due to this procedure TBA-354 identified as a latent next-generation nitroimidazole with highly powerful activity compared to PA-824 with in vitro activity against *M. tuberculosis*, PA-824 has a potentially superior pharmacokinetic profile as compared to this nitroimidazole. Which is currently being administered twice daily as a result of the greater metabolic stability than delamanid when administered at 100 mg/kg/day in acute and chronic murine infection models of TB.[212]

In mid-2005, the first clinical trial of pretomanid (PA-824) was carried out by GATB and the success to develop a new second generation tuberculosis candidate by ACSRC and also takes over the entire second generation Med Chem program in 2006. Independent research article to understand the mechanism of pretomanid was published by ACSRC and the US national institute of Allergy Infectious Diseases in 2008. In 2009, SN-31354 currently known as TBA-354 preferred as second generation tuberculosis drug and lately in October 2011, International committee was allowed to proceed to IND filing of TBA-354. TBA-354 was first publicly disclosed in the 52 nd Inter-science Conference on Antimicrobial Agents & Chemotherapy (ICAAC) in San Francisco, September 2012. After the public disclosure of TBA-354, US FDA approved the IND to proceed to clinical trials and the first clinical trials of TBA-354 was begin lately in 2014.[213]

March 11, 2016, TB Alliance announced the withdrawal of tuberculosis drug candidate TBA-354 due to side effects in the initial cohort during the MAD (Multiple Ascending Dose) studies, which is designed to test the pharmacokinetics and tolerability of ascending doses of TBA-354 in healthy volunteers. As a result, the TB Alliance together with its scientific advisors made the decision to stop the clinical trial and the clinical development program of TBA-354 due to observed side effects and pharmacokinetic data of TBA-354 generated in this cohort.[214]

9. Conclusion

In this review, it can be concluded that already existing first-ling and second-line anti-tuberculosis drugs are enormously used which were discovered way back in the 1950s. Due to meager success attempt rate obtained in the development of novel anti-tuberculosis drugs in the recent past, a lot of problems like DR-TB, MDR-TB, XDR-TB, and recently emerging threat TDR-TB are prevailing. So, to cure these problems there occurs an urgent need for the development of new anti-TB drugs which can be used for reducing problems as seen before.

Taking it to consideration about problems faced by TB, delamanid (OPC-67683) and bedaquiline (TMC-207) have been recently approved by FDA for their potent activity against MDR-TB and effective usage against TB treatment. Also, it was seen that there is certain novel anti-TB drug candidate in the pipeline, which is quite effective for TB of looking at the present scenario of problems faced by patients.

Finally, tuberculosis emerging worldwide threat day by day with developing resistance against existing anti-TB drugs. So, stop this TB burden worldwide, there is an urgent need to discover novel anti-TB drug candidates which have a novel mechanism of action against TB bacteria and it should help to shorten the current TB treatment.

Conflicts of interest

The authors have none to declare.

Acknowledgements

The authors are thankful to the Department of Chemistry, Gujarat University, Ahmedabad, for providing the required infrastructural facilities and University Grants Commission Info Net and INFLIBNET, Gujarat University, for providing e-source facilities. One of the authors (M.S.V) (RGNF-2014-15-ST-GUJ-57277) is thankful to Rajiv Gandhi National Fellowship for financial assistance.

REFERENCES

1. Zimmer C. *Tuberculosis is Newer than Thought, Study Says.* New York Times; 2014, August 21.
2. Gutierrez MC, Brisse S, Brosch R, et al. Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. *PLoS Pathog.* 2005;1:5.
3. http://www.cdc.gov/tb/events/worldtbday/history July 20/16: 2016 [accessed 24.07.16].

4. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *JAMA*. 1999;282:677–686.
5. Kumar V, Abbas AK, Fausto N, Mitchell RN. *Robbins Basic Pathology*. 8th ed. Saunders Elsevier; 2007:516–522.
6. Bichun LA, Pedrosa MM, Trippel SJ. *Molecular Biology of Mycobacterium tuberculosis*. Connecticut: Springer; 1996.
7. Brennan PJ. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis*. 2003;83:91–97.
8. Cole S, Brosch R, Parkhill J, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*. 1998;393:537–544.
9. Clemens DL, Horwitz MA. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. *J Exp Med*. 1996;184:1349–1355.
10. Akif M, Chauhan R, Mande SC. Expression, purification, crystallization and preliminary X-ray crystallographic studies of *Mycobacterium tuberculosis* thioredoxin reductase. *Acta Crystallogr Sect D: Biol Crystallogr*. 2004;60:777–779.
11. Manganelli R, Dubnau E, Tyagi S, Kramer FR, Smith I. Differential expression of 10 sigma factor genes in *Mycobacterium tuberculosis*. *Mol Microbiol*. 1999;31:715–724.
12. World Health Organization. *Global tuberculosis report*. 2015 In: *http://www.who.int/tb/publications/global_report/en/*.
13. World Health Organization. *Tuberculosis Fact Sheet*. 2016. Available from: http://www.who.int/gtb/publications/factsheet/index.htm [accessed 25.07.16].
14. Gopi PG, Subramani R, Santha T, Chandrasekaran V. Estimation of burden of tuberculosis in India for the year 2000. *Indian J Med Res*. 2005;122:243.
15. Almeida D, Rodrigues C, Udwadia ZF, et al. Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. *Clin Infect Dis*. 2003;36:152–154.
16. Pablos-Méndez A, Gowda DK, Frieden TR. Controlling multidrug-resistant tuberculosis and access to expensive drugs: a rational framework. *Bull World Health Organ*. 2002;80:489–495.
17. World Health Organization. *Report. Global Tuberculosis Control. Epidemiology, Strategy and Financing*. Geneva: WHO; 2010.
18. Gandhi NR, Nunn P, Dheda K, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet*. 2010;375:1830–1843.
19. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. *Lancet Infect Dis*. 2010;10:621–629.
20. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. Totally drug-resistant tuberculosis in India. *Clin Infect Dis*. 2012;54:579–581.
21. Migliori GB, Loddenkemper R, Blasi F, Raviglione MC. 125 years after Robert Koch's discovery of the tubercle bacillus: the new XDR-TB threat. Is ''science'' enough to tackle the epidemic? *Eur Respir J*. 2007;29:423–427.
22. (a). World Health Organization. *Global Tuberculosis Report*. 2013. ISBN 978924 1564656;Ć(b). World Health Organization. *Global Tuberculosis Report*. 2012. ISBN 978924 1564502.
23. Diacon AH, Pym A, Grobusch M, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med*. 2009;360:2397–2405.
24. Schatz A, Waksman SA. Effect of streptomycin and other antibiotic substances upon *Mycobacterium tuberculosis* and related organisms. *Exp Biol Med*. 1944;57:244–248.
25. When I use a word. I mean it. *Br J Med*. 1999;319 (October):972.
26. Timothy MD. *Tuberculosis: Diagnosis and Treatment*. Wallingford, Oxfordshire: CABI; 2011:219.
27. Calvori C, Frontali L, Leoni L, Tecce G. Effect of rifamycin on protein synthesis. *Nature*. 1965;207:417–418.
28. Mani C, Selvakumar N, Narayanan S, Narayanan PR. Mutations in the rpo B gene of multidrug resistant *Mycobacterium tuberculosis* clinical isolates from India. *J Clin Microbiol*. 2001;39:2987–2990.
29. Rattan A, Kalia A, Ahmad N. Multidrug-resistant *Mycobacterium tuberculosis*: molecular perspectives. *Emerg Infect Dis*. 1998;4:195.
30. Sachan AS, Gupta RK, Katoch VM, Mishra K, Jakhmola P. Detection of Rifampicin resistant mutant gene in *Mycobacterium tuberculosis* by line probe assay. *Indian J Tuberc*. 2002;39:209–211.
31. Somoskovi A, Parsons LM, Salfinger M. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*. *Respir Res*. 2001;2:164–168.
32. Caws M, Duy PM, Tho DQ, Lan NT, Hoa DV, Farrar J. Mutations prevalent among rifampin and isoniazid resistant *Mycobacterium tuberculosis* isolates from a hospital in Vietnam. *J Clin Microbiol*. 2006;44:2333–2337.
33. Traore H, Fissette K, Bastian I, Devleeschouwer M, Portaels F. Detection of rifampicin resistance in *Mycobacterium tuberculosis* isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. *Int J Tuberc Lung Dis*. 2000;4:481–484.
34. Comas I, Borrell S, Roetzer A, et al. Whole genome sequencing of rifampicin resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations in RNA polymerase genes. *Nat Genet*. 2011;44:106–110.
35. Bernstein J, Lott WA, Steinberg BA, Yale HL. Chemotherapy of experimental tuberculosis. V. Isonicotinic acid hydrazide (nydrazid) and related compounds. *Am Rev Respir Dis*. 1952;65:357–364.
36. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature*. 1992;358:591–593.
37. Banerjee A, Dubnau E, Quemard A, et al. inhA, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science*. 1994;263:227–229.
38. Vilchèze C, Morbidoni HR, Weisbrod TR, et al. Inactivation of the inhA-encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the FASI end products and cell lysis of *Mycobacterium smegmatis*. *J Bacteriol*. 2000;182:4059–4067.
39. Slayden RA, Lee RE, Barry CE. Isoniazid affects multiple components of the type II fatty acid synthase system of *Mycobacterium tuberculosis*. *Mol Microbiol*. 2000;38:514–525.
40. Lee AS, Lim IH, Tang LL, Telenti A, Wong SY. Contribution of kasA analysis to detection of isoniazid-resistant *Mycobacterium tuberculosis* in Singapore. *Antimicrob Agents Chemother*. 1999;43:2087–2089.
41. Piatek AS, Telenti A, Murray MR, et al. Genotypic analysis of *Mycobacterium tuberculosis* in two distinct populations using molecular beacons: implications for rapid susceptibility testing. *Antimicrob Agents Chemother*. 2000;44:103–110.
42. Argyrou A, Vetting MW, Aladegbami B, Blanchard JS. *Mycobacterium tuberculosis* dihydrofolate reductase is a target for isoniazid. *Nat Struct Mol Biol*. 2006;13:408–413.
43. Argyrou A, Jin L, SiconilfiBaez L, Angeletti RH, Blanchard JS. Proteomewide profiling of isoniazid targets in *Mycobacterium tuberculosis*. *Biochemistry*. 2006;45:13947–13953.
44. Hazbón MH, Brimacombe M, Bobadilla del Valle M, et al. Population genetics study of isoniazid resistance mutations and evolution of multidrug resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2006;50:2640–2649.

45. Cardoso RF, Cardoso MA, Leite CQ, et al. Characterization of ndh gene of isoniazid-resistant and susceptible *Mycobacterium tuberculosis* isolates from Brazil. *Mem Inst Oswaldo Cruz*. 2007;102:59–61.
46. Ando H, Kitao T, MiyoshiAkiyama T, Kato S, Mori T, Kirikae T. Downregulation of KatG expression is associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol*. 2011;79:1615–1628.
47. Ando H, MiyoshiAkiyama T, Watanabe S, Kirikae T. A silent mutation in mabA confers isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol*. 2014;91:538–547.
48. Dalmer O, Walter E, Firma E. Merck in Darmstadt. Verfahren zur Herstellung von Abkömmlingen der Pyrazinmonocarbonsäure. Patentiert im Deutschen Reiche vom. 1934; 8:1936.
49. Yeager RL, Munroe WG, Dessau FI. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. *Am Rev Respir Dis*. 1952;65:523–546.
50. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis*. 1999;3:S231–S279.
51. Somner AR, British Thoracic Association. A controlled trial of six months chemotherapy in pulmonary tuberculosis: first report: results during chemotherapy. *Br J Dis Chest*. 1981;75:141–153.
52. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle*. 1985;66:219–225.
53. Boshoff HI, Mizrahi V, Barry CE. Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. *J Bacteriol*. 2002;184:2167–2172.
54. Zhang Y, Wade MM, Scorpio A, Zhang H, Sun Z. Mode of action of pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetics by pyrazinoic acid. *J Antimicrob Chemother*. 2003;52:790–795.
55. Zhang Y, Permar S, Sun Z. Conditions that may affect the results of susceptibility testing of *Mycobacterium tuberculosis* to pyrazinamide. *J Med Microbiol*. 2002;51:42–49.
56. Hu Y, Coates AR, Mitchison DA. Sterilising action of pyrazinamide in models of dormant and rifampicin-tolerant *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2006;10:317–322.
57. Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat Med*. 1996;2:662–667.
58. Fyfe PK, Rao VA, Zemla A, Cameron S, Hunter WN. Specificity and mechanism of *Acinetobacter baumanii* nicotinamidase: implications for activation of the front-line tuberculosis drug pyrazinamide. *Angew Chem Int Ed Engl*. 2009;48:9176–9179.
59. Seiner DR, Hegde SS, Blanchard JS. Kinetics and inhibition of nicotinamidase from *Mycobacterium tuberculosis*. *Biochemistry*. 2010;49:9613–9619.
60. Konno K, Feldmann FM, McDermott W. Pyrazinamide susceptibility and amidase activity of Tubercle Bacilli 1, 2. *Am Rev Respir Dis*. 1967;95:461–469.
61. Scorpio A, Lindholm-Levy P, Heifets L, et al. Characterization of pncA mutations in pyrazinamideresistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1997;41:540–543.
62. Petrella S, Gelus-Zietal N, Maudry A, Laurans C, Boudjelloul R, Sougakoff W. Crystal structure of the pyrazinamidase of *Mycobacterium tuberculosis*: insights into natural and acquired resistance to pyrazinamide. *PLoS ONE*. 2011;6:15785.
63. Shi W, Zhang X, Jiang X, et al. Pyrazinamide inhibits trans-translation in *Mycobacterium tuberculosis*. *Science*. 2011;333:1630–1632.
64. Feuerriegel S, Koser CU, Richter E, Niemann S. *Mycobacterium canettii* is intrinsically resistant to both pyrazinamide and pyrazinoic acid. *J Antimicrob Chemother*. 2013;68:1439–1440.
65. Shi W, Zhang X, Jiang X, et al. Pyrazinamide inhibits trans translation in *Mycobacterium tuberculosis*. *Science*. 2011;333:1630–1632.
66. World Health Organization. *Anti-Tuberculosis Drug Resistance in the World, Report No. 4*. Geneva, Switzerland: World Health Organization; 2008 In: *http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf*.
67. Thomas JP, Baughn CO, Wilkinson RG, Shepherd RG. A new synthetic compound with antituberculous activity in mice: ethambutol (dextro-2,2′-(ethylenediimino)-di-1-butanol). *Am Rev Respir Dis*. 1961;83:891–893.
68. Karlson AG. The in vitro activity of ethambutol (dextro-2,2′-[ethylenediimino]-di-l-butanol) against tubercle bacilli and other microorganisms 1. *Am Rev Respir Dis*. 1961;84:905–906.
69. Wilkinson RG, Shepherd RG, Thomas JP, Baughn C. Stereospecificity in a new type of synthetic antituberculous agent 1, 2. *J Am Chem Soc*. 1961;83:2212–2213.
70. Wilkinson RG, Cantrall MB, Shepherd RG. Antituberculous agents. III (+)-2,2-(ethylenediimino)-di-1-butanol1, 2 and some analogs. *J Med Chem*. 1962;5:835–845.
71. Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. *Antimicrob Agents Chemother*. 1989;33:1493–1499.
72. Silve G, Valero-Guillen P, Quemard A, Dupont MA, Daffe MA, Laneelle G. Ethambutol inhibition of glucose metabolism in mycobacteria: a possible target of the drug. *Antimicrob Agents Chemother*. 1993;37:1536–1538.
73. Belanger AE, Besra GS, Ford ME, et al. The embAB genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. *Proc Natl Acad Sci U S A*. 1996;93:11919–11924.
74. Telenti A, Philipp WJ, Sreevatsan S, et al. The emb operon, a gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. *Nat Med*. 1997;3:567–570.
75. Deng L, Mikusova K, Robuck KG, Scherman M, Brennan PJ, McNeil MR. Recognition of multiple effects of ethambutol on metabolism of mycobacterial cell envelope. *Antimicrob Agents Chemother*. 1995;39:694–701.
76. Edson RS, Terrell CL. The aminoglycosides. *Mayo Clin Proc*. 1991;66:1158–1164.
77. Comroe Jr JH. Pay dirt: the story of streptomycin: Part II. Feldman and Hinshaw; Lehmann. *Am Rev Respir Dis*. 1978;117:957–968.
78. Kingston W. Streptomycin, Schatz v. Waksman, and the balance of credit for discovery. *J Hist Med Allied Sci*. 2004;59:441–462.
79. Schatz A, Bugle E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Exp Biol Med*. 1944;55:66–69.
80. Hinshaw H, Feldman WH. Streptomycin in treatment of clinical tuberculosis: a preliminary report. *Proc Staff Meet Mayo Clin*. 1945;20:313–318.
81. Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. *Clin Microbiol Rev*. 1995;8:496–514.
82. Waksman SA, Lechevalier HA. Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculosis organisms. American association for the advancement of science. *Science*. 1949;305–307.

83. Metcalfe NH. Sir Geoffrey Marshall (1887–1982): respiratory physician, catalyst for anaesthesia development, doctor to both Prime Minster and King, and World War I Barge Commander. *J Med Biogr*. 2011;19:10–14.
84. Bilgin N, Claesens F, Pahverk H, Ehrenberg M. Kinetic properties of *Escherichia coli* ribosomes with altered forms of S12. *J Mol Biol*. 1992;224:1011–1027.
85. Karimi R, Ehrenberg M. Dissociation rates of peptidyl-tRNA from the P-site of *E. coli* ribosomes. *EMBO J*. 1996;15:1149–1154.
86. Powers T, Noller HF. Selective perturbation of G530 of 16S rRNA by translational miscoding agents and a streptomycin-dependence mutation in protein S12. *J Mol Biol*. 1994;235:156–172.
87. Chang F, Flaks JG. Binding of dihydrostreptomycin to *Escherichia coli* ribosomes: characteristics and equilibrium of the reaction. *Antimicrob Agents Chemother*. 1972;2:294–307.
88. Grisé-Miron L, Brakier-Gingras L. Effect of neomycin and protein S1 on the binding of streptomycin to the ribosome. *Eur J Biochem*. 1982;123:643–646.
89. Lando D, Cousin MA, Ojasoo T, Raymond JP. Paromomycin and dihydrostreptomycin binding to *Escherichia coli* ribosomes. *Eur J Biochem*. 1976;66:597–606.
90. Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature*. 1986;327:389–394.
91. Gravel M, Melancon P, Brakier-Gingras L. Cross-linking of streptomycin to the 16S ribosomal RNA of *Escherichia coli*. *Biochemistry*. 1987;26:6227–6232.
92. Montandon PE, Nicolas P, Schümann P, Stutz E. Streptomycin resistance of *Euglena gracilis* chloroplasts: identification of a point mutation in the 16S rRNA gene in an invariant position. *Nucleic Acids Res*. 1985;13:4299–4310.
93. Frattali AL, Flynn MK, De Stasio EA, Dahlberg AE. Effects of mutagenesis of C912 in the streptomycin binding region of *Escherichia coli* 16S ribosomal RNA. *Biochim Biophys Acta (BBA) – Gene Struct Expr*. 1990;1050:27–33.
94. Leclerc D, Melançon P, Brakier-Gingras L. The interaction between streptomycin and ribosomal RNA. *Biochimie*. 1991;73:1431–1438.
95. Lodmell JS, Gutell RR, Dahlberg AE. Genetic and comparative analyses reveal an alternative secondary structure in the region of nt 912 of *Escherichia coli* 16S rRNA. *Proc Natl Acad Sci U S A*. 1995;92:10555–10559.
96. Pinard R, Payant C, Melançon P, Brakier-Gingras L. The 50proximal helix of 16S rRNA is involved in the binding of streptomycin to the ribosome. *FASEB J*. 1993;7:173–176.
97. Powers T, Noller HF. A functional pseudoknot in 16S ribosomal RNA. *EMBO J*. 1991;10:2203–2214.
98. Santer M, Santer U, Nurse K, et al. Functional effects of a G to U base change at position 530 in a highly conserved loop of *Escherichia coli* 16S RNA. *Biochemistry*. 1993;32:5539–5547.
99. Melancon P, Boileau G, Brakier-Gingras L. Cross-linking of streptomycin to the 30S subunit of *Escherichia coli* with phenyldiglyoxal. *Biochemistry*. 1984;23:6697–6703.
100. Abad JP, Amils R. Location of the streptomycin ribosomal binding site explains its pleiotropic effects on protein biosynthesis. *J Mol Biol*. 1994;235:1251–1260.
101. Hill WE. The ribosome: structure, function, and evolution. *Am Soc Microbiol*. 1990.
102. Raymon LP. *COMLEX Level 1 Pharmacology Lecture Notes*. Miami, FL: Kaplan Inc.; 2011:181.
103. Voet D, Voet JG. *Biochemistry*. 3rd ed. John Wiley & Sons; 2004:1341.
104. Spickler C, Brunelle MN, Brakier-Gingras L. Streptomycin binds to the decoding center of 16S ribosomal RNA. *J Mol Biol*. 1997;273:586–599.
105. *Ethionamide. TB Online. Global Tuberculosis Community Advisory Board*. 2016 [accessed 20.07.16].
106. *Trecator SC Tablet by Wyeth*. 2016. http://www.fda.gov/downloads/Safety/MedWatch/SafetyInformation/SafetyAlertsforHuman_Medical_products/UCM164879.pdf [accessed 16.08.16].
107. Takayama K, Wang L, David HL. Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 1972;2:29–35.
108. Winder FG, Collins PB, Whelan D. Effects of ethionamide and isoxyl on mycolic acid synthesis in *Mycobacterium tuberculosis* BCG. *J Gen Microbiol*. 1971;66:379–380.
109. Winder F, The biology of the Mycobacteria.Ratledge C, Stanford J, eds. *Physiology Identification and Classification*. vol. 1. London: Academic Press; 1982: 353–438.
110. Heym B, Saint-Joanis B, Cole ST. The molecular basis of isoniazid resistance in *Mycobacterium tuberculosis*. *Tuber Lung Dis*. 1999;79:267–271.
111. Barry CE, Slayden RA, Mdluli K. Mechanisms of isoniazid resistance in *Mycobacterium tuberculosis*. *Drug Resist Updat*. 1998;1:128–134.
112. Loewen PC, Klotz MG, Hassett DJ. Catalase—an "old" enzyme that continues to surprise us. *ASM News*. 2000;66:76–82.
113. Winder FG, Collins PB, Whelan D. Effects of ethionamide and isoxyl on mycolic acid synthesis in *Mycobacterium tuberculosis* BCG. *Microbiology*. 1971;66:379–380.
114. Quémard AN, Lanéelle GI, Lacave CH. Mycolic acid synthesis: a target for ethionamide in mycobacteria? *Antimicrob Agents Chemother*. 1992;36:1316–1321.
115. Baulard AR, Betts JC, Engohang-Ndong J, et al. Activation of the pro-drug ethionamide is regulated in mycobacteria. *J Biol Chem*. 2000;275:28326–28331.
116. Vilchèze C, Wang F, Arai M, et al. Transfer of a point mutation in *Mycobacterium tuberculosis* inhA resolves the target of isoniazid. *Nat Med*. 2006;12:1027–1029.
117. Goss WA, Deitz WH, Cook TM. Mechanism of action of nalidixic acid on *Escherichia coli* II. Inhibition of deoxyribonucleic acid synthesis. *J Bacteriol*. 1965;89:1068–1074.
118. Fàbrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. *Microb Biotechnol*. 2009;2:40–61.
119. Cheng AF, Yew WW, Chan EW, Chin ML, Hui MM, Chan RC. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother*. 2004;48:596–601.
120. Sun Z, Zhang J, Zhang X, Wang S, Zhang Y, Li C. Comparison of gyrA gene mutations between laboratory selected Of loxacin resistant *Mycobacterium tuberculosis* strains and clinical isolates. *Int J Antimicrob Agents*. 2008;31:115–121.
121. Rengarajan J, Sassetti CM, Naroditskaya V, Sloutsky A, Bloom BR, Rubin EJ. The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. *Mol Microbiol*. 2004;53:275–282.
122. Tomlinson C. *TB Online – Capreomycin*. 2016 [accessed 18.08.16].
123. Farmer P, Kim JY. Community based approaches to the control of multidrug resistant tuberculosis: introducing "DOTS-plus". *Br Med J*. 1998;317:671.
124. Sutton WB, Gordee RS, Wick WE, Stanfield L. In vitro and in vivo laboratory studies on the antituberculous activity of capreomycin. *Ann N Y Acad Sci*. 1966;135:947–959.

125. Trnka L, Smith DW. Proteosynthetic activity of isolated ribosomes of Mycobacteria and its alteration by rifampicin and related tuberculostatic drugs. In: *Experimental and Clinical Evaluation of the Tuberculostatics Capreomycin, Isoxyl, Myambutok, Rifampicin*. Karger Publishers; 1970:369–379.
126. Gale EF, Cundliffe E, Reynolds PE, Richmond MH, Waring MJ. *The Molecular Basis of Antibiotic Action*. London: Wiley; 1972.
127. Blumberg H, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med*. 2003;167:603.
128. McClatchy JK, Kanes W, Davidson PT, Moulding TS. Cross-resistance in *Mycobacterium tuberculosis* to kanamycin, capreomycin and viomycin. *Tubercle*. 1977;58:29–34.
129. Stanley RE, Blaha G, Grodzicki RL, Strickler MD, Steitz TA. The structures of the anti-tuberculosis antibiotics viomycin and capreomycin bound to the 70S ribosome. *Nat Struct Mol Biol*. 2010;17:289–293.
130. Modolfll J, Vázquez D. The inhibition of ribosomal translocation by viomycin. *Eur J Biochem*. 1977;81: 491–497.
131. Peske F, Savelsbergh A, Katunin VI, Rodnina MV, Wintermeyer W. Conformational changes of the small ribosomal subunit during elongation factor G-dependent tRNA–mRNA translocation. *J Mol Biol*. 2004;343:1183–1194.
132. Johansen SK, Maus CE, Plikaytis BB, Douthwaite S. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2′-O-methylations in 16S and 23S rRNAs. *Mol Cell*. 2006;23:173–182.
133. Maus CE, Plikaytis BB, Shinnick TM. Mutation of tlyA confers capreomycin resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2005;49:571–577.
134. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2005;49:3192–3197.
135. Monshupanee T, Gregory ST, Douthwaite S, Chungjatupornchai W, Dahlberg AE. Mutations in conserved helix 69 of 23S rRNA of *Thermus thermophilus* that affect capreomycin resistance but not posttranscriptional modifications. *J Bacteriol*. 2008;190:7754–7761.
136. Umezawa H, Ueda M, Maeda K, et al. Production and isolation of a new antibiotic: kanamycin. *J Antibiot*. 1957;10:181.
137. Umezawa H. Kanamycin: its discovery. *Ann N Y Acad Sci*. 1958;76:20–26.
138. Garland J. Kanamycin (editorial). *N Engl J Med*. 1958;259:352–353.
139. Wong CH, Hendrix M, Priestley ES, Greenberg WA. Specificity of aminoglycoside antibiotics for the A-site of the decoding region of ribosomal RNA. *Chem Biol*. 1998;5:397–406.
140. Amikacin Pharmacodynamics and Mechanism of Action. https://medipub.blogspot.in/2011/04/amikacin-pharmacodynamics-and_mechanism_24.html 2016; [accessed 26.07.16].
141. Udwadia ZF. MDR, XDR, TDR tuberculosis: ominous progression. *Thorax*. 2012;67:286–288.
142. Matsumoto M, Hashizume H, Tomishige T, et al. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. *PLoS Med*. 2006;3:466.
143. World Health Organization. *The Use of Delamanid in the Treatment of Multidrug-Resistant Tuberculosis Interim Policy Guidance*. 2014. Geneva. http://apps.who.int/iris/bitstream/10665/137334/1/WHO_HTM_TB_2014.23_eng.pdf?ua=1. 2016; [accessed 08.07.16].
144. World Health Organization. The Selection and Use of Essential Medicine Report: 20–24 April. Geneva. 2015. http://www.who.int/medicines/publications/essentialmedicines/Executive-Summary_EML-2015_7-May-15.pdf.2016; [accessed 14.07.16].
145. Matsumoto M. *In vitro activities of OPC-67683 and reference compounds against Mycobacterium tuberculosis standard strains and Mycobacterium bovis strains*. *Otsuka Study No. 019064*. 2003.
146. Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science*. 2005;307:223–227.
147. Ralph AP, Anstey NM, Kelly P. *Mycobacterium tuberculosis* into the 2010s: is the glass half full? *Clin Infect Dis*. 2009;49:574–583.
148. Huitric E, Verhasselt P, Andries K, Hoffner SE. In vitro antimycobacterial spectrum of a diarylquinoline ATP synthase inhibitor. *Antimicrob Agents Chemother*. 2007;51:4202–4204.
149. Koul A, Dendouga N, Vergauwen K, et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. *Nat Chem Biol*. 2007;3:323–324.
150. Lounis N, Veziris N, Chauffour A, Truffot-Pernot C, Andries K, Jarlier V. Combinations of R207910 with drugs used to treat multidrug-resistant tuberculosis have the potential to shorten treatment duration. *Antimicrob Agents Chemother*. 2006;50:3543–3547.
151. Chahine EB, Karaoui LR, Mansour H. Bedaquiline a novel diarylquinoline for multidrug-resistant tuberculosis. *Ann Pharmacother*. 2014;48:107–115.
152. Palomino JC, Martin A. TMC207 becomes bedaquiline, a new anti-TB drug. *Future Microbiol*. 2013;8:1071–1080.
153. Petrella S, Cambau E, Chauffour A, Andries K, Jarlier V, Sougakoff W. Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. *Antimicrob Agents Chemother*. 2006;50:2853–2856.
154. Segala E, Sougakoff W, Nevejans CA, Jarlier V, Petrella S. New mutations in the mycobacterial ATP synthase: new insights into the binding of the diarylquinoline TMC207 to the ATP synthase Cring structure. *Antimicrob Agents Chemother*. 2012;56:2326–2634.
155. Huitric E, Verhasselt P, Koul A, Andries K, Hoffner S, Andersson DI. Rates and mechanisms of resistance development in *Mycobacterium tuberculosis* to a novel diarylquinoline ATP synthase inhibitor. *Antimicrob Agents Chemother*. 2010;54:1022–1028.
156. Gaudillière B, Berna P. *Annual Reports in Medicinal Chemistry*. *Manoj C. Desai, In: editor, 35*. 2000;340–343.
157. Woodcock JM, Andrews JM, Boswell FJ, Brenwald NP, Wise R. In vitro activity of BAY 12-8039, a new fluoroquinolone. *Antimicrob Agents Chemother*. 1997;41:101–106.
158. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society, Centers for Disease Control and Prevention and the Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med*. 2003;167:603–662.
159. Grossman RF, Hsueh PR, Gillespie SH, Blasi F. Community-acquired pneumonia and tuberculosis: differential diagnosis and the use of fluoroquinolones. *Int J Infect Dis*. 2014;18(January):14–21.
160. Leach KL, Brickner SJ, Noe MC, Miller PF. Linezolid, the first oxazolidinone antibacterial agent. *Ann N Y Acad Sci*. 2011;1222:49–54.
161. Brickner SJ, Hutchinson DK, Barbachyn MR, et al. Synthesis and antibacterial activity of U-100592 and U-100766, two

oxalidinone antibacterial agents for the potential treatment of multidrugresistant Gram-positive bacterial infections. *J Med Chem*. 1996;39:673–679.
162. Zurenko GE, Yagi BH, Schaadt RD, et al. In vitro activities of U-100592 and U-100766 novel oxalidinone antibacterial agents. *Antimicrob Agents Chemother*. 1996;40:839–845.
163. Hadjiangelis NP, Leibert E, Harkin TJ. Linezolid: a promising new agent for multidrug resistant tuberculosis treatment. *Am J Respir Crit Care Med*. 2003;167:868.
164. Shinabarger D. Mechanism of action of the oxazolidinone antibacterial agents. *Expert Opin Investig Drugs*. 1999;8:1195–1202.
165. Ippolito JA, Kanyo ZF, Wang D, et al. Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. *J Med Chem*. 2008;51:3353–3356.
166. Sotgiu G, Pontali E, Migliori GB. Linezolid to treat MDR-/XDR-tuberculosis: available evidence and future scenarios. *Eur Respir J*. 2015;45:25–29.
167. Lechartier B, Rybniker J, Zumla A, Cole S. Tuberculosis drug discovery in the post-post-genomic era. *EMBO Mol Med*. 2014.
168. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. *Nature*. 2011;469:483–490.
169. Stover CK, Warrener P, VanDevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature*. 2000;405:962–966.
170. Choi KP, Bair TB, Bae YM, Daniels L. Use of transposon Tn5367 mutagenesis and a nitroimidazopyran-based selection system to demonstrate a requirement for fbiA and fbiB in coenzyme F420 biosynthesis by *Mycobacterium bovis* BCG. *J Bacteriol*. 2001;183:7058–7066.
171. Manjunatha UH, Boshoff H, Dowd CS, et al. Identification of a nitroimidazooxazinespecific protein involved in PA824 resistance in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A*. 2006;103:431–436.
172. Protopopova M, Hanrahan C, Nikonenko B, et al. Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2ethylenediamines. *J Antimicrob Chemother*. 2005;56:968–974.
173. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry 3rd CE. The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J Biol Chem*. 2004;279:40174–40184.
174. Tahlan K, Wilson R, Kastrinsky DB, et al. SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2012;56:1797–1809.
175. Gregory WA, inventor; EI Dupont De Nemours, assignee. p-Oxooxazolidinylbenzene compounds as antibacterial agents. United States patent US 4,461,773, 1984.
176. Louie A, Eichas K, Files K, Swift M, Bahniuk N. Activities of PNU-100480 (PNU 480) alone, PNU 480 plus its major metabolite PNU-101603 (PNU 1603) and PNU 480 plus PNU 1603 in combination with rifampin (RIF) against *Mycobacterium tuberculosis*: comparison with linezolid. ICAAC 51. Abstract A1–1737. 2011. In: *51st Intersci. Conf. Antimicrob. Agents Chemother. Am Soc for Microbiol*. 2011.
177. Williams KN, Brickner SJ, Stover CK, et al. The addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. *Am J Respir Crit Care Med*. 2009;180:371–376.
178. Villemagne B, Crauste C, Flipo M, Baulard AR, Déprez B, Willand N. Tuberculosis: the drug development pipeline at a glance. *Eur J Med Chem*. 2012;51(May):1–6.
179. Williams KN, Stover CK, Zhu T, et al. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. *Antimicrob Agents Chemother*. 2009;53:1314–1319.
180. Cynamon MH, Klemens SP, Sharpe CA, Chase S. Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. *Antimicrob Agents Chemother*. 1999;43:1189–1191.
181. Wallis RS, Jakubiec W, Kumar V, et al. Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. *Antimicrob Agents Chemother*. 2011;55:567–574.
182. Louie A, Eichas K, Files K, Swift M, Bahniuk N. Activities of PNU-100480 (PNU 480) alone, PNU 480 plus its major metabolite PNU-101603 (PNU 1603) and PNU 480 plus PNU 1603 in combination with rifampin (RIF) against *Mycobacterium tuberculosis*: comparison with linezolid. In: *ICAAC 51. Abstract A1-1737*. 2011.
183. Wallis RS, Dawson R, Friedrich SO, et al. Mycobactericidal activity of sutezolid (PNU-100480) in sputum (EBA) and blood (WBA) of patients with pulmonary tuberculosis. *PLOS ONE*. 2014;9:94462.
184. Nikonenko BV, Protopopova M, Samala R, Einck L, Nacy CA. Drug therapy of experimental tuberculosis (TB): improved outcome by combining SQ109, a new diamine antibiotic, with existing TB drugs. *Antimicrob Agents Chemother*. 2007;51:1563–1565.
185. Czock D, Keller F. Mechanism-based pharmacokineticpharmacodynamic modeling of antimicrobial drug effects. *J Pharmacokinet Pharmacodyn*. 2007;34:727–751.
186. Gravestock MB, Acton DG, Betts MJ, et al. New classes of antibacterial oxazolidinones with C-5, methylene O-linked heterocyclic side chains. *Bioorg Med Chem Lett*. 2003;13:4179–4186.
187. Ragno R, Marshall GR, Di Santo R, et al. Anti-mycobacterial pyrroles: synthesis, anti-*Mycobacterium tuberculosis* activity and QSAR studies. *Bioorg Med Chem*. 2000;8:1423–1432.
188. Abstract n.63 submitted to the American Chemical Society Meeting, Anheim CA, March 28–April 01 2004.
189. http://www.newtbdrugs.org/project.php?id=145: 2016; [accessed 01.08.16].
190. Bogatcheva E, Hanrahan C, Nikonenko B, et al. Identification of SQ609 as a lead compound from a library of dipiperidines. *Bioorg Med Chem Lett*. 2011;21 (September):5353–5357.
191. Makarov V, Manina G, Mikusova K, et al. Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. *Science*. 2009;324:801–804.
192. http://www.newtbdrugs.org/project.php?id=202802; 2016 [accessed 31.07.16].
193. Pasca MR, Degiacomi G, Ribeiro AL, et al. Clinical isolates of *Mycobacterium tuberculosis* in four European hospitals are uniformly susceptible to benzothiazinones. *Antimicrob Agents Chemother*. 2010;54(April):1616–1618.
194. Mikusová K, Huang H, Yagi T, et al. Decaprenylphosphoryl arabinofuranose, the donor of the Darabinofuranosyl residues of mycobacterial arabinan, is formed via a two-step epimerization of decaprenylphosphoryl ribose. *J Bacteriol*. 2005;187:8020–8025.
195. Trefzer C, RengifoGonzalez M, Hinner MJ, et al. Benzothiazinones: prodrugs that covalently modify the decaprenylphosphoryl-β-D-ribose 2′epimerase DprE1 of *Mycobacterium tuberculosis*. *J Am Chem Soc*. 2010;132:13663–13665.
196. Okumura R, Hirata T, Onodera Y, Hoshino K, Otani T, Yamamoto T. Dual-targeting properties of the 3-aminopyrrolidyl quinolones, DC-159a and sitafloxacin,

against DNA gyrase and topoisomerase IV: contribution to reducing in vitro emergence of quinolone-resistant *Streptococcus pneumoniae*. *J Antimicrob Chemother*. 2008;62:98–104.
197. Onodera Y, Hirata T, Hoshino K, Otani T. DC-159a, a novel quinolone, showed high inhibitory activity against altered topoisomerases of Streptococcus pneumoniae and *Mycobacterium tuberculosis*, abstract. F1-2126. In: *47th Intersci. InConf. Antimicrob Agents Chemother American Society for Microbiology*. 2007.
198. Sekiguchi JI, Disratthakit A, Maeda S, Doi N. Characteristic resistance mechanism of *Mycobacterium tuberculosis* to DC-159a, a new respiratory quinolone. *Antimicrob Agents Chemother*. 2011;55:3958–3960.
199. Disratthakit A, Doi N. In vitro activities of DC-159a, a novel fluoroquinolone, against *Mycobacterium* species. *Antimicrob Agents Chemother*. 2010;54:2684–2686.
200. Ahmad Z, Minkowski A, Peloquin CA, et al. Activity of the fluoroquinolone DC-159a in the initial and continuation phases of treatment of murine tuberculosis. *Antimicrob Agents Chemother*. 2011;55:1781–1783.
201. Takahashi Y, Igarashi M, Miyake T, et al. Novel semisynthetic antibiotics from caprazamycins A–G: caprazene derivatives and their antibacterial activity. *J Antibiot*. 2013;66:171–178.
202. Working Group on New TB Drugs CPZEN-45, Stop TB Partnership, 2009.
203. Hashizume H, Sawa R, Harada S, et al. Tripropeptin C blocks the lipid cycle of cell wall biosynthesis by complex formation with undecaprenyl pyrophosphate. *Antimicrob Agents Chemother*. 2011;55:3821–3828.
204. Sawa R, Takahashi Y, Hashizume H, et al. Amycolamicin: a novel broad-spectrum antibiotic inhibiting bacterial topoisomerase. *Chem Eur J*. 2012;18:15772–15781.
205. Pethe K, Bifani P, Jang J, et al. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat Med*. 2013;19:1157–1160.
206. https://www.fmhs.auckland.ac.nz/en/faculty/about/news-and-events/news/2012/09/21/promising-new-zealand.html [accessed 29.07.16].
207. Moffett M, McGill MI. Treatment of trichomoniasis with metronidazole. *Br J Med*. 1960;2:910.
208. Mukherjee T, Boshoff H. Nitroimidazoles for the treatment of TB: past, present and future. *Future Med Chem*. 2011;3:1427–1454.
209. Anderson RF, Shinde SS, Maroz A, Boyd M, Palmer BD, Denny WA. Intermediates in the reduction of the antituberculosis drug PA-824, (6S)-2-nitro-6-{[4-(trifluoromethoxy) benzyl] oxy}-6,7-dihydro-5H-imidazo [2,1-b][1,3] oxazine, in aqueous solution. *Org Biomol Chem*. 2008;6:1973–1980.
210. Kmentova I, Sutherland HS, Palmer BD, et al. Synthesis and structure–activity relationships of aza-and diazabiphenyl analogues of the antitubercular drug (6 S)-2-nitro-6-{[4-(trifluoromethoxy) benzyl] oxy}-6,7-dihydro-5 H-imidazo [2,1-b][1,3] oxazine (PA-824). *J Med Chem*. 2010;53:8421–8439.
211. Palmer BD, Thompson AM, Sutherland HS, et al. Synthesis and structure–activity studies of biphenyl analogues of the tuberculosis drug (6 S)-2-nitro-6-{[4-(trifluoromethoxy) benzyl] oxy}-6,7-dihydro-5 H-imidazo [2,1-b][1,3] oxazine (PA-824). *J Med Chem*. 2009;53:282–294.
212. NCT01424670 – ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT01424670 2016; [accessed 05.08.16].
213. Denny WA. TBA-354: a new drug for the treatment of persistent tuberculosis. *Chem New Zeal*. 2015;1:18–22.
214. WGND TBA-354 Profile – Working Group on New TB Drugs http://www.newtbdrugs.org/project.php?id=48 2016; [accessed 24.08.16].

Available online at www.sciencedirect.com

ScienceDirect

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

Original Article

Tuberculosis versus pyogenic meningitis in a Pakistani population

Shujah Saleem Khan [a,*], *Zawar Ali* [b]

[a] *Medecins Sans Frontieres (MSF), Pakistan*
[b] *Northwest General Hospital & Research Centre, Peshawar, Pakistan*

ARTICLE INFO

Article history:
Received 10 October 2016
Accepted 4 January 2017
Available online 24 January 2017

Keywords:
Tuberculosis
Meningitis
CSF analysis
TB meningitis
Bacterial meningitis

ABSTRACT

Background: Research has been going on to formulate diagnostic criteria for TBM. Two criteria that have been studied and validated in high TB prevalence areas are the Youssef criteria (Rule 1) and Thwaites criteria (Rule 2). In our study we aimed to compare the different features of TBM and acute bacterial meningitis.

Methods: This retrospective study was done at Northwest General Hospital & Research Centre (NWGH&RC), Peshawar, Pakistan. Patients who were clinically diagnosed with TB meningitis or bacterial meningitis at the time of presentation were included in the study.

Results: Lab parameters for both groups were compared using independent sample *T* tests.

We plotted ROC curves for Rule 1 and Rule 2. For Rule 1, at cut off value 2 it has a sensitivity of 97.5% and a specificity of 47.2%. For Rule 2, area at cut off value 3.5, sensitivity was 95% and specificity was 23.5%.

We also plotted CSF protein to glucose ratio of our sample on an ROC curve and looked for measures of sensitivity and specificity. At cut off point 2 the sensitivity was 93% and specificity was 66.66%.

Conclusion: It should be noted that although sensitivity for all three indices were high, specificity of all three tests was not very encouraging. We would like to emphasize that these indices can be useful in screening for patients with suspected TBM but they do not have the specificity to act as the sole test for initiation and continuance of therapy.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. Although it typically affects the lungs (pulmonary TB) it can affect any other organ in the body (extra pulmonary TB). TB is a major global health problem with 1.3 million deaths worldwide in 2012. An estimated 8.6 million people developed TB in the same year.[1] TB ranks as the second leading cause of death from an infectious disease worldwide after HIV.[1]

Pakistan has a high TB-burden and ranks sixth among countries worldwide in terms of TB prevalence. Pakistan has an estimated prevalence and incidence of 420,000 and 231 per 100,000 populations, respectively.[1]

Although pulmonary TB is an easily identified disease, the manifestations of extra pulmonary TB are often inconspicuous. Extra pulmonary TB (EPTB) is probably underreported and

* *Corresponding author.*
E-mail address: sskmsf@gmail.com (S.S. Khan).
http://dx.doi.org/10.1016/j.ijtb.2017.01.001

prevalence varies by geographical location and is thought to be related to several factors such as ethnicity and HIV status.[2–4]

It is estimated that central nervous system (CNS) TB constitutes ~1–10% of all forms of TB.[5–7] It accounts for 6.3% of patients with EPTB.[8] Amongst all the different clinical phenotypes of TB, tuberculous meningitis (TBM) is the most severe, with a high mortality and morbidity.[9,10] TBM results from the haematogenous dissemination of *M. tuberculosis* from the primary pulmonary site of infection to the meninges or brain parenchyma, where it forms small tubercles. The tuberculous (Rich) foci may remain asymptomatic for an unspecified period. The bursting, presumably preceded by an unknown immune stimulus of these foci into the arachnoid space, may lead to the development of TBM.[11] Untreated TBM is fatal, and even with treatment, the mortality rate is high.[12] Approximately half of the survivors suffer from long-term neurological sequelae including cognitive impairment, motor deficits, optic atrophy and other cranial nerve involvement.[13–15] Therefore early recognition and treatment of the disease is essential.

