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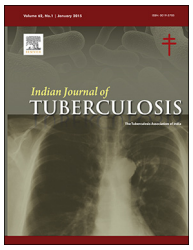

Editorial

Inflammation plays a central role in respiratory diseases, including tuberculosis

Inflammation is the body's response to factors including infection, trauma, and hypersensitivity. Inflammation is an essential component of various respiratory diseases, which is initiated and perpetuated by microorganism pathogens or from the damage or death of host cells. This review highlights about the major role of inflammation in respiratory diseases.

New research suggests that bacteria from gum disease travel through airways and into the lungs.[1] Inflammation is eliminates the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. The classical signs of inflammation are heat, pain, redness, swelling and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.[2] Also, when the lung is exposed to minimal bacterial loads, pathogen clearance operates through innate defenses and the event is generally subclinical. Acute inflammation involves both innate and adaptive defenses resulting in acute infection due to higher loads of bacteria overcoming the local defenses.

Immunity involves innate and adaptive systems: Innate immunity is nonspecific and evokes rapid responses, including inflammation in face of pathogen insults. Adaptive immunity is antigen-specific. It first detects the specific antigen and then mobilizes inflammatory cells to target that particular antigen. The innate and adaptive systems share components and act in concert to defend against pathogens. The lungs are exposed to constant insults from the atmosphere and also to toxic molecules circulating through the pulmonary and bronchial vasculature. Elaborate pulmonary defence mechanisms are needed for survival. These include first-line filtration and removal systems such as the nasal vibrissae, mucociliary escalator, and cough reflex. Secretory immunoglobulin A (IgA) in mucus and surfactant produced by alveolar cells also assist in immunity against pathogens and smaller particles, while resident immune cells within the lung parenchyma await organisms that successfully penetrate the physical barriers. Optimal lung defence requires the coordinated action of multiple cell types.[3]

Defence mechanism in lungs: The airway epithelium is the first site of contact with inhaled agents. Its epithelial cells secrete a variety of substances such as mucins, defensins, lysozyme, lactoferrin and nitric oxide, which nonspecifically shield the respiratory tract from microbial attack.[4] The epithelial cells also produce a number of mediators such as reactive oxygen radicals, cytokines (TNF-α, IL-1β, granulocyte/macrophage colony-stimulating factor [GM-CSF]), and platelet-activating factor to recruit inflammatory cells onto the site of inflammation.[5] The cytokines stimulate arachidonic acid release from membrane lipids, leading to production of eiconasoids, which further stimulate mucus secretion by goblet cells and tissue inflammation.

Inflammatory cells: Dendritic cells are antigen-presenting cells (APCs), which stimulate naïve T cell proliferation. Dendritic cells and macrophages are the first line of defense in recognizing various pathogens.[6] Macrophages role is essential in modulating acute and chronic inflammatory responses. Neutrophils provide second-line defense. They are the first cells to be recruited to sites of infection or injury, and attack fungi, protozoa, bacteria, viruses, and tumor cells. During pulmonary infection, neutrophils migrate out of the pulmonary capillaries and into the air spaces for phagocytosis. Lymphocytes are found throughout the airway and lung parenchyma. There are two major populations of lymphocytes: thymus-dependent T cells and bone marrow-dependent B cells. T lymphocytes provide cell-mediated immunity, while B lymphocytes produce humoral immune responses by synthesizing antibodies (immunoglobulins). They secrete molecules that kill infected cells and tumor cells. Mast cells reside near blood vessels and nerves in tissues throughout the body. They may be activated by a variety of stimuli through various receptors. In the airways, mast cells have receptors for IgE. Once activated, the mast cells produce histamine, leukotrienes, proteases, cytokines, chemokines, and other substances that cause immediate airway inflammation, leading to asthma symptoms. The eosinophil is an important source of major basic proteins, lipid mediators, cytokines, and growth factors, and also secretes mast cell stem cell factor, essential for mast cell growth, activation, chemotaxis, and degranulation.[7]

Respiratory diseases range from mild and self-limiting, such as the common cold, to life-threatening entities like bacterial pneumonia, pulmonary embolism, acute asthma and lung cancer.[8] Other examples of respiratory diseases raising inflammation: bronchitis, asthma, COPD, pulmonary fibrosis, pneumonia, tuberculosis.

Interstitial lung diseases: All interstitial lung diseases affect the interstitium, a part of the lungs anatomic structure. All forms of interstitial lung disease cause thickening of the interstitium. The thickening can be due to inflammation, scarring, or edema. Some forms of interstitial lung disease are short-lived; others are chronic and irreversible. Several types of interstitial lung disease includes, Interstitial pneumonia which is caused by bacteria, viruses, or fungi infecting the interstitium of the lung. A bacterium called *Mycoplasma pneumonia* is the most common cause. Idiopathic pulmonary fibrosis is a chronic, progressive form of fibrosis of the interstitium, its cause is unknown. Nonspecific interstitial pneumonitis is an interstitial lung disease that's often present with autoimmune conditions (such as rheumatoid arthritis or scleroderma). Hypersensitivity pneumonitis is an interstitial lung disease caused by ongoing inhalation of dust, mold, or other irritants. Cryptogenic organizing pneumonia (COP) is pneumonia-like interstitial lung disease but without an infection present. COP is also called bronchiolitis obliterans with organizing pneumonia (BOOP). Acute interstitial pneumonitis is a sudden, severe interstitial lung disease, often requiring life support. Desquamative interstitial pneumonitis is an interstitial lung disease that's partially caused by smoking. Sarcoidosis is a condition causing interstitial lung disease along with lymphadenitis, and sometimes heart, skin, nerve, or eye involvement. Asbestosis is an interstitial lung disease caused by asbestos exposure.