Diagnosis should be based on a culture-positive specimen, histological or strong clinical evidence consistent with active extra pulmonary disease, followed by a clinical decision to treat with a full course of anti-TB chemotherapy.[16]

TBM has remained a diagnostic dilemma. It has a spectrum of non-specific and vague symptoms. Discriminating TBM from other forms of meningitis by clinical features alone is often difficult due to overlapping clinical presentations. The evaluation of cerebrospinal fluid (CSF) is therefore an important parameter.

The standard for diagnosis of TBM is isolation of *M. tuberculosis* from CSF.[17,18] These methods are operator and laboratory expertise dependant and thus have a highly variable sensitivity. Newer methods have been developed such as PCR, ELISA and CSF adenosine deaminase and lactic acid, but these techniques are expensive and usually not available in most hospital settings of a developing country. In practice, in a developing country like Pakistan, the clinical diagnosis and initial treatment of TBM is based on strong clinical suspicion based on clinical features and simple laboratory tests of CSF and blood.

Research has been going on to formulate diagnostic criteria for TBM. There are a few criteria that have been developed. Two criteria that have been studied and validated in high TB prevalence areas are the **Youssef criteria (Rule 1)** and **Thwaites criteria (Rule 2)**.[19,20]

In a recent study from a large tertiary care centre in Pakistan, it was suggested that **CSF protein to glucose (CSF P/G) ratio** could help in establishing a diagnosis of TBM in children.[12]

A CSF P/G ratio of less than 2 was suggestive of TBM.

In this study our primary objective was to compare the different features clinical, radiological and diagnostic laboratory indices of TBM and acute bacterial meningitis. Our secondary objective was to compare the previously validated criterion (Youssef & Thwaites criteria) to our clinical diagnosis and check their validity in our population. A tertiary objective was to assess the potential of CSF protein to glucose ratio as a diagnostic tool in TBM.

2. Methodology

This retrospective study was carried out at the Neurology department of Northwest General Hospital & Research Centre (NWGH & RC), Peshawar, Pakistan. NWGH is a 220 bedded tertiary care hospital in the north of Pakistan, close to the Afghanistan border that receives patients from both countries.

2.1. *Inclusion criteria*

Patients who were clinically diagnosed with TB meningitis or bacterial meningitis at the time of presentation were included in the study. Clinical diagnosis was based on the duration of the illness, previous exposure to TB, previous history of TB, physical examination, blood culture and radiological findings (such as basal enhancement, hydrocephalus and presence of tuberculomas on CT). Patients admitted between August 2011 and September 2013 were included in the study.

2.2. *Bacterial meningitis group*

Patients diagnosed with bacterial meningitis/pyogenic (PM) were started on Ceftriaxone IV and steroids and response to treatment after 72 h was checked in patient files. Patients without a positive response were excluded from the study. Patients in whom a response was not recorded or left the hospital before 72 h were also excluded.

2.3. *TBM group*

Patients with a clinical diagnosis of TBM were included in this group. They were started on a four drug regimen along with steroids according to WHO criteria. Response to treatment at 1 month or more in patient files was checked and only patients with a positive response were included.

3. Results

A total of 72 cases were identified; 42 in the TBM group and 30 in the PM group.

The mean age of the TBM group was 33.07 (standard deviation {s.d.} 19.14) years and that of the PM group were 37.93 (s.d. 22.49) years. Both groups had more male patients with 64% (n = 27) in the TBM group and 53% (n = 16) in the PM group.

3.1. *Clinical features*

Table 1 compares the different clinical signs and symptoms of patients recorded during the first 24 h of admission.

Headache, fever, neck stiffness & vomiting were the commonest features in both groups.

History of weight loss was more common in the TBM group compared to the PM group (47% vs 23%). Patients with TBM were also more likely to report diplopia (16.7% vs 3.3%).

Patients with TBM tested positive more frequently when Brudzinski (40.5%) and Kernig (38.1%) were tested as compared to the PM group (16.7% and 13.3%, respectively).

Table 1 – Signs and symptoms at time of admission.

Signs and symptoms	TBM		PM	
	Count	Percentage	Count	Percentage
Headache	37	88.1%	23	76.7%
Fever	37	88.1%	25	83.3%
Vomiting	29	69.0%	19	63.3%
Photophobia	2	4.8%	1	3.3%
Diplopia	7	16.7%	1	3.3%
Weight loss	20	47.6%	7	23.3%
Paresis/plegia	9	21.4%	7	23.3%
Papilledema	13	31.0%	7	23.3%
Brudzinskis sign	17	40.5%	5	16.7%
Kernig sign	16	38.1%	4	13.3%
Neck stiffness	33	78.6%	18	60.0%

Table 2 – Radiological features.

	n (TBM)	Percentage	*n* (PM)	Percentage	Chi-square/*p*-value
Meningeal enhancement	12	32.4	2	8.0	5.094/0.024
Hydrocephalus	25	67.6	3	12.0	18.6/<0.001
Tuberculoma	4	10	0	0	2.88/0.89
Cerebral oedema	17	45.9	4	16	5.973/0.015
Cerebral Infarct	10	27	5	20	0.402/0.506
Chest X-ray suggestive of TB	7	18.9	2	8	1.433/0.231

Somewhat surprisingly, the frequency of photophobia was low across both groups (TBM 4.8%, PM 3.3%).

3.2. *Radiological features*

Table 2 shows the comparison of radiological features between the two groups.

Radiological features compared in both groups included meningeal enhancement, hydrocephalus, tuberculoma, cerebral oedema, cerebral infarct and chest X-ray suggestive for pulmonary TB.

Meningeal enhancement, hydrocephalus and cerebral oedema were significantly associated with TBM.

3.3. *Lab indices*

Lab parameters for both groups were compared using independent sample *T* tests.

Blood and CSF samples were collected within 12 h of admission and prior to commencement of any antibiotic therapy.

Erythrocyte Sedimentation Rate (ESR) was 21.23(±22.50) s and 32.17(±24.56) s, for the TBM and PM groups respectively {t (51) = 1.62, p = 0.11}.

Total Leucocyte Count (TLC) was 13.07(±14.3) and 11.3(±5.4) for the TBM and PM groups respectively, {t (68) = 0.609, p = 0.545}.

CSF proteins were significantly lower in the TBM group (mean 60.58 ± 37.3 mg/dl) compared to the PM group (114.4 ± 55.1 mg/d) {t (66) = 1.78, $p < 0.001$}.

CSF glucose levels were higher in TBM group compared to PM group, 82.28(±47.1) mg/dl vs 42.88(±27.1) mg/dl {t (40) = 4.010, $p < 0.001$}.

CSF WBCs were 216.71 ± 479.27/mm^3 in PM group and 95.97 ± 406.17/mm^3 in TBM group.

We plotted CSF protein to glucose ratio of our sample on an ROC curve and looked for measures of sensitivity and specificity (Fig. 1). The area under the curve is 0.800, 95% CI

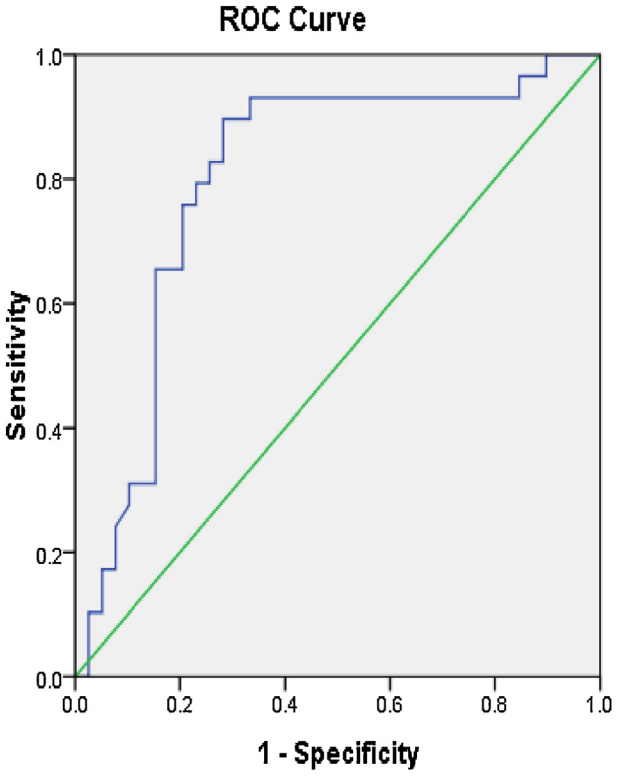


Fig. 1 – ROC curve – CSF protein/glucose ratio.

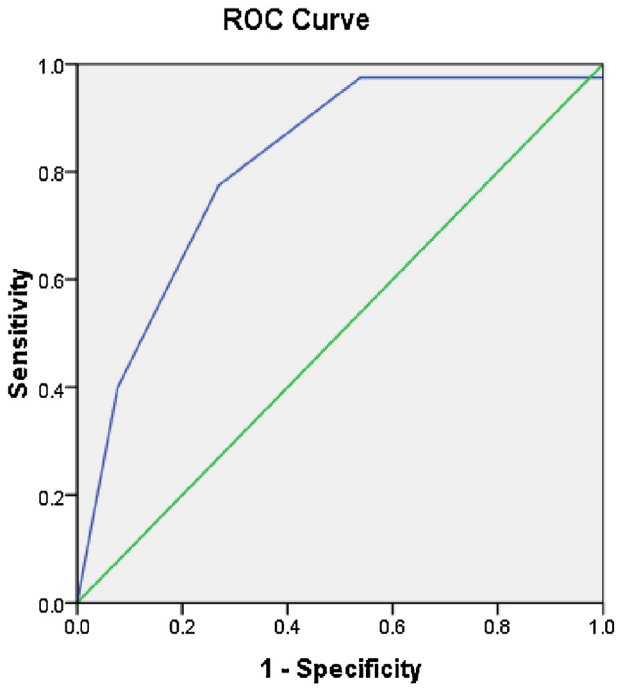


Fig. 2 – ROC curve Rule 1 (youseff criteria).

(0.687–0.913), $p < 0.001$. This analysis was done under the presumption that a lower value signifies a more positive test for TBM. At cut off point 2 the sensitivity was 93% and specificity was 66.66%.

Similarly we also plotted ROC curves for Rule 1 and Rule 2.

For Rule 1 (Fig. 2) area under the curve was 0.814, 95% CI (0.704–0.924) $p < 0.001$.

At cut off value 2 it has a sensitivity of 97.5% and a specificity of 47.2%.

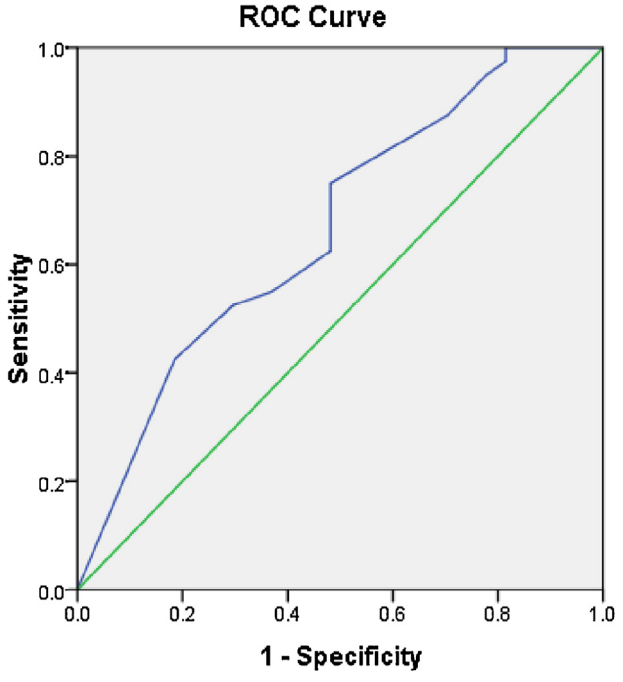


Fig. 3 – ROC Rule 2 – Thwaites crtieria.

For Rule 2 (Fig. 3) area under the curve was 0.666, 95% CI (0.533–0.800) $p = 0.022$.

At cut off value 3.5, sensitivity was 95% and specificity was 23.5%.

4. Discussion

Our study showed the different characteristics of patients presenting with TBM and PM in an endemic area. Clinical signs and symptoms were comparable in both groups. Headache, fever, neck stiffness and vomiting were the most common symptoms in both groups. Weight loss and diplopia were more common in TBM group.

Positive bedside clinical signs of meningeal irritation (Brudzinskis and Kernigs) were more likely to be positive in the TBM group. One reason for this could be the difference in duration from onset of symptoms to hospital presentation between the two groups. In the TBM group the mean duration was 26.1 ± 25.9 days while in the BM group it was 5.9 ± 4.3 days. Another explanation may be inconsistency in examination procedure carried out by different individuals.

The laboratory values in both groups were comparatively analyzed.

Mean ESR, TLC and CSF-WBCs were not significantly different in both groups.

CSF protein values were significantly higher in PM group (114.4 ± 55.1 mg/dl) compared to TBM group (60.58 ± 37.3 mg/dl).

CSF glucose values were significantly higher in the TBM group as compared to the PM group {82.28(±47.1) mg/dl and 42.88(±27.1) mg/dl respectively}. This is somewhat similar to previously observed trends.[21,22]

Comparison of radiological features showed meningeal enhancement ($p = 0.024$), hydrocephalus ($p < 0.001$) and cerebral oedema ($p = 0.015$) to be significantly associated with TBM. Previous studies also show similar findings.[23] It should be noted that a chest X-ray suggestive of pulmonary TB was not significantly associated with any of the groups. This may perhaps be reflective of the endemic nature of TB in our study population.

The two diagnostic rules studied showed encouraging results in our study. Similar to the study by FN Qamar et al.,[12] our study also showed that CSF Protein to glucose ratio can be helpful in diagnosing TBM. Area under the curve of 0.800 shows a strong association compared to clinical diagnosis which was our standard. It yields a high sensitivity (93%) at cut off point of 2. Thwaites criteria has been studied previously and shown encouraging results in some populations.[24–26]

It should be noted that although sensitivity for all three indices were high, specificity of all three tests was not as inspiring.

Sensitivity for Rule 1 was 97.5%, for Rule 2 it was 95% and for CSF P/G ratio it was 93%.

Specificity was highest for CSF P/G ratio at 66.7% and lowest for Rule 2 at 23.5%, while Rule 1 had a specificity of 47.2%.

All these factors can be helpful in diagnosing TBM in endemic settings.

In our clinical setting, PCR or other advanced lab tests cannot be performed either due to the absence of relevant infrastructure or a lack of financial resources. Clinical

diagnosis is routinely done. We would like to emphasize that these indices can be useful in screening for patients with suspected TBM but they do not have the specificity to act as the sole test for initiation and continuance of therapy. They can be used as helpful aids in making a clinical diagnosis and initiating anti tuberculous therapy while results for more specific tests like culture or PCR are awaited. The importance of faster, more specific tests cannot be emphasized enough.

5. Limitations

Limitations of our study were the retrospective nature of it and a lack of long term follow up.

No definitive culture or immunological tests to confirm our clinical diagnosis.

We did not have any data on the HIV status or presence of any other immunodeficiency state in the included subjects. Data and subsequent results/analysis were not available to effectively compare paediatric and adult patients.

We could not evaluate the severity of symptoms at presentation and we believe that would have been helpful in comparing the presentation in both groups. It should be noted that we only included patients who responded to Ceftriaxone IV and ATT in PM and TBM groups respectively, so we may have most likely missed out on resistant cases in both groups.

Ethical approval

The study was approved by the Ethics Review Committee of the hospital.

Informed consent was obtained from all participants included in the study.

Conflicts of interest

The authors have none to declare.

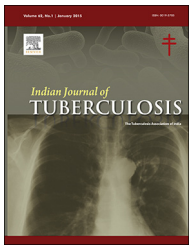
REFERENCES

1. WHO. *Global Tuberculosis Control: Surveillance, Planning, Financing. WHO Report 2012*. Geneva, Switzerland: World Health Organization; 2012.
2. DoH TB: http://www.doh.gov.za/list.php?type=Tuberculosis – See more at: http://www.healthlink.org.za/healthstats/220/data#sthash.sNDUzadp.dpuf. TB Register. Pretoria, South Africa: National Department of Health (TB section). Pretoria, 2012.
3. Gonzalez OY, Adams G, Teeter LD, Bui TT, Musser JM, Graviss EA. Extra-pulmonary manifestations in a large metropolitan area with a low incidence of tuberculosis. *Int J Tuberc Lung Dis*. 2003;7(12):1178–1185.
4. Kingkaew N, Sangtong B, Amnuaiphon W, et al. HIV-associated extrapulmonary tuberculosis in Thailand: epidemiology and risk factors for death. *Int J Infect Dis*. 2009;13(6):722–729.
5. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clin Microbiol Rev*. 2008;21(2):243–261. table of contents.
6. Phypers M, Harris T, Power C. CNS tuberculosis: a longitudinal analysis of epidemiological and clinical features. *Int J Tuberc Lung Dis*. 2006;10(1):99–103.
7. Chatterjee S. Brain tuberculomas, tubercular meningitis, and post-tubercular hydrocephalus in children. *J Pediatr Neurosci*. 2011;6(suppl 1):S96–S100.
8. CDC. *Extrapulmonary Tuberculosis Cases and Percentages by Site of Disease: Reporting Areas, 2005*. Atlanta, GA: Centers for Disease Control and Prevention; 2005.
9. Woldeamanuel YW1. Girma B. *J Neurol*. 2014;261(May (5)):851–865. http://dx.doi.org/10.1007/s00415-013-7060-6 [Epub 2013 Aug 21]. A 43-year systematic review and meta-analysis: case-fatality and risk of death among adults with tuberculous meningitis in Africa.
10. Pehlivanoglu F, Yasar KK, Sengoz G. Tuberculous meningitis in adults: a review of 160 cases. *Sci World J*. 2012;2012:169028. http://dx.doi.org/10.1100/2012/169028 [Epub 2012 Apr 24].
11. Rom WN, Garay SM. *Tuberculosis*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.
12. Farah Naz Qamar AJR, Iqbal S, Humayun K. Comparison of clinical and CSF profiles in children with tuberculous and pyogenic meningitis; role of CSF protein: glucose ratio as diagnostic marker of tuberculous meningitis. *J Pak Med Assoc*. 2013;February:.
13. Thwaites GE, Nguyen DB, Nguyen HD, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med*. 2004;351(17):1741–1751.
14. Hosoglu S, Geyik MF, Balik I, et al. Predictors of outcome in patients with tuberculous meningitis. *Int J Tuberc Lung Dis*. 2002;6(1):64–70.
15. Kalita J, Misra UK, Ranjan P. Predictors of long-term neurological sequelae of tuberculous meningitis: a multivariate analysis. *Eur J Neurol*. 2007;14(1):33–37.
16. Thwaites G, Fisher M, Hemingway C, et al. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect*. 2009;59(3):167–187.
17. Daniel TM. New approaches to the rapid diagnosis of tuberculous meningitis. *J Infect Dis*. 1987;155(4):599–602.
18. Sutlas PN, Unal A, Forta H, Senol S, Kirbas D. Tuberculous meningitis in adults: review of 61 cases. *Infection*. 2003;31 (6):387–391.
19. Thwaites GE, Chau TT, Stepniewska K, et al. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. *Lancet*. 2002;360(9342):1287–1292.
20. Youssef FG, Afifi SA, Azab AM, et al. Differentiation of tuberculous meningitis from acute bacterial meningitis using simple clinical and laboratory parameters. *Diagn Microbiol Infect Dis*. 2006;55(4):275–278.
21. Girgis NI, Sultan Y, Farid Z, et al. Tuberculosis meningitis, Abbassia Fever Hospital-Naval Medical Research Unit No. 3-Cairo, Egypt, from 1976 to 1996. *Am J Trop Med Hyg*. 1998;58 (1):28–34.
22. Thwaites GE, Tran TH. Tuberculous meningitis: many questions, too few answers. *Lancet Neurol*. 2005;4(3):160–170.
23. Ozates M, Kemaloglu S, Gurkan F, Ozkan U, Hosoglu S, Simsek MM. CT of the brain in tuberculous meningitis. A review of 289 patients. *Acta Radiol*. 2000;41(1):13–17.
24. Sunbul M, Atilla A, Esen S, Eroglu C, Leblebicioglu H. Thwaites' diagnostic scoring and the prediction of tuberculous meningitis. *Med Princ Pract*. 2005;14(3):151–154.
25. Torok ME, Nghia HD, Chau TT, et al. Validation of a diagnostic algorithm for adult tuberculous meningitis. *Am J Trop Med Hyg*. 2007;77(3):555–559.
26. Kurien R, Sudarsanam TD, Samantha S, Thomas K. Tuberculous meningitis: a comparison of scoring systems for diagnosis. *Oman Med J*. 2013;28(3):163–166.

Available online at www.sciencedirect.com

ScienceDirect

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



Original Article

Meso level multi-disciplinary approach for reduction of pre-treatment loss to follow-up in Revised National Tuberculosis Control Program, Delhi, India

Nandini Sharma [a], *Shivani Chandra* [b], *Meera Dhuria* [c,*], *Charu Kohli* [d], *Kamal Kishore Chopra* [e], *Nishi Aggarwal* [f], *Kuldeep Sachdeva* [g]

[a] *Director Professor, Department of Community Medicine, Maulana Azad Medical College, 2 Bahadurshah Zafar Marg, New Delhi 110002, India*
[b] *WHO Consultant, State TB Office, Delhi Government Dispensary, Gulabi Bagh, New Delhi 110002, India*
[c] *Ex-Epidemiologist, New Delhi TB Centre, JLN Marg, New Delhi 110002, India*
[d] *Department of Community Medicine, Maulana Azad Medical College, 2 Bahadurshah Zafar Marg, New Delhi 110002, India*
[e] *Director, New Delhi TB Centre, JLN Marg, New Delhi 110002, India*
[f] *Statistician, New Delhi TB Centre, JLN Marg, New Delhi 110002, India*
[g] *Ex-Addl DG TB, Central TB Division, 523, C-wing, Nirman Bhawan, New Delhi, India*

ARTICLE INFO

Article history:
Received 29 May 2016
Accepted 11 May 2017
Available online 13 June 2017

Keywords:
Loss to follow up
Tuberculosis
India

ABSTRACT

Background: Universal access to tuberculosis (TB) care services emphasizes early detection and initiation of treatment for all pulmonary TB patients. Pre-treatment loss to follow-up patients needs to be actively tracked and treated to break the chain of transmission in the community.
Objectives:

1) To examine the various reasons for pre-treatment loss to follow-up among new sputum positive cases diagnosed under the Revised National TB Control Program in Delhi.
2) To propose an intervention model to reduce pre-treatment loss to follow-up based on provider's feedback and health seeking behavior of patients.

Materials and methods: A questionnaire based cross sectional study of a sample of 340 patients who were pre-treatment loss to follow-up was conducted from November 2011 to March 2012 in Delhi. Qualitative study involved focused group discussions with para-medical providers using a topic outline guide, patients were interviewed using semi-structured questionnaire and brainstorming of program managers to elicit reasons, suggestions and health seeking behavior among those who were pre-treatment loss to follow-up.
Results: Preference for private practitioners (64.4%), lack of trust in government health system (26.7%), inconvenient time of Directly Observed Treatment (DOT) centre (18.5%) and wrong patient address (14%) were the main reasons for pre-treatment loss to follow-up. Paramedical provider's opinion elicited in focused group discussion was that there is an

* *Corresponding author.*
E-mail address: miradhuria@gmail.com (M. Dhuria).
http://dx.doi.org/10.1016/j.ijtb.2017.05.010

increased tendency of pre-treatment loss to follow-up in drug addicts and home-less patients. Brainstorming with program managers revealed that a lack of trust in allopathic system of medicine and human resource constraints were the leading causes of pre-treatment loss to follow-up.

A Meso level multi disciplinary model with community participation through Resident Welfare Associations (RWAs) has been designed based on the above findings. The model suggests mutual collaboration between government and non government agencies for promotion of International Standards of TB care in private clinics, de addiction services and social welfare schemes through RWAs.

Conclusion: There is a need for Advocacy Communication and Social Mobilization on a large scale. Collaboration with Resident Welfare Associations (RWAs) and with practitioners from alternate systems of medicine should be encouraged.

1. Introduction

Sputum positive pulmonary tuberculosis (TB) patients who are diagnosed but do not initiate anti-tuberculosis treatment are termed as pre-treatment loss to follow-up.[1] Revised National TB Control Programme (RNTCP) has been implemented in the State of Delhi since 1997 and there has been a steady decline in TB burden in the community. The mortality rate for Delhi has come down considerably and presently is less than 2%.[2] In Delhi state, 24,665 cases were new smear positive cases out of total 55,260 cases registered in year 2015.[3] Some patients do not register for treatment and are lost from the system. They may either attend the private sector for treatment or may not take treatment at all. Such cases may continue to transmit infection in the community.[4] Pre-treatment loss to follow-up is therefore a potentially serious problem as one smear positive patient infects 10–15 persons in a year, 10% of whom develop the disease in due course.[2] This assumes greater importance as we face an increasing threat of multi drug resistant (MDR) and extensively drug resistant (XDR) TB.

One of the major policy decisions taken by RNTCP in the year 2010 is to change the focus of the new sputum positive case detection objective of at least 70% to the concept of universal access to good quality care for TB patients. There is now global consensus that the twin objectives of 70/85 alone is not enough to achieve adequate reduction of TB transmission and reduction in disease burden at the pace with which epidemiological impact is expected. Also, some studies suggest mortality remains higher than expected, including post TB treatment mortality. Hence it is extremely important for TB control programmes to focus on early and complete detection of all TB cases including smear positive and smear negative TB cases. This essentially means that more attention is to be paid to the processes involved in case detection and case holding and strategies to ensure that all TB patients have access to early case detection and effective treatment.[5] Therefore, there is an urgent need to address this problem of pre-treatment loss to follow-up and create appropriate modalities for addressing it for achieving universal access to TB care. This study was done with the objective to determine the reasons for pre-treatment loss to follow-up among new sputum positive cases diagnosed under the Revised National TB Control Program in Delhi and to devise an intervention model to reduce pre-treatment loss to follow-up based on patients' and providers' feedback and program implementation strategies.

2. Materials and methods

This was an analytical cross sectional study incorporating both quantitative and qualitative methods conducted over a period of 5 months from November 2011 to March 2012. Delhi has been divided into nine regions/zones for implementation of RNTCP. Each zone is further divided into Chest clinics for program implementation. Delhi has 24 such clinics. Each Chest Clinic is equivalent to a District TB Centre with District TB Officer as in-charge. A Chest Clinic has Designated Microscopy Centers (DMCs) and Directly Observed Treatment (DOT) centers which provide diagnostic services and intermittent DOT therapy respectively. The sample size was calculated taking into consideration the pre-treatment loss to follow-up rate of 10% among the anticipated population with 95% confidence interval and 5% absolute precision. The sample size came out to be 340 patients (with a design effect of 2). For the present study, from each zone, one district was selected by simple random sampling method to give representation to each zone. The sampling units from each selected district of nine region/zones were selected using probability proportion to size method. For qualitative study, one focus group discussion (FGD) per selected district was held. One brainstorming session was conducted with the program managers.

2.1. Study instruments

A pre-tested, semi-structured questionnaire containing items on socio-demographic data and reasons for pre-treatment loss to follow-up including treatment history, etc. was used to collect data by trained field investigators for quantitative survey. Focused Group discussions (FGDs) were conducted with providers (medical and paramedical personnel).

Brainstorming session was conducted with the program managers i.e. District TB Officers (DTO) who are in-charge of the Chest Clinics. FGDs were included because it was felt that the research could gain valuable insights from interactions and discussions conducted with a group of people who were directly involved in care of TB patients and were the first point of contact in the program. The objective of such group work was to develop lively discussions among the group that would lead to an informative exchange of views about the profile of patients who were pre-treatment loss to follow-up, reasons for it, operational constraints in managing pre-treatment loss to follow-up retrieval. The group that was focused was paramedical staff viz. Lab technicians (LTs) and TB health visitor (TBHVs). The information was collected using topic outline guide. Nine FGDs, one at each of the selected chest clinics were conducted. Sample saturation was achieved in the fifth FGD, however, all districts were covered. Brainstorming session was conducted with the District TB Officers and 24 out of 25 DTOs participated.

The problem of pre-treatment loss to follow-up was studied from the perspective of the District Tuberculosis Officers who manage the operational aspects of the program and have a vast experience in the field. The technique used was brainstorming, to ascertain their perception about pre-treatment loss to follow-up viz.; definition of pre-treatment loss to follow-up, reasons for pre-treatment loss to follow-up, actions taken or to be taken to prevent pre-treatment loss to follow-up etc. This was done to identify key cause and effect relationships that can be leveraged to improve the overall performance of the process, that is case holding of those found to be sputum positive. The critical evaluation was done after the session. Onsite supervision of data collected by field investigators was also conducted by Principal investigator and co-investigators.

2.1.1. *Ethical considerations*

The institutional ethical review committee approved the study protocol. Written informed consent was obtained from the study subjects.

2.1.2. *Data analysis*

Quantitative data: Data was entered in MS excel and analyzed using SPSS ver 17. Data was analyzed for socio demographic features, determinants of pre-treatment loss to follow-up; background characteristics i.e. income, education, occupation, residence, health education given at DMC etc.

Qualitative: Detailed analysis of reasons for pre-treatment loss to follow-up was conducted using thematic analysis. The ideas that were generated during brainstorming were organized in the form of a cause and effect diagram (fishbone diagram).

3. Results

3.1. *Characteristics of study subjects*

A total of 98 (28.8%) subjects were between 15 and 24 years of age and 88 (25.8%) were aged 25–35 years as shown in Fig. 1. Maximum subjects, 248 (72.94%) belonged to the state of Delhi.

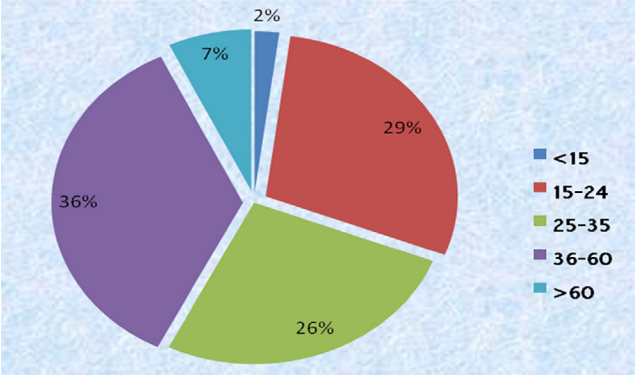


Fig. 1 – Age wise distribution of study subjects.

Table 1 – Demographic characteristics of study subjects.

Characteristic	Frequency (%)
Gender	
Male	266 (78.4)
Female	74 (21.6)
Religion	
Hindu	270 (79.41)
Muslim	67 (19.71)
Sikh	3 (0.88)
Occupation	
Unemployed	31 (9.1)
Retired	6 (1.7)
Others	43 (12.64)
Student	62 (18.23)
Employed	195 (57.3)
Marital status	
Married	227 (66.76)
Unmarried	112 (32.94)
Divorced	1 (0.29)
Total	340

Only 92 (27.06%) patients stated their permanent address as being outside Delhi. These patients however had been continuously residing in Delhi for more than 1 year on the day of coming to the DMC clinic. A majority 266 (78.4%) were males, Hindus 270 (79.41%) and married 227 (66.76%). 31 (57.3%) of the subjects were employed, 6 (9.1%) retired and 62 (18.23%) were students (Table 1). More than one-fourth, 100 (29.41%) patients were tobacco smokers, 4 (1.18%) were alcohol users and 96 (28.2%) patients were both alcohol users and tobacco smokers.

Approximately, 275 (80.8%) of the patients had to travel a distance less than 2 km to avail services at a DOT center. Only 63 (18.5%) had to travel more than 5 km to reach DOT center.

All 100%, 340 subjects reported that they had received health education regarding TB when they visited the DMC for sputum testing. For most of the patients 337 (99%), pre-treatment loss to follow-up retrieval action was taken by the TBHVs within 1–3 days of but they were not able to bring them under the program for treatment.

3.2. *Reasons for pre-treatment loss to follow-up*

The reasons for pre-treatment loss to follow-up were assessed from the patients' and providers' perspective.

3.2.1. *Patients*

When the reasons for pre-treatment loss to follow-up were assessed, out of the total 340 patients, 35 (10.3%) had died. These deaths had occurred within nine months of diagnosis. Treatment in all these cases was taken from private practitioners.

Maximum number; 218 (64.1%) of subjects did not take treatment from RNTCP. However, they reportedly took treatment from private practitioners (allopathic doctors/chemists). The maximum proportion, (81, 23.8%) of such pre-treatment loss to follow-up were those from chest clinics situated near Haryana and Uttar Pradesh border. They also reported various reasons for seeking treatment from private sector after being diagnosed under RNTCP (Table 2).

The exact details of type and source of private treatment could not be elicited but few patients reported seeking treatment from local Registered Medical Practitioners and chemists. More than one tenth (12.9%) patients felt they were too sick to travel to RNTCP center.

Table 3 shows reasons for pre-treatment loss to follow-up with respect to socio demographic characteristics. A significantly higher proportion of patients with per capita monthly income between Rs. 5000/- and Rs. 9999/- were not taking any treatment.

3.2.2. *Service providers*

The results of the brainstorming session have been organized in the form of a cause and effect diagram. The major categories shown as bones (vertical lines) in the fishbone diagram are patient limitations, logistic reasons, lack of faith in allopathic system, social causes, problems of special groups and human resource constraints (Fig. 2). The individual causes are listed along each bone as branches. Analysis of causes has been done under various categories.

Patient limitations: Patient's perception of his illness, lack of awareness about diagnosis, and accessibility accounted for patients' limitations according to the DTOs. They felt that some patients were not convinced about their illness as the symptoms were either mild or the patient was in the "denial" mode due to stigma associated with TB. At times, the patients believed that TB occurs only in those individuals who have a family member afflicted with TB and were therefore, in denial. Patients who had co-morbid conditions like Hypertension/Diabetes mellitus usually considered TB and its treatment as less serious. Few patients were unaware of the results of sputum examination, as they never came back for giving the second sputum sample for testing at DMC, although their first sample was positive for acid-fast bacilli. These also got labeled as pre-treatment loss to follow-up.

As per guidelines, patients are expected to report to the DOT center for taking drugs, therefore, for patients employed in unorganized sector, visiting the DOT center during the scheduled working hours posed a problem. They informed that female patients have to deliver their role of care takers at home especially with kids or elderly at home, therefore, visiting DOT center every alternate day is a constraint for them. These factors lead the patients to opt for the convenience of private treatment according to the program managers.

Social reasons: Stigma, lack of faith in government system and the quality of drugs were the social reasons cited by DTOs. They said patients were reluctant to visit DOT centers for treatment for fear of disclosure of their disease status. Patients also did not have faith in the government run health system and doubted the quality of free drugs available at the DOT centers. They also had faith in the diagnostic facilities provided under RNTCP at DMC as compared to local laboratories as proved by the fact that they visited the DMC for sputum examinations (twice, on most of the occasions) but once the results were provided to them, they did not take treatment under DOTS. General Practitioners also refer patients who seek treatment from them to DMC for sputum testing but later treat them.

Table 2 – Reasons for pre-treatment loss to follow-up among study subjects.

	Frequency (%)
No treatment/status unknown	
Refusal to start Anti TB Treatment/not interested in treatment	18 (5.2)
Symptoms mild, do not call for initiation of Anti TB Treatment	7 (2.0)
Died & treatment status unknown	11 (3.2)
Go to village but treatment status unknown	44 (12.94)
Taking treatment from non RNTCP source	
Private treatment (Allopathic doctors/chemists)	218 (64.12)
(i) No trust/faith in govt. system	91 (41.74)[a]
(ii) Too sick to take treatment	44 (20.18)[a]
(iii) No faith in quality of medicines being provided	1 (0.45)[a]
(iv) Work related problems	58 (26.60)[a]
(v) Inconvenient timings	63 (28.89)[a]
(vi) Too old	1 (0.45)[a]
(vii) Loss of wages	31 (1.37)[a]
(viii) Inappropriate behavior by staff at public health facilities	7 (3.21)[a]
(ix) Stigma associated with TB	9 (4.12)[a]
(xi) Lack of family support	21 (9.63)[a]
(xii) Died & were taking treatment from private. practitioner	24 (7.1)[a]
Gone to village & taking private treatment	37 (16.97)[a]
Ayurvedic treatment	5 (1.4)

[a] Percentages out of $n = 218$.

Table 3 – Major reasons for pre-treatment loss to follow-up by socio-demographic characteristics of study subjects.

	Patients not taking any treatment/treatment status unknown				Patients taking some treatment		
	Do not want to take treatment *n*-18	Symptoms mild/no need for treatment *n*-7	Gone to village, treatment status unknown *n*-44	Died and treatment status unknown *n*-11	Private treatment[a] *n*-218	Gone to village and took private treatment *n*-37	Taking ayurvedic treatment *n*-5
Gender**							
Male (*n*-266)	14 (77.8)	4 (57.1)	43 (97.7)	9 (81.9)	164 (75.2)	27 (73)	5 (100.0)
Female (*n*-74)	4 (22.2)	3 (42.9)	1 (2.3)	2 (19.1)	54 (24.8)	10 (27)	0 (0.0)
Age group							
Less than 15 yrs (*n*-7)	1 (5.6)	0 (0)	1 (2.2)	1 (9.0)	3 (1.4)	1 (2.7)	0 (0.0)
15–24 (*n*-98)	5 (27.8)	2 (28.6)	10 (22.7)	4 (36.4)	65 (29.8)	12 (32.4)	0 (0.0)
25–35 (*n*-90)	3 (16.66)	2 (28.6)	17 (38.6)	3 (27.2)	51 (23.4)	11 (29.7)	3 (60.0)
36–60 (*n*-121)	6 (33.3)	3 (42.9)	17 (38.6)	3 (27.2)	81 (37.2)	11 (29.7)	0 (0.0)
More than 60 yrs (*n*-24)	3 (16.66)	1 (14.2)	0 (0)	0 (0.0)	18 (8.2)	0 (0)	2 (40.0)
Education**							
Primary (*n*-64)	4 (22.2)	1 (14.3)	13 (29.5)	3 (27.3)	29 (13.3)	12 (32.4)	2 (40.0)
Secondary (*n*-140)	4 (22.2)	2 (28.6)	19 (43.1)	5 (46.4)	97 (44.5)	13 (35.1)	0 (0.0)
Higher secondary (*n*-81)	4 (22.2)	1 (14.3)	4 (9.1)	2 (18.2)	61 (27.9)	9 (24.3)	0 (0.0)
Graduate (*n*-13)	0 (0.0)	0 (0.0)	2 (4.5)	0 (0.0)	10 (4. 6)	1 (2.7)	0 (0.0)
Illiterate (*n*-42)	6 (33.3)	3 (42.9)	6 (13.7)	1 (9.1)	21 (9.6)	2 (5.4)	3 (60.0)
Income** (Per capita per month, in rupees)							
Less than 5000 (*n*-38)	0 (0.0)	0 (0.0)	4 (9.1)	4 (36.4)	24 (11.0)	6 (16.2)	0 (0.0)
5000–9999 (*n*-141)	9 (50.0)	3 (42.9)	29 (66.0)	7 (63.6)	84 (38.5)	10 (27.0)	0 (0.0)
More than 10,000 (*n*-161)	9 (50.0)	5 (71.4)	11 (25.0)	0 (0.0)	110 (50.5)	21 (56.8)	5 (100.0)

[a] Patients who have died but were under treatment from private practitioners as informed by family members.
** *p*-Value < 0.05.

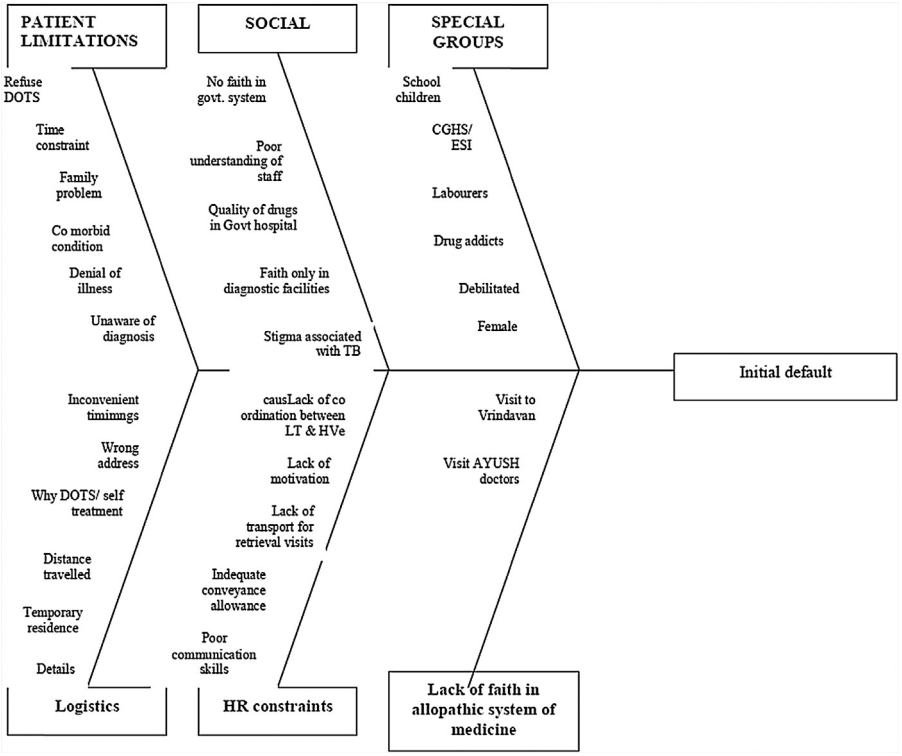


Fig. 2 – Fish bone diagram for cause and effect for pre-treatment loss to follow-up.

Lack of faith in allopathic system of medicine: People have the perception that allopathic medicines only control rather than cure the disease and they also lead to adverse drug reactions. Therefore, patients resort to treatment from doctors of other Indian System of Medicine like Ayurveda, homeopathy, etc. Some patients feel that TB is a result of ''bad'' air. So, to have a change in environment, they visit Vrindavan (Old TB sanatorium/faith healers exist in Vrindavan) for ''fresh clean air.''

Logistic reasons: Working hours of DOT centers, alternate day DOT, distance traveled by patients, lack of regular transport facility and trickling of patients from neighboring states for health care seeking were identified as logistic reasons leading to pre-treatment loss to follow-up. At the grass root level, the program has its functional units as DMCs and DOT centers catering to specific areas. DOT centers located at public health facilities have fixed timings (8 am–2 pm) that may be inconvenient for different sections due to reasons related to working hours. Patients tend to give wrong addresses pertaining to area catered by a particular DMC for getting their sputum microscopy done. But these patients become untraceable when the initial home visit for address verification is undertaken, as addresses provided by them do not exist.

In semi urban and peripheral areas of Delhi, patients have to travel approximately 5–8 km to reach DOT centers. At few places in the outskirts of Delhi, patients have to walk long distances on foot as no direct bus route or rickshaw is available for them to travel to the concerned DMC. In few Chest Clinics (bordering Haryana), people come for sputum testing at DMC and after getting the results travel back to their native state from where no feedback is received with regard to initiation of ATT for such patients. This was a major reason for pre-treatment loss to follow-up among many patients.

Some patients feel that they are responsible enough to continue with treatment on their own and supervised therapy at DOT centers is not required. Therefore, they refuse to start treatment under DOTS. During pre-treatment loss to follow-up retrieval visits, it was found that patients were traveling to their village due to personal reasons viz. marriage, illness, death in the family at village, etc.

HR constraints: Non-cooperative staff at DOT centers, poor patient–provider interaction, poor communication skills of TBHV/LT, lack of coordination between HV and LT, inadequate motivation of staff for conducting default retrieval, long distance between DOT center and catchment area, lack of transport facility for health visitor to make retrieval visits were the major issues related to HR highlighted by the DTOs. It was also observed by them that elicitation of address of the patient by LT/HV might not be skillful. DTOs also opined that the conveyance allowance for the TB health visitor is considered inadequate by them resulting in decreased motivation of staff for conducting home visits.

Problems of special groups: Drug addicts, alcohol users, daily wagers, school going children, affluent patients and patients registered under Central Government Health Service Scheme (CGHS) and Employee State Insurance (ESI) constituted special groups whose needs required special initiatives to retain them in the program. Drug addicts and alcohol users have psychological problems and need special counseling. For daily wagers and laborers working in the unorganized sector, the

timings of DOT centers were inconvenient because of their odd working hours (leave early in morning and come back late at night).