Pneumonia is an inflammation of the lung parenchyma. Consolidation of the lung tissue may be identified by physical examination and chest X-ray. Numerous factors, including environmental contaminants and autoimmune diseases, as well as infection, may cause pneumonia. *Streptococcus pneumoniae* is the most common agent of community-acquired acute bacterial pneumonia. An exception is the viral pneumonia caused by influenza viruses, which can have a high mortality in the elderly and in patients with underlying disease. Pneumonia occurs when lung defense mechanisms are diminished or overwhelmed. The pneumococcal vaccine should be given to patients at high risk for developing pneumococcal infections, the elderly and any patients immuno-compromised through disease or medical therapy. In addition, Bronchitis and bronchiolitis involve inflammation of the bronchial tree. Bronchitis is usually preceded by an upper respiratory tract infection or forms part of a clinical syndrome in diseases such as influenza, rubeola, rubella, pertussis, scarlet fever and typhoid fever. Chronic bronchitis with a persistent cough and sputum production appears to be caused by a combination of environmental factors, such as smoking, and bacterial infection with pathogens such as H *influenzae* and S *pneumoniae*. Patients with chronic bronchitis have an increase in the number of mucus-producing cells in their airways, as well as inflammation and loss of bronchial epithelium. Viral infections are treated with supportive measures. Respiratory syncytial virus infections in infants may be treated with ribavirin, amantadine and rimantadine. Asthma is an ongoing disease of the bronchial tubes, where the airways overreact to external factors like smoke, air pollution, and allergens. The bronchial tubes become narrower due to the ensuing inflammation in the tissue lining the airways. This then produces the symptom of dyspnea, where the patient complains of trouble breathing and has difficulty moving air in and out of the lungs. In both acute and chronic asthma, we can observe a chronic inflammation which has consolidating characteristics in a tense patient who has an overreaction to stimuli.[9] COPD is another inflammatory disease where both the airways and lung tissue are affected. This can manifest as a combination of chronic obstructive bronchitis and emphysema, where the former is the result of chronic inflammation of the bronchial tubes and the latter is due to breakdown of the alveoli. COPD patients have difficulty moving air in and out of the lungs in addition to poor oxygen exchange.

Mycobacterium tuberculosis is the organism that is the causative agent for tuberculosis (TB). Infection with *M. tuberculosis* (MTB) is accompanied by an intense local inflammatory response which may be critical to the pathogenesis of tuberculosis. Microscopically, the inflammation produced with TB infection is granulomatous, with epithelioid macrophages and Langhans giant cells along with lymphocytes, plasma cells, maybe a few PMNs, fibroblasts with collagen, and characteristic caseous necrosis in the center. The inflammatory response is mediated by a type IV hypersensitivity reaction. This can be utilized as a basis for diagnosis by a TB skin test. An acid fast stain (Ziehl–Neelsen or Kinyoun's acid fast stains) will show the organisms as slender red rods. The most common specimen screened is sputum, but the histologic stains can also be performed on tissues or other body fluids. Culture of sputum or tissues or other body fluids can be done to determine drug sensitivities. The genetic factors which determine the expression of inflammatory markers affect the onset of the disease and its treatment. The susceptibility to infection, progression to active or latent form and dissemination to the other sites are governed by the inflammatory responses generated by the host.

Pulmonary fibrosis is a chronic lung disease that is due to scarring or thickening of the lungs, which affects oxygen exchange. Often, the etiology for pulmonary fibrosis is unknown. COPD is most commonly caused by tobacco smoke. Asthma triggers can range from allergens, infections, cold air, or smoke. Pulmonary fibrosis can pose a more difficult problem, as sometimes there may be a source but oftentimes the disease is idiopathic. Therapies for lung disease can be effective in symptomatic treatment but not curative. Treatment initially consists of: corticosteroids, beta agonists, leukotriene modifier, receptor antagonists, methylxanthines like theophylline. In the early stages of lung disease, these medications can be given as monotherapy, but as the disease progresses, treatment is more likely to consist of multiple medications as well as supplemental oxygen and pulmonary rehabilitation.

Animal model of lung diseases: A classic mouse model of lung disease comprises of using the chemotherapeutic glycopeptide bleomycin to induce interstitial pulmonary fibrosis like that in humans. This is the most commonly used experimental model for experimentally induce pulmonary fibrosis. For asthma, ovalbumin is a frequently used allergen

for inducing allergic pulmonary inflammation like that found in asthma. To study acute lung exacerbation and injury that is common in chronic lung disease sufferers, bacterial lipopolysaccharide (LPS) is used to mimic such acute events. Other models are **Bleomycin model of Lung Disease** – pulmonary fibrosis, **Ovalbumin model of Lung Disease** – acute or chronic asthma, **LPS model of Lung Disease** – emphysema and COPD.[9]

In recent years, there has been progress in use of stem cells in respiratory diseases as they act as both immuno-modulators and anti-inflammatory.

In conclusion, various strategies have been adopted to intervene in pulmonary immune responses. In addition to looking at the cytokines, cytokine receptors, and cell-surface molecules, cellular signal transduction and gene activation have been targeted for therapy. Novel insights into targeted immune modulation could lead to clinical trial developments and new treatment strategies. It is apparent that to understand the mechanism of upcoming future modalities of treatment. Also a comprehensive diagnostic approach to chronic disorders is much needed. Moreover, all risk factors should be approached with lifestyle modifications (e.g., smoking cessation, weight loss, physical activity), and every associated chronic co-morbid disorder should be treated simultaneously. Summarily, Knowledge and understanding of the possible mechanisms and pathways underlying the inflammatory responses have led to the development of therapy and drug designs leading to better clinical care to patients.

Lastly, we can say that inflammation is at the base of all respiratory diseases including TB. In addition to giving specific therapy and antibiotics as per the etiology, inflammation has also to be tackled to manage the disease.

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Viewpoint

Too little too late: Waiting for TB to come

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ABSTRACT

There is a new paradigm that preventing tuberculosis (TB) and addressing the reservoir of latent TB infection in combination with curing all TB cases is essential to accelerate the decline of TB rates and ending TB by 2050. However, complacency and incremental change eludes radical policy transformation needed to meet global targets. This essay explores current attitudes, policy disparities between high and lower burden settings, and what changes are needed to remove the obstacles to progress.

Achieving TB elimination requires a direct attack on the reservoir of latent infection, with a drug or a vaccine (or both) that is effective against established infection. For instance, if just 8% of people infected with M. tuberculosis are fully and permanently protected each year, incidence would fall to 90 per million by 2050 with no other intervention.[1] – Chris Dye

Imagine a garden with patches of dandelion weed. Would you pull the weeds only after it has developed its classic fluffy seed head with half of it blown in the wind? Or, would you use a comprehensive approach and pull all dandelion weeds out, those that have bloomed or seeded and those not yet bloomed? The scenario is similar to a comprehensive disease control strategy, but until this year tuberculosis (TB) control has been nothing more than passive case finding of those who are ill enough to have symptoms and have already spread TB, each to at least 10 people. This secures TB's future in society.

1. Policy disparities: low-intermediate vs. high burden countries

Tuberculosis (TB) is a curable and preventable disease, yet the ancient scourge continues to persist and grow in drug-resistant strength. Today, the global cure rate of drug resistant TB is no better than sunshine, fresh air and grandmother's chicken soup. Airborne transmission of multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) go unchecked from the shameful fraction of cases that are passively detected. Yet, we forget or ignore that acquired drug resistance can be prevented if those with latent TB infection (LTBI) never get the disease.

Preventive treatment of LTBI is a proven strategy that has maximum benefit when targeting the screening and treatment to those at highest risk of progression, especially in congregate or geo-hotspots and yet, prevention is not recommended for at-risk groups in high burden settings. Paradoxically, the WHO

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launched guidelines on the management of LTBI in 2015 for 115 countries with a TB incidence of less than 100 cases per 100,000 population.[2] Ironically, TB prevention would make a much bigger impact in high-burden settings and yet India, a country that contributes the most in the world, is left out.

The WHO also recommends comprehensive contact screening and prevention in low and intermediate burden countries,[2] yet high burden settings like India rely on limited testing and prevention to those living with HIV or pediatric contacts under 5. This sliver of prevention is too small to make any impact on case rate decline. Further, this pick and choose approach confuses healthcare workers performing this duty as well as the very people exposed to active TB. It provides a perception that those not screened for LTBI are not valued, or are invulnerable from acquiring TB. Even worse, it implies that TB is somehow a weak pathogen that can be easily controlled with limited measures.

Paradoxically, screening and prevention of persons living with diabetes, the largest contributing cause of TB in India and countries outside of Africa, has a negative recommendation from the WHO despite poorer active treatment response, higher relapse, and death rates compared to persons without diabetes.[3,4] Persons with diabetes living in high-TB burden settings do not have a right to know their TB infection status, thereby missing the opportunity of TB prevention, and to stop smoking and gain better control their blood glucose through medication, weight loss, diet and exercise.

Despite a modern artillery of studies on TB risk factors and epidemiologic tools that include genotyping, cloud-based surveillance and geo-mapping, local TB programs of high burden countries do not target populations for active case finding and prevention. Instead, high-burden programs ignore the profiles of their own cases and like robots, use global recommendations that prohibit TB screening, active case finding and prevention except to persons living with HIV or contacts under age 5.

Despite effective methods that use combined symptom screening and TB testing as a non-stigmatizing means to find subclinical and symptomatic cases as well as LTBI, active case finding study pilots focus on poorly sensitive methods such as isolated symptom review as triggers for sputum collection or chest X-ray.[5] Mass chest X-ray (CXR) screening can be effective in finding active TB; however, non-TB findings can be costly and stigmatizing to work up while LTBI, the seedbed, is totally neglected.

Finally, preventive treatment has been reduced from a daily 9–12 month isoniazid (INH) regimen to a 12-dose once a week INH-rifapentine (3HP) 3-month regimen.[2] With equivalent efficacy to 9 months of INH, a better safety profile and significantly better adherence rates, the 3HP regimen is rapidly replacing INH as the regimen of choice in US TB programs. Additionally, for more than a decade, more accurate Food and Drug Administration (FDA)-approved blood tests have been available. Like the tuberculin skin test, interferon gamma release assays (IGRAs) are aids to diagnose TB's carrier state. Unlike the skin test, IGRAs are not impacted by prior Bacillus Calmette–Guérin (BCG) vaccination or most non-tuberculous mycobacterial bacilli, and they require only one patient visit instead of two to get a result.[6] In large prospective LTBI prevalence trials in China and Vietnam comparing an IGRA to tuberculin skin tests (TST) head to head, a profound reduction in LTBI rates and a statistically significant higher progression rate were found with the IGRA.[7–9]

2. Distorted reality

While treatment of active TB cuts the line of transmission, it has no impact on the seeds of infection that have already fallen, ensuring the fate of future TB disease. Hence, ''waiting for TB to come'' is an innocent assault on the very principles of disease control by allowing an airborne pathogen to fester and spread until consumption brings them to death or the doctor. ''Even if TB transmission is interrupted completely in 2015, reactivation and relapse of old infections would still generate more than 100 cases per million population in 2050.''[1]

It is also illogical to not prevent disease in individuals and families who are living on the margins of society, who cannot afford to be sick while barely having enough for the minimum necessities of food and shelter. From a non-public health viewpoint, passive case finding undermines our Hippocratic oath of doing no harm as it encourages advanced disease and decreases the chance of cure while increasing morbidity and mortality. From a public health perspective, it is illogical to not actively pursue disease diagnosis and prevention among those patients with known risks that are causing syndemics, or surges in rates of disease.

Business as usual is not working. India provides 25% of the world's 10.4 million TB cases and despite the decline in deaths from TB in India, it contributes one third of the 1.4 million global TB deaths annually.[10] Pediatric TB rates remain high and serve as a sentinel for transmission. Drug resistant strains and incurable drug resistance continue to grow and spread, becoming part of the seedbed of tomorrow's disease. Yet, most Indian providers wait for TB to come. They do not understand the importance of the LTBI reservoir or how to diagnose it. They believe that if LTBI is treated, patients will get infected again so there is ''no use''. It is also misunderstood that treatment of LTBI could cause drug resistance. India's annual infection rate is estimated at 1.5%, hardly a high chance for general reinfection, and the bacilli burden in LTBI is much too small to harbor wild drug resistant mutants required for drug resistance to emerge. A WHO systematic review showed no evidence of acquired resistance from LTBI treatment.[2] Finally, there is a perception that LTBI treatment toxicity is similar to active TB treatment which is utterly false.

It is now clear that a comprehensive strategy of active case finding, effective treatment of all cases and attacking the reservoir of LTBI with preventive treatment is essential to achieving TB elimination. The WHO includes TB prevention in their strategic pillars to the End TB Strategy and multiple modeling studies confirm the need to address LTBI in order accelerate the decline of TB.[1,11,12] The new National Strategic Plan for Tuberculosis Elimination of the Revised National TB Control Program (RNTCP) acknowledges that scaling up TB preventive therapy is important to meet the goals of ending TB in India; yet disappointingly, it only expands screening and prevention to a mere sliver of persons with LTBI: individuals with silicosis, individuals on immunosuppressive drugs and high-risk adult contacts that are not well defined. According to

a WHO modeling study, the critical mass for accelerating the decline to end TB by 2050 is effectively treating 14% of those with LTBI along with effectively treating all TB cases.[1] This amounts to approximately 73 million persons of the estimated 40 percent of the Indian population with LTBI. This is a huge number but one that can be used to estimate the true cost of eliminating TB in India.

3. Changing the passive mindset to true action and change

It is unimaginable to have approached severe acute respiratory syndrome (SARS) or Ebola with the "wait until sick" TB approach. Why should it be any different for TB? The complacency of our TB community is mind boggling since TB kills someone every 18 seconds, more people globally every year than any other infectious agent. Our obstacles to a true call to action mimic the stealth slow nature of the TB pathogen itself. Its timescale from infection to disease is variable, unpredictable and long. It could be decades before disease emerges. Instead of thinking of it as a time bomb or land mine, we behave on a slow time scale by acting incrementally, repeating studies and collecting evidence as if time itself will solve the problem. Our obsession on the affordability and cost-effectiveness of interventions make us forget the unrelenting toll and cost caused by TB's kill rate, pervasiveness, individual financial impact, lifelong morbidity and acquired drug resistance. How can we not afford to prevent TB? We must accept the fact that if we truly want to achieve the goal of eliminating TB it will be expensive. It will cost billions, but how is that different to other investments in that price range such as the Indian space program that is considered a bargain with an annual budget in 2014 of 1.2 billion USD.