Middle class and upper class patients report to DMC only for diagnosis but prefer to seek treatment from local practitioners. For school children – DOTS seems to be unfriendly because of timings and stigma attached to the disease. In CGHS and ESI, doctors prefer daily regimen over intermittent DOTS therapy.

3.2.3. *FGDs with the TBHVs and LTs*

FGDs were conducted with the objective of eliciting knowledge, perception and understanding of TBHVs and LTs regarding pre-treatment loss to follow-up. Most of the TBHVs and LTs had correct knowledge regarding definition of pre-treatment loss to follow-up. Various definitions given by them were:

''*Patient is sputum positive but doesn't receive treatment*''

''*Patient is diagnosed to be suffering from TB but not put on treatment (ATT)*''

''*Patient is sputum positive but neither referred nor put on treatment*''

''*Any patient who comes from any village or outside and return back after diagnosis*''. ''*Sometimes, people come for initial checkup but do not come back for proper treatment*'' ''*Patients who get their sputum tested from DMC but go to ESI, CGHS for treatment*''

According to the paramedics working in peripheral areas of Delhi, many patients' who visit DMC are migrants and tend to provide incomplete address. Eliciting complete address of patients living in slums was also a problem faced by the HW as the ''jhuggis'' (slum dwelling) were not numbered properly. Most of the patients living in a rented accommodation leave for their villages without informing any health functionary if their condition is serious. Paramedics were of the opinion that patients tend to take treatment from private doctors but for investigations visit DMC. As under the program, they cannot refuse sputum testing to anyone who comes to DMC, patients from areas other than their catchment area approach them, provide them ''any local address'' to get themselves tested and during initial home visit, it is found that patient has provided wrong address.

They also felt that patients have the perception that sputum testing undertaken at a particular DMC is better than other DMCs in Delhi (''*Moti nagar mein acchi jaanch hoti hai*''), therefore come from all over Delhi for sputum microscopy.

Another problem cited was of referrals made by nearby hospitals for sputum testing. As patients visiting hospitals may not belong to catchment area of a particular DMC, these patients usually do not report back to DMC after taking their results. The referring hospital does not provide feedback.

Migrants, alcohol users, smokers and drug addicts were more likely to be pre-treatment loss to follow-up. ''*pre-treatment loss to follow-up are migrants, alcoholics or drug addicts*''. Therefore, they are different from rest of the patients and their psychological and treatment needs are different.

''*Alcoholics and smokers don't give up smoking/drinking, but they prefer to give up medicine*''.

''*Many patients become defaulters because of lack of time and job*''.

''*Patients usually live alone in a rented accommodation therefore, go back to their native places as there is no one to take care of them here.*''

Other reasons given were that private doctors convince the patients that everyday/daily medicine is more effective than alternate day therapy under DOTS.

''*ek din khana khaya aur ek din nahin, iss se kaise asar ayega*'' and this is especially true for patients who are failures of Category I. They are convinced that intermittent therapy could not cure their disease and are therefore, not ready to initiate treatment under Category II. These patients are demotivated to start treatment under DOTS and they also tend to negatively publicize the program among their relatives and friends. Patients also visited the DMC for cross checking of results of investigations done elsewhere but did not report back for treatment.

Paramedics of DMC located in a Medical College felt that faculty members/doctors in Medical College were not in favor of intermittent therapy, therefore after investigations at DMC, they started the patient on treatment but did not provide any feedback regarding treatment to the specific DOT/DMC where the sputum was tested. Inconvenience of DOTS was mentioned as a reason for pre-treatment loss to follow-up by many patients to TBHVs during initial home visit.

3.3. *Creating a model to achieve universal access to TB care with community participation*

To achieve the goal of Universal access to TB care, it is important to include this group of suspects (pre-treatment loss to follow-up) as they have approached the program for diagnosis but have not taken treatment and therefore, their treatment status remains unknown. These patients can continue to spread the disease if they are untreated or become MDR if inadequately treated. It is important to ensure that these people are treated appropriately to break the chain of transmission. These pre-treatment loss to follow-ups have contacted the service meaning they are aware of it but need motivation to continue in the program. As evident from the findings of the study this motivation can be enhanced by providing solutions like patient friendly timings for students and daily wagers, catering to communication needs of special groups like smokers, alcohol users and drug addicts, etc. This can only be feasible via Public Private Partnership (PPP) approach with active community participation, as formal health services alone cannot provide all the solutions. Therefore, a model is proposed based on community participation involving civil society organizations, which uses the available schemes in Delhi and describes ways to decrease

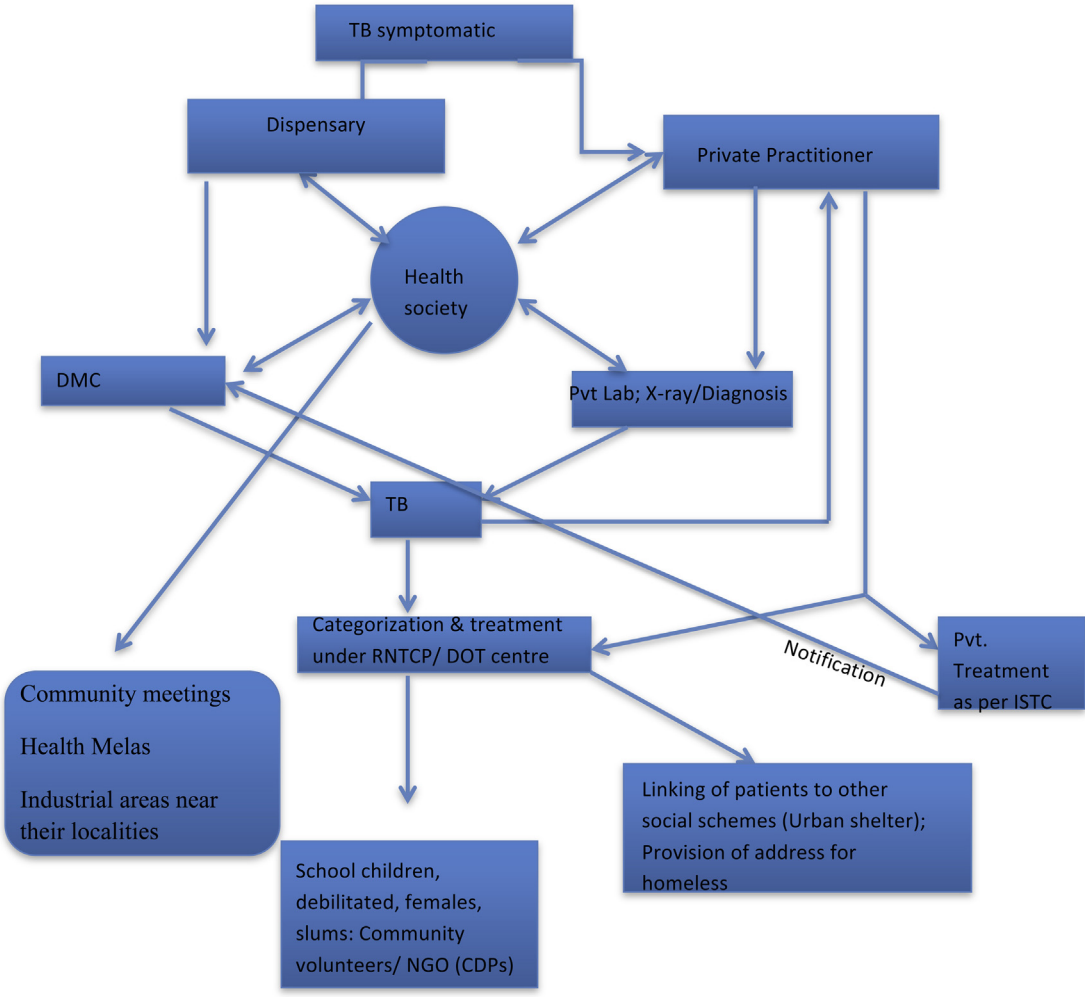


Fig. 3 – Model for involvement of RWAs.

pre-treatment loss to follow-up based on reasons detected. The local civil society organizations have the comparative advantage of a bidirectional influence on community structures as well as governmental institutions; knowledge and understanding of local circumstances and flexibility and adaptability toward local situations and are therefore appropriate.

Most of the registered housing societies have their Resident Welfare Associations (RWAs). RWAs were initially located only in authorized colonies. However, there are unauthorized colonies, slums/resettlement colonies surrounding the authorized ones. People from these areas provide various forms of domestic services to the residents of authorized localities. Residents in authorized colonies can benefit from an improved health status of the domestic service providers by decreased transmission of diseases in their own community. The RWAs as a part of their social responsibility in collaboration with non-governmental organizations (NGOs); health care provision by various national programs is assisted to these disadvantaged communities.

The unauthorized colonies and slums have informal leaders called Pradhans. These local leaders could be the contact persons for all TB patients in their locality. The LT would contact the pradhan after receiving first sputum sample from the patient. This would ensure minimal pre-treatment loss to follow-up due to wrong address. For effective functioning of this model; RWAs, local community leaders of these unauthorized colonies, local NGOs and the program representatives need to come together and a health society needs to be created with a designated nodal officer for health.

Special groups like elderly, drug addicts, alcoholics, smokers, students and daily wagers etc. need flexible timings and counseling in some cases. The program provides community DOTS providers (CDPs) who can be used for such door step DOTS. The partner NGOs with program representatives would provide counseling services and be involved in capacity building of community volunteers for provision of flexible DOTS. The aforementioned described model is depicted in Fig. 3.

Homelessness leading to non-contact ability has been overcome to a great extent in Delhi by its welfare schemes like Urban shelter scheme initiated with RWAs wherein a temporary shelter and nutrition is provided to homeless through NGOs. RNTCP can effectively use it to track any pre-treatment loss to follow-up and ensure compliance as NGOs running these schemes would be part of the envisaged society to be created with RWAs help.

The study results show that those pre-treatment loss to follow-ups who took any treatment after diagnosis from DOT centre, took it from Private practitioners (PPs) or chemists. Private practitioners will be roped in with help of RWAs and their capacity enhanced in TB care by training in International Standards in TB care through Medical Professional bodies. Chemists would also be included. The compulsory notification ensures that all cases coming to a private practitioner will also be known to the Chest Clinic/DMC and standardized care along with reduction of default can be ensured through the health society. This proposed model needs to be tested in an area with high pre-treatment loss to follow-up. All the components are in place and the only need is to ensure an integrated approach by setting up a health society.

4. Discussion

In the current study to assess the reasons for pre-treatment loss to follow-up, 340 patients who were pre-treatment loss to follow-up were interviewed. Brainstorming session was conducted with program managers and FGDs were carried out with the paramedic staff viz. LTs and TBHVs who are the first point of contact for many patients visiting a DMC/DOT centre for sputum testing.

In a study conducted in Uttarakhand, pre-treatment loss to follow-up was seen in 120 (21.6%) patients. The main reasons for pre-treatment loss to follow-up among patients referred for DOTS were limited trust in DOTS (44.8%), adverse effects of previous therapy (41.8%), dissatisfaction with health services (38.7%), local deaths while taking DOTS (28.5%), advice by others against DOTS (25.5%), disbelief in the diagnosis (18.3%) and patient death before starting treatment (4.0%).[6] These are similar to findings of our study except for disbelief in diagnosis. Qualitative study conducted on program managers and paramedic staff has cited belief in diagnosis under RNTCP and distrust in treatment services as reasons for pre-treatment loss to follow-up. In a study conducted in twenty districts of Andhra Pradesh State, India, of the 685 (4.5% of the total diagnosed) who were confirmed pre-treatment loss to follow-ups, 350 (51%) were untraceable, 152 (22%) had died before treatment initiation, 38 (5.5%) were treated privately, 93 (13.5%) had other reasons (e.g., refusal of treatment, chronic case, etc.) and no data were available for 52 (8%).[7]

Some patients were reported to be very sick to go to DOT centre for treatment. For such cases, there is provision of community DOT providers where any member of local community or even a family member can act as DOT provider. Improvements in address recording may assist efforts to retrieve these patients for treatment. All the cases were traced by health workers at their address when they did not report for the further management. Neighbors, village *pradhans (head)*, nearby shopkeepers were approached for eliciting information about them. This process needs to be strengthen under the program. In Delhi, same can be accomplished with help of RWAs as has been done in the suggested model. Additional evaluations are needed for improved counseling of TB suspects to prevent pre-treatment loss to follow-up, and of reasons for death before treatment initiation. NGO partners with help of RWAs and health society in the proposed model will take care of counseling for special groups.

The results of a study conducted in Ho Chi Minh City, Vietnam to determine the extent of pre-treatment loss to follow-up in the National TB Program (NTP), revealed that pre-treatment loss to follow-up is mainly caused by some patients' negative perceptions of working procedures and/or treatment strategy in the NTP. The majority of these patients were treated in private clinics after pre-treatment loss to follow-up from the NTP.[8] These findings corroborate with our study as pre-treatment loss to follow-ups were seeking treatment outside RNTCP, from private practitioners. The TB control programs needs to improve patients' perceptions of the treatment strategy and develop more user-friendly services that enable more patients to access treatment and reduce the risk of patients receiving substandard treatment in the private sector.

After qualitative analysis, the major possible reasons for pre-treatment loss to follow-up were: shifted to village, died, seeking treatment from private practitioners, seeking treatment from Indian systems of Medicine. The results of the qualitative study highlighted patient limitations, problems of special groups, social causes, logistic reasons, lack of faith in allopathic system of medicine and human resource constraints as the major causes. Almost one-fifth had given wrong addresses. This also reflected in the qualitative findings of the study as patients had trust in diagnostic facilities but did not initiate treatment because of lack of faith in the government system and the quality of drugs. Another study from South Africa reported an pre-treatment loss to follow-up rate of 17%.[9]

Another study was conducted in West Bengal among 132 patients who were pre-treatment loss to follow-up. It was found that pre-treatment loss to follow-up rate was 23.5%. Age, literacy, employment, marital status, smoking habits, alcohol consumption and pre treatment counseling were significantly associated more among males than females. Reasons given were busy with jobs, temporary vocational migration were some reasons reported for pre-treatment loss to follow-up.[10]

Similar study done in Puducherry stated factors for pre-treatment loss to follow-up. Patient-related factors for pre-treatment loss to follow-up were long distance to the health facility, lack of support from the family members, being advised against alcohol consumption while taking treatment, monetary constraints, job constraints, not convinced about results by the health facility, stigma related to TB, and lack of awareness regarding TB. Health system-related factors were unpleasant experience with the health system, lack of dissemination of adequate information regarding further course of action to the patients, and non-availability of the laboratory staff.[11]

Another study stated that majority of the non-adherent patients were in the age group of 30–60 years, residents of rural area (72%), illiterate (76.3%) and from lower or upper lower socio-economic status (76.3%). Maximum patients (36.5%) stopped their ATT during 3rd month. Adverse effects of ATT (54.8%) was the most important reason followed by feeling of early improvement (32.8%) and then family problem (31%), loss of work (20.4%), and stigma related (19.3%).[12]

Patient's perception about the disease as mild and seriousness about other co morbid conditions also led to

ignorant behavior regarding initiation of anti tuberculosis treatment. There were special groups like daily wagers, laborers, students (one fifth of the pre-treatment loss to follow-ups were students) for whom the accessibility with respect to working hours made DOTS inconvenient and increased preference for private treatment. Around who may face problem in visiting DOT centers due to timings or stigma attached to it. More than one fourth of the patients were smokers and about one third were smokers as well as alcohol users. This is a difficult group to handle as they lack insight and it has been seen that case holding is a problem in such cases. Moreover, it is difficult to convince them to start treatment as stated by the paramedics working in the program.

5. Conclusion and recommendations

There is a need for Advocacy Communication and Social Mobilisation on large scale and also for enhancing the counseling skills of health workers posted at DMCs to motivate patients to start treatment under DOTS. There is a need to build a public private partnership (PPP) model for inclusion of private practitioners in the program and train them on standardized TB care as per guidelines of *International Standards of TB Care* (ISTC) as most of the pre-treatment loss to follow-up were seen to be taking treatment from private practitioners. For identification of local practitioners in the community, the services of Resident Welfare Associations (RWAs) can be utilized. Sensitization and training workshops for the private practitioners can be conducted in collaboration with the local medical associations. A mechanism should be evolved to include the practitioners from alternate systems of medicine i.e. Ayurveda Unani Siddha Homeopathy (AYUSH) in the PPP model and encourage their active participation. Intensive Information Education Communication (IEC) campaign to explain that the potency of drugs given free of cost at DOT centres is as good as one available in the market should be launched. Sensitization of the chemists for not giving anti TB drugs over the counter has already been started in Delhi and should be continued.

Conflicts of interest

The authors have none to declare.

Acknowledgement

The study was funded by RNTCP-Delhi.

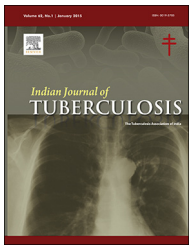
REFERENCES

1. MacPherson P, Houben RM, Glynn JR, Corbett EL, Kranzer K. Pre-treatment loss to follow-up in tuberculosis patients in low- and lower-middle-income countries and high-burden countries: a systematic review and meta-analysis. *Bull World Health Organ*. 2014;92(February (2)):126–138.
2. Dhingra VK, Aggarwal N, Chandra S, Vashist RP. TB mortality trends in Delhi after implementation of RNTCP. *Indian J Tuberc*. 2009;56:77–81.
3. TB Statistics for India – National & State Statistics. Available from: http://www.tbfacts.org/tb-statistics-india/; Accessed 21.04.17.
4. Gopi PG, Chandrasekaran V, Subramani R, Narayanan PR. Failure to initiate treatment for TB patients diagnosed in a community survey and at health facilities under DOTS program in a District of South India. *Indian J Tuberc*. 2005;52:153–156.
5. Universal Access to TB Care. Available from: http://www.searo.who.int/india/tuberculosis/topic/universal_access_care/en/; Accessed 09.04.16.
6. Mehra D, Kaushik RM, Kaushik R, Rawat J, Kakkar R. Initial default among sputum-positive pulmonary TB patients at a referral hospital in Uttarakhand, India. *Trans R Soc Trop Med Hyg*. 2013;107(September (9)):558–565.
7. Babu BS, Satyanarayana AVV, Venkateshwaralu G, et al. Initial default among diagnosed sputum smear-positive pulmonary tuberculosis patients in Andhra Pradesh, India. *Int J Tuberc Lung Dis*. 2008;12(9):1055–1058.
8. Buu TN, Lönnroth K, Quy HT. Initial defaulting in the National Tuberculosis Programme in Ho Chi Minh City, Vietnam: a survey of extent, reasons and alternative actions taken following default. *Int J Tuberc Lung Dis*. 2003;7(8):735–741.
9. Botha E, den Boon SD, Lawrence KA, et al. From suspect to patient: tuberculosis diagnosis and treatment initiation in health facilities in South Africa. *Int J Tuberc Lung Dis*. 2008;12:936–941.
10. Mandal A, Basu M, Das P, Mukherjee S, Das S, Roy N. Magnitude and reasons of initial default among new sputum positive cases of pulmonary tuberculosis under RNTCP in a district of West Bengal, India. *South East Asia J Public Health*. 2014;4(1):41–47.
11. Pillai D, Purty AJ, Prabakaran S, Singh Z, Soundappan G, Anandan V. Initial default among tuberculosis patients diagnosed in selected medical colleges of Puducherry: issues and possible interventions. *Int J Med Sci Public Health*. 2015;4(7):957–960.
12. Asati A, Nayak S, Indurkar M. A study on factors associated with non-adherence to ATT among pulmonary tuberculosis patients under RNTCP. *Int J Med Sci Clin Invent*. 2017;4(3):2759–2763.

Available online at www.sciencedirect.com

ScienceDirect

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



Original Article

Decadal impact of Directly Observed Treatment Short course program on age and gender among New Infectious Tuberculosis cases in Delhi

Tanu Anand [a,*], *Nandini Sharma* [b], *Shivani Chandra* [c], *G.K. Ingle* [d], *Shekhar Grover* [e]

[a] *Assistant Professor, Department of Community Medicine, North DMC Medical College, Delhi-07, India*
[b] *Director Professor, Department of Community Medicine, Maulana Azad Medical College, New Delhi-02, India*
[c] *WHO Consultant*
[d] *Director Professor & Head, Department of Community Medicine, Maulana Azad Medical College, Delhi-02, India*
[e] *Scientist B, National Institute of Cancer Prevention & Research, Noida, India*

ARTICLE INFO

Article history:
Received 10 August 2015
Accepted 11 May 2017
Available online 27 May 2017

Keywords:
New sputum positive cases
Revised National Tuberculosis Control Programme
Epidemiological transition
Gender
DOTS

ABSTRACT

Background: Burden of tuberculosis in India remains enormous. The Revised National Tuberculosis Control Programme (RNTCP), based on the Directly Observed Treatment Short course (DOTS) strategy, was launched in 1997 in India. The question of what DOTS has or has not accomplished over the past 15 years is a central technical question.

Objectives: To assess the decadal impact of DOTS strategy on some epidemiological factors such as age and gender of new sputum positive (NSP) TB patients in Delhi.

Material and methods: Secondary Data for Delhi was obtained from the state wise performance of RNTCP (Annual Summary) for the year 2001 and year 2012. Data was analyzed in Microsoft Excel 2007.

Results: The population of Delhi covered under DOTS has considerably increased over the decade. The case detection rate has also shown a considerable increase from 196/100,000 population in 2001 to 306/100,000 population at the end of Quarter 3 of 2011. The number of NSP male and female patients have increased in all age groups from 2001 to 2011 except in 25–34 years age group. NSP male patients on DOTS aged 15–44 years showed a left ward shift in increase, a significant right ward shift was noted in increase in female NSP patients of similar age group.

Conclusions: The decadal assessment of DOTS in Delhi on TB epidemiology has pointed towards beginning of epidemiological transition in TB control in India.

* *Corresponding author at*: Assistant Professor, Department of Community Medicine, North DMC Medical College, Hindu Rao Hospital, Delhi-110007, India. Tel.: +98 11028964.
E-mail address: drtanu.anand@gmail.com (T. Anand).

http://dx.doi.org/10.1016/j.ijtb.2017.05.009

1. Background

Tuberculosis (TB) continues to remain a major public health problem. TB is one of the top 10 causes of death worldwide.[1] According to World Health Organization Global Tuberculosis Report 2016, 10.4 million people fell ill with TB and 1.4 million died from TB in 2015.[2] India, China along with Russian Federation is accounting for 45% of the world's TB cases.[2] India is the highest TB burden country with World Health Organisation (WHO) statistics for 2015 giving an estimated incidence figure of 2.84 million cases of TB for India out of a global incidence of 10.4 million cases.[2] Thus, evidently burden of tuberculosis in India remains enormous despite the availability of treatment that will cure tuberculosis.

The Revised National Tuberculosis Control Programme (RNTCP), based on the Directly Observed Treatment Short course (DOTS) strategy, began as a pilot in 1993 and was launched as a national programme in 1997 in India. The RNTCP has made great progress in the last decade with universal expansion of DOTS strategy in 2006 and achievement of the global target of 70% case detection while maintaining the treatment success rate of more than 85% since 2007.[3] Despite this progress, TB incidence and mortality in the country continue to be high. Given that DOTS will likely continue to occupy a central place in tuberculosis control efforts in coming years, the question of what DOTS has or has not accomplished over the past 15 years is a central technical question.[4]

The present paper aims to assess the decadal impact of DOTS strategy on some epidemiological factors such as age and gender of new sputum positive (NSP) TB patients in Delhi. There is a differential risk of developing TB by age. It is common to see a J-shaped curve of TB incidence rates by age, with higher rates in younger children from infancy to pre-adolescence. Rates increase abruptly during the adolescent years and remain high throughout adulthood, with a tendency to increase as age progresses.[5] In the population where the transmission has been stable or increasing, the incidence rate is higher in children mostly because of recent infection or re-infection. As transmission falls, the case load shifts to older adults mainly because of reactivation of LTBI at later ages.[6] Evidence from studies in US and Europe re-affirms this fact that with improvement in tuberculosis control, there is an upward shift in median age of occurrence.[7]

Gender wise analysis of tuberculosis incidence shows the prevalence of TB to be higher among males as compared to females in India.[8] The reasons for these differences are likely to result from various factors, including access to care, ethnicity, the influence of the HIV co-epidemic, as well as other biological, social and cultural variables.[5] The importance of a gender perspective on current policies regarding disease prevention and treatment is slowly being recognized. Introduction of health system reforms have shown to impact the gender specific notification rates in several countries.[9,10] Age and gender wise trends in TB in India are known. But there is paucity of evidences assessing the impact of programmatic measures on these factors with respect to occurrence of tuberculosis. Therefore, we seek to examine the differences in epidemiological trends of TB from 2001 to 2011.

2. Material and methods

Settings: Delhi is a Metropolitan city State with a population of over 17 million. It is estimated that 40% of its population is infected with TB and the occurrence of new TB cases is 209/100,000 population/year.[11] The state has been implementing DOTS since 1997 and on the two international goals of RNTCP, Delhi program has been rated as the best performing in the country consecutively for five years (2005 to 2009) amongst the States and Union Territories of India.[11]

Methods: We sought to assess the decadal impact of DOTS program in Delhi among NSP TB patients primarily on two factors: age and gender. Since the definition of sputum smear positive case has largely been consistent across time and place and since smear positive cases have been the primary target of DOTS strategy since its inception,[4,12] we limited our analysis of data to this cadre of patients only. The performance of RNTCP is monitored on the basis of Treatment success rate and NSP Case Detection rate. Therefore, impact of DOTS strategy on these two key dimension of tuberculosis control was also assessed.

Data for Delhi was obtained from the state wise performance of RNTCP (Annual Summary) for the year 2001 and year 2012.[13,14] Data was analyzed in Microsoft Excel 2007.

3. Results

Table 1 shows that the population of Delhi covered under DOTS has considerably increased over the decade. The case detection rate has also shown a considerable increase from 196/100,000 population in 2001 to 306/100,000 population at the end of Quarter 3 of 2011. Success rate and sputum conversion rate of NSP patients have, however, seen a meagre rise only.

Fig. 1 depicts the age wise distribution of NSP male patients on DOTS in the year 2001 and 2011. The diagram shows that the number of NSP male patients on DOTS in each age group

Table 1 – Comparison of indicators of Case Finding, Smear Conversion and Treatment outcome in Delhi in year 2001 and 2011.

S. No.	RNTCP indicators	2001	2011
1.	Population covered under DOTS (in lakhs)	138	170
2.	Annual Total detection rate (per lakh population)	196	306
3.	Total cases treated	26,380	52,206
4.	3 month conversion rate of new sputum positive patients (%)	88	89
5.	Success rate of New Sputum positive patients (%)	83	85

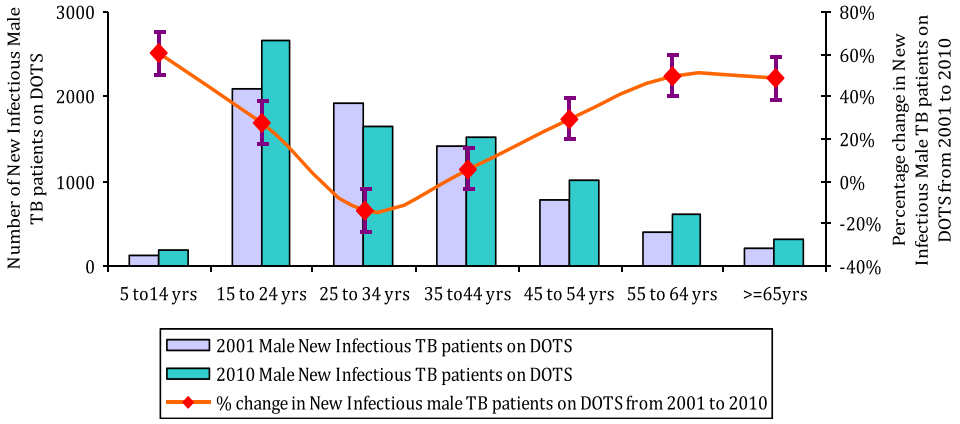


Fig. 1 – Age wise distribution and percentage change in NSP male TB patients on DOTS from 2001 to 2011.

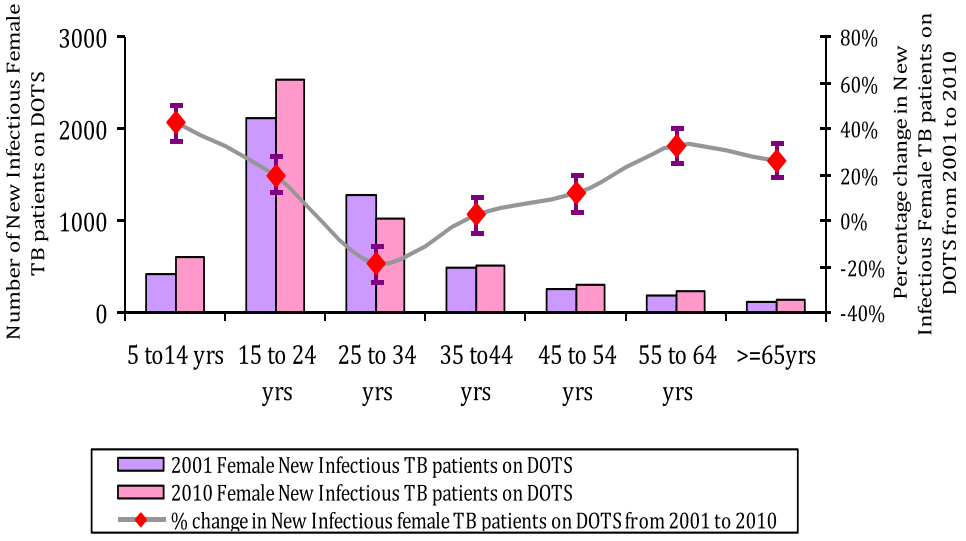


Fig. 2 – Age wise distribution and percentage change in NSP female TB patients on DOTS from 2001 to 2011.

have increased in 2011 except in the age group of 25–34 years when compared with 2001. Similarly, percentage change in NSP male patients show a V shaped curve with age and dip in the age group of 25–34 years only. The maximum number of patients were aged 15–24 years.

Table 2 – Decadal impact of DOTS program on age and gender among NSP TB cases in Delhi (2001–2011).

Decadal impact of DOTS program on age and gender among new infectious TB cases in Delhi (2001–2011)			
Patient characteristics	*p*-Value with proportion test		
	0–14 yrs	15–44 yrs	>45 yrs
Male	0.42**↑	6.25*↓	3.13*↑
Female	1.35**↑	4.35*↑	1.08**↑

↑ Right shift in proportional increase in number of TB cases between 2001 and 2011.
↓ Left shift in proportional increase in number of TB cases between 2001 and 2011.
* Significant; $p < 0.05$.
** Not significant; $p > 0.05$.

Fig. 2 reveals that the number of NSP female patients have increased in all age groups from 2001 to 2011 except in 25–34 years age group. Percentage change in NSP female patients on DOTS shows a declining trend with age up to 25–34 years, after which there is increase till 64 years. The maximum number of patients on DOTS in both the years were from 15–24 years age group.

Table 2 shows that there was non-significant increase in number of male and female patients aged 0–14 years from 2001 to 2011. While NSP male patients on DOTS aged 15–44 years showed a left ward shift in increase, a significant right ward shift was noted in increase in female NSP patients of similar age group. There was significant increase in proportion of male patients aged >45 years over the decade while proportional increase in female patients of similar age group was not significant.

4. Discussion

DOTS is currently the most essential operational strategy for tuberculosis control. Delhi is the first state to implement DOTS

on 100% basis.[11] It is pertinent to assess the accomplishment of DOTS program over the past 15 years of its implementation. The current secondary data analysis provides empirical evidences of expansion of DOTS strategy in the state that led to improvement in treatment success rates. While the cure rates are at par with global targets with TB control, it is 3 point less than the national average of 88%. The possible reasons for not much improved cure rate in Delhi could be the fact that large proportion of patients are seen in the private sector.[15–17] Survey conducted among private practitioners (PPs) in different parts of Delhi clearly show that existing practices of management of TB patients are largely inappropriate.[18] This leads to not only poor cure rates but also amplifies the problem of drug resistance. Thus, there is a need to strengthen the involvement of private sector in efforts for tuberculosis control.

Besides treatment success rate, New Smear positive case detection rate is also used to monitor the performance of RNTCP. A considerable increase noted in the case detection rate in Delhi point towards the strong surveillance system in place in the state over the decade. India's Revised National TB Control Programme recommends screening of all household contacts of smear-positive pulmonary tuberculosis cases for tuberculosis (TB) disease and provision of isoniazid preventive therapy (IPT). They recommended that contact tracing could increase the identification of active pulmonary TB case through tracking down the household contacts of newly diagnosed TB cases. Such pro-active strategies have proven to be effective in increasing case detection and consequently reducing TB incidence and burden.[19]

Age wise analysis showed a decrease in the number of patients both males and females in the age group of 25–34 years over the decade with relative shift towards a higher age groups. This may be correlated to changing demographics of India. It also points towards an epidemiological shift in tuberculosis as reported in the industrialized world in the mid 20th Century. It is one of the epidemiologic characteristic of declining incidence and transmission of infection in the country.[20]

Age specific gender wise analysis has shown a 'gender reversed pattern' over the time. Interestingly, both sexes have shown a dip in the age group of 25–34 years. At the same time, while the number of male patients of productive group have shown a significant decline over the decade, a significant increase in number of female patients receiving DOTS treatment have been noted. This is a positive finding considering the fact that the gap in notification rates between male and female reported cases seems to start after puberty. Sex and gender differences across different regions may impact upon the tuberculosis notification rates.[21] Considering the fact that Tuberculosis is a social disease, there is growing consensus that progress in tuberculosis control in the low and middle-income world will require not only investment in strengthening tuberculosis control programs, diagnostics, and treatment but also action on the social determinants of tuberculosis.[22] Interventions from outside the health sector—specifically, in social protection and urban planning—have the potential to strengthen tuberculosis control.[22]

Delhi Government has more than 40 social programs spread across 9 different departments. One of the major steps towards participatory governance has been the roll out of 'Bhagidari' program by Government of Delhi in the year 2000 with Resident Welfare Associations for improvement in education, health and civic amenities. Bhagidari is a Citizen Government partnership, which has re-engineered the process of service delivery through a unique Public Private Community Participation to make the systems more responsive to the citizens. The Bhagidars represent local residents and vulnerable groups. Through the mechanism of Bhagidari, all the social protection schemes have received enhanced advocacy and outreach among the beneficiaries who include TB patients and their families. In 2008, Mission Convergence scheme also known as Samajik Suvidha Sangam was rolled out by Delhi Government for collaboration with civil society and various government departments. Under the Mission Convergence module, Gender Resource Centre-Suvidha Kendra was set up all over Delhi which acts as the first point of contact for the vulnerable communities. In addition to advocacy, these centres provide a database of vulnerable households and rolled out smartcards (e-cards) for easy access to cash benefits. Vulnerability has been defined based on place, social and occupational aspects. E-cards are being used across participating programs and multiple ministries and have been linked with Unique Identification Number. This scheme has been linked with Bhagdari program for effective outreach of services to all citizens. Information about the several schemes accessed by the patients and their families is captured through TB treatment cards. The database of vulnerable TB patients is shared with the team of Bhagidari and Mission convergence District Resource Centres for improving outreach of social welfare schemes to patients and their families and for improving coordination between relevant ministries for specific interventions.

Thus, increasing number of female patients receiving DOTS could be attributed these social protection schemes particularly for women and other vulnerable groups. However, evaluation and effectiveness of such interventions on TB epidemiology needs to be investigated further. To conclude, decadal assessment of DOTS in Delhi on TB epidemiology has pointed towards beginning of epidemiological transition in TB control in India. Increasing number of female patients receiving treatment as compared to males point towards changing gender roles and effect the empowerment schemes can have on disease epidemiology. There are gaps in our understanding of social determinants in driving the TB epidemic, which needs to be assessed.

Conflicts of interest

The authors have none to declare.

Authors' contributions

Dr. Tanu Anand, Assistant Professor, Department of Community Medicine, North DMC Medical College, Delhi-07, contributed in preparation of protocol, literature search, data collection, analysis and its interpretation, and drafting the report.

Dr. Nandini Sharma, Director Professor, Department of Community Medicine, Maulana Azad Medical College, New Delhi-02, conceptualized the idea for this study. She guided in preparation of protocol, questionnaire, data collection, analysis writing and reviewing of the report.

Dr. Shivani Chandra, WHO Consultant, prepared the protocol, literature search, data collection and drafted the report.

Dr. G.K. Ingle, Director Professor & Head, Department of Community Medicine, Maulana Azad Medical College, New Delhi, guided in preparation of protocol, questionnaire, and reviewing of the report.

Dr. Shekhar Grover, Scientist B, NICPR, did literature search and contributed in data analysis.

REFERENCES

1. WHO. *Media Centre: Factsheet No. 104 Tuberculosis. [Internet]*. 2017. Available from: http://www.who.int/mediacentre/factsheets/fs104/en/ Accessed 09.05.17.
2. WHO. *Global Tuberculosis Report 2016*. Geneva, Switzerland: WHO; 2016.
3. TBC India. *About RNTCP. [Internet]*. 2007. Available from: http://www.tbcindia.nic.in/rntcp.html Accessed 09.05.13.
4. Obermeyer Z, Abbott-Klafter J, Murray CJL. Has the DOTS strategy improved case finding or treatment success? An empirical assessment. *PLoS ONE*. 2008;3(3):e1721. http://dx.doi.org/10.1371/journal.pone.0001721.
5. Bierrenbach A. *Background Paper 9: Estimating the burden of TB by age and sex: Availability of data, gaps and next steps. [Internet]*. 2008. Available from: http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/tf_17march10_bg_9_estimating_tb_by_age_sex.pdf Accessed 09.05.13.
6. Datiko DG. *Improving Tuberculosis Control in Ethiopia: performance of TB control programme, community DOTS and its cost-effectiveness. [Internet]*. 2011. Available from: http://bernt.b.uib.no/files/2011/01/Daniel-Datiko-PhD-thesis-2011.pdf Accessed 20.05.13.
7. Reider HL. Epidemiology of tuberculosis in Europe. *Eur Resp J Suppl*. 1995;20:620s–632s.
8. Sharma PP, Kumar A, Singh P. A study of gender differentials in the prevalence of tuberculosis based on NFHS-2 and NFHS-3 data. *IJCM*. 2010;35(2):230–237.
9. Soltan V, Henry AK, Crudu V, Zatuseveski I. Increasing tuberculosis case detection: lessons from the Republic of Moldova. *Bull World Health Organ*. 2008;86(1):71–76. http://dx.doi.org/10.2471/BLT.06.038265.
10. Stebbing M. Gender and TB: perspectives from Nepal. In: *Proceedings of a Conference on Poverty, Power and TB*. 2000.
11. Dotsdelhi.org. *DOTS Services in Delhi. [Internet]*. 2009. Available from: http://www.dotsdelhi.org/program-performance.php Accessed 10.05.13.
12. WHO. *Global Tuberculosis Control: Surveillance, Planning, Financing*. Geneva: WHO; 2007.
13. RNTCP. MoHFW. *TB India 2002: Part 3. [Internet]*. 2002. Available from: http://www.tbcindia.nic.in/pdfs/TB%20India%202002-Part3.pdf Accessed 01.05.13.
14. RNTCP. MoHFW. *TB India 2013. [Internet]*. 2013. Available from: http://www.tbcindia.nic.in/pdfs/TB%20India%202013.pdf Accessed 01.05.13.
15. Uplekar M, Juvekar S, Morankar S, Rangan S, Nunn P. Tuberculosis patients and practitioners in private clinics in India. *Int J Tuberc Lung Dis*. 1998;2:324–329.
16. Krishnan A, Kapoor SK. Involvement of private practitioners in Tuberculosis control in Ballabgarh, Northern India. *Int J Tuberc Lung Dis*. 2006;10(2):264–269.
17. Kapoor SK, Raman AV, Sachdeva KS, Satyanarayana S. How did the TB patients reach DOTS services in Delhi? A study of patient treatment seeking behavior. *PLoS ONE*. 2012;7(8):e42458. http://dx.doi.org/10.1371/journal.pone.0042458.
18. Singla N, Sharma PP, Singla R, Jain RC. Survey of knowledge, attitudes and practices for tuberculosis among general practitioners in Delhi, India. *Int J Tuberc Lung Dis*. 1998;2:384–389.
19. Abebe M, Doherty M, Wassie L, et al. TB case detection: can we remain passive while the process is active? *Pan Afr Med J*. 2012;11:50.
20. Powell KE, Farer LS. The rising age of the tuberculosis patient: a sign of success and failure. *J Infect Dis*. 1980;142(6):946–948.
21. WHO. *Gender and Tuberculosis. [Internet]*. 2003. Available from: http://www.who.int/gender/documents/en/TB.factsheet.pdf Accessed 21.05.13.
22. Hargreaves JR, Boccia D, Evans CA, Adato M, Petticrew M, Porter J. The social determinants of Tuberculosis: from evidence to action. *Am J Public Health*. 2011;101(4):654–662.

Available online at www.sciencedirect.com

ScienceDirect

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

Original Article

Predictors of adverse outcome in patients of tuberculous meningitis in a multi-centric study from India

Renu Gupta [a,*], *Suman Kushwaha* [b], *Rajeev Thakur* [c], *Nupur Jalan* [d], *Pumanshi Rawat* [e], *Piyush Gupta* [f], *Amitesh Aggarwal* [g], *Meena Gupta* [h], *Vikas Manchanda* [i]

[a] *Assistant Professor, Dept. of Microbiology, Institute of Human Behaviour and Allied Sciences (IHBAS), Dilshad Garden, Delhi 95, India*
[b] *Associate Professor, Dept. of Neurology, IHBAS, Delhi 95, India*
[c] *Prof and Head, Dept. of Microbiology, IHBAS, Delhi 95, India*
[d] *Senior Research Fellow, Dept. of Microbiology, IHBAS, Delhi 95, India*
[e] *Research Assistant, Dept. of Microbiology, IHBAS, Delhi 95, India*
[f] *Professor, Dept. of Pediatrics, University College of Medical Sciences, Dilshad Garden, Delhi 95, India*
[g] *Asst. Professor, Dept. of Medicine, University College of Medical Sciences, Dilshad Garden, Delhi 95, India*
[h] *Formerly - Prof and Head, GB Pant Hospital, Delhi, India; Presently - Senior Consultant, Paras Hospital, Gurgaon, Haryana 122002, India*
[i] *Formerly - Assistant Professor, Chacha Nehru Bal Chikitsalya, Delhi, India; Presently - Assistant Professor, Department of Microbiology, Maulana Azad Medical College, Delhi, India*

ARTICLE INFO

Article history:
Received 10 September 2016
Accepted 15 March 2017
Available online 13 June 2017

Keywords:
Adverse outcome
Mortality
Neurological sequelae
Predictors
Tuberculous meningitis

ABSTRACT

Introduction: This study aimed to investigate the factors which may predict mortality and neurological disability at one year follow up in patients of tuberculous meningitis (TBM) in India.
Methodology: Patients with TBM were prospectively enrolled from July 2012 to September 2014 from four tertiary care hospitals of Delhi. The demographic characteristics, clinical features and laboratory findings were collected and patients were followed up till 1 year. These were analyzed by univariate and multivariate multinomial logistic regression analysis to identify predictors of adverse patient outcome at 1 year follow up.
Results: Out of 478 patients enrolled, 391 patients could be followed up to 1 year. Sixty-four patients (16.3%) died and 150 patients (39%) survived with one or more neurological disability. Altered sensorium, motor deficit, cranial nerve palsy, seizures, isolation of *M. tuberculosis* and presence of multi-drug resistance were independently associated with any adverse outcome (death or disability) but by multivariate analysis only motor deficit, altered sensorium and isolation of *M. tuberculosis* on culture produced a statistically significant model for prediction of patient outcome.
Conclusion: The three-predictor model with motor deficit, altered sensorium and isolation of *M. tuberculosis* produced a statistically significant model with correct prediction rate of 60.4%. These three variables predicted death with odds ratio of 39.2, 6.7 and 2.1 respectively in comparison to recovery whereas only motor deficit and isolation of *M. tuberculosis* predicted neurological disability at 1 year with odds ratio of 3.9, 2.4 respectively.