A comprehensive TB control and prevention approach will require new champions who will boldly take on human rights and TB prevention as the new paradigm and demand that advocacy, mass education, educational and treatment centers of excellence, and adequate program funding are prioritized. Rapid scale up and investment in TB programs are needed from local, provincial, national and international funders. Surveillance systems and bidirectional data sharing between national and local programs need to be enhanced to target and determine interventions and track their progress.[13]

A shift from dullness and complacency to action will require looking in the mirror of truth. "Waiting for TB to come" is neither a convenient or affordable option. It is a rationed approach and not a logical disease control choice. Let's not kid ourselves: our goal of TB elimination by 2050 is but a pipe dream without aggressively draining the infection reservoir of LTBI. We have a choice to rise up and prevent TB now or just stay with business as usual, preoccupied with the usual excuses. . .while waiting for TB to come.

Conflicts of interest

The author has none to declare.

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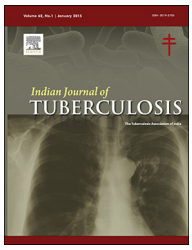

Review article

Modalities to monitor the treatment response in tuberculosis

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ABSTRACT

Considering the global epidemic of drug resistance in *Mycobacterium tuberculosis*, early and accurate diagnosis as well as prompt initiation of antitubercular therapy (ATT) forms the mainstay of tuberculosis control programs. Patients on ATT may develop treatment failure due to diverse reasons including emergence of drug resistance in the host during the course of therapy. Monitoring the timely response to treatment in such cases has a significant role in rapid identification of drug resistant strains and institution of change of regimen to further decrease the morbidity and mortality associated with the disease. Furthermore, availability of faster surrogate end points to assess treatment efficacy, disease activity, cure, and relapse is one of the crucial requirements for undertaking innovative clinical trials related to TB. The article presents here the compilation of currently available methods for monitoring the treatment response in pulmonary as well as extrapulmonary TB.

1. Introduction

Tuberculosis has been a major killer disease known since antiquity. As an important public health disease, tuberculosis continues to uphold its position worldwide, especially in the developing countries. According to the most recent WHO global tuberculosis report, the TB incidence has fallen by an average of 1.5% per year since 2000 and is now 18% lower than the level of 2000.[1] Despite the global success of tuberculosis control programs in reducing the incidence, year 2014 witnessed estimated 9.6 million new TB cases and 1.5 million TB deaths all over the world. India still remains the top most country among the list of high TB burden countries contributing to 23% of the global total, followed by Indonesia (10%) and China (10%).[1]

TB-HIV co-infection and emergence of drug resistant forms of *Mycobacterium tuberculosis* (MTB) are two major factors which continue to impede the progress of TB control campaigns worldwide. TB is the most common opportunistic infection in HIV patients exacerbating the morbidity and mortality.[2] Vice versa, HIV co-infection in TB patients is considered to be the most powerful known risk factor for progression of MTB infection to active disease, increasing the risk of latent TB reactivation twenty-fold. Hence, MTB and HIV are known to act in synergy, accelerating the decline of immunological functions and leading to subsequent death, if untreated.[3]

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Appearance of drug resistant TB is a totally man made problem accountable to the poor patient management, non-adherence to the prescribed regimen, a poor national program or some combination of these three.[4] Multidrug-resistant tuberculosis (MDR-TB) is among the most bothersome elements of the antibiotic resistance pandemic because TB patients that fail treatment have a high risk of death.[5] Globally, 5% of TB cases were estimated to have had MDR-TB in 2014, while on an average, an estimated 9.7% of people with MDR-TB have Extensively drug resistant TB (XDR-TB).[6] Each MDR TB case costs more than 20 times the cost of a simple drug-susceptible TB case which makes it impossible to tackle the problem of drug-resistant TB through treatment alone.[7] Hence, preventing emergence of MDR-TB in the community is more imperative rather than its treatment.

Early diagnosis and prompt initiation of therapy forms the mainstay of tuberculosis control. Rapidly identifying response to treatment and changing the treatment regimen accordingly plays a significant role in prevention of emergence of drug resistance. All patients put on antitubercular treatment should be monitored to assess their response to therapy. Regular monitoring of patients not only facilitates treatment completion but allows the identification and management of adverse drug reactions.[8] The estimated treatment failure rate in patients infected with drug susceptible organisms receiving six-month regimens is 1–4% and the relapse rate is 7% or less.[9] Recurrence of TB, either due to treatment failure or relapse continues to place a significant burden on the patient as well as TB control programs. Furthermore, availability of faster surrogate end points to assess treatment efficacy, disease activity, cure, and relapse is one of the crucial requirement for undertaking innovative clinical trials related to TB.[10]

The current approach to monitor the treatment response in pulmonary tuberculosis patients is by sputum smear microscopy at the end of intensive phase. Smears positive at this stage may indicate; poor adherence, poorly supervised initial phase of therapy, poor quality of anti-TB drugs, improper doses of anti-TB drugs, heavy initial bacillary load or extensive cavitation in the patient, associated co-morbid conditions, development of drug resistance in the infecting organism or the presence of non-viable bacteria that remains visible by microscopy.[8]

Although, smear microscopy is being universally used as a marker to assess the treatment response it categorically do not distinguish between viable and non-viable bacilli as mentioned above. Hence, various other tools are being researched upon to explore the most suitable and definitive measure to analyze positive treatment outcomes in patients. This review describes various methods that have been used for monitoring the treatment response traditionally and newer methods which have the potential to act as surrogate markers for treatment efficacy.

1.1. *Traditional methods*

1.1.1. *Clinical assessment*

Clinical assessment of disease progress is largely subjective. CDC recommends at least monthly clinical evaluations of the patients to identify possible adverse reactions to medications, assess adherence and to determine treatment efficacy.[11] Some of the widely used indicators for clinical progress are disappearance of clinical symptoms, general well-being, ability to resume normal activities, and weight gain. Moreover, it is often the only means available for judging progress in extrapulmonary and smear-negative pulmonary tuberculosis. Weight gain is a valuable indicator in such cases.[12] However, Kennedy et al.[13] found that weight gain during therapy is not a reliable indicator of overall treatment response. On the other hand, radiographic resolution as a marker of treatment response is thought to be inadequate as well because it lags significantly behind clinical improvement. Another limiting factor is the presence of concurrent illness, making assessment of symptomatic response difficult.[14]