* *Corresponding author*. Tel.: +91 9868396833.
E-mail address: renugoyal_123@yahoo.co.in (R. Gupta).

http://dx.doi.org/10.1016/j.ijtb.2017.03.001

1. Introduction

Tuberculous meningitis (TBM) is the most severe clinical manifestation of tuberculosis and leads to significant mortality and morbidity in spite of many advances in diagnosis and treatment modalities.[1] Timely initiation of chemotherapy and active management of complications of TBM has reduced the mortality rate but morbidity is still unacceptably high.[1,2] Though TBM is endemic in India but there is limited data on patient outcome after initiation of anti-tubercular drug treatment (ATT) and there are very few studies which have followed up the patients till treatment completion. British Medical Research Council (BMRC) staging for evaluation of disease severity is extensively used to predict patient outcome but is not built on multivariable approach.[3,10] It is still challenging to predict patient outcome on the basis of different clinical and laboratory parameters exhibited by the patients.[3–11] Some earlier studies have evaluated the association of different combinations of clinical, neuro-imaging and laboratory variables in limited number of patients (19–100) with prediction of disease outcome as either good or poor, death or recovery, neurological sequelae or no sequelae with clubbing of neurological sequelae in recovery or poor patient outcome depending upon scoring systems. The clubbing of neurological sequelae with either the recovery or death may lead to some degree of bias, e.g. patients with focal neurological deficit like optic atrophy cannot be clubbed with either recovery or death.[3,11]

This multicentric study aimed to analyze the demographic, clinical and laboratory variables in patients diagnosed as TBM on prediction of mortality and neurological sequelae separately at 1 year follow up by multinomial logistic regression technique so as to determine the effect of each predictor variable on the outcome with and without controlling for confounding.

2. Methodology

2.1. Settings

The patients for this study were prospectively recruited (purposive sampling) from Department of Neurology, Institute of Human Behaviour and Allied Sciences and GB Pant Hospital, Dept of Medicine and paediatrics, Guru Tegh Bhadur Hospital and Department of Paediatrics, Chacha Nehru Bal Chikitsalaya, Delhi, India from July 2012 to September 2014 after obtaining ethical approval from all the Institutes (IHBAS/ethics/2011/010, MAMC/(30)/2/2012/197, MAMC/(35)/1/2013/70, UCMS/2012/23/3). Informed written consent was obtained from all patients recruited in the study. All the diagnostic testing was done in Dept. of Microbiology, Institute of Human Behaviour and Allied Sciences, Delhi.

The consecutive patients diagnosed as TBM according to consensus TBM criteria of Marais et al. and decided for initiation of ATT were included in the study ($n = 520$).[12] The patients with absolute contraindications to lumbar puncture, with significant pre-existing neurological deficit, seizure disorder, mental retardation, cerebral palsy were not included in the study. A total of 42 patients were excluded later because of the reasons mentioned in Fig. 1.

2.2. Clinical evaluation and diagnosis

2.2.1. Clinical history

The history for duration of illness, fever, signs of meningeal irritation (headache vomiting, neck stiffness), altered sensorium and seizures was taken. All the patients were subjected to detailed neurological examination which included assessment of level of consciousness by Glassgow Coma Scale, signs of meningeal irritation, cranial nerve involvement, fundus examination, motor, sensory deficits and any other neurological signs. Screening was done to rule out the dissemination of tuberculosis to other parts of the body. All the clinical details were recorded in pre-designed performa.

All the patients were staged according to disease severity as per BMRC guidelines: Stage 1 included patients in prodromal phase with no definite neurological symptoms, Stage 2 included patients with signs of meningeal irritation with slight or no clouding of sensorium and minor (cranial nerve palsies) or no neurological deficit, Stage 3 included patients with severe clouding of sensorium, convulsions, focal neurological deficit and/or involuntary movements.[3]

Other medical details included history of past tuberculosis, contact with TB patients, human immuno deficiency virus (HIV) co infection and any other chronic illness.

2.2.2. Laboratory investigations

Besides routine laboratory investigations, lumbar puncture was done in all the clinically suspected patients and 2 ml of cerebro spinal fluid (CSF) was collected and subjected to cytology, biochemistry, smear microscopy, bacterial cultures ((BACTEC MGIT 960, Becton Dickinson, Sparks, MD, USA)) and conventional polymerase chain reaction (PCR) (IS6110 gene, PalmCycler, Genetix Biotech Asia Pvt. Ltd).[13]

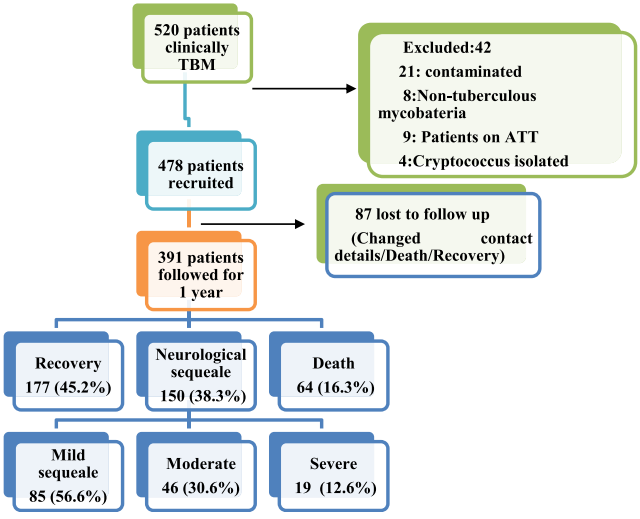


Fig. 1 – Flow chart of patients recruited in the study with clinical outcome.

2.2.3. *Neuroimaging*

Magnetic resonance imaging (MRI) brain/contrast enhanced computerized tomography (CT) head was done in all patients. CT chest and abdomen was done in selected patients with clinical suspicion of dissemination of tuberculosis.

2.2.4. *Management*

All the included patients were admitted, managed for complications and treated by daily treatment regimen of anti-tubercular drugs as per standard treatment guidelines.[14,15]

2.2.5. *Monitoring and follow up*

All the patients were followed up once a month for 1 year. During follow up, patients were clinically evaluated and liver function test of all the patients were done to see any side effects due to ATT. MRI/CT scan was done only if it was essential for clinical management. Response to treatment was judged by improvement in sensorium, neurological disability and constitutional symptoms like fever, headache, appetite, weight. Patient outcome was recorded as complete recovery, with neurological sequelae (if presence of altered sensorium, cranial nerve palsy, extrapyramidal movements, focal neurological deficit, mental retardation, optic atrophy, and/or tone abnormalities) or death.

2.3. *Statistical analysis*

Patient outcome were grouped into three categories as complete recovery, neurological sequelae, death and was analyzed for significance at 1 year follow up. Loss to follow up was adjusted by increasing the study period for enrolment of patients for further three months. The study was as per protocol analysis and all those patients who were lost to follow up were not included in analysis. The data was analyzed by the SPSS software version 21 (SPSS Inc., Chicago, IL, USA) Quantitative data was analyzed using mean and ranges and 95% confidence intervals (CI). Qualitative data was expressed as proportion of total number of patients. All the independent variables were analyzed by both chi square and trend chi square test (Pearson's' linear by linear in SPSS). $p < 0.05$ was considered statistically significant. All the significant variables were analyzed independently as well as in blocks by multinomial logistic regression (forward step wise) to predict the best model for adverse patient outcome.[16]

3. Results

A total of 520 patients were recruited in this study for management of TBM, 42 patients were excluded for the reasons given in Fig. 1 leaving 478 patients. Out of these only 391 patients could be followed up till 1 year and out of these 64 patients (16.4%) died, 150 had any neurological sequelae (38.3%) and 177 (45.2%) patients had complete recovery (Fig. 1).

The details for demographic, clinical features and laboratory results in relation to patient outcome along with significance of association are shown in Table 1 in numbers and percentages.

The independent multinomial logistic regression analysis of all the significant variables with patient's recovery, any neurological deficit and death showed altered sensorium, motor deficit, seizures and isolation of multi-drug resistant (MDR) *M. tuberculosis* were independent predictors of death as compared to recovery whereas motor deficit, cranial nerve palsy, seizures and isolation of *M. tuberculosis* on culture were independent predictors of neurological disability at 1 year compared to recovery (Table 2).

The multinomial logistic regression of all significant predictors together, revealed that the three-predictor model with altered sensorium, motor deficit and isolation of *M. tuberculosis* produced a statistically significant improvement over the constant only model, $\chi^2(6, N = 391) = 141.65, p = <0.001$ with −2 log likelihood ratio of 62.7 and correct prediction rate of 60.4%. The parameter estimate table shows the logistic coefficient for each predictor variable for death and neurological squeal in reference to recovery (Table 3). Motor deficit, altered sensorium and culture isolation of *M. tuberculosis* played a statistically significant role in predicting death with respect to recovery. A TBM patient with presence of motor deficit was 40 times more likely to die than complete recovery keeping other factors constant and patient with altered sensorium was 6.7 times more likely to die than completely recovery. Isolation of *M. tuberculosis* increased the likelihood of death to twice as compared to recovery. For prediction of neurological deficit vs. recovery both presence of motor deficits and positive culture increased the likelihood of neurological disability to 4, 2 times as compared to recovery keeping other factors constant.

4. Discussion

This study is unique as prediction of patient outcome was assessed as death in reference to recovery and neurological sequelae in reference to recovery by multinomial logistic regression analysis in a cohort of 391 patients who could complete follow up till 1 year without clubbing mild neurological sequelae in recovery group and severe sequelae with death. The mortality rate in our study was 16.3% and one or more neurological disability was present in 39% of patients at one year follow up. The earlier published studies have reported mortality rates varying from 15% to 60% and neurological disability of 27–50% but all these studies have treated patients with directly observed thrice a week regimen treatment regimen and have given mortality and morbidity rates at different point of times either at discharge, 6 weeks post treatment, at 6 months or 1 year follow up making it difficult to directly compare these studies with the present study.[3–11] The relative lower mortality in this study could be due to efficient early diagnosis, initiation of treatment in the right time frame, daily treatment regimen, availability of dedicated Intensive care units and neurosurgical expertise at one centre and regular follow up.[3,4,9,10]

In this study majority of patients presented in stage 3 disease which may be due to delayed access to tertiary care hospitals and poor health seeking behaviour in developing countries which is similar to studies from other developing countries.[4,9–11] Presence of motor deficit, seizures, altered

Table 1 – Analysis of socio-demographic, clinical and laboratory parameters with patient outcome.

Parameter (no. positive)	Death N (%)	Neurological sequelae N (%)	Recovery N (%)	Pearson's p value	Linear by linear association (trend χ^2)
	N = 64	N = 150	N = 177		
Age (Mean: 27.8 ± 17.27 years)					
<18 year (n = 138)	29 (45.3)	42 (28)	68 (38.4)	0.369	0.993
>18 year (n = 253)	35 (54.7)	108 (72)	109 (61.6)	0.300	0.866
Males	28 (43.7)	62 (41.3)	93 (52.5)	0.112	0.093
Females	36 (56.2)	88 (58.5)	84 (47.4)		
Clinical features					
HIV positivity (n = 12)	3 (4.6)	2 (1.3)	7 (3.9)	0.280	0.831
Contact with TB person (n = 105)	16 (25)	43 (28.6)	46 (25.9)	0.270	0.190
Past history of TB (n = 58)	8 (12.5)	26 (17.3)	24 (13.5)	0.380	0.327
Duration of illness (Mean 42.7 ± 62.3 days)					
0–7 days (n = 100)	24 (37.5)	30 (20)	47 (26.5)	0.047	0.328
8-30days (n = 167)	23 (35.9)	67 (44.6)	77 (43.5)	0.417	0.422
30 and above (n = 123)	17 (26.5)	53 (36)	53 (29.9)	0.378	0.946
Disease stage (3 vs. 2 + 1) (n = 306)	63 (98.4)	125 (83.3)	118 (66.6)	<0.001	<0.001
Fever (n = 304)	46 (71.8)	114 (76)	144 (81.3)	0.238	0.092
Meningeal irritation (n = 249)	36 (56.2)	95 (63.3)	118 (66.6)	0.330	0.149
Extra meningeal tuberculosis (n = 65)	14 (21.9)	25 (16.6)	26 (14.6)	0.417	0.523
Altered sensorium (n = 197)	49 (76.5)	68 (45.3)	80 (45.1)	<0.001	<0.001
New onset seizure (n = 147)	34 (53.1)	77 (51.3)	36 (20.33)	<0.001	<0.001
Neurological deficit (n = 150)	60 (93.7)	59 (39.3)	31 (17.5)	<0.001	<0.001
Motor deficit (n = 120)	50 (78.1)	49 (32.6)	21 (11.8)	<0.001	<0.001
Cranial nerve palsy (n = 92)	10 (6.4)	48 (32)	34 (19.2)	0.007	0.673
CSF findings					
TLC: 100–500 (n = 144)	20 (31.2)	60 (40)	64 (36.1)	0.463	0.732
500 and above (n = 65)	12 (18.7)	24 (16)	29 (16.3)	0.879	0.740
Lymphocyte > 50% (n = 324)	52 (81.2)	126 (84)	146 (82.4)	0.873	0.947
Protein > 100 mg/dl (n = 251)	39 (60.1)	97 (64.6)	115 (64.9)	0.735	0.831
Sugar < 50 mg/dl (n = 218)	38 (59.3)	83 (55.3)	97 (54.8)	0.812	0.577
Microbiological findings					
Smear positivity (n = 23)	4 (6.3)	10 (6.7)	9 (5.1)	0.825	0.628
Culture positive (n = 170)	29 (45.3)	81 (54)	60 (33.8)	<0.001	0.011
PCR positive (n = 283)	46 (71.8)	118 (78.6)	119 (67.2)	0.070	0.174
Confirmed diagnosis (287)	46 (71.8)	120 (80)	121 (68.3)	0.057	0.214
MDR (n = 9)	6 (9.3)	1 (0.6)	2 (1.1)	<0.001	0.006
Only INH resistant (n = 24)	3 (4.6)	14 (9.3)	7 (3.9)	0.520	0.906

Table 2 – Univariate multinomial logistic regression for significant predictors.

Parameter	Death vs. recovery		Neurological sequelae vs. recovery	
	p value	Exp(B) (95% CI)	p value	Exp(B)
Altered sensorium	<0.001	3.96 (2.0–7.5)	0.980	1.005 (0.650–1.5)
Motor deficit	<0.001	26.53 (12.6–56.0)	<0.001	3.604 (2–6.3)
Cranial nerve palsy	0.525	0.78 (0.36–1.6)	0.008	1.979 (1.2–3.2)
Seizures	<0.001	4.43 (2.4–8.1)	<0.001	4.1 (2.5–6.7)
Culture positive	0.106	1.616 (0.90–2.80)	<0.001	2.289 (1.5–3.5)
MDR	0.018	7.6 (1.4–40.2)	0.412	0.362 (0.032–4.0)

$p < 0.05$ significant; Exp(B): odds ratio.

sensorium and isolation of MDR *M. tuberculosis* were found to be independent risk factors for death whereas motor deficit, seizures, cranial nerve palsy and positive *M. tuberculosis* culture were independently associated with neurological disability. Motor deficit increased likelihood of death to 26 times and for neurological disability to 3.6 times as compared to recovery. New onset Seizures were associated with fourfold risk of death as well as neurological disability. Previous studies have also reported that motor deficit and seizures are markers of severe intra cerebral damage and are much more frequently associated with death or disability.[3,10,11,17–19] Altered sensorium was found as a significant independent risk factor for death (odds ratio (OR): 3.9) but not for neurological disability. Earlier studies have also reported impairment of sensorium as an important determinant of death.[3,10–12] Cranial nerve palsies were significantly associated with twofold risk of persistent

Table 3 – The parameter estimates for death and neurological sequelae in reference to recovery by multivariate multinomial logistic regression.

Parameter		*B*	Std. Error	Wald	Sig.	Exp(*B*)	95% confidence interval for Exp(*B*)	
							Lower bound	Upper bound
Death	Intercept	−4.074	0.481	71.760	<0.001			
	Culture positive	0.765	0.366	4.365	0.037	2.14	1.048	4.401
	Motor deficit	3.671	0.418	77.085	<0.001	39.28	17.313	89.157
	Altered sensorium	1.903	0.397	23.005	<0.001	6.70	3.081	14.589
Neurological sequelae	Intercept	−0.896	0.206	18.970	<0.001			
	Culture positive	0.887	0.236	14.085	<0.001	2.42	1.528	3.858
	Motor deficit	1.367	0.300	20.773	<0.001	3.92	2.179	7.062
	Altered sensorium	0.118	0.237	0.247	0.619	1.12	0.707	1.791

The reference category is recovery; Exp(*B*): odds ratio.
$R^2 = 0.35$ (Nagelkerke). Model $\chi^2(6) = 141.65$, $p < 0.001$.

neurological impairment rather than death which was quite similar to findings of Hosoglu et al. and Misra et al. who also showed cranial nerve palsy is associated with threefold risk of neurological deficit.[11,12] Confirmed diagnosis of TBM has been shown to be a predictor of adverse patient outcome in earlier studies but in the present study confirmed diagnosis by microscopy and/or culture and/or PCR did not emerge as a significant contributor to death or neurological sequelae.[18] However, patients who were positive for *M. tuberculosis* culture had twofold higher chances of residual neurological disability than recovery.

Infection with MDR *M. tuberculosis* has been shown to be a strong predictor of death due to slow or non-clearance of MDR organisms from CSF.[20] In this study isolation of MDR *M. tuberculosis* was independently associated with risk of death as 6 out of 9 patients with MDR died within 2 months of initiation of first line ATT. Out of these 5 patients died before the availability of drug susceptibility test results implying the urgent need of early detection of drug resistant strains. One patient died after 1 months of initiating second line drug treatment. Mono resistance to Isoniazid (INH) did not increase the risk of adverse patient outcome in the present study and earlier published studies have also shown conflicting association of mono resistance to INH with poor patient outcome.[20,21]

Age, duration of illness, previous history of tuberculosis, contact with TB patients and presence of extra meningeal tuberculosis, HIV positive serology, hydrocephalus, protein >100 mg%, absence of headache at presentation, presence of brain infarcts were not found to be significant predictors for either death or neurological sequelae in this study in contrast to some earlier published studies.[8,11,21–24]

In multiple multinomial logistic regression, altered sensorium, motor deficit and isolation of *M. tuberculosis* produced a significant model for prediction of patient outcome. Presence of motor deficit (OR: 39.2), altered sensorium (OR: 6.7) and culture isolation (OR: 2.1) had a statistically significant relationship to distinguish death from recovery and presence of motor deficit and isolation of *M. tuberculosis* in culture increased the likelihood of neurological disability to 4, 2 times as compared to recovery. Presence of seizures and isolation of MDR *M. tuberculosis* were not included in final model as seizures displayed co linearity with altered sensorium and inclusion of MDR *M. tuberculosis* and cranial nerve palsy did not contribute to any improvement in significance.

There were two main limitations of this study: (1) The loss to follow up was 18% (statistically acceptable range, 5–20%) and could not be avoided due to inherent nature of the disease which requires long, frequent follow up and partly because of lower socio economic strata of patients who present to public health facilities for treatment finding it difficult to afford repeated visits due to long distance travel/loss of wages or some other reasons. We could not contact these patients due to change of contact details furnished during admission. We tried to adjust for this loss by doing as per protocol analysis rather than intention to treat analysis and increasing the duration of study for 3 months (initially study was planned for 2 years) to recruit more number of patients. (2) Out of 520 CSF samples collected from 4 different Institutes, 21 samples grew non-significant pathogens like, Yeast cells, diphtheroids, Gram negative bacteria in pure or mixed growth suggestive of some extraneous contamination. Ideally, this should not have happened but it reality it is impossible to avoid this problem of contamination due to some pre-analytical problems involving sample collection, transport or processing. A repeat lumber puncture could not be obtained for analysis so these patients were excluded from the study.

5. Conclusion

Our findings suggest that presence of motor deficit, seizures, altered sensorium and isolation of MDR *M. tuberculosis* are independent risk factors for death whereas motor deficit, seizures, cranial nerve palsy and positive *M. tuberculosis* culture are independently associated with neurological disability at 1 year. By controlling the effect of variable which can act as confounders we found that the three-predictor model with altered sensorium, motor deficit, and culture isolation of *M. tuberculosis* produced a statistically significant model with correct prediction rate of 60.4%. Presence of motor deficit, altered sensorium and culture positivity emerged as significant predictors of death in comparison to recovery whereas presence of motor deficit and culture positivity predicted neurological disability at 1 year.

Conflicts of interest

The authors have none to declare.

Acknowledgments

We are thankful to Indian Council of Medical Research for providing financial support for this work. We also acknowledge the support of all lab personnel of the Dept. of Microbiology, IHBAS for providing support in carrying out the study. We acknowledge Dr. Sarbjeet Khurana, Associate professor Epidemiology for critically reviewing and providing inputs.

REFERENCES

1. Marx GE, Chan ED. Tuberculous meningitis: diagnosis and treatment overview. *Tuberc Res Treat*. 2011;24:1–9.
2. Schoeman J, Wait J, Burger M, et al. Long-term follow up of childhood tuberculous meningitis. *Dev Med Child Neurol*. 2002;44:522–526.
3. Alarcon F, Moreira J, Rivera J, Salinas R, Duenas G, Ende JV. Tuberculous meningitis: do modern diagnostic tools offer better prognosis prediction? *Indian J Tuberc*. 2013;60:5–14.
4. Yasri S, Wiwanitkit V. Tuberculous meningitis on directly observed thrice a week regime. *Ann Indian Acad Neurol*. 2015;18:129.
5. Iype T, Pillai AK, Cherian A, et al. Major outcomes of patients with tuberculous meningitis on directly observed thrice a week regime. *Ann Indian Acad Neurol*. 2014;17:281–286.
6. Sharma SR, Lynrah KG, Sharm N, Lyngdoh M. Directly observed treatment, short course in tuberculous meningitis: Indian perspective. *Ann Indian Acad Neurol*. 2013;16:82–84.
7. Dewan P, Chadha TK, Kaur IR, Gupta P. Early outcome of intermittent directly observed treatment-short course, for tuberculous meningitis in children: a descriptive analysis. *Astrocyte*. 2014;1:3–8.
8. Kaur H, Sharma K, Modi M, et al. Prospective analysis of 55 cases of tuberculosis meningitis (TBM) in North India. *J Clin Diagn Res*. 2015;9:15–19.
9. Kalita J, Misra UK. Outcome of tuberculous meningitis at 6 and 12 months: a multiple regression analysis. *Int J Tuberc Lung Dis*. 1999;3(3):261–265.
10. Hosoglu S, Geyik MF, Balik I, et al. Predictors of outcome in patients with tuberculous meningitis. *Int J Tuberc Lung Dis*. 2002;6(1):64–70.
11. Misra UK, Kalita J, Roy AK, Mandal SK, Srivastava M. Role of clinical, radiological, and neurophysiological changes in predicting the outcome of tuberculous meningitis: a multivariable analysis. *J Neurol Neurosurg Psychiatry*. 2000;68:300–303.
12. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: A uniform case definition for use in clinical research. *Lancet Infect Dis*. 2010;10:803–812.
13. Mycobacteria.Winn Jr W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, eds. et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology* 6th ed. Philadelphia: Lippincott, Williams & Willkins; 2006:1064–1124.
14. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect*. 2009;59:167–187.
15. Thwaites. Guy E. The diagnosis and management of tuberculous meningitis. *Pract Neurol*. 2002;2(5):250–261.
16. Conduct and Interpret a Multinomial Logistic Regression Statistics. Available at www.statisticssolutions.com/mlr/ Accessed 23.8.15.
17. Hsu P-C, Yang C-C, Ye J-J, Huang P-Y, Chiang P-C, Lee M-H. Prognostic factors of tuberculous meningitis in adults: a 6-year retrospective study at a tertiary hospital in northern Taiwan. *J Microbiol Immunol Infect*. 2010;43(2):111–118.
18. Jin G, Xiao H, Wu F, et al. Prognostic factors of tuberculous meningitis: a single centre study. *Int J Clin Exp Med*. 2015;8(3):4487–4493.
19. Lu CH, Chang WN, Chang HW. The prognostic factors of adult tuberculous meningitis. *Infection*. 2001;29:299–304.
20. Thwaites GE, Lan NT, Dung NH, et al. Effect of antituberculosis drug resistance on response to treatment and outcome in adults with tuberculous meningitis. *J Infect Dis*. 2005;192:79–88.
21. Vinnard C, Winston CA, Wileyto EP, MacGregor RR, Bisson GP. Isoniazid-resistant tuberculous meningitis, United States, 1993–2005. *Emerg Infect Dis*. 2011;17(3):539–542.
22. Bandyopadhyay SK, Bandyopadhyay R, Dutta A. Profile of tuberculous meningitis with or without HIV infection and the predicators of adverse outcome. *West Indian Med J*. 2009;58(6):589–592.
23. Croda MG, Vidal JE, Hernandez A, Dal Molin T, Gualberto FA, Penalva de Oliveira A. Tuberculous meningitis in HIV-infected patients in Brazil: clinical and laboratory characteristics and factors associated with mortality. *Int J Infect Dis*. 2010;14:e586–e591.
24. Karande S, Gupta V, Kulkarni M, Joshi A, Rele M. Tuberculous meningitis and HIV. *Indian J Pediatr*. 2005;72:755–760.

Available online at www.sciencedirect.com

ScienceDirect

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

Original Article

Predominance of Central Asian and European families among *Mycobacterium tuberculosis* isolates in Kashmir Valley, India

Gulnaz Bashir [a,*], Tehmeena Wani [a], Pragya Sharma [b], V.M. Katoch [c,d], Rubina Lone [e], Azra Shah [f], Kiran Katoch [b], D.K. Kakru [a], Devendra Singh Chauhan [b]

[a] Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, India
[b] Department of Microbiology and Molecular Biology, NJIL& OMD (ICMR), Tajganj, Agra, India
[c] NASI-ICMR Chair on Public Health Research, Rajasthan University of Health Sciences, Jaipur, India
[d] Former Secretary, Former Director General, Department of Health Research (Ministry of Health and Family Welfare), Indian Council of Medical Research, New Delhi, India
[e] Department of Microbiology, SKIMS Medical College, Bemina, Srinagar, India
[f] Department of Pathology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, India

ARTICLE INFO

Article history:
Received 8 November 2016
Accepted 11 May 2017
Available online 17 May 2017

Keywords:
Mycobacterium tuberculosis
Drug susceptibility testing
Spoligotyping
MIRU-VNTR
Kashmir Valley

ABSTRACT

Background: As there are no data available regarding the strains of *Mycobacterium tuberculosis* circulating in Kashmir Valley, India, the current study aimed at describing the genetic diversity of *M. tuberculosis* strains in this region, by spoligotyping and 12-locus-based MIRU-VNTR typing (Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat).
Methods: Sputa from 207 smear positive cases with newly diagnosed pulmonary tuberculosis were subjected to culture for *M. tuberculosis*. Eighty-five isolates confirmed as *M. tuberculosis* were subjected to drug susceptibility testing and molecular typing by spoligotyping and MIRU-VNTRs.
Results: Drug susceptibility results of 72 isolates revealed 76.3% as fully sensitive while 5.5% as multidrug resistant (MDR). Spoligotyping of 85 isolates detected 42 spoligotypes with 50 isolates (58.8%) clustered into seven spoligotypes. SIT26/CAS1_Del was the major spoligotype (23, 27%) followed by SIT127/H4 (12, 14.1%); CAS lineage (37.6%) was predominant, followed by Haarlem (25.8%) and ill-defined T clade (23.5%). MIRU-VNTR analysis displayed 82 MIRU patterns from 85 strains, including 3 small clusters and 79 unique. MIRU 26 was found to be the most discriminatory locus.
Conclusions: Kashmir Valley has CAS as the predominant lineage of *M. tuberculosis* similar to the rest of the Indian sub-continent, while it is peculiar in having Euro American lineages such as Haarlem and ill-defined T clade.

* *Corresponding author at*: Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, J&K, India. Tel.: +91 01942401013x2163; mob: +91 09419081260.
E-mail address: gulnaz.bashir@skims.ac.in (G. Bashir).

http://dx.doi.org/10.1016/j.ijtb.2017.05.004

1. Introduction

Tuberculosis (TB) is a disease of antiquity and continues to remain a major public health problem in the developing world. Globally, in 2015 there were an estimated 10.4 million incident cases of TB with 1.8 million TB deaths. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa had the largest numbers of cases. India, with the incidence of 2.8 million has the distinction of being a country with the highest burden of TB disease in the world.[1] Despite the high TB burden in India, there are limited data available pertaining to the epidemiological typing of the strains circulating in various parts of the country. The situation is further complicated by the lack of available genotypic epidemiological tools that would allow contact tracing and identification of transmission patterns within the country.

In the past, drug susceptibility tests (DSTs) and phage typing were the only biomarkers available for epidemiological studies of *Mycobacterium tuberculosis*, and both of which have serious limitations. Drug susceptibility results can change in a strain as it acquires resistance to one or more antimicrobials during treatment. Bacteriophage typing of *M. tuberculosis* is of limited value for epidemiologic studies as only a few phage types can be recognized and thus, it cannot adequately distinguish between different strains.[2]

Nucleic acid-based genotyping methods based on the variations in sequence in bacterial genomes allow us to accurately distinguish between different strains of *M. tuberculosis*. The most widely used methods of genotyping employ the IS*6110* element to fingerprint strains. However, IS*6110* fingerprinting is of limited use because a significant proportion (40–44%) of *M. tuberculosis* isolates from several regions of India have been reported to either lack or have low copy numbers of IS*6110*.[3] Spacer oligotyping (spoligotyping) is a polymerase chain reaction (PCR)-based fingerprinting method that detects the presence or absence of 43 defined spacers (36–41 bp) in the direct-repeat (DR) sequences in the genomes of the members of the *M. tuberculosis* Complex (MTC).[4] Although, less discriminatory than IS*6110* RFLP typing, spoligotyping is more rapid and easier to perform. In addition, it has been demonstrated that the results are highly reproducible.[5] A high resolution typing method based on the Variable Number Tandem Repeats (VNTRs) of Mycobacterial Interspersed Repetitive Units (MIRUs) based on 12 specific intergenic regions of the *M. tuberculosis* genome has been proposed.[6] With a discriminatory power close to IS*6110* fingerprinting, MIRU-VNTR is a PCR-based method that differentiates between strains by identifying the number and length of exact tandem repeats present in an isolate, independently of the IS*6110* polymorphisms.[7]

Although some studies have been conducted in other parts of India, nothing is known about the genetic diversity of *M. tuberculosis* in Kashmir Valley till date. The Kashmir Valley, belonging to the state of Jammu and Kashmir, is spread over 16,000 km^2 and consists of 10 districts (Fig. 1). It is culturally, geographically and climatically distinct from the rest of India. Hence, there is a need to know the prevalence of various genotypes in this region. The current study aimed at describing, along with the pattern of drug resistance the genetic diversity of *M. tuberculosis* strains isolated from cases of pulmonary TB, in Kashmir Valley.

2. Material and methods

2.1. Study population

The study was conducted in the department of Microbiology, SKIMS in collaboration with the National JALMA Institute for Leprosy and other Mycobacterial Diseases (NJIL & OMD) Agra, where molecular typing was done. A total of 300 sputum samples from pulmonary TB cases belonging to Cat I – previously untreated cases were collected during 3 years, from May 2007 till May 2010. These patients attended the Designated Microscopy Centres (DMCs) of various District Tuberculosis Centres of Kashmir. Consent was taken from all

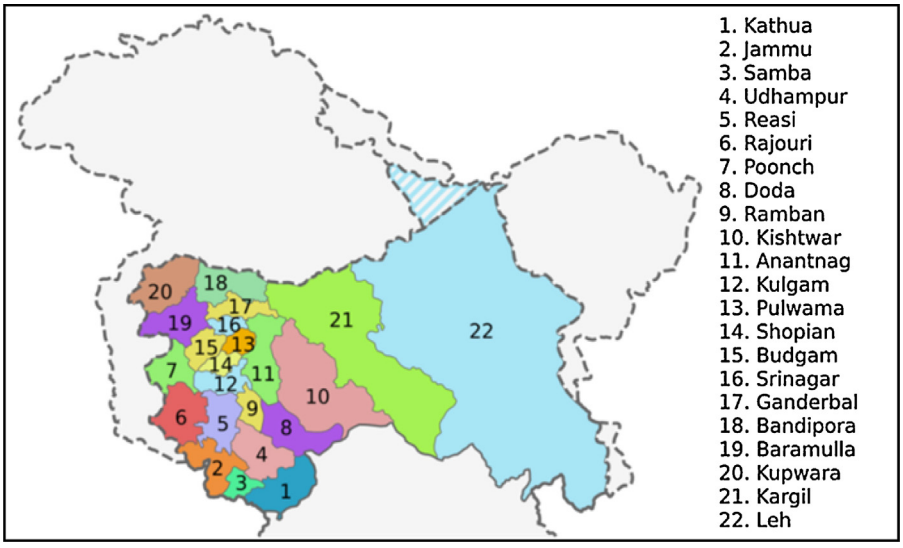


Fig. 1 – J&K is divided into 3 divisions; Jammu with 10 districts (1–10), Kashmir Valley with 10 districts (11–20) and Ladakh with 2 districts (21–22).

the patients and the study was approved by the Institute's ethics committee. Due to lack of systematic sampling in our study, it is difficult to interpret our study sample in terms of representative of the whole region; hence it is arbitrarily designated as a 'convenience sample.'

2.2. Culture and DST

The sputum samples which were positive on smear microscopy were transported in equal volume of 1% cetylpyridinium chloride (CPC)[8] to the Mycobacteriology laboratory of SKIMS where after decontamination[8] they were subjected to repeated Ziehl Neelsen staining and culture for AFB. Culture was done on two bottles of Lowenstein Jensen (LJ) media with glycerol and one bottle of LJ medium with sodium pyruvate, which were incubated at 37 °C and read daily for the first week to detect any non-tubercular mycobacterium and then weekly for 8 weeks. No growth after 8 weeks of incubation was treated as negative.

2.3. Identification of M. tuberculosis

Phenotypic methods viz.; colony morphology, growth rate, pigmentation properties, standard biochemical tests viz. niacin, nitrate reduction, catalase at 68 °C, tween 80 hydrolysis and growth on LJ with PNB (500 μg/ml) were used for the identification of *M. tuberculosis*.[9]

2.4. DSTs

Species confirmation was followed by drug susceptibility testing against rifampicin (RIF, 40.0 μg/ml), ethambutol (ETM, 2.0 μg/ml), streptomycin (STM, 4.0 μg/ml), and isoniazid (INH, 0.2 μg/ml) by using the standard 1% proportion method on LJ.[10] MDR was defined as an isolate at least resistant to INH and RIF. Isolates were preserved on LJ medium at 4 °C and in glycerol at −20 °C.

2.5. Genotyping of M. tuberculosis isolates by spoligotyping and MIRU-VNTR

All genotyping methods were performed at NJIL & OMD, Agra in two batches in 2007 and 2011. DNA extraction was done by suspending a loopful of bacterial colonies in 400 μl 1× Tris–(ethylene diamine tetra acetic acid) buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and inactivated at 80 °C for 20 min. These were then snap chilled in ice. Bacterial DNA was extracted by the standard cetyl-trimethyl ammonium bromide method.[11] The pellet of DNA was dried at room temperature, suspended in 1× TE buffer and stored at −20 °C until further use.

2.5.1. Spoligotyping

Spoligotyping was performed by using commercially available Kit (Isogen Bioscience, BV, Maarssen, The Netherlands) as described by Kamerbeek et al.[4] and according to the manufacturer's instructions. The 43 spacers between the DRs in the target region were amplified by using DRa biotinylated at the 5′ end and DRb primers. The PCR product was hybridized to a membrane containing 43 oligonucleotides derived from the spacer sequences of *M. tuberculosis* H37Rv and *M. bovis* BCG P3 by reverse line blotting. *M. tuberculosis* H37Rv and *M. bovis* BCG P3 were used as positive control for spoligotyping and autoclaved purified water was used as a negative control.

Spoligotyping results obtained were entered in a binary format and were converted into octal code and analyzed by using the SITVIT2 proprietary database of the Pasteur Institute of Guadeloupe, which is an updated version of the previously released SpolDB4 database (available at http://www.pasteurguadeloupe. fr:8081/SITVIT Demo). At the time of comparison, it contained genotyping data on more than 100,000 MTC strains isolated from 160 countries of patient origin.

2.5.2. MIRU-VNTR genotyping

MIRU-VNTR genotyping was performed by amplifying 12 hyper variable MIRU Loci (MIRU 2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39 and 40) described by Supply et al.[12] PCR mixture was prepared using the Hot start Taq DNA Polymerase kit (Qiagen, Hilden, Germany). PCR amplicons were subjected to electrophoresis on 2% agarose gel with 100 bp DNA ladder. The number of repeats was calculated by using the 'Quantity One (4.4.0)' gel documentation software from BIO-RAD. The allelic diversity of the strains was determined by using the Hunter Gaston Discriminatory Index (HGDI).[13]

2.6. Phylogenetic analysis

The evolutionary relationships among all the observed spoligotypes were studied by drawing phylogenetic tree (Fig. 2) with the tools available at www.miru-vntr.plus.org.

3. Results

3.1. Studied population

Of the 300 samples, 93 samples in various phases of incubation were lost due to faulty incubation, hence were not included in the analysis. Of the 207 samples, 123 were confirmed as *M. tuberculosis* by the phenotypic methods. Of these 123 *M. tuberculosis* strains, 85 were included in the study as both spoligotyping and MIRU-VNTR results were available for them, out of which DST results were available for 72 only. Age of these 85 patients ranged between 10–90 years with a mean age of 50 years. Of these 85 patients, 39.58% belonged to 15–30 year age group. The majority of these patients (67.70%) were in the 15–45 year age group, compared with 26.04% in the >45 year age group. Males predominated with a male-to-female ratio of 1.23. Of the 10 districts sampled, 20 patients belonged to Srinagar district followed by 19 from Anantnag, 17 from Baramulla, 8 from Kupwara, 5 from Kulgam, 4 each from Pulwama, Budgam, Shopian and 2 each from Bandipora and Ganderbal.

3.2. Drug resistance patterns

Of the 85 *M. tuberculosis* isolates susceptibility results to the four first line drugs (Table 1) were available only in 72 as 11 isolates were lost due to contamination. Of the 72 isolates, 55 (76.3%) were fully sensitive, 8 (11.1%) were monoresistant to

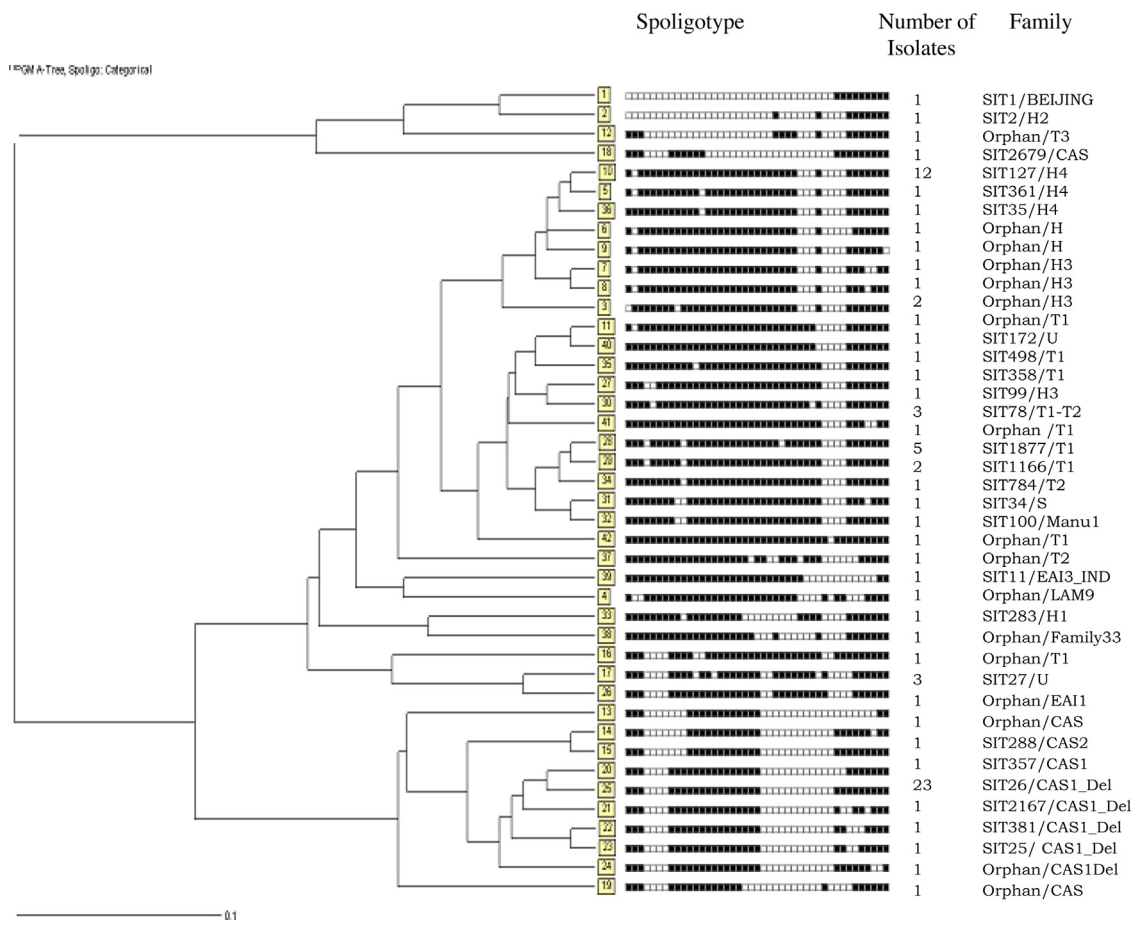


Fig. 2 – Dendrogram based on 42 spoligopatterns of 85 *M. tuberculosis* isolates from Kashmir Valley, India.

STM and 2(2.7%) each were monoresistant to INH and RIF. Four (5.5%) were MDR, 1(1.4%) was resistant to INH and STM.

3.3. *Distribution of phylogenetic clades*

Results of spoligotyping were available for 85 isolates (Fig. 2). Comparison of the results with STVIT web database showed that these isolates belonged to 42 spoligotypes and fifty isolates (60%) were clustered into seven spoligotypes. SIT26/CAS1_Del was the major spoligotype (23, 27%) followed by SIT127/H4 (12, 14.1%), SIT1877/T1 (5, 5.8%), SIT27/U, SIT78/T1-T2 (3, 3.53% each), SIT1166/T1 (2, 2.3%) and Orphan/H3 (2, 2.3%). Sixteen isolates were found to be orphan, 6 belonged to T family, 4 to Haarlem, 3 to CAS, 1 each to EAI1, Family 33, LAM 9. Two MDR strains belonged to SIT26/CAS1_Del while one each belonged to SIT127/H4 and SIT498/T1.

These 85 *M. tuberculosis* isolates were distributed into eight families. These include, in decreasing order: CAS 37.6%, Haarlem 25.8%, ill-defined T clade 23.5%, Ural 4.7%, EAI 2.3%, S, Manu, Beijing 1.2% each, 2.5% others. The single Beijing strain was isolated from a patient belonging to the Anantnag district of Kashmir Valley.

The MIRU-VNTR analysis detected a total of 82 MIRU-VNTR patterns from 85 strains using 12 MIRU-VNTR locus set,

Table 1 – Distribution of spoligotypes in different drug susceptibility profiles of *M. tuberculosis* (*n* = 72) from Kashmir Valley.

Resistance patterns	No. of isolates	No. of isolates (%) by genotypes						
		CAS	Haarlem	T	Ural	EAI	S	Others
Monoresistance								
INH	2	1/72 (1.4)	1/72 (1.4)					
STM	8	2/72 (2.7)	4/72 (5.5)	1/72 (1.4)				1/72 (1.4)
RIF	2		2/72 (2.7)					
MDR	4	2/72 (2.7)	1/72 (1.4)	1/72 (1.4)				
Resistance to INH + STM	1		1/72 (1.4)					
Susceptible to all	55	23/72 (32)	10/72 (13.8)	14/72 (19.4)	3/72 (4.2)	2/72 (2.7)	1/72 (1.4)	2/72 (2.7)

INH: isoniazid, STM: streptomycin, RIF: rifampicin, MDR: multidrug resistant.

including 3 small clusters and 79 unique. Allelic diversity for each locus was calculated in order to determine the discriminatory power of these loci in a combined group for the *M. tuberculosis* population studied. Based on their discriminatory index (HGDI), 5 loci (MIRU02, MIRU04, MIRU20, MIRU23, and MIRU24) showed poor discriminatory power (HGDI < 0.3). Three loci (MIRU 27, MIRU 39 and MIRU 40) discriminated the isolates moderately ($0.3 \leq$ HGDI ≤ 0.6). Lastly, 4 loci (MIRU 10, MIRU 16, MIRU 26 and MIRU31) were highly discriminating (HGDI > 0.6). In this study, MIRU 26 was found to be the most discriminatory locus in order to differentiate between strains (HGDI of 0.843). Conversely, locus MIRU-24 was found to be the least discriminatory with an HGDI of 0.026. MIRU 26 exhibited the maximum variability with 12 alleles. Other loci MIRU 10 and 31 were moderately variable with 8 alleles, MIRU 4 and 16 with 6 alleles, MIRU 23 and 40 with 5 alleles, MIRU 39 with 4 alleles, MIRU27 and MIRU 39 with 3 alleles respectively whereas MIRU 20 and 24 were least variable with 2 alleles.

In comparison to 7 spoligotypes clustering 50 *M. tuberculosis* isolates, MIRU-VNTR typing generated 47 profiles. MIRU-VNTR grouped six isolates into three clusters, including two isolates in each group. Isolates from each group shared identical spoligotype (SIT127/H4, SIT1877/T1, SIT26/CAS1_Del). Only four isolates, two each from SIT26 and SIT127 showed similarity at 11 MIRU Loci. The rest of the isolates showed variation at two or more loci. 12-Locus MIRU-VNTR typing showed greater resolving power as compared to spoligotyping.

4. Discussion

This report, first of its kind, from the Kashmir Valley, gives a preliminary account of the population structure of *M. tuberculosis* in the region. Studies on molecular epidemiology of *M. tuberculosis* help unravel the disease transmission dynamics and highlight the most prevalent strains circulating in a particular geographical region and thus help in surveillance and contribute toward the success of an ongoing TB control program.

The predominant lineage in this study was SIT26/CAS1_-Delhi ($n = 23$, 27% of all isolates). CAS1-Delhi family is essentially localized in the Middle-East and Central Asia, more specifically in South-Asia, (21.2%), and preferentially in India (75%).[14,15] It is also reported in other countries of this region such as Iran, and Pakistan.[16,17] In China, CAS1 type is almost exclusively detected in Xinjiang Uygur Autonomous region.[18] Geographically, the Xinjiang region is adjacent to several Central Asian countries, and they share similar cultural traditions. Historically, those countries, along with Kashmir, are all located along what was called the ''Silk Road'' that was the backbone of trading between the Far East and the Middle East in history.[19] Movements of people and merchants along this road may have played a major role in the transmission of this strain type between those countries.[20] Reportedly, this is a predominant spoligotype in Northern India,[3,21–25] but it has been found at a lower frequency (7.4% of all strains) in a study from Mumbai[15] and southern part of India.[26] The presence of the CAS lineage in a big cluster may indicate successful circulation of this lineage within this population and the presence of this lineage throughout the country with a greater percentage in north India suggest successful adaptation of this family in the Indian population. Detecting clusters alongside with unique patterns of strains belonging to the CAS lineage (Fig. 2) in the Kashmiri population may reflect ongoing evolution events.

The second most prevalent lineage present is Haarlem (H) lineage, a 4th sub-lineage (H4) characterized by the absence of spacers 29–31 (prototypes SIT127 and/or SIT 777). The distribution proportion of SIT127/H4, the second-largest spoligotype found in our study is 14.1% ($n = 12$) of all isolates. More than 60% of SIT127 isolates are localized in Armenia, Austria, Finland, Georgia, Iran, and Russia[27] and the distribution pattern is 17% in Iran, 3% in Iraq, and 0.7% in Saudi Arabia.[28] Ramazanzadeh et al. found the highest and lowest rates of occurrence of the Haarlem family of *M. tuberculosis* in worldwide population in Hungary in 2006 (66.20%) and in China in 2010 (0.8%), respectively.[29] The Haarlem genotype is ubiquitous worldwide[5] and represents about 25% of the isolates in Europe, Central America, and the Caribbean, suggesting a link to the post-Columbus European colonization.[30]

The ill-defined T clade was about 23.5% with spoligotype SIT1877/T1 (5, 5.8%), SIT78/T1-T2 (3, 3.53%), SIT1166/T1 (2, 2.3%) most prevalent. These findings are in contrast to the reports from other areas of the Indian subcontinent[3,21,22,24,25] where CAS, EAI, Manu and Beijing lineages are predominant compared to these Euro American lineages. The population structure of Kashmir could be the possible reason for finding European lineages (Haarlem and ill-defined T) in higher percentages as Kashmir has witnessed a series of occupations in the past by various invaders from Europe, Afghanistan and Central Asia. Not simply that, to spread the message of Islam in Kashmir various Sufi orders like the Suharwardi, Kubravi, Naqshbandi and Quadri, arrived in Kashmir from Persia, Central Asia, and Central and North India. These people settled in Kashmir, and completely intermingled with the indigenous population.

The low prevalence (1.2%) of Beijing spoligotype (SIT1) in the present study is in contrast to that reported from other parts of North India (3.8–9.6%).[3,21,24,31]

Drug susceptibility results of 72 isolates showed, 55 (76.3%) to be fully sensitive, 8 (11.1%) mono resistant to STM, 2 (2.7%) each mono resistant to INH and RIF, 4 (5.5%) MDR, 1 (1.4%) resistant to INH and STM. Four MDR-TB isolates belonged to three lineages: CAS (2.7%), Haarlem and ill-defined T clade (1.4% each). The single Beijing isolate was sensitive to all the four first line drugs. The major limitation of the present study is the small sample size and therefore, it is not representative of the population at large. Overall antimicrobial susceptibilities were similar to those reported from other parts of India with higher mono resistant to STM[32] and primary MDR similar to other parts of India.[33] INH resistance has been reported 41% in a study by Maurya et al.[34] A study by Malhotra et al.[35] from Jaipur, India found that primary drug resistance to INH was 13.6%, and to RIF 6.8%, both were higher than our findings.

Although the 85 isolates of *M. tuberculosis* had been differentiated into 42 patterns, including 7 clusters, by spoligotyping, MIRU-VNTR typing using 12 MIRU-VNTR loci set differentiated them into 3 small clusters and 79 unique MIRU-VNTR genotypes. We found MIRU locus 26 to be highly

polymorphic with an allelic diversity of 0.843. The discriminatory index for other MIRU loci-10, 16, 31, 40, 39, 27, 2, 4, 23, 20 and 24 were 0.842, 0.797, 0.780, 0.621, 0.598, 0.388, 0.248, 0.201, 0.185, 0.175, 0.026. Basil et al.,[3] Sharma et al.[23] and Stavrum et al.[25] also found MIRU 26 to be the most discriminatory locus for isolates belonging to the CAS1_Delhi sub-lineage. Three small clusters, including two isolates each were clustered by spoligotyping also, two belonged to SIT127/H4 and one to SIT1877/T1 family. No epidemiological linkage could be found among the isolates as they belonged to different districts. No clustering was seen among resistant isolates by both methods. MIRU locus 24 has been associated with classification of 'modern' and 'ancestral' lineages. In our study, all isolates of CAS, Haarlem, Beijing, T lineages had one repeat confirming their 'modern' lineage and EAI lineages had allele 2 confirming them to be 'ancestral'.

The observations of our study emphasize the complex diversity of circulating *M. tuberculosis* strains in Kashmir Valley that could reflect the different transmission pathways occurring within the Valley. Besides, it has been suggested that particular lineage of the *M. tuberculosis* might be adapted to the specific human population requiring established, facilitated typing schemes suitable for each geographical setting. This understanding of the dynamics of *M. tuberculosis* strains will provide novel insights into the *M. tuberculosis* population structure and how it relates to the epidemiology of TB in the Valley and beyond. Due to the small numbers of isolates in our study, statistical analysis would not be significant and was not performed. This study was done in a few health centers in this region, so our findings here may not be representative of the entire state.

Limitations of the study: The major limitation is the small sample size, which is not a true representative of the population. Due to unrest in the Kashmir Valley in the summers of 2008–2010, transport of samples from various districts became difficult, many samples reaching the central laboratory after 7 days of collection in CPC. This lead to increased contamination and decreased recovery of *M. tuberculosis* from these samples. Of the 300 samples processed, 93 samples in various phases of incubation were lost due to fault in an incubator which went unchecked for a few days because of complete shutdown in the Valley. These samples were excluded from the study. For logistic reasons, sputum samples were collected only from patients who were smear positive at a DMC located in the public health system. Smear negative and patients diagnosed outside Revised National Tuberculosis Control Program were not included. Also, (Human immunodeficiency virus) testing of patients was not undertaken to exclude HIV – the biggest single risk factor for developing TB, in view of its low prevalence in Kashmir Valley and logistic reasons. Future studies are needed that will take care of these limitations and give a true representative data from Kashmir Valley.

5. Conclusion

Kashmir Valley is similar to the rest of the Indian subcontinent in having CAS as the predominant lineage of the *M. tuberculosis*, while it is peculiar in having Euro American lineages like Haarlem and ill-defined T clade. Our results show the discriminatory potential of spoligotyping in conjunction with MIRU typing with MIRU having a better discriminatory power than spoligotyping alone.

Funding

This work was supported by the Indian Council of Medical Research (No. 5/8/5/3/2004-ECD-I).

Conflicts of interest

The authors have none to declare.

REFERENCES

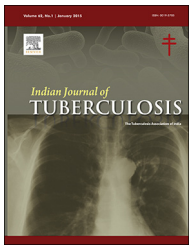
1. World Health Organization. *WHO Global Tuberculosis Report-2016. Tuberculosis Report 2016*. Retrieved March 25, 2017. www.who.int/tb/publications/global_report/en/-who-global.
2. De Riemer K, Daley CL. The molecular epidemiology of tuberculosis. In: Madkour M, ed. et al. *Tuberculosis*. Berlin, Heidelberg: Springer-Verlag; 2004:57–74.
3. Basil MV, Kumar S, Arora J, et al. Comparison of spoligotyping, mycobacterial interspersed repetitive units typing and IS*6110*-RFLP in a study of genotypic diversity of *Mycobacterium tuberculosis* in Delhi, North India. *Mem Inst Oswaldo Cruz*. 2011;106:524–535.
4. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol*. 1997;35:907–914.
5. Kremer K, van Soolingen D, Frothingham R, et al. Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: inter laboratory study of discriminatory power and reproducibility. *J Clin Microbiol*. 1999;37:2607–2618.
6. Mazars E, Lesjean S, Banuls A-L, et al. High-resolution minisatellite-based typing as a portable approach to global analysis of *Mycobacterium tuberculosis* molecular epidemiology. *Proct Natl Acad Sci USA*. 2001;98:1901–1906.
7. Skuce RA, McCorry TP, McCarroll JF, et al. Discrimination of *Mycobacterium tuberculosis* complex bacteria using novel VNTR-PCR target. *Microbiology*. 2002;148:519–528.
8. Smithwick RW, Stratigos CB, David HL. Use of cetylpyridinium chloride and sodium chloride for the decontamination of sputum specimens that are transported to the laboratory for the isolation of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 1975;1:411–413.
9. Vestal AL. *Procedure for Isolation and Identification of Mycobacteria*. Atlanta, GA: US Department of Health, Education and Welfare, Centre for Disease Control (CDC). Publication No. CDC 77-8230; 1977.
10. Canetti G, Fox W, Khomenko A, et al. Advances in techniques in testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ*. 1969;41:21–43.
11. van Soolingen D, de Haas PEW, Hermans PWM, Groenen PMA, van Embden JDA. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 1993;31:1987–1995.

12. Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. *Mol Microbiol.* 2000;36:762–771.
13. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpsons's index of diversity. *J Clin Microbiol.* 1998;26:2465–2466.
14. Vijaya-Bhanu N, van Soolingen D, van Embden JDA, Dar L, Pandey RM, Seth P. Predominance of a novel *Mycobacterium tuberculosis* genotype in the Delhi region of India. *Tuberculosis.* 2002;82:105–112.
15. Kulkarni S, Sola C, Filliol I, Rastogi N, Kadival G. Spoligotyping of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in Mumbai, India. *Res Microbiol.* 2005;156:588–596.
16. Gascoyne-Binzi DM, Barlow RE, Essex A, et al. Predominant VNTR family of strains of *Mycobacterium tuberculosis* isolated from South Asian patients. *Int J Tuberc Lung Dis.* 2002;6: 492–496.
17. Farnia P, Mohammadi F, Masjedi MR, et al. Evaluation of tuberculosis transmission in Tehran: using RFLP and spoligotyping methods. *J Infect.* 2004;49:94–101.
18. Pang Y, Zhou Y, Zhao B, et al. Spoligotyping and drug resistance analysis of *Mycobacterium tuberculosis* strains from national survey in China. *PLoS ONE.* 2012;7:e32976.
19. Elisseeff V. *The Silk Road; Highway of Culture and Commerce*. Paris: UNESCO; 2000.
20. Pannell CW. China Gazes West: Xinjiangs growing Rendezvous with Central Asia. *Eur Geogr Econ.* 2011;52: 105–118.
21. Singh UB, Suresh N, Bhanu NV, et al. Predominant tuberculosis spoligotypes, Delhi, India. *Emerg Infect Dis.* 2004;10:1138–1142.
22. Singh UB, Arora J, Suresh N, et al. Genetic biodiversity of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in India. *Infect Genet Evol.* 2007;7:441–448.
23. Sharma P, Chauhan DS, Upadhyay P, et al. Molecular typing of *Mycobacterium tuberculosis* isolates from a rural area of Kanpur by spoligotyping and mycobacterial interspersed repetitive units (MIRUs) typing. *Infect Genet Evol.* 2008;8:621–626.
24. Mathuria JP, Sharma P, Prakash P, Samaria JK, Katoch VM, Anupurba S. Role of spoligotyping and IS*6110*-RFLP in assessing genetic diversity of *Mycobacterium tuberculosis* in India. *Infect Genet Evol.* 2008;8:346–351.
25. Stavrum R, Myneedu VP, Arora VK, Ahmed N, Grewal HMS. In-depth molecular characterization of *Mycobacterium tuberculosis* from New Delhi-predominance of drug resistant isolates of the 'Modern' (TbD1) type. *PLoS ONE.* 2009;4:e4540.
26. Narayanan S, Gagneux S, Hari L, et al. Genomic interrogation of ancestral *Mycobacterium tuberculosis* from south India. *Infect Genet Evol.* 2008;8:474–483.
27. Brudey K, Driscoll J, Rigouts L, et al. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 2006;6:6–23.
28. Ahmed MM, Mohammed SH, Nasurallah HAA, Ali MM, Couvin D, Rastogi N. Snapshot of the genetic diversity of *Mycobacterium tuberculosis* isolates in Iraq. *Int J Mycobacteriol.* 2014;3:184–196.
29. Ramazanzadeh R, Roshani D, Shakib P, Rouhi S. Prevalence and occurrence rate of *Mycobacterium tuberculosis* Haarlem family multi-drug resistant in the worldwide population: a systematic review and meta-analysis. *J Res Med Sci.* 2015;20:78–88.
30. Duchene V, Ferdinand S, Filliol I, Guegan JF, Rastogi N, Sola C. Phylogenetic reconstruction of *Mycobacterium tuberculosis* within four settings of the Caribbean region: tree comparative analyse and first appraisal on their phylogeography. *Infect Genet Evol.* 2004;4:5–14.
31. Suresh N, Singh UB, Arora J, et al. *rpoB* gene sequencing and spoligotyping of multidrug resistant *Mycobacterium tuberculosis* isolates from India. *Infect Genet Evol.* 2006;6: 474–483.
32. Kalo D, Kant S, Srivastava K, Sharma AK. Pattern of drug resistance of *Mycobacterium tuberculosis* clinical isolates to first-line antituberculosis drugs in pulmonary cases. *Lung India Off Organ Indian Chest Soc.* 2015;32:339–341.
33. Sharma SK, Kaushik G, Jha B, et al. Prevalence of multidrug-resistant tuberculosis among newly diagnosed cases of sputum-positive pulmonary tuberculosis. *Indian J Med Res.* 2011;133:308–311.
34. Maurya AK, Singh AK, Kumar M, et al. Changing patterns and trends of multidrug resistant tuberculosis at referral centre in Northern India: a 4-year experience. *Indian J Med Microbiol.* 2013;31:40–46.
35. Malhotra B, Pathak S, Vyas L, et al. Drug susceptibility profiles of *Mycobacterium tuberculosis* isolates at Jaipur. *Indian J Med Microbiol.* 2002;20:76–78.

Available online at www.sciencedirect.com

ScienceDirect

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



Original Article

Clinical and cytological features in diagnosis of peripheral tubercular lymphadenitis – A hospital-based study from central India

Vivek Gupta [a,*], *Arvind Bhake* [b]

[a] *Assistant Professor, Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha 442002, India*
[b] *Professor and Head, Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha 442002, India*

ARTICLE INFO

Article history:
Received 30 March 2016
Accepted 24 November 2016
Available online 13 February 2017

Keywords:
Clinical features
Peripheral tubercular lymphadenitis
Cytological features

ABSTRACT

Background: Tuberculosis lymphadenitis is difficult to diagnose clinically, and often the laboratory confirmation is not available in resource-poor countries. We describe here the symptoms, clinical characteristics, and results of cytological analysis in peripheral tuberculous lymphadenitis patients.

Methods: One hundred and fifty-six patients with peripheral lymph node for cytological evaluation presenting to Department of Pathology, Acharya Vinoba Bhave Rural Hospital, Wardha, India were included in this study.

Results: Sixty-nine cases were tuberculous lymphadenitis, with female to male ratio of 1.3:1. One or more constitutional symptoms were present in 59.4% of patients, with 89.9% of lymph nodes ≥2 × 2 cm and the most common site of involvement was cervical lymph node (70.3%). The lymph nodes were multiple (85.5%), either discrete or matted. Cytomorphologically, hemorrhagic aspirate was observed in 29 cases, well-formed epithelioid cell granuloma with caseous necrosis was seen in 34 cases, and Zeihl Neelsen staining was positive in 45 cases. Correlation between character of aspirate and cytomorphological pattern was found highly significant.

Conclusion: These data suggest that constitutional symptoms and clinical and cytological features help in diagnosing cases of peripheral tubercular lymphadenitis and also open new frontiers to further research that affects the cytological features of these cases.

1. Introduction

The incidence of extrapulmonary tuberculosis (EPTB) is on rise over the last few years. Peripherial tuberculous lymphadenopathy is the most common form of extrapulmonary tuberculosis[1–3] and accounts for 25–60% of all EPTB cases, in regions where mycobacterial infection is highly prevalent and presents commonly in lymphnodes draining the head and neck.

* *Corresponding author*. Tel.: +91 9420245648.
E-mail address: dr_vivek_gupta@yahoo.com (V. Gupta).

Abbreviations: EPTB, extrapulmonary tuberculosis; TBLN, tuberculous lymphadenitis; FNAC, fine needle aspiration cytology; ZN, Ziehl Neelsen; SPSS, software package for statistical analysis; AFB, acid fast bacilli.

http://dx.doi.org/10.1016/j.ijtb.2016.11.032
0019-5707/

In India, 43% of tuberculous lymphadenitis (TBLN) cases are diagnosed on clinical grounds alone without laboratory confirmation, as these facilities are often not available. The conventional methods of diagnosis for tuberculosis like sputum examination of acid-fast bacilli and chest X-ray are fairly accurate in detecting the active pulmonary component of the disease. However, they are not useful for detecting extrapulmonary components.

Fine needle aspiration cytology (FNAC) is usually the first line of investigation in the diagnosis of tuberculous lymphadenitis and has a high diagnostic yield (97%)[4–6]; however, laboratory facilities are not available at all centers in developing countries. We were interested in reviewing the clinical parameters of TBLN lymphadenitis and the morphological changes observed on cytology.

The aim of the present study was to describe the symptoms, clinical characteristics, and results of cytology analysis in tuberculous lymphadenitis patients to assess their diagnostic value in cohort of patients attending Acharya Vinoba Bhave Rural Hospital.

2. Materials and methods

2.1. Study site

The study is a retrospective descriptive study which reviewed all the peripheral lymph node FNAC samples during the period of April 2014–July 2015. The present study was conducted in Acharya Vinoba Bhave Rural Hospital attached to Jawaharlal Nehru Medical College, Sawangi, Wardha. Acharya Vinoba Bhave Rural Hospital is a 1206-bedded referral and teaching hospital in Wardha in central India which receives patients from the district of Wardha and provides care of approximately over 1,000,000 patients annually with inflow of patients from many deprived villages in the periphery.

2.2. Patients

All the patients underwent FNAC on an Out-Patient Department basis or were admitted to wards. The patients who presented with persistent peripheral lymph node persisting for ≥2 weeks despite a course of oral antibiotics were referred to the pathology department for cytological evaluation. The patients were included both as clinically suspected and unsuspected lymphadenopathy for tuberculosis.

2.3. Fine needle aspirates

FNAC was done with 23 gauze needle, under aseptic condition and the material was aspirated using 20 ml disposable syringe attached to Franzen handle. Nature of aspirate was characterized as sticky, purulent, hemorrhagic, and cheesy white. The aspirate taken from each case for cytological examination and was smeared on slides, 2 air dried and 1 wet fixed. The smears were stained with May Grunwald Giemsa, Ziehl Neelsen (ZN) stain on air-dried smear and papanicolaou stain on alcohol fixed smears. Cytomorphological typing was done on smears stained with Papanicolaou and grouped into three categories[7]: Type 1 as epithelioid granuloma with caseous necrosis. In addition to epithelioid cells, the smear contained clumps of amorphous debris or caseous necrotic material. Lymphocytes, Langhans giant cells, and neutrophils may be found; Type 2 as epithelioid granuloma without caseous necrosis having groups of epithelioid cells along with a variable number of lymphoid cells. Foreign body or Langhans giant cells may or may not be present; and Type 3 as necrotic materials with marked degeneration and variable polymorphonuclear infiltration without epithelioid granuloma and described as tubercular abscess.

2.4. Ethical considerations

Ethical clearance was obtained from Institutional Ethical committee of Datta Meghe Institute of Medical Science (Deemed University) as per reference letter number: DMIMS (DU)/IEC/2014-15/863, to carry out the research work in Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi, Wardha. The decision to do aspiration cytology was based on clinical demand and not for the sake of participation in the study. Written informed consent was obtained from each patient.

2.5. Statistical analysis

Data entry and analysis were done using SPSS 11.5 for Windows. Analysis using cross-tabulation was performed to assess relationships among variables. The Pearson Chi-square test was used to compare differences in the different morphological categories and nature of aspirate. Differences were considered statistically significant if $p \leq 0.05$.

3. Results

A total of 156 patients with peripheral lymphadenopathy suspected to be of tubercular origin were studied and FNAC was performed. Out of 156 cases, 70 cases were diagnosed as reactive lymphadenitis, 5 cases with other diagnosis, and 69 cases were of TBLN. The remaining 12 cases were inadequate for opinion. Among the 69 cases, female to male ratio was 1.3:1. The mean age of females was 29.9 ± 15.18 years with 75% of the patients being aged between 18 and 42 years. The mean age of males was 30.5 ± 12.57 with 75% of the patients being aged between 19 and 39 years. The mean age of female and male was not statistically significant.

The most common site for FNAC was cervical in 70.3%, supraclavicular 11%, submandibular 10%, axillary 7.4%, and inguinal 1.3%. Table 1 summarizes the clinical characteristics of 69 patients with tubercular lymphadenopathy. Of the lymph nodes diagnosed, 62 (89.9%) were of size ≥2 × 2 cm and most of them presented as multiple, either discrete or matted (59, 85.5%) and solid (55, 79.7%) lymph nodes. Constitutional symptoms were present in 41 (59.4%) patients.

Cytomorphological findings in 69 cases were categorized as: Well-formed epithelioid cell granulomas, giant cells, and caseous necrosis (34 cases), epithelioid cell granulomas but no caseous necrosis (18 cases), and caseous necrosis but no granulomas (17 cases). 45 cases were ZN-positive with maximum positivity, with 26/34 (76.5%) seen in smears showing both granuloma and necrosis followed by smears having necrosis (68.9%) and only three cases (16.7%) of granuloma. Cytological features and ZN staining for Acid-fast bacilli (AFB) are shown in Table 2.

Cytomorphological features in purulent aspirate (26 cases) were epithelioid cell granulomas, with caseation necrosis in 12/26 cases and necrosis only seen in 14/26 cases. AFB positivity was seen in 18/26 cases. Cytomorphological features in hemorrhagic aspirate (29 cases) were epithelioid cell granuloma, with caseation necrosis in 18/29 cases and epithelioid cell granuloma only in 10/29 cases. Cheesy aspirate was seen in 14 cases only. Characteristics of aspirate and cytomorphological patterns are shown in Table 3.

Characteristics of aspirate showed tendency to correlate with cytomorphological patterns, and p value was found to be <0.0001 (highly significant). Type 2 cytomorphological pattern was found to be more common in cheesy aspirates. Type 1 pattern was most commonly associated with hemorrhagic aspirate and type 3 with purulent aspirate.

A total of 70 patients had diagnosis of reactive lymphadenitis on FNAC. Female:male ratio was 4:5 and the mean age of presentation among females was 22.8 ± 13.4 years and among males it was 29 ± 18.6 years. Out of 70 patients with reactive lymphadenitis, 65 (93%) presented as single lymph node with size $<2 \times 2$ cm and the remaining 5 (7%) had multiple lymph nodes of 1×1 cm. The reactive lymphadenitis in 60 (86%) was not associated with any symptoms and the remaining 10 (14%) cases had only fever as a presenting symptom.

The types of aspirates that were associated with diagnosis of reactive lymphadenitis were as follows: particulate and sticky in 60 (85.7%) cases, hemorrhagic in 8 (11.5%) cases, and purulent and cheesy 1 (1.4%) each.

All the 70 samples for reactive lymphoid hyperplasia underwent ZN staining and were negative for AFB.

None of these patients underwent repeat FNAC. Histopathological diagnosis was available in 13 cases, out of which 2 cases were diagnosed as Tubercular lymphadenitis and 11 cases were diagnosed as reactive lymphadenitis. All the cases correlated well with the diagnosis of FNAC in TBLN and reactive lymphadenitis category.

4. Discussion

Tuberculous lymphadenitis is one of the most common manifestations of extrapulmonary tuberculosis. Cervical lymphadenitis, caused by *Mycobacterium tuberculosis*, is generally considered to have its origin in the lymphatic spread of organisms from a primary pulmonary focus, but in a minority of cases, it can originate from a primary focus in the mouth, tonsils, oropharynx, or tissues of the head and neck.[8] The diagnosis of tuberculosis is often difficult, given that symptoms and signs might be non-specific, the collection of bacteriologic specimen's problematic and bacteriologic yields low. In patients with peripheral tuberculous lymphadenitis, however, clinical signs are usually apparent, and fine needle aspiration (FNAC) provides excellent bacteriologic yields.[6,9] Although the diagnostic value of FNAC has been demonstrated in resource-limited settings,[6,9] to date it remains underutilized as a routine diagnostic modality in most endemic areas.

Among the diagnosed TBLN, the mean age of presentation was 29.9 for females and 30.5 for males. Ratio of female to male was 1.3:1 which support other reports that more women than men have tuberculous lymphadenitis.[10,11] Most of the patients presenting with TBLN had lymph nodes of $\geq 2 \times 2$ cm in size and were mostly multiple having solid consistency in this study. The triad of size, multiplicity, and matting helps in reaching the diagnosis of tuberculous lymphadenitis. Presence of constitutional symptoms helps in raising the suspicion of tuberculous lymphadenitis, especially in resource-poor countries like India where diagnosis and treatment was on clinical suspicion alone. Thus, there is a need to improve the clinical criteria used to diagnose tuberculous lymphadenitis.

The most common site was cervical lymph node which is also in accordance with the findings of other studies.[3] Cytomorphologically, the most common category on this study was Type 1 with the presence of epithelioid cell granuloma, giant cell, and necrosis (49.3%) while Mittal et al.[12] reported it to be 47.2% and 50.5%, respectively. The nature of aspirate was predominantly hemorrhagic in i.e., 42% and purulent in 37.7%, Mittal et al [12] reported maximum aspirates to be purulent 48% and particulate 30%. Characteristics of aspirate showed a highly significant correlation with cytomorphological pattern in this study. It is well known that delayed type hypersensitivity reaction is seen in tuberculosis. Morphologically delayed type hypersensitivity is signaled by caseous necrosis in center of granuloma. This type of hypersensitivity is caused by cytokines which lead to symptoms of tuberculosis.[13] It is also proposed that different granuloma formation is a result cell-mediated immune response, and a poor cell-mediated immune response results in scattered epithelioid macrophages with massive necrosis.[14]

Maximum AFB positivity 76.5% was seen in lymph nodes showing both granuloma and necrosis which is also similar to observations of another study.[12] However, in one of the studies,[10] maximum AFB positivity was found in cases having necrosis only. The variation in positivity may be due to difference in sensitivity of staining method.

The reactive lymph in the present study was mostly associated with male patients which is in contrast to the tuberculosis patients where females predominated. These observations reflect biological, hormonal, social, environmental, or behavioral differences between men and women. Biologically, there is a fundamental difference in the immune systems of men and women.[15] The study[15] suggested a hormonal influence on immunity as the underlying cause for the different pattern of disease in women. Socially, in developing countries, women often have a low socioeconomic and nutritional status, which can affect the immune response to the disease making them more susceptible to tuberculosis. Among the reactive lymph nodes, 86% were not associated with any symptoms which is also in contrast to patients of tubercular lymphadenitis where 59.4% had constitutional

symptoms. The clinical manifestations of tuberculous lymphadenitis are thought to be a local manifestation of a systemic disease[16] which is probably the reason for difference in symptoms. The nature of aspirate was particulate and sticky in 85.7% of patients with reactive lymphadenitis which could be due to the presence of immunoglobulins secreted by B cells in contrast to tuberculosis having T cell mediated immune response leading to granuloma formation and necrosis determining the characteristics of aspirate.

This being a hospital-based study, the patient population was partially selected but our cohort included both outpatients and hospitalized patients. However, the study gives an insight into symptoms and clinical characteristics in the most common form of extrapulmonary tuberculosis and it also provides the background for a community study for a better symptom-based diagnostic approach, particularly in resource-limited settings. The laboratory findings like nature of aspirate and cytomorphological spectrum should be interpreted along with the clinical picture to consider a patient for accurate diagnosis. Further, the immune reactions taking place at cellular and molecular levels, affecting the nature of aspirate and cytomorphological spectrum seen as granuloma and caseous necrosis of the affected lymph node, need to be studied.

5. Conclusion

In conclusion, we found that constitutional symptoms and clinical and cytological features help in diagnosing peripheral tubercular lymphadenitis cases and also open new frontiers to further study the immune reactions taking place at cellular and molecular levels, affecting the nature of aspirate and cytomorphological spectrum seen as granuloma and caseous necrosis in these cases.

Authors' contribution

VG carried out the study, contributed to concept and design of study, acquisition of data along with analysis and interpretation. VG drafted the manuscript and reviewed it and also gave final approval for submitting and publication.

AB helped in acquisition of data and interpretation of data. AB helped in drafting and revised the manuscript and also gave approval for the submitting and publication.

Conflicts of interest

The authors have none to declare.

Acknowledgements

We gratefully acknowledge all the consultant and laboratory staff of AVBRH, Jawaharlal Nehru Medical College for their support in data collection. This research work was supported by a grant from the International Society of Infectious Diseases.

Appendices

Tables 1–3.

Table 1 – Clinical characteristics of patients with tuberculous lymphadenitis (*n* = 69 cases).

	No. of instances
Lymph node characteristics	
Size[a]	
<2 × 2 cm	07 (10.1%)
(2–4) × (2–4) × cm	48 (69.5%)
>4 × 4 cm	14 (20.4%)
Characters	
Single	10 (14.5%)
Multiple	
(Discreet)	28 (40.6%)
(Matted)	31 (44.9%)
Solid	55 (79.7%)
Fluctuant	14 (20.2%)
Constitutional symptoms	
Any symptom	41 (59.4%)
Fever	29 (42.0%)
Cough	23 (33.3%)
Night sweat	33 (47.8%)
Fatigue	37 (53.6%)
Weight loss[b]	17 (24.6%)

[a] Transverse diameter of the largest cervical mass.
[b] >10% of body weight in 3 months.

Table 2 – Cytological findings and Zeihl Neelsen Staining for AFB positive cases.

Cytological findings	No. of cases	AFB positive cases
Type 1	34	26
Type 2	18	03
Type 3	17	16
Total	69	45

Table 3 – Nature of aspirate and cytomorphological spectrum.

Nature of aspirate	Type 1	Type 2	Type 3	Total
Purulent	12	00	14	26
Hemorrhagic	18	10	01	29
Cheesy	04	08	02	14
Total	34	18	17	69

REFERENCES

1. Elder NC. Extrapulmonary tuberculosis. A review. *Arch Fam Med*. 1992;1:91–98.
2. Mert A, Tabak F, Ozaras R, Tahan V, Ozturk R, Aktuglu Y. Tuberculous lymphadenopathy in adults: a review of 35 cases. *Acta Chir Belg*. 2002;102:118–121.
3. Al-Serhani AM. Mycobacterial infection of the head and neck: presentation and diagnosis. *Laryngoscope*. 2001;111:2012–2016.
4. Ergete W, Bekele A. Acid fast bacilli in aspiration smears from tuberculosis patients. *Ethiop J Health Dev*. 2002;14(1):99–104.

5. Arora B, Arora DR. Fine needle aspiration cytology in diagnosis of tuberculous lymphadenitis. *Indian J Med Res*. 1990;91:189–192.
6. Handa U, Palta A, Mohan H, Punia RP. Fine needle aspiration diagnosis of tuberculous lymphadenitis. *Trop Doct*. 2002;32:147–149.
7. Parvez M, Mohiuddin M, Zahid MH, Ahmad F, Haq JA. Diagnosis of tubercular lymphadenitis by PCR of fine needle aspirates. *Ibrahim Med Coll J*. 2012;6(2):46–49.
8. Miller FLW, Cashman JM. The natural history of peripheral tuberculous lymphadenitis associated with a visible primary focus. *Lancet*. 1955;1:1286–1289.
9. Lau S, Ignace W, Kwan S, Yew W. Combined use of fine needle aspiration cytologic examination and tuberculin skin test in the diagnosis of cervical tuberculous lymphadenitis. *Arch Otolaryngol Head Neck Surg*. 1991;117:87–90.
10. Paliwal N, Thakur S, Mullick S, Gupta K. FNAC in tuberculous lymphadenitis: experience from a tertiary level referral centre. *Indian J Tuberc*. 2001;58:102–107.
11. Purohit MR, Mustafa T, Mørkve O, Sviland L. Gender difference in the clinical diagnosis of tuberculous lymphadenitis – a hospital based study from central India. *Int J Infect Dis*. 2009;13:600–605.
12. Mittal P, Handa U, Mohan H, Gupta V. Comparative evaluation of fine needle aspiration cytology, culture, and PCR in diagnosis of tuberculous lymphadenitis. *Diagn Cytopathol*. 2010;39(11):822–826.
13. Sharma S, Bose M. Role of cytokines in immune response to pulmonary tuberculosis. *Asian Pac J Allergy Immunol*. 2001;19:213–219.
14. Ridley DS, Ridley MJ. Rational for histological spectrum of tuberculosis. A basis of classification. *Pathology*. 1987;19:186–192.
15. Bothamley G. Sex and gender in the pathogenesis of infectious tuberculosis. A perspective from immunology, microbiology and human genetics. In: Diwan VK, Winkvist A, eds. *Gender and tuberculosis. NHV report*. Göteborg, Sweden: Nordic School of Public Health; 1998:41–53.
16. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. *Medicine (Baltimore)*. 2005;84:350–362.

Available online at www.sciencedirect.com

ScienceDirect

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

Original Article

Diagnosis of clinically suspected and unsuspected tubercular lymphadenopathy by cytology, culture, and smear microscopy

Vivek Gupta [a,*], *Arvind Bhake* [b]

[a] *Assistant Professor, Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha 442002, India*
[b] *Professor and Head, Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha 442002, India*

ARTICLE INFO

Article history:
Received 7 July 2016
Accepted 1 November 2016
Available online 13 February 2017

Keywords:
Tubercular lymphadenopathy
Clinically suspected and unsuspected
Cytology
Culture

ABSTRACT

Background: Tubercular lymphadenopathy (TBLN) accounts for 20–40% cases of extrapulmonary tuberculosis. But the common presenting symptoms of tuberculosis like fever, cough, weight loss, fatigue, and night sweats are not always associated with tuberculosis lymphadenopathy, thereby, making its diagnosis difficult. Our aim was to study if Fine Needle Aspiration Cytology (FNAC) combined with Zeihl Neelsen stain and culture for *Mycobacterium tuberculosis bacilli* could improve the diagnostic accuracy in patients clinically suspected and unsuspected for tubercular lymphadenitis.

Methods: The study was conducted at Department of Pathology, Acharya Vinoba Bhave Rural Hospital, Jawaharlal Nehru medical College, Wardha, India. One hundred and twenty-nine patients with enlarged lymph node for more than two weeks duration were evaluated. All the patients were subjected to cytology, smear, and culture examination of their lymph node aspirate.

Results: Age range for the patients was from 1 to 74 years (mean 30.49 ± 16.69) and F:M ratio was 1:1.18. Most common site of involvement was cervical lymph node. 48 patients were diagnosed as TBLN, out of which 19 patients had no associated symptoms and 28 patients had one or more presenting symptoms of tuberculosis. Fever was the most common presenting symptoms. Pediatric age group patients were more commonly associated with symptoms than adults (p value = 0.000). Culture and ZN stain were positive in 32 and 10 cases respectively among TBLN. Additionally, culture was positive in 20 patients diagnosed as reactive lymphoid hyperplasia.

Conclusion: Cytology combined with culture improves the diagnostic accuracy in cases with enlarged lymph nodes, suspected or unsuspected for tuberculosis.

* *Corresponding author*. Tel.: +91 9420245648.
E-mail address: dr_vivek_gupta@yahoo.com (V. Gupta).

http://dx.doi.org/10.1016/j.ijtb.2016.11.014

1. Introduction

Tuberculosis (TB) is one of the greatest killers worldwide and continues to threaten the human race since time immemorial, not only due to its effects as a medical problem, but also by its impact as a social and economic tragedy. TB accounted for 9.6 million new cases and 1.5 million deaths in 2014. It is among the top 5 causes of death among women aged 15–44 years. Over 95% of TB deaths occur in low- and middle-income countries. In 2014, India is among the top six countries having largest number of incident cases of Tuberculosis.[1]

TB can occur as pulmonary tuberculosis or extrapulmonary tuberculosis (EPTB). EPTB tuberculosis accounts for one-fifth of all the cases of tuberculosis[2] and can involve lymph nodes, pleurae, meninges, pericardium, skeleton, gastrointestinal tract, genitourinary tract, and can even be miliary TB. Tubercular lymphadenopathy (TBLN) accounts for 20–40% cases of EPTB.[3] The common presenting symptoms of TB like fever, cough, weight loss, fatigue, and night sweats are not always associated with TBLN. Patients may often present with only enlarged lymph node. They are investigated by Fine Needle Aspiration Cytology (FNAC) or biopsy for histopathology to find out the cause of lymphadenopathy. In these cases, conventional, diagnostic methods like Ziehl Neelsen (ZN) staining and culture for lymph node aspirates or biopsy can help to provide the evidence of tuberculosis by detecting *Mycobacterium tuberculosis bacilli*.[4,5] But a diagnostic difficulty is met when there is absence of associated symptoms or when the FNAC results are not suggestive of TBLN.

With this background, we aimed to study if FNAC combined with tubercular bacilli detection techniques (ZN stain and culture) could improve the diagnostic accuracy in patients clinically suspected and unsuspected for tubercular lymphadenitis.

2. Materials and methods

2.1. Study site and design

This cross-sectional study was conducted at Acharya Vinoba Bhave Rural Hospital attached to Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Science (Deemed University), Sawangi, Wardha, during the period from September 2015 to June 2016.

2.2. Study participants

Patients with enlarged lymph nodes of more than two weeks duration clinically suspected and unsuspected for tubercular lymphadenopathies were included.

2.3. Fine needle aspirates and culture

FNAC was done with 23 gauze needle, under aseptic condition and material was aspirated using 20 ml disposable syringe attached to Franzen handle. The aspirate obtained was divided into two parts: one taken for cytological examination where smears were prepared, two air dried and one wet fixed in each cases; the second part was treated with N-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) for decontamination and taken for culture by inoculating on Lowenstein-Jensen (LJ) slants.[6]

The cytological criteria (for FNAC) for diagnosis of possible TBLN were presence of epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis.[7,8] Culture was observed for eight weeks after inoculation for each case.

2.4. Ethical considerations

Ethical clearance was obtained from the institutional ethics committee. Informed consent was taken from all patients and those unwilling to participate in study were excluded.

2.5. Statistical analysis

Data entry and analysis was done using SPSS 11.5 for Windows. Analysis using cross-tabulation was performed to assess relationships among variables. The Pearson Chi square test was used to compare differences in the presence of symptoms in different age groups. Differences were considered statistically significant if $p \leq 0.05$.

3. Role of funding source

The research work was funded by International Society of Infectious Diseases, USA. The funder has no role in study design, data collection, data analysis, data interpretation or writing of report.

4. Results

A total of 129 cases were included in this study with age range from 1 year to 74 years (mean 30.49 ± 16.69). The female to male ratio was 1:1.18. Cervical lymph node was the most commonly involved lymph node. A total of 23 cases were in pediatrics age group (≤14 years) and 107 patients were in adult age group (>14 years). On cytopathology, 48 patients were diagnosed with TBLN, 63 patients with reactive lymphoid hyperplasia, 1 patient with suppurative lymphadenitis, and 17 cases with malignancy.

Out of 48 cases diagnosed as TBLN, history of exposure through family members was present in three patients and 3/48 patients had previous partial or full treatment of tuberculosis. Fever was the most common presenting symptom involving 19 (39.3%) patients, followed by weight loss in 6 (12.5%) patients, and cough or loss of appetite in 3 (6.3%) patients. Six (12.5%) patients had more than two symptoms and 4 (8%) patients had more than three symptoms whereas 19 (40%) patients had no symptom suggestive of tuberculosis (Fig. 1).

Out of the 23 pediatric patients, cytological diagnosis of tubercular lymphadenitis was made in 5 (22%) patients and none had exposure to or previous history of tuberculosis. Fever was present in 3 (13%) patients and one patient had cough. The remaining 19 did not have any symptoms.

Table 1 – Demonstration of tubercular bacilli by smear and/or culture.

Smear/culture for AFB	Cytological diagnosis		
	Tubercular lymphadenopathy	Reactive lymphoid hyperplasia	Others
Smear positive	10	0	0
Culture positive	32	20	0
Smear and culture negative	06	43	17
Total (129)	48	63	18

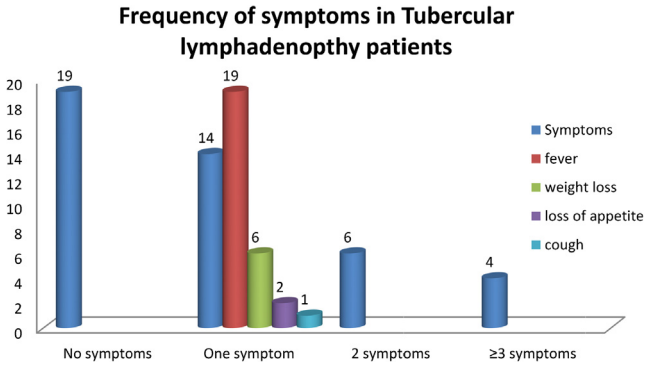


Fig. 1 – Frequency of symptoms in tubercular lymphadenopathy patients.

Out of 107 adult patients, cytological diagnosis of tubercular lymphadenitis was made in 43 (40%) patients. 6 patients had exposure by one of the family members or previous history of tubercular treatment. Fever was present in 16 (37%) patients and was the most common presenting symptom, loss of weight was present in 6 patients, and 3 patients had one or other symptoms like cough or loss of appetite. Eighteen patients had no symptoms suggestive of lymphadenitis of tubercular origin.

The presence or absence of symptom differed significantly in both the age groups, p value <0.05 (0.0005), that is, lymph node enlargement in pediatrics age group was more likely to be associated with symptoms when compared with adults.

Culture alone was positive in 32 (66.7%) cases and Zeihl Neelsen stain for acid fast bacilli was positive in 10 (21%) cases of tubercular lymphadenitis (Table 1). Culture was positive in 20 (32%) patients diagnosed with reactive lymphoid hyperplasia on cytology, suggesting that some cases are missed on routine cytology due to lack of evidence of granuloma formation or other cytomorphological features suggestive of tubercular lymphadenitis.

Histopathological examination was available only in 6 cases with TBLN and reactive lymphoid hyperplasia. The findings of cytopathology and histopathology were similar for the available cases.

5. Discussion

TBLN is the most common form of EPTB whose diagnosis faces many diagnostic challenges. The problem in diagnosis keeps the clinician in perplexing situation whether to start the anti-tubercular treatment or not. The present study of 129 patients with enlarged lymph nodes had female:male ratio of 1:1.18, which is in accordance with the other studies.[9,10] Cervical lymph node was the most common presenting symptom in TBLN patients and was found in accordance with findings of Verma et al.[9] and Gadre et al.[10] The enlarged lymph nodes as found in our study were associated with past history of treatment or history of contact in 6/48 patients whereas Verma et al.[9] reported 34/100 cases that had exposure to tuberculosis.

In our study, only 24/48 patients had one or more symptoms associated with tuberculosis whereas other studies report 21/35 patients with any symptoms of tuberculosis.[11] Fever was the most common presenting symptom in our study, and is in accordance with the study of Verma et al.[9]

Cytology accurately diagnosed 48 tubercular lymphadenitis cases. Culture sensitivity was found to be 67% whereas Gadre et al.[10] reported it to be 70%. Culture also detected bacilli in 20 (32%) patients of reactive lymphoid hyperplasia patients suggesting that even if bacterial load is not large enough to trigger immune response for granuloma formation, tuberculosis cannot be excluded in these patients. In the study by Gadre et al.[10] six culture positive cases were reported in non-tubercular lymphadenitis group whereas Reddy et al.[12] reported 23% of culture positive cases among nonspecific lymphadenitis/reactive lymphadenitis diagnosed on cytology.