1.1.2. *Microscopic examination of sputum smears*

Sputum smear microscopy has a fundamental role in monitoring the response to treatment of infectious cases of pulmonary tuberculosis.[12] Diminishing numbers of AFB during the treatment followed by smear-negative status indicates treatment success, while increasing numbers of AFB in the later phase of treatment are an indication of failure. As recommended by WHO and IUATLD, Revised National Tuberculosis Control Program (RNTCP) of India, follows a scheme for monitoring progress during treatment in smear positive patients on three occasions: at the end of intensive phase (2 months for new cases and 3 months for re-treatment cases), two months into continuation phase (at the end of 4 and 5 months for new and re-treatment cases respectively) and at the end of treatment. Smears, those are positive at the end of intensive phase should be retested again at the end of extended intensive phase (3 months in new and 4 months in re-treatment cases).[15] In addition to monitoring individual patient's response to treatment, smear examinations showing a conversion rate at 2–3 months is a good operational indicator. It reflects the capacity of the program to maintain patients on treatment, obtain sputum samples, and eliminate sources of infection, and it is an early surrogate of the treatment outcome indicator.[12]

Although simple and relatively inexpensive method, sputum smear microscopy carries a number of limitations questioning its reliability. The poor sensitivity of smear microscopy, particularly in patients with limited pulmonary involvement or immunosuppression, leads to under diagnosis of the disease. Furthermore, it also lacks specificity for MTB disease, as non-tuberculous mycobacteria (NTM) and various other bacteria can stain AFB positive. Additionally, excretion of dead bacilli after treatment may give a false sense of smear positivity while the samples are actually culture negative.[16]

1.1.3. *Viability stains as an alternative to acid fast staining*

Tuberculosis and other mycobacterial infections such as leprosy are characterized by the persistence of nonviable acid-fast bacilli at the site of disease and in clinical samples, regardless of effective treatment.[17] Recently it has been hypothesized that, assessing viability of tubercle bacilli on sputum smear microscopy can predict the concentration of culturable *M. tuberculosis* indicating early therapeutic response to antitubercular treatment.[18] Fluorescein diacetate (FDA) is a fatty acid ester which is otherwise non fluorescent, but gets fluoresced after being enzymatically hydrolyzed by

acetyltransferases possessed by live mycobacterial cells, thus highlighting viable (Fluorescent) bacilli. Contrastingly, ethidium bromide is a fluorescent dye which enters the dead cells to get intercalated between bases of DNA molecules and thus stains dead bacilli as red orange color under UV light.[19] The viability assays using FDA have been used to assess viability of *M. leprae*, Nontuberculous Mycobacteria and MTB >30 years ago.[19,20] It has also been evaluated in few field studies against culture as a means of detecting tuberculosis treatment failure, with variable accuracy.[21,22] When compared to culture, FDA was found to have moderate sensitivity (83.7% [95% confidence interval {CI}, 70.3–92.6]) and low (66.1% [59.5–72.2]) specificity.[22] However, the good negative predictive value (94.8% [90.1–97.8]) and negative likelihood ratio (0.2) suggest using this method to rule out treatment failure in settings without access to culture.[22]

1.1.4. *Culture conversion*

While sputum smear microscopy is frequently the only diagnostic modality available for the diagnosis and monitoring of pulmonary tuberculosis patients worldwide, culture on solid or in liquid media is the recognized 'gold standard'.[16] As per WHO, new patients who remain sputum smear-positive at the end of the intensive phase should submit another specimen for smear microscopy the following month. If that specimen is also smear-positive, culture and DST should be undertaken, i.e. at the end of 3 months. While in previously treated patients, if the sputum smear is positive at the end of intensive phase (3 months), culture and DST should be performed without further investing on smear microscopy. This allows a result to be available earlier than the fifth month of treatment.[8] Treatment is considered as a failure if the patient is found to harbor MDR-TB at any point of time. Similarly, in patients on MDR/XDR regimen, sputum culture conversion is a more reliable marker than smear conversion. Patients are considered culture converted after having two consecutive negative cultures taken at least one month apart. Use of liquid culture is preferred over solid culture for follow up cases based on resources available.[7] However, sputum culture takes a long time, especially with the use of solid media, is prone to contamination, is expensive, requires a sophisticated infrastructure with proper containment facilities as well as highly trained staff and is rarely available in high-burden settings.[10,23]

1.2. *Newer modalities for assessment of treatment response*

1.2.1. *Colorimetric assays*

Colorimetric assays are based upon use of oxidation–reduction indicator dyes which changes their color in the presence of metabolically active cells of MTB. Number of these dyes; Alamar blue,[24] MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide),[25,26,27] XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2h-tetrazolium-5-carboxanilide),[28] Resazurin[29] and nitrate reductase assay(NRA)[30] has been used in past to assess the viability of *M. tuberculosis* in presence of antitubercular drugs in both direct[31,32] and indirect,[26,27,33] drug sensitivity testing formats. Farnia et al. (2004)[34] evaluated the prospect of using oxidation reduction indicators like Alamar blue and Malachite green for monitoring the treatment of tuberculosis. The sensitivities of 95% and 93% and specificities of 93% and 92% were observed, respectively, for Alamar Blue assay and Malachite Green assay as compared to culture on LJ. The mean time required to get a positive signal by Alamar Blue assay was 9 days, and that of Malachite Green culture medium was 11 days. Another study by Rojas-Ponce et al.[35] evaluated a new method using 2,3-diphenyl-5-thienyl-(2)-tetrazoliumchloride (STC) coupled with Nitrate reductase assay (NRA), labeled as STC-NRA method as a potential marker to assess treatment progress. The time to detection (TTD) was 14 days as compared to MGIT which was just 7 days. Further, the TTD was increased with duration of anti-tuberculosis treatment, highlighting the value of this method in monitoring treatment success. A systematic review and metaanalysis of all colorimetric methods concluded that these methods are highly sensitive and specific for the rapid detection of rifampicin and isoniazid resistance in culture isolates with respective 7 and 14 days of average time to have first results.[36] They are simple and inexpensive and hence carry great expectations to be a test of choice for evaluation of treatment progress in resource constrained settings.

1.2.2. *Immunological biomarkers*

TB-specific host biomarkers for diagnosis of active tuberculosis and monitoring treatment response have been identified as important priorities for TB research and have been explored in some recent studies.[37] CD4+T cells are the predominant cell type producing IFN-γ in response to MTB infection.