Smear positivity was seen in 10 (21%) cases of TBLN diagnosed on cytology in our study. Reddy et al.[12] found the same to be 18% and Gadre et al.[10] found smear alone to be positive in 2/54 patients.

6. Conclusion

The above study diagnosed TBLN by cytology and by bacillary demonstration using smear microscopy and culture in patients with or without symptoms of tuberculosis in both pediatric and adult age groups. Based on these findings, it can be concluded that cytology combined with culture can improve the diagnostic accuracy and that culture of lymph node aspirates should be a mandatory diagnostic procedure for patients with enlarged lymph nodes suspected or unsuspected for tuberculosis. However, further studies are needed to investigate the immune mechanism and its correlation with tubercular bacilli, so that the patients missed for tubercular lymphadenitis on cytology but providing evidence on culture can be diagnosed accurately.

7. Limitations

The limitation of the study was that the *Mycobacterium* species identification by biochemical test could not be done, which would have helped in therapeutic approach.

Authors' contributions

All the authors had full access to all the data in the study and share the final responsibility for the decision to submit for publication.

VG carried out the study, contributed towards concept and design of study, acquisition of data along with analysis and interpretation. VG drafted the manuscript and reviewed it and also gave final approval for submitting and publication.

AB helped in acquisition of data and interpretation of data. AB helped in drafting and revised the manuscript and also gave approval for the submitting and publication.

Conflicts of interest

The authors have none to declare.

Acknowledgements

The authors gratefully acknowledge all the consultant and laboratory staff of AVBRH, Jawaharlal Nehru Medical College for their support in data collection. This research work was supported by a grant from the International Society of Infectious Diseases.

REFERENCES

1. *World Health Organization Global Tuberculosis Report*. 2016. Available from: http://www.who.int/mediacentre/factsheets/fs104/en/ Accessed 15.08.16.
2. Sharma SK, Mohan A. Extra pulmonary tuberculosis. *Indian J Med Res*. 2004;120:4316–4353.
3. Gupta PR. Difficulties in managing lymph node tuberculosis. *Lung India*. 2004;21:50–53.
4. Waard JH, Robledo J. Conventional diagnostic methods. In: Palomino JC, Leao SC, Ritacco V, eds. *Tuberculosis* 1st ed. 2007; 401–424 [chapter 12].
5. Gopinath K, Singh S. Multiplex PCR assay for simultaneous detection and differentiation of *Mycobacterium tuberculosis*, *Mycobacterium avium* complexes and other Mycobacterial species directly from clinical specimens. *J Appl Microbiol*. 2009;107:425–435.
6. Ghariani A, Jaouadi T, Smaoui S, et al. Diagnosis of lymph node tuberculosis using GeneXpert MTB/RIF in Tunisia. *Int J Mycobacteriol*. 2015;4:270–275.
7. Sen R, Marwah N, Gupta KB, Marwah S, Arora R, Jain K. Cytomorphological patterns in tuberculous lymphadenitis. *Indian J Tuberc*. 1999;46:125–127.
8. Mittal P, Handa U, Mohan H, Gupta V. Comparative evaluation of fine needle aspiration cytology, culture and PCR in diagnosis of tuberculous lymphadenitis. *Diagn Cytopathol*. 2011;39:822–826.
9. Verma P, Jain A, Patra SK, Gandhi S, Sherwal BL, Chaudhary M. Evaluation of Polymerase Chain reaction (PCR) using hupB gene in diagnosis of tuberculous lymphadenitis in fine needle aspirates. *Indian J Tuberc*. 2010;57:128–133.
10. Gadre DV, Singh UR, Saxena K, Bhatia A, Talwar V. Diagnosis by tubercular cervical lymphadenitis by FNAC, microscopy and culture. *Indian J Tuberc*. 1991;38:25–27.
11. Marais BJ, Wright CA, Schaff HS, Gie RP, Hesseling AC. Tuberculous lymphadenitis as a cause if persistent cervical lymphadenopathy in children from a tuberculosis-endemic area. *Pediatr Infect Dis J*. 2006;25:34–38.
12. Reddy VCK, Aparna S, Prasad CE, et al. Mycobacterial culture on Fine Needle Aspirates – a useful tool in diagnosing tuberculous lymphadenitis. *Indian J Med Microbiol*. 2008;26:259–261.

Available online at www.sciencedirect.com

ScienceDirect

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

Original Article

Effective communication approaches in tuberculosis control: Health workers' perceptions and experiences

Sriram Arulchelvan *, *Rengan Elangovan*

Anna University, Department of Media Sciences, CEG Campus, Anna University, Guindy, Chennai 600025, India

ARTICLE INFO

Article history:
Received 13 September 2016
Accepted 1 November 2016
Available online 16 December 2016

Keywords:
TB
Interpersonal communication
IEC
DOTS
Mobile phones

ABSTRACT

Background: Health workers' experiences and understanding of the myths, misconceptions, beliefs about TB, and patients in the community (and effective communication methods) can be useful in designing effective IEC materials and strategies.
Objective: To study the perceptions and experiences of health workers regarding TB disease, patients, and effective communication strategies in TB control.
Methods: A survey was conducted among health workers involved in Directly Observed Treatment Short (DOTS) course. Data regarding general health beliefs, prevalent myths and misconceptions about TB in their respective localities, knowledge level among patients, and utilization of various communication strategies were collected.
Results: There is a significant increase in knowledge about TB during DOTS among patients, as observed by about half of the health workers. TB patients are aware about how TB spreads to others and their responsibilities. Regular interaction with patients is required for treatment adherence. Two thirds of the health workers believe that media-mix strategy can be very effective in creating awareness among the patients as well as the public. Health workers realized that the video player facility on their mobile phones is useful for showing health-related videos.
Conclusion: A combination of mass media and interpersonal communication could be effective for TB control. Face-to-face communication with community members, patient-provider discussions, and information through television could be very effective techniques. Exclusive communication materials should be designed for family members of the patients. Smart phones can be used for effective implementation of TB control programs.

1. Introduction

Tuberculosis (TB) remains the number one killer infectious disease affecting people in many developing countries. In 2015, out of the global annual incidence of 9.6 million TB cases, 2.5 million occurred in India accounting for one-fourth of the global TB burden.[1] In India, TB kills 2 people every 3 min, which is nearly 1000 every day.[2] A recent health report states that TB cases are on the rise in some parts of India.[3]

TB control programs heavily rely on a strong infrastructure and an effective Information, Education, and Communication (IEC) strategy. IEC can play a major role in TB control by generating awareness, encouraging timely self-reporting, and improving adherence to Directly Observed Treatment Short

* *Corresponding author at*: Assistant Professor, Department of Media Sciences, Anna University, Chennai 25, India. Tel.: +91 9444819958.
E-mail address: arulchelvansriram@gmail.com (S. Arulchelvan).
http://dx.doi.org/10.1016/j.ijtb.2016.11.017

course (DOTS) among patients, all of which are major determinants of the success of the program.

Treatment default rate decreased in Vietnam and Peru, and the treatment rate in these countries surpassed 85% mainly through their intense and sustained communication programs.[4] People do not know the correct mode of TB transmission and have misconceptions about the disease.[5] One of the causes of TB patients disengaging from treatment is health-care professional's failure to listen and respond to the patient's misconceptions regarding TB treatment and the disease.[6] Increased provider-patient contact can have a positive impact on the treatment completion.[7]

Public education and awareness raising programs designed to counteract myths and to encourage greater inclusion of people who have TB are essential elements of any effort to combat TB stigma.[8] Varied communication strategies are needed for different target groups and settings. Print messages had less influence on the poor and disadvantaged populations of the city according to a study based in New Delhi on the impact of IEC campaign.[9]

This paper attempts to study the perceptions and experiences of the TB health workers, as they are important stakeholders of all TB control programs. They play an important role attending to the treatment needs of the patients and the public on a daily basis. Thus, their perspectives and observations of the disease, patients, and communication strategies become significant to understand the health-seeking behavior of community members and they might be useful in designing effective IEC materials and strategies.

2. Methodology

The areas chosen for the study are Chennai and Salem districts of Tamil Nadu state in South India. Chennai is a metropolitan city consisting more of urban population while Salem town has a mix of urban, semirural, and rural people. These two places were selected as they were some of the high TB burden districts of the state[10] representing all sections.

Health workers' category includes senior treatment supervisors (TB), community DOTS providers and lab technicians who administer DOTS to the patients at the TB centers regularly. Data from 110 health workers were collected through a structured questionnaire in their vernacular language, i.e., Tamil (because all the health workers are Tamil natives) using stratified sampling technique. It had queries about general health beliefs, prevalent myths and misconceptions about TB among people in the community, knowledge level of TB patients, various communication strategies, and base-level constraints being faced in TB control. This cross-sectional study was conducted from May to October 2015. The questionnaire was pretested on 5% of the population.

3. Results and discussion

3.1. Profile of health workers

More than half of health workers who participated in the study were graduates (bachelor degree holders). Women health workers had equal representation. About one-fourth of them had more than 10 years of work experience whereas 39.1% and 36.4% had less than 5 years and 5–10 years of experience, respectively. Most of them were working in primary health centers and city corporation hospitals while 17.3% were community DOTS providers.

3.2. Health literacy level

The majority of health workers (71.8%) state that the overall health literacy level in their respective areas is satisfactory followed by low-level health literacy (21.8%) as shown in Table 1. Health literacy is related to treatment-seeking behavior. People with low health literacy level cannot act on the health information even if they have access to it. General awareness about hygiene and healthy behaviors is important for preventing and treating TB.

3.3. Myths and superstitions about general health

There are many myths and misconceptions prevalent in the communities related to health. According to 70% of health workers, ''people believes that treatment through injections is more effective than drugs'' (Table 1). A study done in Pakistan shows that patients may question the efficacy of the pills or think that only injections are ''medicine'' corroborating the above observation.[11] As many as 51% say that people think of private hospitals as better places for taking treatment than government hospitals. A very small percentage of 6.4% observed home remedies being followed among people. The widespread belief among community members that having drips of glucose at the hospital is good for a speedy recovery from many diseases was observed by a few health workers.

These general misconceptions and superstitions regarding health have to be addressed in the TB IEC materials and other public health programs. Overall increase in general health awareness will make TB communication efforts easier and

Table 1 – General health beliefs and misconceptions about TB (N-110).

Variable	*n* (%)
General health literacy levels	
Low	24 (21.8)
Satisfactory	79 (71.8)
High	7 (6.4)
Myths and superstitions about general health[a]	
Injections are better than drugs	77 (70)
Not applying oil can lead to poor eyesight	3 (2.7)
Home remedies work better than allopathic medicines	56 (50.9)
Private hospitals are better than Government hospitals	7 (6.4)
Other beliefs	4 (3.6)
Myths and superstitions regarding TB[a]	
Hereditary disease	63 (57.3)
People get TB due to past sins	10 (9.1)
Women are more prone to it	4 (3.6)
Home remedies/traditional treatment is better	19 (17.3)
Eating beef meat cures TB	48 (43.6)
Others	11 (10)

[a] Multiple response.

effective. People in the community tend to ask health visitors about other general health problems during TB health camps and house visits to TB patients.

3.4. Myths and superstitions regarding TB

About 57% of health workers state that TB is still considered to be a hereditary disease by people. 43.6% have noticed a widespread belief in the community that the intake of beef meat can cure TB. There is also a popular notion that traditional treatment systems and home remedies are better, as mentioned by 17.3%. TB is due to past sins and women are being more prone to it are other myths, as stated by 9.1% and 3.6%, respectively (Table 1). Smoking and drinking alcohol regularly are also believed to be the causes of TB. The notion that only poor people seem to be affected by the disease is also observed among people.

Merely stating that TB is an infectious disease will not clear these widespread, deep-rooted beliefs. Influential community members, elderly people, and religious leaders could be more effective in making a health initiative successful, as observed in the polio eradication program in Uttar Pradesh.[12]

3.5. Knowledge gain during treatment

An increase in knowledge about the disease during treatment among patients was seen by about half the health workers (Table 2). For a better cure rate and adherence, proper understanding of the disease and treatment period is necessary. Health workers' attitudes, their IPC, and a good IEC environment with posters, booklets and pamphlets, and TB-related videos on television at the DOTS centers can help this knowledge gain. It will help to bring new patients for treatment.

Table 2 – Knowledge of TB and reasons for treatment default among patients (N-110).

Variable	*n* (%)
Knowledge gain about TB during treatment	
Yes	56 (50.9)
No	46 (49.1)
Awareness about infecting others	
Yes	52 (56.4)
No	48 (43.6)
Knowledge of HIV-TB co-infection	
Poor	51 (46.4)
Good	47 (42.7)
Confusion	12 (10.9)
Reasons for not completing DOTS[a]	
Side effects of the drugs	45 (40.9)
Feeling better after 2 to 3 months	94 (85.4)
Non availability of drugs	6 (5.4)
apathy of health workers	6 (5.4)
Other reasons	12 (10.9)
Usage of communication materials meant for family members	
Yes	
No	20 (18.2)
There are very few	46 (41.8)
	44 (40)

[a] Multiple response.

3.6. Awareness among patients about infecting others

More than half of (56.4%) the health workers say that there is awareness among patients that it can spread to others from them. Knowledge about the transmission route of TB has to be further reinforced through interpersonal communication (IPC) with patients as their immediate family members; neighbors in the community are at greater risk of getting infected. Patients have to be educated about covering their mouths while sneezing and coughing to avoid further spread.

3.7. Knowledge of TB and HIV coinfection

Knowledge about TB and HIV coinfection is poor according to 46.4% of the health workers (Table 2). About 11% think that there is confusion regarding this aspect of TB among people and patients. Imparting this knowledge clearly is important as TB's association with HIV/AIDS might increase the stigma surrounding TB. Misunderstanding and stigma may affect the self-reporting of cases as well as passive case detection by health workers.

3.8. Reasons for not completing DOTS

Main reasons for not completing DOTS treatment by patients include feeling better with disappearing of the symptoms after a few weeks (85.4%), side effects of the drugs (40.9%), nonavailability of drugs (5.4%), and apathy of health workers (5.4%) as reported by health workers (Table 2). Work pressure, alcoholism, confusion and misunderstanding about the disease, and depression are cited to be other reasons.

The importance of treatment and its adherence should be explained to the patients repeatedly through IEC materials and counseling. The common side effects such as vomiting and nausea should also be addressed. There are very few exclusive IEC materials for the patients.

3.9. IEC materials for family members of TB patients

Only 18.2% have used some sort of IEC materials meant for family members of the patients. Family members play a key role in persuading patients to complete DOTS. They should be aware of the transmission route as they may also get infected with TB from them. They should know that touching the patients or sharing utensils with them does not spread the infection.

3.10. Engaging beneficiaries (cured patients) to create awareness

More than three-fourth of health workers think that engaging cured patients in the communication program can be an effective method to create general awareness among the public (Table 3). Some TB posters and brochures have photographs of former patients showing them before and after the treatment. In addition to their pictures, they can be part of television programs narrating their treatment experiences. Including

Table 3 – Communication strategies to spread awareness among public and patients (N-110).

Variable	*n* (%)
Engaging cured patients to create awareness as an effective method	
Yes	85 (77.3)
No	25 (22.7)
Effective media for creating TB awareness[a]	
Wall posters/banners	23 (20.9)
Fliers/pamphlets	17 (15.4)
Radio	17 (15.4)
Television	40 (36.4)
Mobile phones	7 (6.4)
IPC through Health workers	26 (23.6)
All the above	65 (59.1)
Other IEC activities[a]	
Rallies	17 (15.4)
Public meetings	41 (37.3)
Human chains	8 (7.3)
Celebrating world TB day	21 (19.1)
Collateral media	17 (15.4)
Patient-provider meetings	30 (27.3)
All the above	66 (60)

[a] Multiple response.

them in rallies, music concerts, and street plays has been proven to be effective in Mexico and Peru.

3.11. *Effective media for creating TB awareness*

Television (36.4%) and IPC (23.6%) seem to be the most effective media for TB communication in the communities (Table 3). Wall posters and banners (20.9%) also play an important role. Fliers and radio are considered to be a useful medium (15.4%) followed by mobile phones (6.4%).

Two-thirds of health workers think of media-mix strategy as effective. Communication programs are impactful when consistent messages are conveyed through a mix of mass media and IPC. This approach helps emphasize and reinforce messages, and enables the program to reach different sectors of the population, who may be more receptive to one form of communication over the other. A similar approach was used by Vietnam to disseminate consistent messages through national television, radio, local radio stations, print materials, billboards, community gatherings, theater shows, video spots in clinics and home visits (HC insights 2004).[4]

3.12. *Other IEC activities for awareness generation*

Among various communication means shown in Table 3, public meetings and patient-provider meetings can be very effective as reported by 37.3% and 27.3% of the health workers respectively followed by world TB day celebrations (19.1%), rallies (15.4%) and human chain formation by people (7.3%). Collateral media (utility products such as T-shirts, caps, and key chains with short printed messages on them) distributed during events could be useful (15.4%). All of the above strategies can be effective according to the majority (60%) of them.

Well-organized public meetings can address and reach a large audience. Patient-provider meetings can break the formal power barrier leading to mutual understanding and this healthy relationship could achieve better adherence rate among patients. Strategies like rallies, human chain and celebrating World TB day can create more visibility for the disease and draw the attention of policy makers and bureaucrats.

3.13. *Audio–visual aids used by health workers*

Flip charts and video projectors are the most common audio–visual aids on the field used by 53.6% and 40% of health workers, respectively. Mobile units such as jeeps/autorickshaws (30.9%) and megaphones (11.8%) are used for making announcements (Table 4). Audio recordings are used by about 11% in the outdoors. People in the villages see the jeep with mike set as a government symbol and immediately associate it with the usual polio or other health campaigns.

3.14. *Frequency of calls made to patients*

About two-thirds of the health workers call at least 5–15 patients a day from their mobile phones. About 12% make more than 15 calls to different patients followed by 10% making less than 5 calls. Mobile phones can be an inexpensive medium to contact the defaulting patients. Health workers can also make calls to the family members of patients in case of default. Alarm facilities and SMS text messages can be used as reminders for medication. Mobile phones can play a useful role in DOTS treatment.

3.15. *Showing films/clips on mobile phones*

From Table 4, it is clear that only 11.8% have shown video health clips on their mobiles to the patients, whereas the vast

Table 4 – Audio visual aids and mobile phones usage by health workers (N-110).

Variable	*n* (%)
Use of audio-visual aids in the field[a]	
Megaphones	13 (11.8)
Audio recordings	12 (10.9)
Flip chart	59 (53.6)
Video screenings	44 (40)
Announcements through microphone from a moving vehicle	34 (30.9)
Number of calls made to patients in a day	
Nil	14 (56.4)
Less than 5	11 (10)
5–15	72 (65.5)
More than 15	13 (11.8)
Most discussed issues on the mobile phone[a]	
Side effects of the drugs	70 (63.6)
Food and nutrients	35 (31.8)
Counseling	15 (13.6)
Disease	45 (40.9)
Showing films/clips on mobile phones to people	
Yes	13 (11.8)
No	97 (88.2)

[a] Multiple response.

majority of health workers (88.2%) have not used this facility. This feature on mobile phones can be put to good use in the field for health education. The use of smart phones having many features is less among health workers.

4. Conclusion

The findings of the study suggest that interpersonal channels such as face-to-face communication, regular interaction among health workers and patients, public gatherings, discussions/patient-provider meetings and television could be very effective for creating TB awareness. Flip-charts, microphone announcements and video screenings are popular and regularly used by health workers in rural communities. Misconceptions, myths and superstitions about TB and TB/HIV coinfection should be reinforced through multiple channels and messages. Though there is an increase in the knowledge levels among patients lack of awareness about infecting others was also observed. There are very few communication materials for TB patients and family members. Their information needs such as side effects of the medication and nutrient food should be addressed in them.

Conflicts of interest

The authors have none to declare.

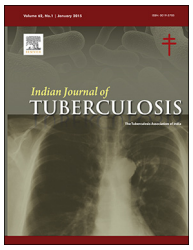
REFERENCES

1. WHO. *WHO Global Tuberculosis Report*. World Health Organization Publication; 2015. http://www.who.int/tb/publications/globalreport/gtbr12_main.pdf Accessed 15.07.16.
2. TBC. *Key facts and concepts*. TBC Publication; 2015. http://www.tbcindia.nic.in/ Accessed 15.03.16.
3. Umashankar K. *TB Cases on Rise in Chittoor District*. The Hindu; 2016. http://www.thehindu.com/todays-paper/tp-national/tp-tamilnadu/tb-cases-on-the-rise/article4549469.ece Accessed on 11.07.16.
4. Thuy DO, Llanos-Zavalga F, Huong NTM, Poppe P, Tawfik Y, Church-Balin C. *The Role of Health Communication in Achieving Global TB Control Goals – Lessons from Peru, Vietnam and Beyond. Health Communication Insights Summary*. Baltimore: Health Communication Partnership based at Johns Hopkins Bloomberg School of Public Health/Center for Communication Programs; 2016. http://www.stoptb.org/assets/documents/countries/acsm/Summary.pdf Accessed on 15.07.16.
5. Sreeramareddy C, HarshaKumar H, Arokiasamy J. Prevalence of self-reported tuberculosis, knowledge about tuberculosis transmission and its determinants among adults in India: results from a nation-wide cross sectional household survey. *BMC Infect Dis*. 2013;13:13–16.
6. Steyn M, vandermerwe N, Dick J, Borcherds R, Wilding R. Communication with TB patients: a neglected dimension of effective treatment. *Curationis*. 1997;20:53–56.
7. Menegoni L. Conceptions of tuberculosis and therapeutic choices in Highland Chiapas, Mexico. *Med Anthropol Q*. 1996;10:381–401.
8. WHO. *Advocacy, Communication and Social Mobilization to fight Tb. A 10-Year Framework for Action 2006*. ASCM Framework for Action 2006–2007; 2006. Available from http://whqlibdoc.who.int/publications/2006/9241594276_eng.pdf. Accessed on 12.06.13.
9. Sharma N, Taneja DK, Pagare D. The impact of an IEC campaign on tuberculosis awareness and health seeking behavior in Delhi, India. *Int J Tuberc Lung Dis*. 2005;91:259–265.
10. Revised National TB Control programme Annual Status Report. *TB India 2015 Central TB Division*. 2015. Available from: http://tbcindia.nic.in/showfile.php?lid=3166 Accessed on 06.03.15.
11. Khan A, Walley J, Newell J, Imdad N. Tuberculosis in Pakistan: socio-cultural constraints and opportunities in treatment. *Soc Sci Med*. 2000;50:247–254.
12. Singhal A. Adaptive micro and macro communication strategies to eradicate polio in India: social mobilization, opinion leadership and inter-personal influence at unprecedented scale. *Int J Commun Soc Res*. 2013;1.

Available online at www.sciencedirect.com

ScienceDirect

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



Original Article

A cross-sectional study to assess the stigma associated with tuberculosis among tuberculosis patients in Udupi district, Karnataka

R. Shivapujimath *, *A.P. Rao*, *A.R. Nilima*, *D.M. Shilpa*

Manipal University, Department of Public Health, Manipal, India

ARTICLE INFO

Article history:
Received 6 August 2016
Accepted 26 October 2016
Available online 15 December 2016

Keywords:
Stigma
Tuberculosis
Social determinants
DOTS
Karnataka

ABSTRACT

Background: For decades, tuberculosis and other communicable diseases like human immunodeficiency virus/acquired immune deficiency syndrome, leprosy, etc., have been associated with stigma and discrimination by the society; this can interfere with the lifestyle and disease management among these patients.

Objective: To assess the stigma experienced by tuberculosis patients and to find the factors associated with stigma.

Methods: A cross-sectional study was conducted among 209 sputum-positive and sputum-negative tuberculosis patients. Convenient sampling was used to identify the patients. A predesigned, pretested proforma from Explanatory Model Interview Catalogue developed by World Health Organization was used for data collection.

Results: The study revealed that out of 209 respondents, 51.2% of the respondents experienced some form of stigma. Majority of the patients have received only primary education and 71.3% of the respondents were males. Most of the patients were under category 1 of Directly Observed Treatment Short course. Age, education, and smear status of the patient were found to be associated with stigmatization ($P < 0.05$), whereas factors like gender, income, occupation, family history, and marital status were found to be not significantly associated with stigmatization.

Conclusion: Effective counseling measures are recommended for tuberculosis patients with advancing age and education which can help reduce stigmatization and thereby improve quality of life.

1. Introduction

One of the top 10 causes of global mortality is tuberculosis (TB).[1] Approximately one-fourth of the population is infected with the TB bacillus, and in the year 2014, 9.8 million people developed the disease and 1.5 million people died due to it worldwide.[2] In 2014, an estimated 1 million children became ill with TB and 140,000 children died of TB.[3] The situation has improved over the past two decades for the people with only

* *Corresponding author.*
E-mail address: dr.rajesh.s88@gmail.com (R. Shivapujimath).

http://dx.doi.org/10.1016/j.ijtb.2016.10.002

TB disease, whereas the situation has worsened for others over the past two decades owing to the human immunodeficiency virus/acquired immune-deficiency syndrome epidemic.[1] TB is a leading killer of human immunodeficiency virus (HIV)-positive people; in 2015, 1 in 3 deaths among HIV patients was due to TB.[2] Globally in 2014, an estimated 480,000 people developed multidrug-resistant TB (MDR-TB), with the emergence of multidrug resistance in almost all the parts of India, following deterioration of the health infrastructure. The World Health Organization statistics for 2014 give an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9 million.[2] In 2014, a total of 43,689 people were diagnosed for smear-positive TB out of 61,328 people who were registered for treatment.[3] Mortality and morbidity rates are always high with TB, although it is almost curable with uninterrupted and appropriate treatment.[4] Most specialists recognize the integral part of patient adherence in endeavors to control the disease. Health-seeking behavior and knowledge about the causes of TB among groups and individuals are critical, and may influence the transmission of the disease.[5] There are beliefs and practices that may delay diagnosis, and in this way increasing the risk of spread of disease in the society.[1]

Numerous communicable diseases, for example, TB, HIV/acquired immune deficiency syndrome (AIDS), and leprosy, are connected with stigma and segregation, which enormously affect the sufferers.[4] The effect is felt at home, in the work environment, and in the community. Discrimination has critical contemplations for the arrangement of well-being and clinical practice. It adds to torment in different ways, and may defer care-seeking behavior and treatment, prompting delayed transmission of communicable illness, drug resistance, or intricacies that expand treatment costs for a treatable health issue.[5] Regardless of the presence of an effective cure for TB, TB rate in high-burden nations suggests obstructions to successful determination, treatment, and cure.[6] Evidence proposes that sociocultural variables and TB-related stigma may restrain patients from looking for care or finishing a full course of treatment, increasing morbidity and mortality because of TB, and aggravating its spread inside groups.[7]

The social stigma is perceived as a critical boundary for fruitful consideration of individuals affected by TB. TB has been and is still considered as a 'messy infection,' 'a capital punishment,' or as influencing 'liable individuals'.[8–10]

2. Methods

A cross-sectional study was conducted in Udupi district of Karnataka state between January and June 2016. All the patients registered under Directly Observed Treatment Short course (DOTS) for anti-TB treatment in all the three Taluks of Udupi district were included in the study. Patients who were not willing to participate, critically ill, and transferred out cases were excluded. The semi-structured interview schedule was adopted from Explanatory Model Interview Catalogue (EMIC) developed by WHO/Special Programme for Research and Training in Tropical Diseases as a research instrument. Pretesting and validation of the tool was done among 20 patients under anti-TB treatment. Twelve of the patients were found to be stigmatized among the total. The interview schedule consisted of sociodemographic profile, review of record, illness experience-related questions, and stigma-related questionnaire. The stigma-related questionnaire consisted of 22 items. Responses were coded on a 0–3 ordinal scale (0 = no, 1 = uncertain, 2 = possibly and 3 = yes). Items were scored on a 4 point Likert scale (3–0 with 3 = yes, 2 = possibly, 1 = uncertain and 0 = no). Maximum obtainable score was 66 and minimum score was 0. The participants scoring 33 and above were considered as stigmatized, and the participants scoring below 33 were considered as not stigmatized. Total patients registered under DOTS for the last quarter of 2015–2016 were 376. At 43% prevalence rate of stigma, and 95% confidence interval, and 80% power, the sample size was taken as 377. After obtaining the ethical clearance from the Institutional Ethical Committee (IEC 836/2015) and permission from the Joint Director of state TB cell and District Tuberculosis Control Officer (DTO), in the presence of Senior Treatment Supervisor (STS), after obtaining the informed consent, interview was conducted as per convenience of the STS by visiting the houses of TB patients.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) 15 for windows (SPSS Inc., Chicago, IL). Statistical test of significance (Chi-square test) was used to find out the association between stigmatization and various clinical and sociodemographic variables.

3. Results

Among the 209 respondents, majority of the TB patients (24.40%) were found in the age group of 38–47 years while 18.18% were found in the age group of 28–37 years. Most of the TB patients have received only primary education (26.3%). Most of the participants were found to be males (71.3%), and nearly 29.7% of the TB patients were daily wage workers. Treatment pattern of the TB patients is shown in Table 1. More than 50% of the respondents had a family income of Rupees 5000–10000 per month.

Out of the 209 respondents, 51.2% of the respondents were stigmatized. Among 149 males, 76 (51%) of them reported that they were stigmatized, and out of 60 females, 31 (51.7%) of them reported; however, they were stigmatized and there was no significant association between gender and stigma experienced ($P = 0.931$).

About 81% of the respondents opined that TB will be cured, before the start of the treatment, and 92% of them said it is curable, after the start of the treatment. About 84.7% of them said that their friends were supportive if the disease status was not disclosed, while 84.7% of them said that their neighbors were supportive after disclosing the disease status. Majority (88%) of the participants reported that they were not invited for any social functions, and 98.08% of them were not attending any social functions.

In this study, it was found that 18.7% of the patients stopped/discontinued treatment. The most common reason they opined was loss of respect or being put to shame by the surrounding community. There was significant association between stigmatization and age ($P = 0.011$), education ($P = 0.007$), and smear status ($P = 0.045$).

Table 1 – Sociodemographic variables and clinical profile.

Characteristics	n (%)	Stigmatized n (%)	P value
Age in Years			
18–27	36 (17.22)	27 (75.0)	0.011*
28–37	38 (18.1)	20 (52.6)	
38–47	51 (24.4)	23 (45.0)	
48–57	35 (16.7)	20 (57.1)	
58–67	28 (13.3)	9 (32.1)	
68–77	21 (10.0)	8 (38.0)	
Sex			
Male	149 (71.3)	76 (51.0)	0.931
Female	60 (28.7)	31 (51.7)	
Marital status			
Unmarried	39 (18.7)	23 (58.9)	0.342
Married	169 (80.9)	84 (49.7)	
Divorced	0 (0)	0 (0)	
Widow	1 (.5)	0 (0)	
Education			
Illiterate	38 (18.2)	13 (34.2)	0.007*
Primary	55 (26.3)	29 (52.7)	
High school	54 (25.8)	26 (48.1)	
Pre University	53 (25.4)	31 (58.5)	
Degree	9 (4.3)	8 (88.9)	
Family Income monthly (Rs)	37 (17.7)	17 (45.9)	0.84
0–5000	116 (55.5)	55 (47.4)	
5001–10000	33 (15.7)	33 (62.5)	
>10000			
DOTS option			
Category 1	155 (74.2)	82 (52.9)	0.065
Category 2	40 (19.1	15 (37.5)	
MDR-TB	14 (6.7)	10 (71.4)	
Smear status			
Sputum positive	162 (77.5)	89 (54.9)	0.045*
Sputum negative	47 (22.5)	18 (38.3)	
Treatment phase			
Intensive phase	155 (74.2)	81 (52.3)	0.603
Continuous phase	54 (25.8)	23 (48.1)	
Previous history of TB			
Yes	47 (22.5)	23 (48.9)	0.725
No	162 (77.5	84 (51.9)	
Family history of TB			
Yes	52 (24.9)	27 (51.9)	0.904
No	157 (75.1)	80 (51.0)	

* Significant at P-value <0.05.

4. Discussion

The majority of the study respondents were male (71.3%), and 28.7% were female. About 80.9% of the respondents were married. More than one-fourth (26.3%) of the respondents had only primary education, 25.8% had high school education, 25.4% had pre-university education, and 18.2% of them were illiterate. Similar results have been reported by Suleiman et al. in the study conducted in Gezira state, Sudan, which shows that 58.19% of respondents were males and 41.81% were females. 54.66% of the respondents were married, while 57.41% of the respondents had intermediate education. 23.52% of the respondents were illiterate.[1]

This study also shows that 74.2% of the respondents belonged to category 1 treatment, while 19.1% of them belonged to category 2 treatment. About 22.5% of the respondents had previous history of TB and 24.9% of them had family history of TB. This is similar to the findings of the study conducted by Aryal et al. in Nepal, in which 73.33% belonged to category 1 and 13.33% of them belonged to category 2. 65% of the respondents had previous history of TB and 50% of them had family history of TB.[6]

This study's results show that more than half of the participants were stigmatized (51.02%). This was found to be lesser than the study conducted by Aryal et al. in Nepal, in which 63.3% of respondents were stigmatized,[6] and also in the study conducted by Jittimanee et al. in Thailand, wherein 65% of the respondents were stigmatized.[11]

There was no significant difference across genders in relation to stigma experienced (0.931). Similar findings have been reported in the study conducted by Chandrasekaran and Muniyandi in South India.[7]

This study has limitations; because of nature of sampling and nonparticipation of TB patients, it was possible to interview only 55.4% of the patients. Complete enumeration of the patients would have yielded better results. Presence of STS at the time of interview might have influenced the answers of the respondents. Another limitation was that we could not interview the patients who were not registered under DOTS.

This study revealed that age and educational status influenced stigmatization among TB patients in the district. Improved awareness regarding impact of adequate treatment and follow-up can bring about a change in behavior and attitude of patients as well as community and this can effectively enhance the quality of life of TB patients by reducing stigmatization. DOTS providers and community health workers can help discharge these duties to improve the awareness of the family and social support of TB patients.

Conflicts of interest

The authors have none to declare.

Acknowledgements

The researchers are deeply indebted to Professor Mitchell G. Weiss of Swiss Tropical Institute, Department of Public Health, Switzerland for providing the EMIC. We also acknowledge Dr. G.N. Rajani (DTO, Udupi district) for allowing us to conduct the study, State TB cell, Karnataka for providing funds for the project, and also to Mr. Sandeep (STS, Udupi Taluk), Mr. Hariprasad (STS, Karkala Taluk), and Mr. Gurudas (STS, Kundapura Taluk) for accompanying with us for every interview.

REFERENCES

1. Ahmed Suleiman MM, Sahal N, Sodemann M, El Sony A, Aro AR. Tuberculosis stigma in Gezira State, Sudan: a case–control study. *Int J Tuberc Lung Dis*. 2013;17(3):388–393.
2. World Health Organization. *Global Tuberculosis Control*. WHO; 2008. Available from: http://www.who.int/entity/TB/publications/global_report/en/index.html [accessed 8.02.16].
3. World Health Organization. Tuberculosis – TB in South-East Asia – Epidemiology. Available from: http://www.searo.who.int/en/Section10/Section2097/Section2100_10639.htm [accessed 28.11.15].
4. Abebe G, Deribew A, Apers L, et al. Knowledge, health seeking behavior and perceived stigma towards tuberculosis among tuberculosis suspects in a rural community in Southwest Ethiopia. *PLoS ONE*. 2010;5(10):1–7.
5. Ganapathy S, Thomas BE, Jawahar MS, Selvi KJA, Weiss M. Perceptions of gender and tuberculosis in a south Indian urban community. *Indian J Tuberc*. 2008;55:9–14.
6. Aryal S, Badhu A, Pandey S, et al. Stigma related to tuberculosis among patients attending DOTS clinics of Dharan municipality. *Kathmandu Univ Med J*. 2012;10(37):48–52.
7. Chandrasekaran V, Muniyandi M. Psycho-social dysfunction: perceived and enacted stigma among tuberculosis patients registered under revised national tuberculosis control programme. *Indian J Tuberc*. 2008;55:179–187.
8. Atre SR, Kudale AM, Moranker SN, Rangan SG, Weiss MG. Cultural concepts of tuberculosis and gender among the general population without tuberculosis in rural Maharashtra. *Trop Med Int Med*. 2004;9(11):1228–1238.
9. Long NH, Johansson E, Diwan VK, Winkvist A. Fear and social isolation as consequences of tuberculosis in Vietnam: a gender analysis. *Health Policy*. 2001;58(1):69–81.
10. Weiss MG, Somma D, Karim F, et al. Cultural epidemiology of TB with reference to gender in Bangladesh, India and Malawi. *Int J Tuberc Lung Dis*. 2008;12(7):837–847. Available from: http://www.who.int/tdr/publications/documents/cultural-epidemiology-TB.pdf.
11. Jittimanee SX, Nateniyom S, Kittikraisak W, et al. Social stigma and knowledge of tuberculosis and HIV among patients with both diseases in Thailand. *PLoS ONE*. 2009;4(7):e6360. http://dx.doi.org/10.1371/journal.pone.0006360.

Available online at www.sciencedirect.com

ScienceDirect

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

Forum

A rare case of tension pneumatocele

Pankaj Madhukar Gholap *, *S. Anuroop Shankar*

Department of Pulmonary Medicine, Government Medical College and Hospital, Nagpur, India

ARTICLE INFO

Article history:
Received 2 March 2016
Accepted 30 September 2016
Available online 28 December 2016

Keywords:
Tension pneumatocele
Lung herniation

1. Brief report

A 48-year-old, married farmer, a known case of type 2 diabetes mellitus, presented with right-sided chest pain, which was continuous and gradually progressing in severity since 15 days, shortness of breath on exertion that was gradually progressing in intensity for 15 days, fever, and cough with yellowish purulent expectoration for 15 days. After two days of admission, the patient complained of sudden onset of swelling over the right side of the chest. The pulse rate was 102/min, blood pressure was 100/60 mm Hg, respiratory rate was 32/min, and SpO_2 was 78% in room air and improved to 94% after supplemental oxygen. Total leukocyte count was 22,000/cmm with 90% neutrophils and 10% lymphocytes; random blood sugar level was 330 mg/dl, urine ketones were nil, and liver function tests and renal function test were within normal limits. Sputum culture sensitivity report showed *Staphylococcus aureus* sensitive to Linezolid and resistant to Vancomycin and Methicillin. Serial chest X-ray showed expanding cavity/pneumatocele; high-resolution computed tomography (HRCT) of the chest; HRCT chest was suggestive of a tension pneumatocele, which expanded and herniated into the subcutaneous plane. A final diagnosis of Staphylococcal pneumonia with tension pneumatocele formation herniating into the subcutaneous plane was made. The patient was treated with Inj. Linezolid with supplemental oxygen and CT-guided percutaneous catheter placement using a pigtail catheter for decompression of pneumatocele.

Pulmonary pneumatoceles are thin-walled, air-filled cysts that develop within the lung parenchyma. They can be single, emphysematous lesions or more often multiple, thin-walled, air-filled, cyst-like cavities. Acute pneumonia with pneumatocele complication is commonly caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*,

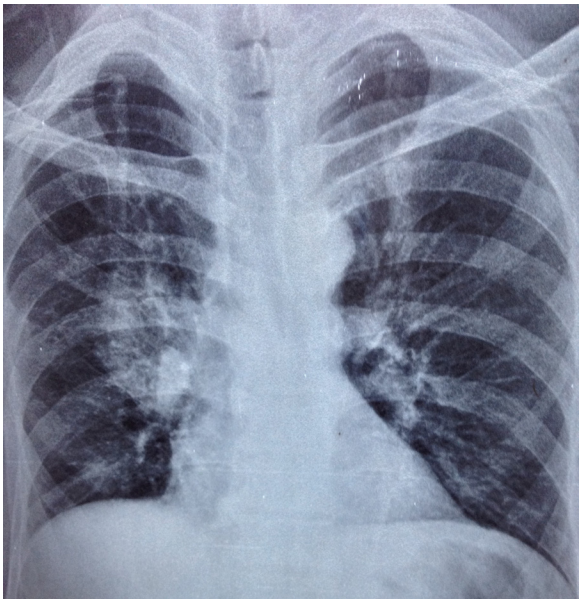


Fig. 1 – Chest X-ray showed small right lung cavitating nodule.

* *Corresponding author*. Tel.: +91 8600043422.
E-mail address: drpankajgholap@gmail.com (P.M. Gholap).

http://dx.doi.org/10.1016/j.ijtb.2016.09.020

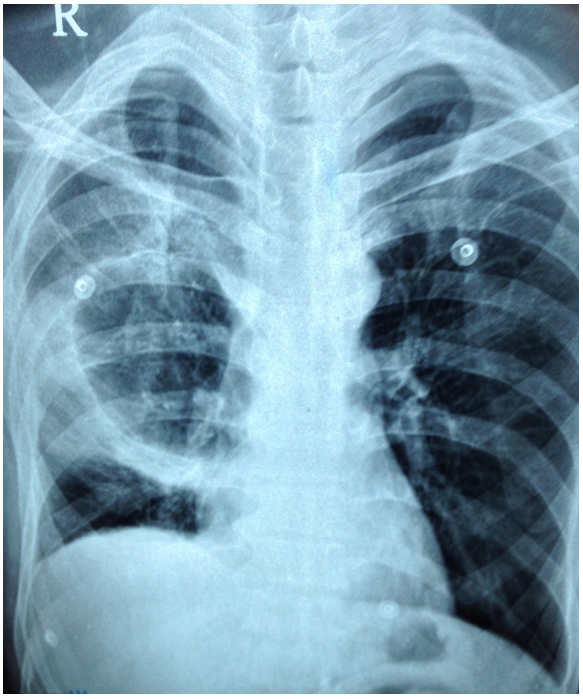


Fig. 2 – Second X-ray chest PA view after 2 days showing thin-walled cavity in right parahilar area.

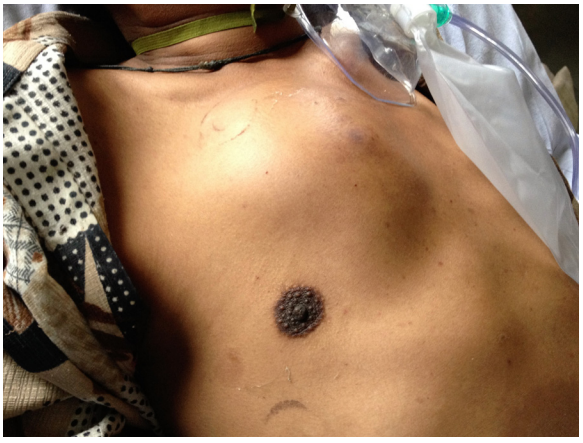


Fig. 3 – Swelling over anterior chest wall.

group A streptococci, *Serratia marcescens*, *Klebsiella pneumoniae*, Adenovirus, *Pneumocystis jiroveci* pneumonia, and tuberculosis. Pneumatoceles are more common in young children less than 4 years of age,[1] indicating the incidence decreases with age (Figs. 1–6).

Antibiotics are the first-line therapy for asymptomatic pneumatoceles. Most pneumatoceles are asymptomatic and resolve naturally with conservative management,[2] and invasive treatment is required only for those with complications like tension pneumatocele cardiovascular and respiratory distress. For the management of postinfectious multiple pneumatoceles, image-guided decompression using catheters or chest tubes is indicated to relieve the acute symptoms.[3] Thoracotomy and resection of the diseased lung, tube thoracostomy, and multiple, blind percutaneous venous catheter drainage are the other options for management. On

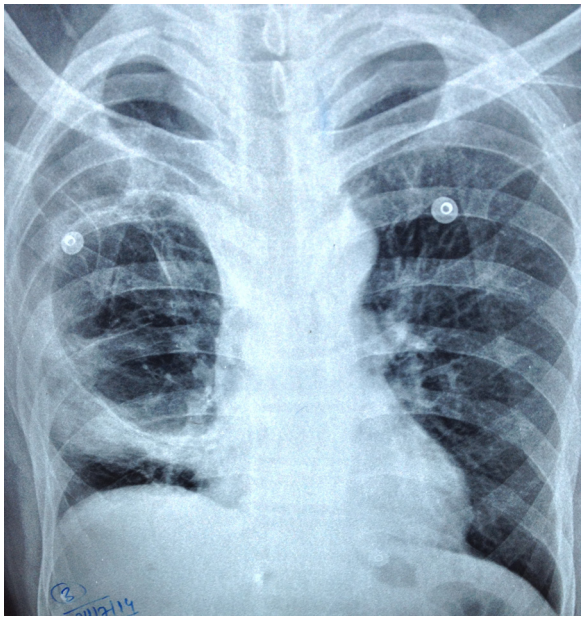


Fig. 4 – Follow-up X-ray showed progressive increase in the size of the cavity.