Recently, Adekambi et al.[38] identified immune activation markers expressed on IFN-γ producing CD4+ cells after stimulation with MTB specific ESAT6 and CFP10 antigens, such as CD38, HLA-DR and Ki-67. Human CD38 is a transmembrane glycoprotein and HLA-DR is an MHC class II cell-surface receptor involved in antigen presentation. Both CD38 and HLA-DR are early immune markers and their expression is highly induced on the surface of antigen specific T cells reflecting T cell activation in response to microbial infection or vaccination. A nuclear protein, Ki-67 shows selective expression in cycling cells and hence has been widely used as an intracellular proliferation marker. These markers accurately classified individuals with active TB and latent TB and also distinguished individuals with untreated TB from those who had successfully completed anti-TB treatment and correlated with decreasing mycobacterial loads during treatment.[38] Several other exploratory studies have evaluated the diagnostic potential of cytokine biomarkers other than interferon-gamma for monitoring anti-tuberculous therapy. Among the other cytokines, TNF-α, IL-2, IL-6, IL-10 and IL-12 were the most extensively investigated as a biomarker to monitor tuberculosis treatment.[39]

1.2.3. *Molecular biomarkers*

1.2.3.1. *Xpert MTB/RIF system*. Xpert MTB/RIF assay is an automated cartridge based nucleic acid amplification test (CB-NAAT) based on real time amplification of mycobacterial nucleic acid and has a potential to simultaneously diagnose MTB and rifampicin resistance. Role of this assay as a possible biomarker to monitor the treatment outcome has been

extensively evaluated by Friedrich et al.[10] When compared with a combined reference standards of smear and culture methods, the Xpert MTB/RIF assay had high sensitivity (97%) but poor specificity (49%) for monitoring the treatment outcome. The high sensitivity is probably due amplification of killed bacilli which either remained intact or the free DNA released from the dead bacilli during treatment. Hence, detection of *M. tuberculosis* DNA in sputum with the Xpert MTB/RIF assay in its current format cannot be used as a biomarker of disease activity.

1.2.3.2. *Detection of mycobacterial mRNA in sputum.* Molecular studies based on detection of DNA, although sensitive in diagnosing tuberculosis, are not useful in monitoring response to therapy since *M. tuberculosis* DNA persists well beyond the time points that cultures are negative, i.e., 1–2 months.[40,41] This is likely due to continued shedding of intact dormant or dead tubercle bacilli from the focus of infection and due to an inherent resistance of DNA to degradation when sequestered within macrophages. On the contrary, levels of rRNA which is present in large amount in the cells may decline slowly in the presence of effective drug therapy despite being more labile than DNA.[42]

mRNA is a marker of cell viability and hence quantification of *MTB* mRNA in sputum is a promising tool for monitoring response to antituberculosis therapy and evaluating the efficacy of individual drugs. It has been demonstrated that, the levels of *MTB fbpB* mRNA (encoding fibronectin-binding protein, antigen 85B)[41,43] and *hspX* (encoding alpha-crystalline homologue protein) mRNA[41] decline rapidly in conjunction with *MTB* colony counts after initiation of a rifampin-based standard drug therapy. Additionally, newer assays demonstrating *icl mRNA* (encoding isocitrate lyase) and *rrnA*-P1 mRNA (noncoding ribosomal promoter region), have also been evaluated as a marker of bactericidal and sterilizing activity in sputum.[44] Amongst them, *icl* mRNA was determined to be the best marker since it is expressed in the sputum at high levels and has strong correlation with CFU counts in the patients receiving INH monotherapy as well as fluoroquinolone monotherapy, indicating its potential to measure sterilizing activity of anti-TB drugs. However, *icl* mRNA may not be as useful as *fbpB* mRNA marker for studying early bactericidal activity.[44]

1.2.3.3. *Change in whole blood transcriptional signature.* The sequence of RNA reflects exact sequence of the DNA from which it was transcribed. Consequently, by analyzing the entire collection of RNA sequences in a cell (the transcriptome) and comparing it with the transcriptomes of different types of cells, researchers can gain a deeper understanding of what constitutes a specific cell type, how that type of cell normally functions, and how changes in the normal level of gene activity may reflect or contribute to disease. In the past two decades, it has become possible to analyze the transcritptomes of various cell types with the widespread use of mRNA microarray technology. This involves profiling the expression levels of thousands of genes simultaneously to constitute a transcriptional signature.[45] Recently it has been demonstrated that a whole blood transcriptional signature can distinguish active TB from latent TB and other diseases.[46] Moreover, Berry et al.[46] for the first time demonstrated that significant transcriptional changes could be detected after two months following initiation of successful anti tubercular treatment. This signature reverted back to that of healthy individuals after completing treatment. The findings were substantiated by subsequent studies on patients from high burden TB countries like South African countries.[46–48] Furthermore, Bloom et al. 2012,[47] have shown a significant blood transcriptional response to anti-TB treatment to occur rapidly, as early as 2 weeks, suggesting that blood transcriptional signatures could be used as early surrogate biomarkers of successful treatment response. Another advantage conferred by these tests is, since they are blood based tests, they are devoid of requirement for sputum sample which is sometimes difficult to obtain.[45,47]

Even though these tests are more specific, they demand validation of the published findings among larger cohorts with inclusion of patients known to have treatment failures.[47] Similarly, their performances in diverse population groups need to be ascertained so that the transcriptional signatures can be better defined and are appropriate to 'real-world' situations.

1.2.4. *Other biomarkers*

Various other biomarkers have been explored to predict durable (non-relapsing) tuberculosis cure. They include biomarkers which can be detected from samples other than sputum like serum, whole blood or even urine. Serum prealbumin (PA) is a kind of plasma protein synthesized by the hepatocytes with a short biological half-life and is considered as a useful indicator in assessing individual's recent nutritional intake and current nutritional state. Luo et al.[49] has demonstrated its usefulness as an objective marker for assessment of improvement in nutritional status in tuberculosis patients who were put on treatment, thus, indirectly monitoring treatment response. Acute phase reactant and immune system activation markers like CRP, Beta$_2$ Microglobulin (β_2M) and Neopterin have also been assessed as the fast and effective markers of treatment monitoring at the point-of care setting. CRP showed the most significant decrease by 2 months of treatment ($p < 0.0001$) whereas levels of β_2M and Neopterin showed little change by 2 months but a significant decrease by 6 months of treatment ($p = 0.0002$ and $p < 0.0001$ respectively).[50] Amongst the other activation markers, soluble intercellular adhesion molecule (sICAM) 1, soluble urokinase plasminogen activator receptor and procalcitonin have also demonstrated significant decrease in levels following treatment of TB.[51]

Detection of small fragments of *M. tuberculosis* IS6110 DNA specifically termed as transrenal DNA (tr-DNA), which is thought to arise because of apoptosis of host cells and demonstration of lipoarabinomannan in urinary specimens have also been researched upon demonstrating varying sensitivities. Urinary biomarkers have the potential to serve as both diagnostic and prognostic markers especially in extrapulmonary TB, paucibaliiary disease a pediatric tuberculosis.

The comparative analysis of both traditional as well as newer methods have been summarized in Tables 1 and 2.

Table 1 – Traditional methods for TB treatment monitoring.

S.No.	Method	Advantages	Disadvantages	References
1	Clinical assessment	Useful in extrapulmonary and smear negative pulmonary tuberculosis	Subjective; influenced by concurrent illness	11–14
2	Smear microscopy	Simple, inexpensive, good operational indicator for TB control programs	Poor sensitivity and specificity	12,15,16
3	Viability stains	Easy to perform, rapid, Good negative predictive value	Moderate sensitivity and specificity, requirement of fluorescent microscope and reagents	18–22
4	Culture conversion	Highly specific hence considered as 'The gold standard'	Longer turn around time, expensive, requires containment facilities	10,16,23

Table 2 – Newer and promising approaches for TB treatment monitoring.

S.No.	Method	Advantages	Disadvantages	References
1	Colorimetric assays	Simple, inexpensive, rapid compared to culture	Need to be substantiated by further studies	34–36
2	Immunological markers	Blood based tests format hence useful for patients with weakened responses or children; potential to discriminate between active TB and LTBI in few assays excluding INF-γ as a marker	Cost associated with use of flow cytometer is the major limitation	38,39
3	Molecular markers			
	Xpert MTB/RIF assay-	Rapid (2 h), Good sensitivity (97%)	Poor specificity (49%) as dead bacilli can also be amplified in the assay	10
	Detection of mycobacterial mRNA from sputum	Decreasing levels after antitubercular treatment has strong correlation with bacterial load in sputum	Larger longitudinal studies are required to validate the reliability of the tests	45–47
	Change in whole blood transcriptomal signature	Obviates the need for obtaining sputum sample; response to treatment can be seen as early as 2 weeks of therapy		49–51

2. Role of therapeutic drug monitoring in the treatment of tuberculosis

Therapeutic drug monitoring (TDM) in the form of measurement of plasma concentration of drug is an objective way to determine the dose of a drug. It is a standard clinical technique used to monitor treatment of many diseases including infectious diseases. TDM can be a helpful supplementary tool, in addition to other tools used to monitor anti-tubercular treatment.[52,53]

The various measures used to determine pharmacokinetic-pharmacodynamic (PK/PD) of antitubercular drugs include (a) the duration of time of a drug concentration remains above the minimum inhibitory concentration (T > MIC), (b) the ratio of the peak drug concentration relative to the MIC (C_{max}/MIC) and (c) the ratio of the area under the concentration–time curve at the end of the dosing interval relative to the MIC (AUC_{0-24}/MIC).[54,55] However, the estimation of AUC in patients is not possible practically as it requires several blood samples (minimum of six to seven samples). Another approach is limited sampling strategies (LSS), applied to some TB drugs like linezolid, moxifloxacin, amikacin and kanamycin.[56–58]

For TDM of first-line antitubercular drugs, C_{max} has been targeted the most. Many of the first line agents reach their peak concentration within 1–3 h of administration. The post-dose blood draw at 2 h and 6 h is usually considered for TDM. On a few occasions, 2 h drug concentration alone could not show delayed C_{max} values. Hence, in patients with abnormal 2 h concentration, 6 h blood draw can be useful to distinguish between delayed absorption and malabsorption.[54]

The blood samples are usually collected in plain red top vacuum tubes. For most of the TB drugs, except the injectable drugs, heparin-containing green top vacuum tubes also can be used.[52] The post-dose blood sample is centrifuged and serum is immediately frozen. The commonly used method for drug-serum estimation are high-performance liquid chromatography, gas chromatography and more recently tandem mass spectrophotometry.[54]

One of the concerns in TDM of antitubercular drugs is specimen storage and transport. The application of dried blood spot (DBS) analysis instead of serum analysis may overcome this concern. For DBS analysis, blood obtained by single-use lancets is dropped directly on DBS paper, generally cellulose-or a cotton-based filter. The paper is left to dry at room temperature and then store in a plastic bag with desiccant packages. Another advantage of DBS is that it requires less blood volume, which reduces its biohazardous risk as compared to conventional serum sampling and makes it more applicable to the pediatric population.[59,60]

The advantages TDM in the treatment of tuberculosis are given in Box 1. The indications of TDM in the treatment of tuberculosis can be summarized as follows:

- Slow clinical response to TB treatment

  Rationale: The pharmacokinetics of antitubercular drugs are variable. The risk of poor outcome is more in the patients

Box 1. Advantages of TDM in the treatment of tuberculosis.

- To check patient compliance to antitubercular drugs.
- To shorten the response time and treatment completion.
- To check therapeutic response by correlation plasma concentration of antitubercular drugs with clinical and bacteriological data.
- To adjust the dose during drug–drug interaction.
- To monitor treatment response in patients with diabetes and HIV due to risk of poor drug absorption.

who are slower to respond clinically. Hence, it has been advocated to consider TDM in such patients or in those whose condition is worsening.[61,62]

- Patients with TB and HIV

  Rationale: India is the second highest country in terms of number of estimated HIV associated with TB in the world. Ass per TB India 2017 report, an estimate of 1.1 lakh HIV associated TB occurred in 2015.[63] The coexistence of TB and HIV affects the treatment outcome of tuberculosis. The risk of shortened survival rate and higher rate of recurrence of TB is observed more in patients with HIV-positive than HIV-negative patients.[64,65] The survival rate of HIV-positive patient varies according to smear positive status and regimen. It has been observed that the survival rate in higher in smear-positive cases and lowest with rifampicin-based regimens.[66] It is recommended that the antitubercular treatment should be started in HIV co-infected patients along with antiretroviral therapy irrespective of CD4 count. The concomitant therapy is associated with many complexities. These complexities are due pathophysiological changes in HIV positive patients and altered pharmacokinetics of drugs. The absorption of antitubercular drugs, in particular rifampicin, is affected in HIV patients, due to various forms of enteropathy.[67,68] In a study conducted at Tuberculosis Research Center, Chennai, it has been concluded that in addition to malabsorption of rifampicin in HIV patient, the bioavailability isoniazid is affected more in rapid acetylators. This study also observed that the absorption of pyrazinamide and ethambutol was also reduced.[69] In addition, the polypharmacy required while treating both the diseases increase the risk of toxicity that eventually lead to poor adherence to the treatment, poor treatment outcome and increased cases of drug-resistance tuberculosis. Rifampicin-based antitubercular regimen and efavirenz-based antiretroviral regimen considered as first-line regimen while treating co-infected patients of TB/HIV. Rifamycins, in particular rifampicin, are potent CYP450 enzyme inducers leading to subtherapeutic concentration of anti-retroviral drugs, especially atazanavir and ritonavir and hence reduces their therapeutic effectiveness in standard doses.[70] Rifampicin is also an inducer of adenosine triphosphate (ATP) binding cassette transporter P-glycoprotein, which lead to decreased bioavailability of concomitantly administered antiretroviral drugs.[70] Isoniazid inhibits CYP2A6 enzyme and may actually increase the concentration of efavirenz while treating co-infected patient with slow CYP2B6 metabolizer genotype, which may result into efavirenz toxicity.[54] Considering these issues, it is essential to optimize treatment of both the diseases and balance efficacy and toxicity. Thus, TB/HIV co-infected patients are ideal candidates for TDM.