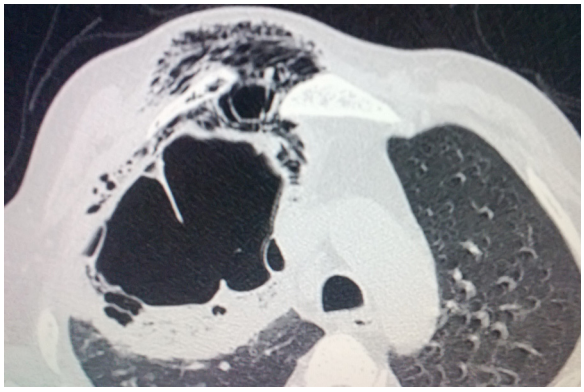


Fig. 5 – HRCT thorax showing tension pneumatocele with lung herniation in subcutaneous plane transverse view.

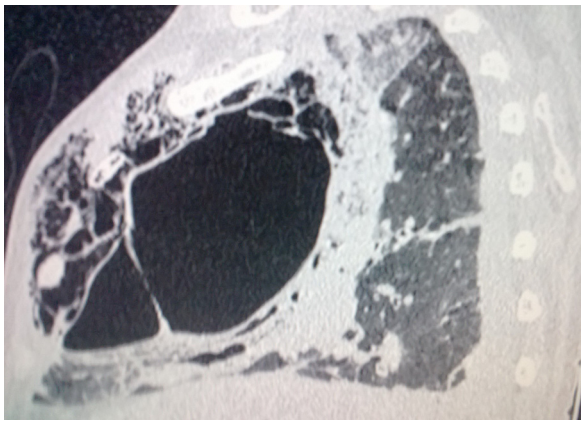


Fig. 6 – HRCT thorax showing tension pneumatocele with lung herniation in subcutaneous plane lateral view.

searching on PubMed, we found only one case of giant pneumatocele in an adult with AIDS (Acquired Immmunodeficiency Syndrome) and *Pneumocystis jiroveci* pneumonia.[4] Even after percutaneous drainage, the prognosis in such cases is poor due to massive alveolar destruction.[5]

Conflict of interest

The authors have none to declare.

REFERENCES

1. Kunyoshi V, Cataneo DC, Cataneo AJM. Complicated pneumonias with empyema and/or pneumatocele in children. *Pediatr Surg Int*. 2006;22(2):186–190.
2. İmamoğlu M, Çay A, Koşucu P, et al. Pneumatoceles in postpneumonic empyema: an algorithmic approach. *J Pediatr Surg [Internet]*. 2016;40(7):1111–1117. http://dx.doi.org/10.1016/j.jpedsurg.2005.03.048.
3. Zuhdi MK, Spear RM, Worthen HM, Peterson BM. Percutaneous catheter drainage of tension pneumatocele, secondarily infected pneumatocele, and lung abscess in children. *Crit Care Med*. 1996;24(2):330–333.
4. Yao CW, Shen TC, Tu CY. Giant pneumatocele with lung herniation. *Intern Med*. 2013;52(20):2377–2378. PMID: 24126406.
5. Konishi M, Amimoto M, Yoshimoto E, et al. AIDS-related *Pneumocystis carinii* pneumonia with disappearance of cystic lesions after treatment. *Intern Med [Internet]*. 2002;41(10):896–898. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12413019.

Available online at www.sciencedirect.com

ScienceDirect

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

Case Report

A rare presentation of disseminated tuberculosis: Prostatic abscess

Ajay Verma, Anubhuti Singh, Kislay Kishore, Surya Kant *

Department of Respiratory Medicine, King George's Medical University, Chowk, Lucknow 226003, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 19 October 2016
Accepted 31 October 2016
Available online 13 February 2017

Keywords:
Genito-urinary TB
Disseminated TB
Protatic abscess

ABSTRACT

Involvement of the prostate by tuberculosis (TB) occurs rarely and tuberculosis prostate abscess is an even rarer occurrence. It has been reported in immunocompromised patients, mainly human immunodeficiency virus seropositive individuals. We are reporting a case of tuberculosis prostatic abscess in an immunocompetent patient with relapse of TB.

1. Introduction

Genito-urinary tuberculosis (GUTB) is one of the common forms of extra-pulmonary tuberculosis (EPTB) in the world. The organs, which are frequently involved in urinary TB, are kidneys, ureters and bladder while organs frequently involved by genital TB are fallopian tubes, uterus and ovaries in females and epididymis and testis among males. Involvement of prostate and seminal vesicles is rare and more so in immune-competent individuals. We are reporting such a case of TB prostatic abscess in an immunocompetent patient with relapse of TB. This case highlights the fact that unexplained urinary symptoms or persistent pyuria in patients with any history of TB or radiographs suggestive of active or inactive disease should lead to an evaluation for GUTB.

2. Case

A 35-year-old, non-diabetic, non hypertensive, non smoker male was referred to neurosurgery department from a private practitioner with left sided upper and lower limb weakness for one month and altered sensorium for 15 days. At admission, patient's Glasgow Coma Scale (GCS) was E_4 V_T M_5. Magnetic resonance imaging (MRI) brain revealed contrast-enhancing ring shaped lesions in right pons suggestive of tuberculoma. Patient was given supportive treatment and opinion was sought from our side.

Mantoux revealed 25 mm induration and chest X ray (CXR) showed right-sided pleural effusion (Fig. 1). Patient was unable to raise sputum. Under ultrasonography (USG) guidance, ~250 ml straw-coloured pleural fluid was aspirated. It was exudative, lymphocyte predominant with adenosine

* *Corresponding author at*: Professor and Head, Department of Respiratory Medicine, King George's Medical University, Chowk, Lucknow 226003, Uttar Pradesh, India.
E-mail address: skantpulmed@gmail.com (S. Kant).

http://dx.doi.org/10.1016/j.ijtb.2016.10.006

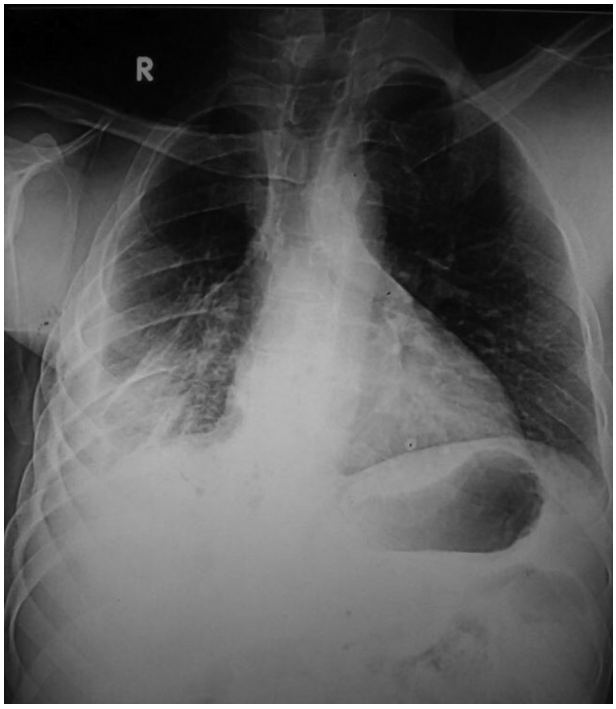


Fig. 1 – Chest radiograph posterio-anterior (PA) view showing right side plural effusion and infiltrates in lower zone.

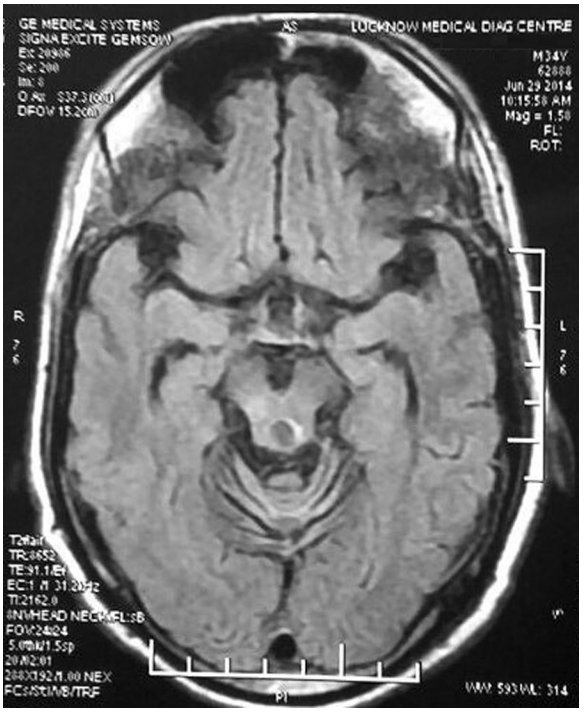


Fig. 2 – Magnetic Resonance Imaging (MRI) brain showing few focal rounded areas of signal intensity alteration suggestive of calcified nodules in pons.

deaminase (ADA) of 46 IU/ml. Patient was started on 4-drug anti-tuberculosis treatment (ATT) including rifampicin, isoniazid, ethambutol and pyrazinamide as per weight with the diagnosis of disseminated TB. GCS improved to E_4 V_T M_6. He was discharged on Ryle's Tube feeding, tracheostomy in situ and per-urethral catheter.

On two months follow-up visit, patient responded significantly to ATT. There was improvement in left hemiparesis, gaining a weight of ~10 kg; tracheostomy was closed and GCS was E_4 V_4 M_6. With adjuvant physiotherapy, limb power improved to 4/5. He started self-feeding and self voiding. He was shifted to continuation phase with 3 drugs after 6 months. Follow-up MRI after one year revealed reduction in size of lesions and after 18 months revealed few focal areas of calcification in pons without any significant enhancement around them and communicating hydrocephalus (Fig. 2). CXR showed clearing of pleural effusion. There was residual weakness on the right side of body, but otherwise, patient had improved. ATT was stopped in view of clinico-radiological improvement. He was lost to follow-up after that.

For the past two months, patient developed high grade fever with chills. There was no burning micturition. Complete blood count (CBC) revealed leukocytosis and peripheral blood smear for malarial parasite was negative. Urine routine and microscopic (R/M) examination showed no abnormality. Despite antibiotics, patient had persistent fever. Patient later developed whitish discolouration of urine.

A repeat urine R/M was done which showed 30 pus cells/high power field (hpf) and urine Gram (G) stain showed *E. coli*. Patient was advised USG whole abdomen which revealed hepatosplenomegaly and an anechoic structure with a size of 4.6 cm × 3.5 cm posterior to the urinary bladder (Fig. 3). For further demarcation, CT kidney ureter and bladder (KUB) was performed, showing enlarged left seminal vesicle with abscess and hypodense areas in prostatic parenchyma suggestive of abscess (Fig. 4). Trans-rectal USG (TRUS) was done, which showed 30 cc prostatic collection. TRUS-guided aspiration of pus from prostatic abscess was performed under local anaesthesia (LA) and sent for Gram stain, pyogenic culture and acid fast bacilli (AFB) stain. G stain revealed few pus cells, no micro-organisms and culture was sterile. AFB were

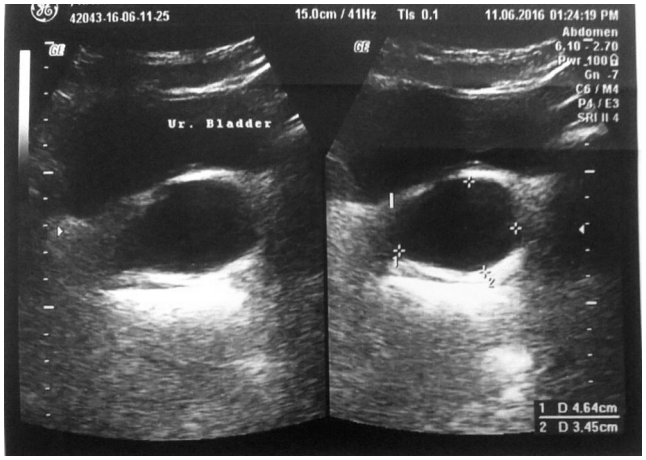


Fig. 3 – Ultra-sonographic image of lower abdomen showing an anechoic structure measuring 4.6 × 3.5 cm posterior to bladder suggestive of prostatic abscess.

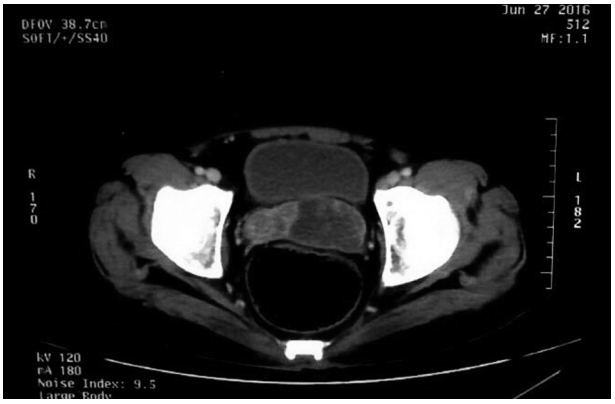


Fig. 4 – Computed Tomography (CT) of KUB region showing a relatively well-defined heterogenous lesion in prostatic region. Solid component of the lesion shows moderate contrast enhancement and cystic C.

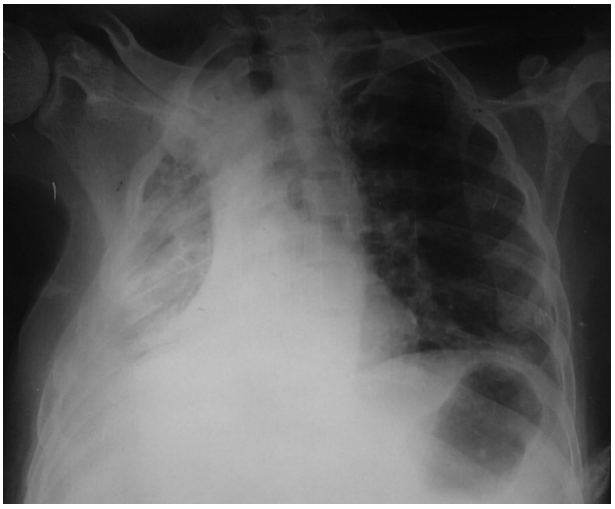


Fig. 5 – Chest radiograph PA view showing right-sided volume loss with obliteration of right costo-phrenic angle.

detected in the pus, and the patient was referred back to our side.

CXR showed right-sided volume loss (Fig. 5) and USG thorax showed pleural thickening. Repeat aspiration of pus was advised for molecular testing by cartridge-based nucleic acid amplification test (CB-NAAT), which detected mycobacterium tuberculosis (Mtb) sensitive to rifampicin. In view of drug sensitive (DS) TB, 5-drug ATT, including streptomycin was initiated.

3. Discussion

Extra-pulmonary TB (EPTB) accounts for around one sixth of the global TB burden. The important sites of involvement of EPTB are lymph nodes, pleura, genito-urinary (GU) system, abdomen and central nervous system (CNS). GUTB affects 2–20% of patients of pulmonary TB (PTB) and accounts for 30–40% of all EPTB cases worldwide.[1] After the Human Immunodeficiency Virus-Acquired Immuno-deficiency Syndrome (HIV-AIDS) epidemic of 90s, there has been a perceptible rise in cases of GUTB, with kidney being the commonest site of involvement.[2]

TB of the prostate is a rare entity but can occur as disseminated TB in AIDS patients. Usually, it is an incidental finding in biopsy specimens following a trans-urethral resection.[3] In a 10-year study conducted at Mayo clinic, Minnesota, U.S.A., among 2599 patients of prostatitis, only 5 had TB.[4] TB prostatitis and epididymo-orchitis have also been rarely reported secondary to intra-vesical Bacilli-Calmette Guerin (BCG) immunotherapy for superficial urinary bladder cancer.[5]

The commonest route of infection is hematogenous spread in cases of disseminated TB.[6] During primary infection with TB, the bacilli get disseminated through bloodstream and lie dormant in various organs of the body. According to Wallgren's timetable of natural progression of TB disease, miliary and meningeal types of TB occur within first 6 months of primary infection, followed by pleural involvement in the next 6–12 months.[7] Post primary pulmonary disease and skeletal TB occur 1–5 years later while GUTB and skin disease manifest usually after 5–15 years. The latent period between pulmonary infection and clinical uro-genital TB is, on an average, 22 years.[6]

The symptoms of TB prostate are non-specific. Patients may present with symptoms of prostatic enlargement such as nocturia, frequency and dysuria. Per rectal examination is done usually within normal limits, with slight enlargement and nodularity.[4] Reduction in semen volume usually indicates extensive prostatic disease or ejaculatory duct obstruction.[8]

Urine examination by Ziehl Neelsen (ZN) staining for AFB has high specificity but sensitivity is only around 50% for diagnosis of GUTB.[9] In Indian studies, culture of urine samples for detection of mycobacterium gives positive results in around 30–40% cases.[10] Hence, a negative culture report should be followed by a polymerase chain reaction (PCR) study. It gives faster results (in 24–48 h) and requires only a few bacilli for detection. Compared with bacteriological, histological or clinic-radiological diagnoses, it is 94.3% sensitive and 85.7% specific.[11]

The important radiographic diagnostic modalities for prostate TB include trans-urethral ultra-sonography (TRUS), computed tomography (CT) and MRI. On TRUS, the most common feature is hypoechogenicity.[12] CT pelvis shows gland enlargement and low density lesions on both sides.[13] CT is also required for visualization of seminal vesicle.[14]

The treatment of TB prostate is similar to any EPTB, with ATT for 6–12 months, depending on clinical response. But, certain studies advocate prolongation of ATT for 2 years.[15]

GUTB is difficult to diagnose, presenting with long-standing, non-specific symptoms. This case highlights that when a patient with PTB or EPTB presents with unexplained urinary symptoms, GUTB should be ruled out.

4. Conclusion

This case highlights the fact that in a TB endemic country such as India, GUTB should always be kept in the differential when a patient with past history of TB presents with non-specific urinary symptoms.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Figueiredo AA, Lucon AM, Junior RF, Srougi M. Epidemiology of uro-genital TB worldwide. *Int J Urol*. 2008;15:827–832.
2. Becker JA. Renal tuberculosis. *Urol Radiol*. 1988;10:25–30.
3. Wolf LE. Tuberculosis abscess of prostate in AIDS. *Ann Intern Med*. 1996;125:156.
4. O'Dea MJ, Moore SB, Greene LE. Tuberculosis prostatitis. *Urology*. 1978;11:483–485.
5. Aust TR, Massey JA. Tuberculosis prostatic abscess as a complication of intra-vesical BCG immunotherapy. *Int J Urol*. 2005;12:920–921.
6. Figueiredo AA, Lucon AM. Urogenital TB: update and review of 8961 cases from the world literature. *Rev Urol*. 2008;10 (3):201–217.
7. Wallgren A. The time-table of tuberculosis. *Tubercle*. 1948;29:245.
8. Paick J, Kim SH, Kim SW. Ejaculatory duct obstruction in infertile men. *BJU Int*. 2000;85:720–724.
9. Mortier E, Pouchot J, Girard L, et al. Assessment of urine analysis for diagnosis of tuberculosis. *Br Med J*. 1996;312:27–28.
10. Das P, Ahuja A, Gupta SD. Incidence, etio-pathogenesis and pathological aspects of GUTB in India: a journey revisited. *Indian J Urol*. 2008;24:356–361.
11. Hemal AK, Gupta NP, Rajeev TP, et al. PCR in clinically suspected GUTB: comparison with intra-venous urography, bladder biopsy and urine AFB culture. *Urology*. 2000;56:570–574.
12. Singh P, Yadav MK, Singh SK, Lal A, Khandelwal N. Case series: diffusion weighted MRI appearance in prostatic abscess. *Indian J Radiol Imaging*. 2011;21:46–48.
13. Wang J, Chang T. TB of the prostate: computed tomography appearance. *J Comput Assit Tomogr*. 1991;15: 269–270.
14. Kim SH, Urogen TB. In: Pollock HM, McClennan BL, Dyer RK, eds. et al. *Clinical Urography*. Philadelphia: WB Saunders; 2000:1193–1228.
15. PasternackF M.S.. Rubin RH, Urinary tract TB. In: Schrier RW, ed. *Diseases of the Kidney and Urinary Tract* 7th ed. Philadelphia: Lippincott William & Wilkins; 2001: 1017–1037.

Available online at www.sciencedirect.com

ScienceDirect

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

Case Report

Bilateral tuberculous otitis media: An unique presentation

Dhiraj Bhatkar, Ketaki Utpat, Unnati Desai, Jyotsna M. Joshi *

Department of Pulmonary Medicine, T. N. Medical College, B. Y. L. Nair Hospital, Mumbai, India

ARTICLE INFO

Article history:
Received 11 October 2016
Accepted 31 October 2016
Available online 27 December 2016

Keywords:
Tuberculous otitis media
Chronic suppurative otitis media

ABSTRACT

Tuberculous otitis media (TOM) is an uncustomary variety of tuberculosis (TB) usually seen secondary to pulmonary tuberculosis or associated with it. It is characterized by indolent and heterogeneous presentations. Diagnosis warrants clinical, radiological, and microbiological amalgamation. It is hence challenging and frequently delayed leading to disabling complications. Opportunate suspicion, timely diagnosis and appropriate antituberculosis therapy are the key to successful management. We report a unique case of bilateral TOM occurring in an immunocompetent adult.

1. Introduction

Tuberculosis is an extremely rampant infectious disease prevalent since antiquity and continues to mystify clinicians across the globe due to its innumerable presentations. It is mainly classified into pulmonary and extrapulmonary forms, with the pulmonary variety accounting for majority of the cases.[1] Extrapulmonary disease is characterized by insidious presentations, gradual evolutions, and paucibacillary lesions.[2] Common sites include lymph nodes and pleurae,[2] with the middle ear being an extremely rare site of affection. Tuberculous otitis media (TOM) is a rare polymorphic condition with nonspecific manifestations. It is more commonly encountered in the pediatric age group and is generally unilateral.[3] The pathology progresses inexorably if the diagnosis is delayed leading to grave vital sensory organ damage and life threatening complications. We herein present an off-center case of bilateral TOM occurring in an immunocompetent adult highlighting the necessity of cognizance about the entity among clinicians.

2. Case report

A 75-year-old woman presented with bilateral ear discharge since 4–6 months. It was associated with progressive hearing loss and ear discomfort. The patient had a past history of abdominal tuberculosis 50 years back for which she had been treated with anti tuberculous therapy (ATT). Systemic examination was normal. Hematological and biochemical blood parameters were normal. Enzyme-linked immunosorbent assay (ELISA) for human immunodeficiency virus (HIV) was nonreactive. Her audiometric evaluation revealed moderate–severe mixed hearing loss in left ear and severe mixed hearing loss in right ear. High resolution computed tomography (HRCT) of temporal bone showed bilateral mastoiditis with abnormal soft tissue in mastoid air cells involving bilateral middle ear surrounding ossicular chains (Fig. 1). Pus swabs were collected from both ears and subjected for GeneXpert and acid-fast bacilli (AFB) smear. The test detected Mycobacterium Tuberculosis in both ears, and it was sensitive to rifampicin. Smears for AFB were also positive (+1) by the Ziehl–Neelsen method.

* *Corresponding author at*: Professor and Head, Department of Pulmonary Medicine, T. N. Medical College and B. Y. L. Nair Hospital, Mumbai 400008, India. Tel.: +91 022 23027642/43.
E-mail address: drjoshijm@gmail.com (J.M. Joshi).

http://dx.doi.org/10.1016/j.ijtb.2016.10.005

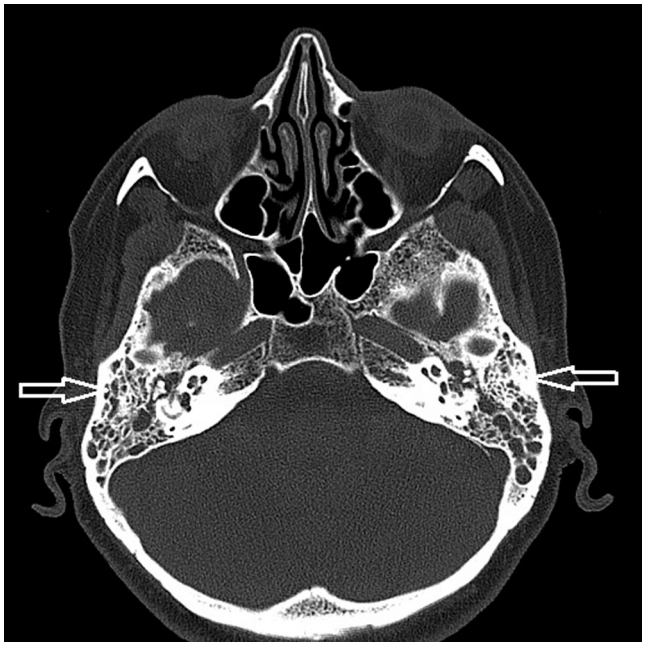


Fig. 1 – High resolution computed tomography (HRCT) of temporal bone showing bilateral mastoiditis with abnormal soft tissue in mastoid air cells involving bilateral middle ear surrounding ossicular chains.

Baseline chest X-ray was normal. The patient was initiated on ATT as a retreatment case according to Revised National Tuberculosis Control Program for a duration of 8 months. The patient improved clinically with minimal residual occasional ear discharge. Repeat ear swabs smear after 2 months of ATT (at end of intensive phase) were negative for AFB. Follow-up HRCT of temporal bone showed improvement in bilateral chronic otitis media. The patient was shifted to continuation phase of ATT in view of clinicoradiological response and microbiological smear conversion.

3. Discussion

Chronic suppurative otitis media (CSOM) is chronic inflammation of the middle ear and mastoid cavity clinically presenting as recurrent otorrhea due to a tympanic perforation persisting for a minimum one month duration.[4] A multitude of host, infectious, environmental, and allergic factors may contribute to its development either in isolation or in combination. Infectious culprits predominantly involve gram-positive and -negative bacteria and viruses.

However, Mycobacterium Tuberculosis (MTB) infection culminating in CSOM has been very rarely reported. The incidence of TOM is lilliputian and is variably reported as 0.05–0.9% of all CSOMs.[5] The pathogenesis involves one of the following three main mechanisms[6]: spread of infection through the eustachian tube, blood–borne dissemination or direct implantation to the external auditory canal, and a tympanic membrane perforation. Primary infection chiefly occurs and manifests in children with no identifiable foci elsewhere in the body. Adult disease is usually due to reactivation of a quiescent focus of infection. In adults, TOM is generally seen in immunocompromised individuals.[5] Our case, however, was an immunocompetent adult. The affection is predominantly unilateral,[3] with only a handful of cases reported with bilateral disease[7] till date like ours. The hallmark symptom is otorrhea, which is usually painless[8] owing to infiltration of nerve endings by tubercles. The consistency of the discharge ranges from thick and mucoid to thin and watery.[3] Multiple tympanic membrane perforations[8,9] due to build up of granulation tissue and caseous necrosis is the rule. Hearing loss can be conductive (90%), sensorineural (8%) or mixed (2%), and maybe incommensurate with the extent of the disease.[9] There may be associated periauricular lymphadenopathy. Our case presented with the cardinal symptoms of otorrhea and hearing deficit. Evaluation requires pure tone audiometry to assess degree of hearing loss. Radiological assessment shows findings like ossicular resorption, soft tissue attenuation filling the mastoid cavity,[2] sclerosis of mastoid cortex, and opacification of the middle ear.[10] Our case demonstrated the finding of soft tissue in mastoid on imaging. Definitive diagnosis requires microbiological and cultural diagnosis. Bacteriological tests may have limited sensitivity due to various hindrances such as coexisting microorganisms, interfering effect of topical applications,[10] and low bacillary load. However, an earnest attempt should always be made to obtain a microbiological diagnosis. This can be aided by rapid diagnostic tests like GeneXpert; a World Health Organization (WHO) accredited cartridge-based nucleic acid amplification test with an ability to detect Mycobacteria along with detection of drug susceptibility to rifampicin, which is of commendable utility in extrapulmonary tuberculosis.[11] In our case too, GeneXpert result aided in clinching the diagnosis.

The condition is, however, often diagnosed very late. Various reasons for this delay in diagnosis include dearth of knowledge about this condition among physicians leading to poor suspicion, variable clinical picture and diagnostic pitfalls.[10] This may culminate in perilous complications such as irrevocable hearing loss, facial palsy, periauricular fistulas, labyrinthitis, subperiosteal abscess, petrous apicitis, and intracranial extension of infection.[3,10] Medical modality with ATT is the mainstay of treatment with exemplary cure rates.[10] Surgery has a nugatory role and it is limited to complications like subperiosteal abscess[5] and sequelae such as bony seqestrae. We therefore emphasize a due index of suspicion for this entity and reiterate the need for an expedient diagnosis and prompt therapy.

Conflicts of interest

The authors have none to declare.

REFERENCES

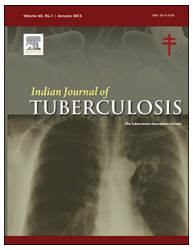
1. Dienye PO, Ndukwu GU. Tuberculous otitis media in an adult in a primary care setting: a case report. *Asian Pac J Trop Med*. 2010;754–756.
2. Djeric D, Tomanovic N, Boricic I. Tuberculous otitis media-diagnosis and treatment of four cases. *Int Adv Otol*. 2013;9:255–259.

3. Saberwal AA, Velankar HK, Dabholkar YG, Shetty AK, Shinde D. Tuberculous otitis media-revisited with 3 interesting case reports. *J Evolut Med Dent Sci.* 2013;2:9436–9440.
4. Vij T, Prashar Y, Jain D. An updated review on otitis media. *IJRRPAS.* 2014;4:922–934.
5. Quaranta N, Petrone P, Michailidou A, et al. Tuberculous otitis media with facial paralysis: a clinical and microbiological diagnosis – a case report. *Case Rep Infect Dis.* 2011;2011:932608.
6. Karkera GV, Shah DD. Silent mastoidosis-tuberculous aetiology presenting as facial nerve palsy. *Indian J Otolaryngol Head Neck Surg.* 2006;58:108–110.
7. Saleh E, Alnemare AK, Alzuraiqi B, Abbas M. Bilateral tuberculous otitis media; a rare case report. *Saudi J Otorhinolaryngol Head Neck Surg.* 2016;18:31–33.
8. Bhardwaj P, Mohan C, Srivastava A. Tuberculous otitis media with facial paralysis. *Int J Adv Integr Med Sci.* 2016;1:69–71.
9. Pandey AK, Singh VP, Maithani T, Dey D. Tuberculous otitis media. *Indian Med Gazette.* 2011;501–505.
10. Araujo MF, Pinheiro TG, Raymundo IT, et al. Tuberculous otitis media. *Int Adv Otol.* 2011;7:413–417.
11. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *J Clin Microbiol.* 2011;49:2540–2545.

Available online at www.sciencedirect.com

ScienceDirect

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



Case Report

Concurrent intramedullary spinal cord and multiple intracranial tuberculomas with tuberculous optic neuritis: A rare case report

Lulup Sahoo *, *Ashok Kumar Mallick, Geeta Mohanty, Kali Prasanna Swain, Soumyadarshan Nayak, Ashwini Kumar Sahu*

Department of Neurology, S.C.B. Medical college, Cuttack, India

ARTICLE INFO

Article history:
Received 23 October 2016
Accepted 31 October 2016
Available online 28 December 2016

Keywords:
Tuberculous meningitis
Tuberculoma
Optochiasmatic arachnoiditis
Optic neuritis

ABSTRACT

Tuberculosis (TB) remains a worldwide burden, with a large majority of new active TB cases occurring in underdeveloped and developing countries. Tuberculous meningitis (TBM) is one of the common infections of central nervous system. Other manifestations include intracranial tuberculoma, tubercular brain abscess, spinal tuberculoma, and granulomatous arachnoiditis. Visual impairment in TBM may be due to optic neuritis, optochiasmatic arachnoiditis (OCA), tuberculoma in the chiasmatic region or in the optic pathways, chorioretinitis, secondary to hydrocephalus and increased intracranial pressure, and finally due to ethambutol toxicity. We report a case of young girl with concurrent spinal cord intramedullary tuberculoma and multiple intracranial tuberculomas with TBM and bilateral visual impairment due to tuberculous optic neuritis.

1. Introduction

Tuberculosis (TB) remains a worldwide burden, with a large majority of new active TB cases occurring in underdeveloped and developing countries. Tuberculous meningitis (TBM) is still one of the common infections of central nervous system (CNS) and poses significant diagnostic and management challenges, more so in the developing world.[1] About 10% of patients, who have TB, develop CNS disease.[2] Tuberculous disease of the CNS usually appears as TBM or a space-occupying tuberculoma. Paraplegia due to TB is often secondary to Pott's disease, a common cause of myelopathy in countries, where TB is prevalent. However, TB can also cause two other forms of myelopathy, quite rare and not part of Pott's paraplegia: spinal tuberculomas and granulomatous arachnoiditis resulting in paraplegia.[3] Concurrent occurrence of intracranial tuberculomas along with intramedullary spinal tuberculoma is very rare. Impairment of vision is a devastating complication of TBM, which may be due to various causes. Tuberculous optic neuritis is a very rare condition leading to visual impairment.

We report a rare case of young girl diagnosed to have spinal cord intramedullary tuberculoma and concurrent multiple intracranial tuberculomas with TBM and bilateral visual impairment due to tuberculous optic neuritis.

* *Corresponding author.*
E-mail address: lulupsahoo@gmail.com (L. Sahoo).

http://dx.doi.org/10.1016/j.ijtb.2016.10.007

2. Case

A 22-year-old female presented to our hospital with fever, headache and vomiting for 15 days. Two days following admission, she developed weakness and numbness of both lower limbs with retention of urine. One day later, she complained sudden onset painless diminution of vision in both eyes. There was history of contact with patients with TB. There was no history of seizure. Examination revealed complete blindness in both eyes with absent perception of light. On optic disc examination, there was hyperemia with blurred margin bilaterally. Bilateral pupils were dilated and not reactive to light. Bilateral lateral recti were restricted. There was spastic weakness of both lower limbs (grade 3/5) with brisk reflexes and bilateral extensor plantar response. All primary sensory modalities were impaired at and below T 10 spinal segment. There was presence of neck rigidity and kernig sign.

The X-ray chest was normal. The cerebrospinal fluid (CSF) examination revealed 36 cells/cmm with predominant lymphocytes with protein 560 mg/dl and sugar 29 mg/dl. The erythrocyte sedimentation rate (ESR) was 42 mm in first hour. T1 contrast magnetic resonance imaging (MRI) of brain showed multiple ring enhancing lesions in both supra and infratentorial regions with leptomemingeal enhancement suggesting tuberculomas with associated meningitis (Fig. 1). There were no exudates in brain or around optic chiasma. MRI of spine showed an intramedullary lesion at D7-8 level which was hyperintense on T2W images and on T1 contrast showed homogenous contrast enhancement suggestive of tuberculoma (Fig. 2). MRI spine also showed vertebral end plate erosion at D4, D7 and D8 vertebra level. Visual evoked potential (VEP) showed no wave formation in both eyes, suggesting severe optic pathway disease.

With the above clinical and neuroimaging features, the patient was diagnosed as a case of intracranial and spinal cord tuberculoma with tubercular meningitis and bilateral optic neuritis. Her treatment was started with four anti tubercular drugs (isoniazid, rifampicin, pyrazinamide, levofloxacin). Ethambutol was not given as she had already developed optic neuritis. She was also treated with intravenous methyl prednisolone one gram for five days followed by oral steroid. The four-drug therapy was given for two months followed by two drugs (isoniazid, rifampicin), which was planned for next 18 months. She showed sustained improvement with resolution of meningitis and improvement in power. At four weeks, fundoscopy showed pale disc bilaterally (Fig. 3). At six-month follow-up, she had normal power in both lower limbs. But,

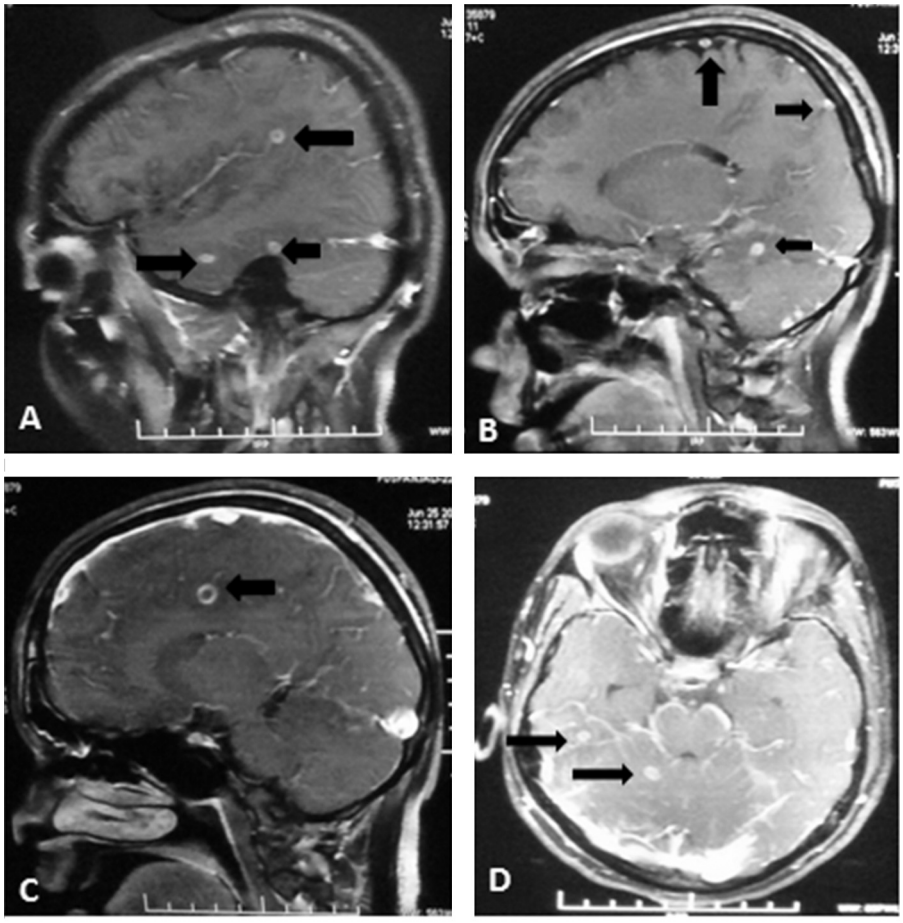


Fig. 1 – (A–D) T1-weighted contrast magnetic resonance imaging (MRI) of brain showing multiple ring enhancing lesions in both supra and infratentorial regions (arrow) with leptomeningeal enhancement suggestive of tuberculomas with associated meningitis.

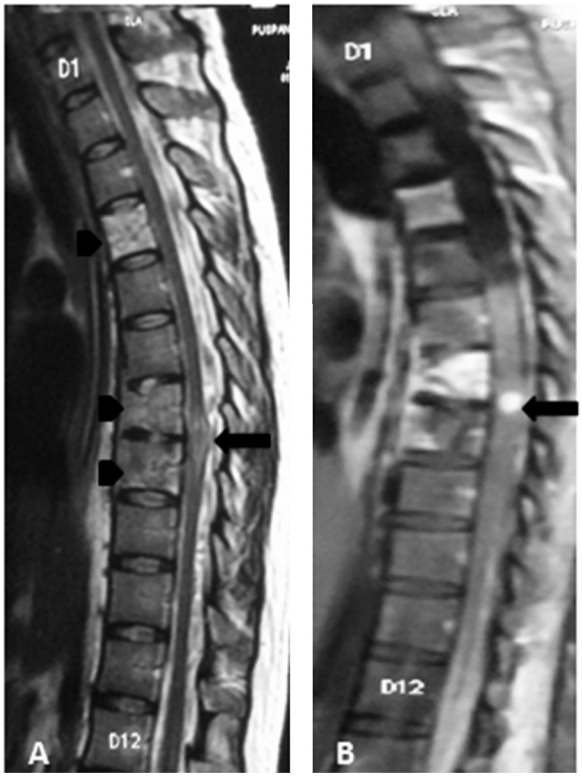


Fig. 2 – (A) T2-weighted saggittal MRI of spine showing an intramedullary hyperintense lesion at D7-8 level (arrow) along with vertebral end plate erosion at D4, D7 and D8 vertebra level (arrow head). (B) T1-weighted postcontrast image showing an homogeneously enhancing lesion at D7-8 level suggestive of intramedullary tuberculoma.

there was no significant improvement in her vision. Her visual acuity at six-month follow-up was only presence of perception of light.

3. Discussion

CNS TB presents mainly as TBM, tuberculoma and rarely as tubercular brain abscess. TB meningitis is the commonest form. TBM occurs in persons of all ages. In developed countries, TBM has become a disease of the elderly due to reactivated disease, whereas subjects from areas where the prevalence of TB is still high are much younger, and meningitis is usually associated with primary infection.[3] A common cause of myelopathy in developing countries, where TB is prevalent, is Pott's disease, caused by spinal cord compression due to abscess, granulomatous tissue, or bony displacement.[4] Two other forms of myelopathy secondary to TB that are less common and different from Pott's paraplegia include tuberculomas within the spinal cord, as well as in intra- and extradural locations,[3,4] and encasing granulomatous arachnoiditis (radiculomyelopathy) with cord compression and vasculitis of spinal cord vessels.[5] Tuberculoma in the brain is not very common, while an intramedullary tuberculoma of the spinal cord is extremely rare, with reported ratio of 1:42.[6] Although tuberculomas can occur at any age, 86% of patients with intracranial tuberculoma are below the age of 25 years.[7] Intracranial tuberculoma is the result of haematogenous spread from a primary focus which is characteristically the lung. The focus may be quite small and, therefore, may not be visible on routine chest radiographs.[8]

Impairment of vision in TBM can be due to a variety of causes, including primary involvement of optic nerve by tuberculous lesion leading to optic neuritis, optochiasmatic arachnoiditis (OCA), tuberculoma in the chiasmatic region or in the optic pathways, chorioretinitis, secondary to hydrocephalus and increased intracranial pressure, and finally due to ethambutol toxicity.[9] Sinha et al.[10] reported that 27% of TBM patients had decreased vision, and the main causes were OCA and optochiasmal tuberculoma. The prognosis in most of the cases with impairment of vision in TBM remains poor; therefore, it is important to identify predictors of occurrence. Sinha et al. identified papilledema, cranial nerve paralysis, raised CSF protein, and OCA on MRI to be the predictors of deterioration of vision.[10] Tuberculous optic neuritis is a very rare entity, and only few cases have been reported worldwide.

Our case demonstrated that, concurrent spinal and intracranial tuberculoma may coexist and usually responds well to antitubercular therapy (ATT). Tubercular optic neuritis is a very rare but devastating complication of TB with poor vision recovery. So, it should be treated aggressively with high dose intravenous steroid along with ATT.

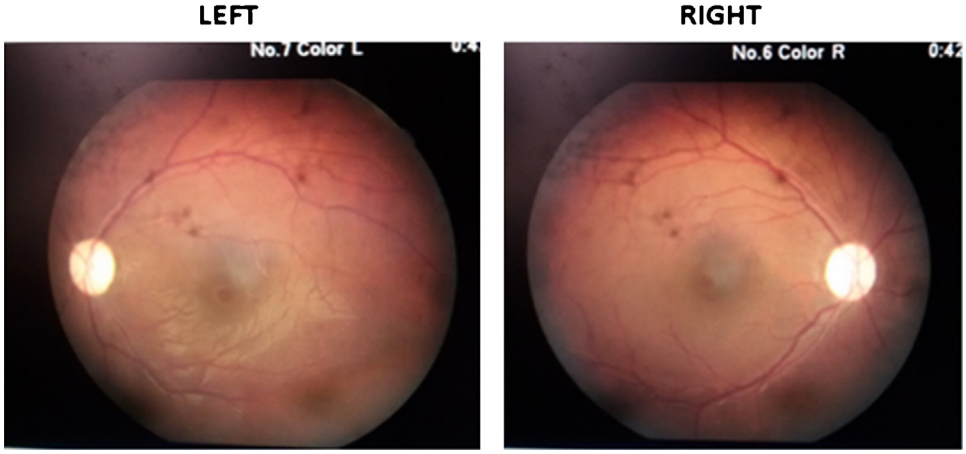


Fig. 3 – Fundoscopy showing bilateral pale disc suggestive of optic atrophy.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Murthy JM. Management of intracranial pressure in tuberculous meningitis. *Neurocrit Care*. 2005;2:306–312.
2. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA*. 1999;282:677–686.
3. Vlcek B, Burchiel KJ, Gordon T. Tuberculous meningitis presenting as an obstructive myelopathy. *J Neurosurg*. 1984;60:196–199.
4. Mantzoros SC, Brown AP, Dembry L. Extraosseous epidural tuberculoma: case report and review. *Clin Infect Dis*. 1993;17:1032–1036.
5. Freilich D, Swash M. Diagnosis and management of tuberculous paraplegia with special reference to tuberculous radiculomyelitis. *J Neurol Neurosurg Psychiatry*. 1979;42:12–18.
6. MacDonnell AH, Baird RW, Bronze MS. Intramedullary tuberculomas of the spinal cord: case report and review. *Rev Infect Dis*. 1990;12:432–439.
7. Boluk A, Turk U, Aribas E, Kokrek Z. Intracranial tuberculoma: clinical and MRI findings. *J Turgut Ozal Med Cent*. 1998;5(3):180–184 [Turkish].
8. Whelan M, Stern J. Intracranial tuberculoma. *Radiology*. 1981;138:75–81.
9. Gourie-Devi M. Optochiasmatic arachnoiditis and neurotuberculosis: prognostic indicators and therapeutic strategies. *Neurol India*. 2010;58:714–715.
10. Sinha MK, Garg RK, Anuradha HK, et al. Vision impairment in tuberculous meningitis: predictors and prognosis. *J Neurol Sci*. 2010;290:27–32.

Available online at www.sciencedirect.com

ScienceDirect

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

Case Report

Tuberculosis occurred on an atlantoaxial synovial pannus during juvenile idiopathic arthritis treated with TNF inhibitors

Khadija Baccouche[a,*], *Rihab Moncer*[a], *Safaa Belghali*[a], *Zeineb Alaya*[a], *Wissem Hachfi*[b], *Hela Zeglaoui*[a], *Elyes Bouajina*[a]

[a] *Department of Rheumatology, Farhat Hached Hospital, Sousse 4000, Tunisia*
[b] *Department of Infectious Diseases, Farhat Hached Hospital, Sousse 4000, Tunisia*

Available online 10 January 2017

Long-term extension studies and observational drug registers have revealed an increased risk of tuberculosis in patients treated with anti-tumor necrosis factor agents, particularly in those treated with monoclonal antibodies. To our knowledge, no suboccipital localization has been reported [1,2].

We report a case of a 21 years old patient followed for idiopathic arthritis juvenile for 12 years and treated with anti TNF alpha. He had since one year an atlantoaxial synovial pannus and he recently, since two months, complained of inflammatory neck pain initially attached to its inflammatory disease. Neurological exam was normal. In front of the pain persistence after a bolus of steroids, and the appearance of dysphagia, a CT scan of the cervical spine showed a C1–C2 dislocation with vertical destruction of dens process (Fig. 1). Imaging magnetic resonance showed a large inflammatory mass of the cervical occipital junction complicated of anterior compressive collections on the hypopharynx and posterior compressive collections on the brainstem with cranial settling (Fig. 2). A tuberculine intra dermo-reaction and the BK research were negative. The diagnosis confirmation was made by the presence of epithelioid-giant cell granuloma in the pottic lesion. The treatment was based on the anti-tuberculosis drugs for a period of 18 months completed with an orthopedic then surgical stabilization of cervical occipital junction.

Although Pott's disease is the most frequent localization of osteo-articular tuberculosis, the suboccipital localization remains rare and no cases have been found in the literature with TNF inhibitors. A recent meta-analysis of RCTs and long-term extension studies of RA patients found 31 cases of TB occurring during anti-TNF treatment (OR 1.92, 95%CI 0.91–4.03,

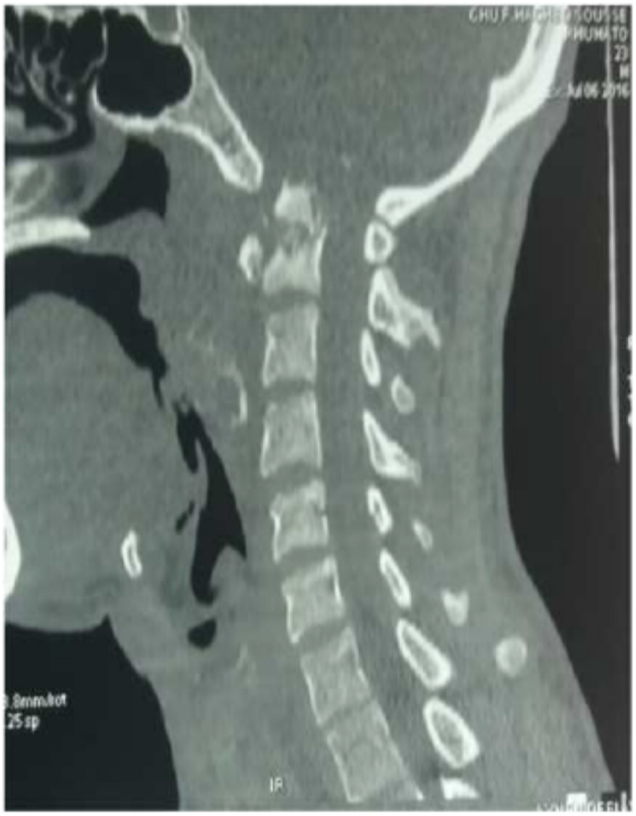


Fig. 1 – CT scan of the cervical spine showed a C1–C2 dislocation with vertical destruction of dens process.

* *Corresponding author*. Tel.: +216 97121205.
E-mail address: bac.khad@yahoo.fr (K. Baccouche).

http://dx.doi.org/10.1016/j.ijtb.2016.10.008

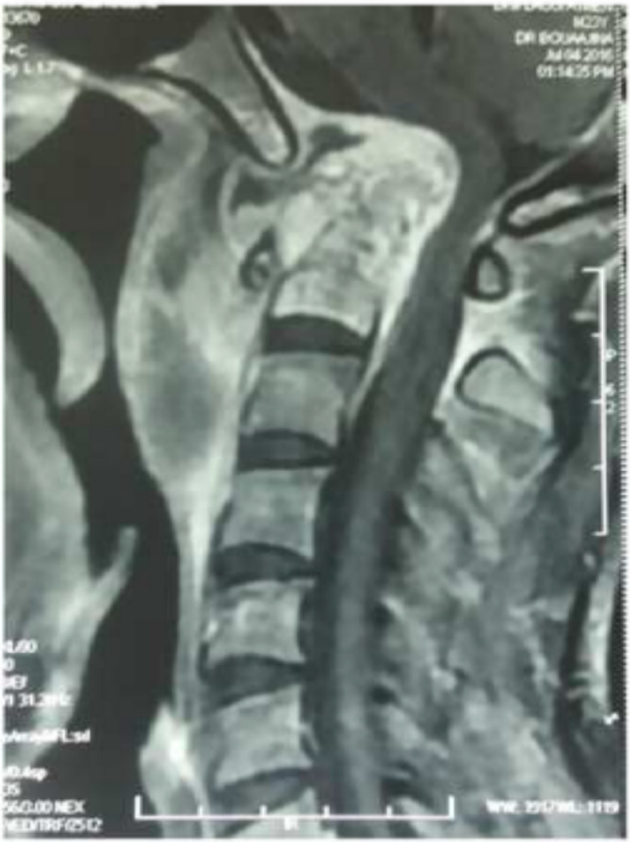


Fig. 2 – Imaging magnetic resonance showed a large inflammatory mass of the cervical occipital junction complicated of anterior compressive collections on the hypopharynx and posterior compressive collections on the brainstem with cranial settling.

P = 0.085) without spinal location [2]. In conclusion, the tuberculosis is common in patients on anti-TNF alpha but reaching the neck-occipital junction is rare. CT and MRI are required for diagnosis and early treatment.

REFERENCES

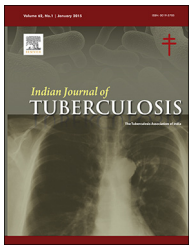
[1]. Toussi 1 SS, Pan N, Walters HM, Walsh TJ. Infections in children and adolescents with juvenile idiopathic arthritis and inflammatory bowel disease treated with tumor necrosis factor-α inhibitors: systematic review of the literature. *Clin Infect Dis*. 2013;57:1318–1330.

[2]. Atzeni F, Batticciotto A, Ignazio F, et al. *Infections and Biological Therapy in Patients with Rheumatic Diseases IMAJ*. Vol. 18. 2016;164–167.

Available online at www.sciencedirect.com

ScienceDirect

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



Letter to the Editor

Analysis of YouTube as a source of information about Tuberculosis

Dear Editor,

The Internet is an often-used source of health-related information with a significant impact in the knowledge, attitudes and beliefs of the general population worldwide.[1] However, the internet-based sources have become a mechanism for the spread of misinformation,[2] especially of high-burden diseases, such as Tuberculosis (TB). Given that TB remains a major public health problem globally,[3] assess the quality and veracity of information from internet-based sources is highly relevant. YouTube is the most popular video sharing platform with over a billion users and 4 billion video views per day in 76 different languages. It is a constantly growing source of freely, unmoderated and unregulated medical information, ranging from public awareness and medical education to misleading or invalid material.[4,5] To the best of our knowledge no study has analyzed the content of uploaded videos about TB. We conducted a descriptive study to characterize the available information about TB from the most widely viewed YouTube videos.

On January 27, 2017, a search in YouTube (www.youtube.com) was conducted using the term ''tuberculosis''. The 100 most viewed videos were reviewed and analyzed. Any duplicate videos were considered as 1, and videos lacking audio were excluded. We extracted the title, number of views prior to review, number of likes and dislikes, upload source, purpose of the video and content. The sources were classified in: consumers (non-professional sources), medical or government professionals, news channels and commercial or advertisement sources. The overall informational and scientific content of videos were rated it as good, fair, or poor using as a reference the current WHO guidelines on TB. All videos were analyzed independently by 3 researchers and a fourth viewer arbitrated any discrepancies.

The videos were watched a total of 9,019,795 times and the number of views per video ranged between 12,973 and 2,084,311. The majority of videos were uploaded by medical or government professionals (48%), followed by consumers (29%), news channels (12%) and commercial or advertisement sources (11%). The purpose of most videos (72%) was to provide information, followed by promote medicinal plants for TB treatment (10%) and communicate patient testimonials (9%). The information content was fair or poor in 53% of all videos and misleading content was identified in 26% of the videos. All videos uploaded by medical and government professionals provided high-quality information. Compared with good quality content videos, fair and poor content videos were more likely to receive likes, and they had a higher number of views (Table 1).

Approximately half of the videos contradicted the reference standard and over a quarter of the videos contain misleading content. The video likes and view counts highlight the rapid spread of TB misinformation on YouTube. Although several videos, especially those uploaded by medical and government professionals are useful adjuncts, YouTube is an inadequate source TB information for patients. Therefore, clinicians should caution their patients to be careful of the information

Table 1 – Ratings and views of most viewed YouTube videos about Tuberculosis.

Quality of videos information	No. of videos	No. of video views, mean (SD)	No. of likes, mean (SD)	No.of dislikes, mean (SD)
Good	47	84,603 (119,017.1)	191.1 (544.9)	9.4 (17.4)
Fair	27	42,412.6 (36,030.0)	130.1 (237.5)	7.70 (13.7)
Poor	26	144,986.7 (398,799.2)	671.0 (794.7)	19.8 (26.9)
Total	100	90,197.9 (223,800.5)	301.3 (798.5)	11.5 (16.8)

from Internet video-sharing sites. There need to be concerted efforts aimed at improving the quality of health-related information contained in YouTube, with more participation of medical professionals in the social media.

1. Con of interest

The authors have none to declare.

REFERENCES

1. Morris K. Tweet, post, share – a new school of health communication. *Lancet Infect Dis.* 2011;11(7):500–501.
2. Ortiz-Martinez Y, Jimenez-Arcia LF. Yellow fever outbreaks and Twitter: rumors and misinformation. *Am J Infect Control.* 2017. http://dx.doi.org/10.1016/j.ajic.2017.02.027. pii:S0196-6553(17)30148-7.
3. Ortiz-Martínez Y. Assessing worldwide research productivity on tuberculosis over a 40-year period: a bibliometric analysis. *Indian J Tuberc.* 2017. http://dx.doi.org/10.1016/j.ijtb.2017.02.003.
4. Azer S. Understanding pharmacokinetics: are YouTube videos a useful learning resource? *Eur Rev Med Pharmacol Sci.* 2014;18:1957–1967.
5. Ortiz-Martinez Y, Ali-Salloum W, González-Ferreira F, Molinas-Argüello J. HIV videos on YouTube: helpful or harmful? *Sex Transm Infect.* 2017. http://dx.doi.org/10.1136/sextrans-2017-053197. pii:sextrans-2017-053197 [Epub ahead of print].

Yeimer Ortiz-Martínez*
Faculty of Health Sciences, University of Sucre, Sincelejo, Colombia

Carlos Hernando Acosta-Fernandez
National University of Colombia, Bogota, Colombia

Diego Andrés Losada-Manchola
Pontifical Xaverian University, Cali, Colombia

Jenifer Astrid Marulanda-Satizabal
Free University of Colombia, Cali, Colombia

*Corresponding author at: Calle 14A #15-75, Barrio Montecarlos, Magangué, Bolívar, Colombia. Tel.: +57 3017124908
E-mail address: yeimer.ortiz@unisucrevirtual.edu.co (Y. Ortiz-Martínez)

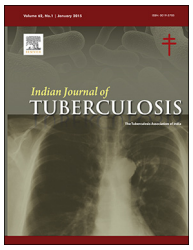
Received 4 March 2017
Available online 16 May 2017

http://dx.doi.org/10.1016/j.ijtb.2017.04.003
0019-5707/

Available online at www.sciencedirect.com

ScienceDirect

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



Perspective

Tuberculosis "the great imitator": False healing and subclinical activity

Keywords:
Tuberculosis
Anti-tuberculosis treatment
Latent tuberculosis
Positron emission tomography

ABSTRACT

Tuberculosis is still being a health problem worldwide despite it being a curable disease. Although the only way to prevent its spread is treating cases of active pulmonary disease, we still do not have reliable markers that help us to evaluate the response to anti-tuberculosis drugs. Currently, a patient with negative conversion in the culture of the sputum is considered as cured; however, several studies have questioned the usefulness of this test given that some individuals persist with data of clinical activity despite their negative culture. A couple of recent studies based on sophisticated imaging techniques confirm the above and show us a broader clinical spectrum of the disease, with false healing and subclinical activity in the affected lung tissue even in the absence of symptoms, forcing us to reconsider the way in which we classify tuberculosis and led us to question the efficacy of the current schemes to treat this illness. Here, we comment the findings of these trials and analyze what is their influence in the view of physicians for future applications in diagnosis and/or therapeutics of tuberculosis.

1. Introduction

Tuberculosis is still being a health problem worldwide. It constitutes the first cause of death attributable to a single pathogen and it is estimated that 10.4 million new cases and 1.4 million deaths occur every year.[1] This disease is spread from person to person through infectious droplets transmitted by coughing, sneezing or phonation. Although it is a condition that with an adequate treatment is curable, it is still a problem in many developing countries due to the difficulty of its diagnosis and the delay in starting treatment. Also, we have complications in determining which patients have an active disease and which ones have a latent infection. Furthermore, although the only way to prevent tuberculosis is to treat active cases correctly, we are still far from evaluating appropriately the response to drugs because of the lack of markers that allow us to assess the success of the treatment and the cure achieved by it. For all these reasons, we have not been able to stop the transmission of tuberculosis and at this time many patients with active disease undiagnosed, untreated, or mistreated are infecting people with whom they have had contact.

To overcome the difficulties mentioned above, in recent years, great efforts have been made to develop new tests for the detection of *Mycobacterium tuberculosis* in various biological samples. While the observation of the growth of bacilli on solid cultures remains being the gold-standard in the diagnosis of active tuberculosis, other methods are being used due of the long time that the bacterium takes to divide in solid media. In most of the low-income countries, smear microscopy with Ziehl–Neelsen staining is the main tool to observe acid-fast bacilli in sputum samples. Nonetheless, fluorescence microscopy with auramine–rodhamine staining and light emitting diode technology are increasingly available in different countries and provide a slight increase in the sensitivity for the detection of mycobacteria compared to conventional microscopy.[2] Fully automated systems that assess bacterial growth in liquid culture now are also available to detect *M. tuberculosis* in clinical samples by adding a mycobacterial growth indicator tuber (BACTEC MGIT Mycobacterial Detection

System, BD), which consist in a fluorescent molecule (ruthenium pentahydrate) that functions as an oxygen sensor and fluoresces following the oxygen reduction induced by aerobically metabolizing bacteria within the medium (modified Middlebrook 7H9 broth). This method offers a faster alternative to the solid culture (1 week for the detection of *M. tuberculosis*) and allows us for the evaluation of drug susceptibility by adding critical concentrations of different anti-tuberculous drugs.[3] In addition, several nucleic acid amplification tests (NAATs) and line probe assays (LiPAs) which simultaneously detect infection and amplify regions of drug resistance now are being used for the diagnosis and the assessment of drug sensibility in patients with smear positive pulmonary disease as well as in subjects with extrapulmonary tuberculosis.[4]

We have also developed better ways to detect subjects with latent tuberculosis. Although the tuberculin skin test (TST) remains a good tool, its high dependence on who reads the test and the possibility of false positive results after the contact with non-tuberculous mycobacteria has made it preferable to use the interferon gamma release assays (IGRAs), which are based on techniques of ELISA/ELISPOT and determine the amount of interferon produced by peripheral blood mononuclear cells (PBMCs) after the exposure to specific antigens of *M. tuberculosis*. In asymptomatic individuals, a positive result in any of the IGRAs currently available indicates previous contact with the bacillus and therefore a latent infection.[5] More recently, IP-10 has also been proposed as a marker to discriminate those subjects with latent tuberculosis.[6]

These methods were designed to facilitate diagnosis and to distinguish between active and latent infection. Nonetheless, some of them such as the fluorescence microscopy, BACTEC MGIT systems and NAATs depend on the presence of bacilli in the sputum and might give false negative results in patients with negative smear pulmonary disease and little amounts of bacilli in other samples. Also, IGRAs and TST do not completely detect all individuals with latent tuberculosis nor fully discriminate them from the patients with the active form of the disease and its usefulness is lower in immunosuppressed subjects. In addition, the clinical definition of latent infection by the absence of symptoms and positive results on IGRAs or TST do not rule out the possibility that all of those patients that are supposedly latently infected might be in fact carrying actively replicating bacilli at the sites of exposure within the lung.

None of the tests mentioned here was conceived and has not been evaluated for the follow-up of the treatment and the determination of the efficacy of it. At present, the way to define if a treatment is successful is based on the negative conversion in the culture of sputum after 8 weeks of anti-tuberculous therapy.[7] Most of the markers that are currently being assessed to determine the response to the treatment are in fact based on this method. However, several studies have questioned its usefulness since a high percentage of supposedly cured patients continue with symptoms and present new or persistent lesions in imaging studies as well as detectable amounts of bacterial genomic material in the sputum sample.[8,9] Moreover, not all patients with active tuberculosis have positive sputum at the time of diagnosis or during the course of treatment, as this requires that the lung lesions become necrotic and drain into a bronchus, which does not always occur due to the heterogeneity of the local immune response in lung tissue and to differences in the state of the host immune system. Therefore, there is an urgent need for markers that help us to assess the response to treatment, especially in the context of accelerated emergence of strains with resistance to multiple drugs.

2. New ways to unmask the "great imitator"

In two recent studies published at the end of 2016, positron emission tomography with 2-deoxy-2-[^{18}F] fluoro-D-glucose ([18F] FDG) combined with computed tomography (PET-CT) were used to follow the behavior of pulmonary lesions of infected patients during treatment with anti-tuberculosis drugs. The technique is based on the increased consumption of [^{18}F] FDG in tissues with high metabolic activity, such as sites with inflammation. In the first of these studies,[10] Malherbe and colleagues enrolled 99 South African and 14 Korean patients with active pulmonary tuberculosis who took anti-tuberculous treatment for 6 months or more. These subjects underwent a PET-CT scan at 1 month, 6 months, and 1 year after the start of treatment. Although most of them showed negative conversion in the sputum culture at 6 months, less than a half had a regression of lung lesions, almost a third of the patients developed new foci of activity during treatment until a year afterwards and even when some took long schemes of 8 months of duration. In addition, relapses occurred two years after initiation of the follow-up and bacterial mRNA was detected in sputum and bronchoalveolar lavage of some patients at six months despite negative culture (the mRNA, being very unstable and short-lived *in vitro*, when found in biological samples could translate persistence of live bacteria).

On the other hand, Esmail and colleagues reported in a brief communication the results of a study of 35 HIV-infected patients who were diagnosed with latent TB by IGRAs and treated with isoniazid as a prevention.[11] All of them underwent PET-CT scan with [^{18}F] FDG prior to initiation of treatment. From the patients studied, 25 had pulmonary anatomical abnormalities and, of those, 10 had evidence of subclinical activity in several regions of the lung even in spite of negative sputum culture. These latter were more likely to develop active tuberculosis during the follow-up period and to show increased consumption of [^{18}F] FDG in the regional lymph nodes of the mediastinum.

These studies add evidence against the assumption that negative conversion in sputum cultures can be used as a marker of bacillus eradication and show us an alternative tool to evaluate the efficacy of current treatments. In addition, when used in clinical trials, PET-CT scan with [^{18}F] FDG could help us to evaluate shorter schedules or to follow up patients with extrapulmonary forms of the disease, for example central nervous system tuberculosis, in which there are still no sufficiently reliable biomarkers to determine the cure and in which even the diagnosis is often complicated. Moreover, the findings of the studies commented here open the possibility of detecting extrapulmonary dissemination during subclinical stages. On the other hand, the results reported by these two groups of researchers force us to rethink the way in which we

classify tuberculosis. Although two clinical forms of disease (latent and active) have been considered for years, is there a heterogeneity of the latent form as previously suggested? or is there a form of subclinical activity different to latency? And if the latter were true, what could be the consequences of treating with a single drug those patients with latent tuberculosis who may actually have an active subclinical disease?

Another important issue that undoubtedly needs to be further analyzed is whether the results of these studies do also call into question the reliability of the new biomarkers that have been developing in recent years, because these markers were evaluated in clinical studies in which the negative conversion of sputum culture was considered as a reference to define treatment success and in which no long-term follow-up has been performed to verify the rate of relapse.[6,12] Additionally, for most trials that are searching new molecules that could help us to differentiate between active and latent infection, it would be necessary to reconsider the criteria by which the ''control group'' of latently infected individuals is enrolled, since from now we cannot ignore the probability of recruiting subjects with active subclinical tuberculosis in future investigations.

3. Challenges

Despite the contributions of studies by Malherbe and Esmail, it remains to be defined whether the findings of mRNA in sputum and bronchoalveolar lavage are because these molecules are also stable *in vivo* or if it truly reflects the persistence of living bacteria. It is also important to mention that the observations of development of new pulmonary lesions, persistent consumption of [^{18}F] FDG and relapse of some patients could be related either with reinfections by different strains because the studies were performed in endemic countries, or with the immune response to antigens released from dead bacteria.

Finally, while these imaging techniques seem to be a new opportunity and a good option, their use in the hospital setting is far from being a routine practice, due to their lack of availability even in developed countries, as well as the risk of radiation exposure and high price. Therefore, its use for now should be limited to clinical trials to evaluate other less-expensive markers applicable in developing countries with high incidence of the disease.

4. Conclusions

It is clear that much work remains to be done to solve the problem of not being able to define with certainty the efficacy of anti-tuberculosis treatment. Even when it has been a great advance in the development of new methods to facilitate the diagnosis of tuberculosis and the discrimination between active and latent infection, more efforts are needed to have markers that allow us to evaluate the cure achieved with pharmacological treatment. However, studies such as those discussed here should stimulate the search for new ways to face the challenge, especially in countries where the disease is still causing great problems to health systems. Although for now the cost of subjecting patients to such sophisticated imaging studies could represent a great effort for many hospitals, the long-term benefit of getting new markers based on ''radiological healing'' could justify the expense, especially in an era in which antibiotics should be used more carefully if we want to avoid the possible future consequences of the increasing emergence of multi-drug resistant strains of *Mycobacterium tuberculosis*.

Funding

The authors are supported by the National Council of Science and Technology of Mexico (CONACyT).

Conflicts of interest

The authors have none to declare.

Acknowledgments

To José Pablo Romero López and Francisco Javier Martínez Hernández at the Department of Immunology, National School of Biological Sciences, National Polytechnic Institute, Mexico City, for their critical reading and comments about the manuscript.

REFERENCES

1. WHO. *Global Tuberculosis Report 2016*. Geneva: World Health Organization; 2016. Available at: http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1 Accessed 29.11.16.
2. Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6(9):570–581.
3. Palomino JC, Martin A, Von Groll A, Portaels F. Rapid culture-based methods for drug-resistance detection in *Mycobacterium tuberculosis*. *J Microbiol Methods*. 2008;75(2):161–166.
4. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. *Curr Opin Infect Dis*. 2009;22(2):174–182.
5. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med*. 2008;149(3):177–184.
6. Wergeland I, Pullar N, Assmus J, et al. IP-10 differentiates between active and latent tuberculosis irrespective of HIV status and declines during therapy. *J Infect*. 2015;70(4):381–391.
7. Horne DJ, Royce SE, Gooze L, et al. Sputum monitoring during tuberculosis treatment for predicting outcome: a systematic review and meta-analysis. *Lancet Infect Dis*. 2010;10(6):387–394.
8. Seon HJ, Kim YI, Lim SC, Kim YH, Kwon YS. Clinical significance of residual lesions in chest computed tomography after anti-tuberculosis treatment. *In J Tuberc Lung Dis*. 2014;18:341–436.

9. Detection of mRNA transcripts and active transcription in persistent *Mycobacterium tuberculosis* induced by exposure to rifampin or pyrazinamide. *J Bacteriol*. 2000;182(22):6358–6365.
10. Malherbe ST, Shenai S, Ronacher K, et al. Persisting positron emission tomography lesion activity and *Mycobacterium tuberculosis* mRNA after tuberculosis cure. *Nat Med*. 2016;22 (10):1094–1100.
11. Esmail H, Lai RP, Lesosky M, et al. Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[^{18}F] fluoro-D-glucose positron emission and computed tomography. *Nat Med*. 2016;22(10):1090–1093.
12. Goletti D, Petruccioli E, Joosten SA, Ottenhoff TH. Tuberculosis biomarkers: from diagnosis to protection. *Infect Dis Rep*. 2016;8(2):6568.

José Alberto Choreño Parra[a,b,*]
[a]*Laboratory of Clinical Immunology I, National School of Biological Sciences, Instituto Politécnico Nacional, Mexico City, Mexico*
[b]*Neuropathology Department, National Institute of Neurology and Neurosurgery "Manuel Velasco Suárez", Insurgentes Sur No. 3877, Tlalpan, La Fama, 14269 Mexico City, Mexico*

Nayeli Martínez Zúñiga
Citlaltepetl Salinas Lara

Neuropathology Department, National Institute of Neurology and Neurosurgery "Manuel Velasco Suárez", Insurgentes Sur No. 3877, Tlalpan, La Fama, 14269 Mexico City, Mexico

**Corresponding author at*: Laboratory of Clinical Immunology I, National School of Biological Sciences, Instituto Politécnico Nacional, Manuel Carpio y Plan de Ayala S/N, Miguel Hidalgo, Santo Tomás 11350, Mexico City, Mexico. Tel.: +521 55 5729 6300
E-mail address: jchorenop1500@alumno.ipn.mx
(J.A. Choreño Parra)

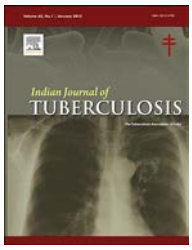
Received 23 December 2016
Available online 31 May 2017

http://dx.doi.org/10.1016/j.ijtb.2017.05.006
0019-5707/

Available online at www.sciencedirect.com

ScienceDirect

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



Abstracts

Household food insecurity is associated with low interferon-gamma levels in pregnant Indian women

Vaidya A, Bhosale R, Sambarey P, Suryavanshi N, Young S, Mave V, Kanade S, Kulkarni V, Deshpande P, Balasubramanian U, Elf J, Gupte N, Gupta A, Mathad JS. *Int J Tuberc Lung Dis*. 2017;**21(7)**:797–803. http://dx.doi.org/10.5588/ijtld.16.0718

Setting: Over 20% of tuberculosis (TB) cases during pregnancy occur in India.
Objective: To determine the association between household food insecurity and interferon-gamma (IFN-γ) levels in pregnancy.
Design: Pregnant women in India were administered the Household Food Insecurity Access Scale (HFIAS) questionnaire and underwent an IFN-γ release assay. Logistic regression was used to identify factors associated with food insecurity.
Results: Of 538 women, 60 (11%) had household food insecurity, 47 (78%) of which were moderate or severe food insecure. After mitogen stimulation, moderate or severe food insecure women had a median IFN-γ concentration of 4.2 IU/ml (IQR 2.2–9.8) vs. 8.4 IU/ml (IQR 3.0–10) in women with no or mild food insecurity ($P = 0.03$). In multivariate analysis, higher IFN-γ concentrations were associated with human immunodeficiency virus infection (OR 1.3, 95%CI 0.51–2.1, $P = 0.001$), and inversely associated with moderate or severe food insecurity (OR −1.6, 95%CI −2.9 to −0.27, $P = 0.02$) and the number of adults in the household (OR −0.08, 95%CI −0.16 to −0.01, $P = 0.03$). There was no association between food insecurity and IFN-γ response to *Mycobacterium tuberculosis* antigen.
Conclusion: Food insecurity in pregnancy is associated with low IFN-γ levels. There was no association between food insecurity and IFN-γ response to *M. tuberculosis* antigen, but our study was underpowered to detect this outcome.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.015

Loss to follow-up among children in pre-ART care under the National AIDS Programme, Tamil Nadu, South India

Devi NP, Kumar AMV, Chinnakali P, Rajendran M, Valan AS, Rewari BB, Swaminathan S. *Public Health Action*;**7(2)**:90–94. http://dx.doi.org/10.5588/pha.16.0112

Setting: Children aged <15 years constitute 7% of all people living with the human immunodeficiency virus (HIV) in India. A previous study from an antiretroviral therapy (ART) centre in south India reported 82% loss to follow-up (LTFU) among children in pre-ART care (2006–2011).
Objective: To assess the proportion of LTFU within 1 year of registration among HIV-infected children (aged < 15 years) registered in all 43 ART centres in the state of Tamil Nadu, India, during the year 2012.
Design: This was a retrospective cohort study involving a review of programme records.
Results: Of 656 children registered for HIV care, 20 (3%) were not assessed for ART eligibility. Of those remaining, 226 (36%) were not ART eligible and entered pre-ART care. Among these, at 1 year of registration, 50 (22%) were LTFU, 40 (18%) were transferred out and 136 (60%) were retained in care at the same centre. The child's age, sex, World Health Organization stage or occurrence of opportunistic infection were not associated with LTFU.
Conclusion: One in five children registered under pre-ART care were lost to follow-up. Stronger measures to prevent LTFU and reinforce retrieval actions are necessary in the existing National HIV Programme.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.016

Comparative study of genotypes of *Mycobacterium tuberculosis* from a Northern Indian setting with strains reported from other parts of India and neighboring countries

Sharma P, Katoch K, Chandra S, Chauhan DS, Sharma VD, Couvin D, Rastogi N, Katoch VM. *Tuberculosis* July 2017;**105**:60–72. http://dx.doi.org/10.1016/j.tube.2017.04.003

This study was carried out to characterize *Mycobacterium tuberculosis* population in Ghatampur, Kanpur, North India, by spoligotyping and Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats (MIRU-VNTRs) typing. A total of 335 isolates were genotyped by spoligotyping and Central Asian (CAS) sub-lineage was the most prevalent, comprising 59.1% of all isolates. Other lineages were: East-African Indian (EAI) (19.10%), T (5.07%), Beijing (3.28%), Manu (2.98%), X (2.68%), S (0.89%), H3 (0.59%), Ural (0.59%), LAM 9 (0.29%) and

unknown (5.37%). This data was compared with 8444 clinical isolates from other parts of India and neighboring countries. Thanks to interrogation of the SITVIT2 database, which shows that China is unique in having a predominance of Beijing lineage; Iran in having an almost equal proportion of Ural and CAS lineages; while the rest of the Middle-East and Indian subcontinent shows a gradient of CAS lineage predominating in the north of tropic of cancer, and the ancestral EAI lineage in South India and South-East Asia. Additionally, 12 loci MIRU-VNTR typing efficiently discriminated 13 spoligotype-defined clusters into 92 patterns; 53 isolates showed >70% homology. It was observed that Beijing lineage strains were more frequently associated with MDR strains (p-value = 0.001). A multi-step application of combination of spoligotyping and MIRU-VNTR typing for analyzing the molecular epidemiology of TB may provide a better means of fingerprinting and studying transmission dynamics.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.017

Drug resistance pattern in pulmonary tuberculosis patients and risk factors associated with multi-drug resistant tuberculosis

Maharjan S, Singh A, Khadka DK, Aryal M. *Journal of Tuberculosis Research* June 2017;**5(2)**:106–17. http://dx.doi.org/10.4236/jtr.2017.52012

Introduction: Anti-tuberculosis drug resistance is a major problem in tuberculosis (TB) control programme, particularly multi-drug resistance TB (MDR-TB) in Nepal. Drug resistance is difficult to treat due to its associated cost and side effects. The objective of this study was to assess the drug resistance pattern and assess risk factor associated with MDR-TB among pulmonary tuberculosis patients attending National Tuberculosis Center.

Methodology: The comparative cross sectional study was conducted at National Tuberculosis Center during August 2015 to February 2015. Early morning sputum samples were collected from pulmonary tuberculosis suspected patients and subjected to Ziehl-Neelsen staining and fluorochrome staining and culture on Lowenstein-Jensen (LJ) medium. Drug Susceptibility test was performed on culture positive isolates by using proportion method. Univariate and multivariate analysis was computed to assess the risk factors of MDR-TB.

Results: Out of 223 sputum samples, 105 were fluorochrome staining positive, 85 were ZN staining positive and 102 were culture positive. Out of 102 culture positive isolates, 37.2% were resistance to any four anti-TB drugs. 11 (28.9%) were initial drug resistance and 28 (43.7%) were acquired drug resistance. The overall prevalence of MDR-TB was 11.7%, of which 2 (5.3%) were initial MDR-TB and 10 (15.6%) were acquired MDR-TB. Univariate and multivariate analysis showed female were significantly associated ($P = 0.05$) with MDR-TB.

Conclusion: Drug resistance TB particularly MDR-TB is high. The most common resistance pattern observed in this study was resistance to both isoniazid and rifampicin. Females were found to be associated with MDR-TB. Thus, early diagnosis of TB and provision of culture and DST are crucial in order to combat the threat of DR-TB.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.018

Time lag between immigration and tuberculosis rates in immigrants in The Netherlands: A time-series analysis

van Aart C, Boshuizen H, Dekkers A, Korthals Altes H. *Int J Tuberc Lung Dis*. 2017;**21(6)**:486–92. http://dx.doi.org/10.5588/ijtld.16.0548

Setting and objective: In low-incidence countries, most tuberculosis (TB) cases are foreign-born. We explored the temporal relationship between immigration and TB in first-generation immigrants between 1995 and 2012 to assess whether immigration can be a predictor for TB in immigrants from high-incidence countries.

Design: We obtained monthly data on immigrant TB cases and immigration for the three countries of origin most frequently represented among TB cases in the Netherlands: Morocco, Somalia and Turkey. The best-fit seasonal autoregressive integrated moving average (SARIMA) model to the immigration time-series was used to prewhiten the TB time series. The cross-correlation function (CCF) was then computed on the residual time series to detect time lags between immigration and TB rates.

Results: We identified a 17-month lag between Somali immigration and Somali immigrant TB cases, but no time lag for immigrants from Morocco and Turkey.

Conclusion: The absence of a lag in the Moroccan and Turkish population may be attributed to the relatively low TB prevalence in the countries of origin and an increased likelihood of reactivation TB in an ageing immigrant population. Understanding the time lag between Somali immigration and TB disease would benefit from a closer epidemiological analysis of cohorts of Somali cases diagnosed within the first years after entry.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.019

Estimating the future burden of multidrug-resistant and extensively drug-resistant tuberculosis in India, the Philippines, Russia, and South Africa: A mathematical modelling study

Sharma A, Hill A, Kurbatova E, van der Walt M, Kvasnovsky C, Tupasi TE, Caoili JC, Gler MT, Volchenkov GV, Kazennyy BY, Demikhova OV, Bayona J, Contreras C, Yagui M, Leimane V, Cho SN, Kim HJ, Kliiman K, Akksilp S, Jou R, Ershova J, Dalton T, Peter Cegielski for the Global Preserving Effective TB Treatment Study Investigators. *The Lancet Infectious Diseases* July 2017;**17(7)**:707–15. http://dx.doi.org/10.1016/S1473-3099(17)30247-5

Background: Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis are emerging worldwide. The Green Light Committee initiative supported programmatic management of drug-resistant tuberculosis in 90 countries.

We used estimates from the Preserving Effective TB Treatment Study to predict MDR and XDR tuberculosis trends in four countries with a high burden of MDR tuberculosis: India, the Philippines, Russia, and South Africa.
Methods: We calibrated a compartmental model to data from drug resistance surveys and WHO tuberculosis reports to forecast estimates of incident MDR and XDR tuberculosis and the percentage of incident MDR and XDR tuberculosis caused by acquired drug resistance, assuming no fitness cost of resistance from 2000 to 2040 in India, the Philippines, Russia, and South Africa.
Findings: The model forecasted the percentage of MDR tuberculosis among incident cases of tuberculosis to increase, reaching 12.4% (95% prediction interval 9.4–16.2) in India, 8.9% (4.5–11.7) in the Philippines, 32.5% (27.0–35.8) in Russia, and 5.7% (3.0–7.6) in South Africa in 2040. It also predicted the percentage of XDR tuberculosis among incident MDR tuberculosis to increase, reaching 8.9% (95% prediction interval 5.1–12.9) in India, 9.0% (4.0–14.7) in the Philippines, 9.0% (4.8–14.2) in Russia, and 8.5% (2.5–14.7) in South Africa in 2040. Acquired drug resistance would cause less than 30% of incident MDR tuberculosis during 2000–40. Acquired drug resistance caused 80% of incident XDR tuberculosis in 2000, but this estimate would decrease to less than 50% by 2040.
Interpretation: MDR and XDR tuberculosis were forecast to increase in all four countries despite improvements in acquired drug resistance shown by the Green Light Committee-supported programmatic management of drug-resistant tuberculosis. Additional control efforts beyond improving acquired drug resistance rates are needed to stop the spread of MDR and XDR tuberculosis in countries with a high burden of MDR tuberculosis.
Funding: US Agency for International Development and US Centers for Disease Control and Prevention, Division of Tuberculosis Elimination.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.020

Care seeking and treatment related delay among childhood tuberculosis patients in Delhi, India

Kalra A. *Int J Tuberc Lung Dis*. 2017;**21(6)**:645–50. http://dx.doi.org/10.5588/ijtld.16.0563
Objective: To examine delays in treatment initiation among child tuberculosis (TB) patients and to identify associated factors.
Method: A multistage cluster random sampling strategy was used to select 175 parents/care givers of childhood TB patients from eight district TB centres covered by the Revised National Tuberculosis Control Programme in Delhi for interview in a cross-sectional survey. Binary logistic regression analysis was used to identify associated factors.
Results: Median estimated patient and health system delay was respectively 3 (range 1–300) and 41 days (range 10–397). Median total delay was 52 days (range 12–553). Among cases with self-reported delay, 64% of care givers thought that the symptoms would subside without treatment. In pulmonary cases, patient's sex, age of the primary care giver, religion and community were associated with patient delay. The child's place of birth and household size were associated with delay among extra-pulmonary TB cases. Type of first provider and number of providers consulted were associated with health system delay. Those who lived at a greater distance from their first health facility (OR 2.2, 95%CI 1.18–4.07) were more likely to experience prolonged patient delay.
Conclusions: As the considerable health system delays were related to the type and number of providers consulted, targeted strategies are required to bring the health system closer to these particularly vulnerable children and their care givers.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.021

Cost-effectiveness of Xpert MTB/RIF for tuberculosis diagnosis in South Africa: A real-world cost analysis and economic evaluation

Vassall A, Siapka M, Foster N, Cunnama L, Ramma L, Fielding K, McCarthy K, Churchyard G, Grant A, Sinanovic E. *The Lancet Global Health* July 2017;**5(7)**:e710–e719. http://dx.doi.org/10.1016/S2214-109X(17)30205-X
Background: In 2010 a new diagnostic test for tuberculosis, Xpert MTB/RIF, received a conditional programmatic recommendation from WHO. Several model-based economic evaluations predicted that Xpert would be cost-effective across sub-Saharan Africa. We investigated the cost-effectiveness of Xpert in the real world during national roll-out in South Africa.
Methods: For this real-world cost analysis and economic evaluation, we applied extensive primary cost and patient event data from the XTEND study, a pragmatic trial examining Xpert introduction for people investigated for tuberculosis in 40 primary health facilities (20 clusters) in South Africa enrolled between June 8, and Nov 16, 2012, to estimate the costs and cost per disability-adjusted life-year averted of introducing Xpert as the initial diagnostic test for tuberculosis, compared with sputum smear microscopy (the standard of care).
Findings: The mean total cost per study participant for tuberculosis investigation and treatment was US$312.58 (95% CI 252.46–372.70) in the Xpert group and $298.58 (246.35–350.82) in the microscopy group. The mean health service (provider) cost per study participant was $168.79 (149.16–188.42) for the Xpert group and $160.46 (143.24–177.68) for the microscopy group of the study. Considering uncertainty in both cost and effect using a wide range of willingness to pay thresholds, we found less than 3% probability that Xpert introduction improved the cost-effectiveness of tuberculosis diagnostics.
Interpretation: After analysing extensive primary data collection during roll-out, we found that Xpert introduction in South Africa was cost-neutral, but found no evidence that Xpert improved the cost-effectiveness of tuberculosis diagnosis. Our study highlights the importance of considering implementation constraints, when predicting and evaluating the cost-effectiveness of new tuberculosis diagnostics in South Africa.
Funding: Bill & Melinda Gates Foundation.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.022

Magnitude and determinants of adverse treatment outcomes among tuberculosis patients registered under Revised National Tuberculosis Control Program in a Tuberculosis Unit, Wardha, Central India: A record-based cohort study

Mundra A, Deshmukh PR, Dawale A. *Journal of Epidemiology and Global Health* June 2017;**7(2)**:111–18. http://dx.doi.org/10.1016/j.jegh.2017.02.002

Introduction: Deaths, defaults, relapses, and treatment failures have made the control of TB difficult across the globe.
Methodology: This study is a record-based follow-up of a cohort of patients registered under Revised National Tuberculosis Control Program in the year 2014 in Wardha Tuberculosis Unit, India. Data was collected from the records available at the District Tuberculosis Office.
Results: Data of 510 patients was analyzed. The sputum conversion rate was 88%. The overall treatment success rate was 81.9%, and rates of any adverse outcome, deaths, defaults, failure, and shift to Category IV regimen were 32.60/100 person years at risk (PYAR), 16.88/100 PYAR, 11.12/100 PYAR, 3.45/100 PYAR, and 1.15/100 PYAR, respectively. The median times for the above outcomes were 81 days, 110 days, 66 days, 118 days, and 237 days, respectively. The cumulative probability of occurrence at 6 months of any adverse outcome, deaths, default, failure, and shift to Category IV regimen was 0.145, 0.056, 0.088, 0.002, and 0.004, respectively. On multivariate analysis, the determinant of any adverse outcome was age >45 years, whereas extrapulmonary disease was protective. The hazard of defaulting was also significantly higher in male patients and those aged >45 years.
Conclusion: Appropriate interventions and program implementation to reduce the adverse treatment outcomes and interruptions will help in improving program performance.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.023

Systematic review and meta-analysis of the nitrate reductase assay for drug susceptibility testing of *Mycobacterium tuberculosis* and the detection limits in liquid medium

Kwak M, Lee W-K, Lim YJ, Lee SH, Ryoo S. *Journal of Microbiological Methods* Available online 8 July 2017. http://dx.doi.org/10.1016/j.mimet.2017.07.001

Recently, the need for rapid, reliable, and low-cost drug susceptibility testing (DST) methods has increased due to the emergence of multidrug-resistant *Mycobacterium tuberculosis*. Colorimetric methods of DST provide results more quickly than standard culture methods and are inexpensive than molecular methods. Thus, colorimetric methods, such as the nitrate reductase assay (NRA), are being recommended. We searched Medline PubMed for reports on the NRA for DST of *M. tuberculosis* written in English and published within the last five years. We selected 20 reports on six major anti-TB drugs and conducted a meta-analysis using Meta-Disc software. The pooled sensitivities for isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, and kanamycin were 95.4%, 96.4%, 91.5%, 93.1%, 99.3%, and 88.4%, and the pooled specificities were 98.5%, 99.2%, 92.9%, 97.8%, 97.4%, and 99.4%, respectively. The area under the summary receiver operator curve for all drugs was 0.9723–0.9952. The time to results (TTR) for the direct and indirect NRAs was 7–28 days and 6–15 days, respectively. Quality assessments were conducted using the quality of diagnostic accuracy studies tool (QUADAS-2) items, and most reports showed good performance. However, ethambutol, streptomycin, and kanamycin showed relatively low sensitivity. We performed a quantitative NRA in liquid media at various inoculum concentrations. The TTR at 4.94×10^6, 1.67×10^4, and 2.27×10^2 CFU/mL was 4, 14, and 14 days, respectively. The minimum absorbance and nitrite concentration for positive samples were 0.8 and 168 μM, respectively. We propose a quantitative standard to determine sample positivity to address the problems with the current standard NRA which is much less expensive than the conventional assay conducted on solid medium.

Conflicts of interest

The authors have none to declare.

http://dx.doi.org/10.1016/j.ijtb.2017.08.024