- Patients with TB and diabetes

  Rationale: Diabetes causes three-fold increase in TB risk and two-fold increase in adverse TB treatment outcome, specifically delay in mycobacterial clearance, treatment failure, death, relapse, and re-infection.[71–73] Two primary reasons identified for such adverse TB treatment outcome in such patients. First, poor glucose control which may reduce efficacy of anti-TB regimen due to dysfunctional immunity to *M. tuberculosis*. Second possible reason could be altered absorption of anti-TB drugs due to disease pathology. Diabetes patients more likely to develop gastroparesis which may affect the absorption of antitubercular drugs (delay or malabsorption).[74] The concentration of isoniazid and rifampicin has been found suboptimal in patients with TB and diabetes.[75] Another reason for suboptimal concentration of anti-TB drug in such patients is heavy bodyweight. Hence, dose optimization is essential as per body weight. Immunity is also compromised in diabetic patients due to chronic hyperglycemia and such patients are more prone for drug toxicity as well.[76] Therefore, it has been advocated to check the drug concentration early in the course of therapy in all diabetics, in the setting where TDM facility is available.

- Special populations – Pediatric age group

  Rationale: The metabolism of isoniazid is rapid in pediatric patients as compared to adults and hence require higher mg/kg dose. In addition, it has been observed that antitubercular drugs achieve low serum concentration in malnourished children compared to those without malnourishment. The co-existence of HIV with TB in children also leads to suboptimal concentration of antitubercular drugs.[77]

- Extrapulmonary tuberculosis

  Rationale: In different forms of extrapulmonary tuberculosis, TB meningitis in particular require dose optimization considering the different determinants like the blood brain barrier for drug penetration, high early mortality and long duration of treatment. The studies showed that isoniazid achieves good concentration in CSF whereas rifampicin being more protein bound and susceptible to alteration in membrane drug transporters, penetrates blood brain barrier poorly. Hence, at standard doses, MIC of rifampicin rarely exceeds. However, high dose of rifampicin is usually not recommended. But, the study of adult patients with TB meningitis in Indonesia showed that high dose intravenous rifampicin during early period of TB meningitis (first two weeks) reduce 6-month mortality with no increase in incidence of adverse effects due to high dose.[78–80]

- Patients with renal failure

  Rationale: The antitubercular drugs that are primarily eliminated by the kidney, like ethambutol, cycloserine, aminoglycosides, are prone to develop toxicity in patients with renal failure. Such patients are ideal candidates for TDM. The patients with renal failure can be categorized into three groups: (a) patients with poor renal functions and who

are not on dialysis; (b) renal failure patients on hemodialysis; (c) renal failure patients on peritoneal dialysis. Such patients have tendency to accumulate anti-Tb drugs which may lead to drug toxicity.[81,82] In patients with renal failure who on dialysis, pre- or post-dialysis sample can be used to assess residual pre-dose drug.[83]

- Patients with hepatic dysfunction

  Rationale: The antitubercular drugs, isoniazid, rifampicin, pyrazinamide, ethionamide, and PAS are primarily cleared by the liver. Hepatic clearance of these drugs cannot be predicted based only on serum test for liver enzymes like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) or bilirubin. Such patients require dose titration based on serum drug concentration.[84] The patients with hepatic dysfunction experience nausea and vomiting and often have malabsorption and decreased clearance of drugs.[53] Therefore, the dose adjustment in required and such patients are also potential candidates for TDM.

3. Concluding remarks

Monitoring the treatment response is a key element in early identification of treatment failure in tuberculosis. It provides a direction to identify the root causes for treatment failure which may be either due to poor compliance or emergence of drug resistance. This approach not only facilitates effective patient management, but also helps in timely identification of programmatic gaps so as to take appropriate actions. Amid the availability of multiple methods to assess the therapeutic response in TB, traditional ways of demonstrating live bacilli from the sputum samples by culture seems to have no equivalent substitute yet. However, long turnaround times, need to have bio containment facility and tendency for contamination limits the use of culture as the most accurate and convenient assessment tool.

Looking at the cost effectiveness as well as convenience, sputum smear microscopy is still considered as a universal marker of treatment response. Sputum smear examination coupled with LED fluorescence microscopy has already increased the sensitivity of TB diagnosis under various national TB control programs. The same set up, in any case can be utilized for treatment monitoring of pulmonary TB patients, simply by adding the viability stains like FDA in the follow up examinations.

Several markers have been evaluated over the past decade; however none has been validated as the most specific and precise. Moreover, many biomarkers are targeted toward monitoring of response in pulmonary tuberculosis and in adult patients. Therefore an urgent need exists to develop the more accurate and universal markers which can help in assessing bacillary clearance from pulmonary as well as extrapulmonary sites and adult as well as pediatric tuberculosis.

Conflicts of interest

The authors have none to declare.

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Original article

A selective and sensitive high performance liquid chromatography assay for the determination of cycloserine in human plasma

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ABSTRACT

Background: Cycloserine (CYC) is a second line antitubercular drug that is used for the treatment of multidrug resistant tuberculosis (MDR-TB) along with other antitubercular agents and is often used in developing countries. Monitoring CYC levels in plasma could be useful in the clinical management of patients with MDR-TB. A high performance liquid chromatography method for the determination of CYC in human plasma was developed.
Methods: The method involved extraction of the sample using solid phase extraction cartridges and analysis of the extracted sample using a reverse phase T3 column (150 mm) and detection at 240 nm with Photo Diode Array (PDA) detector. The chromatogram was run for 15 min at a flow rate of 0.4 ml/min at 30 °C.
Results and conclusion: The assay was specific for CYC and linear from 5.0 to 50.0 μg/ml. The relative standard deviations of within- and between-day assays were less than 10%. Recovery of CYC ranged from 102% to 109%. The interference of other second line anti-TB drugs in the assay of CYC was ruled out. The assay spans the concentration range of clinical interest. The specificity and sensitivity of this assay makes it highly suitable for pharmacokinetic studies.

1. Introduction

Cycloserine (CYC) is an antibiotic produced by *Streptomyces garyphalus* and *Streptomyces orchidaceus* and is an analog of amino acid D-alanine. It inhibits enzymes D-alanine synthetase.[1,2] CYC is a second line antitubercular drug that is used for the treatment of multidrug resistant tuberculosis (MDR-TB) along with other antitubercular agents and is often used in developing countries.[3] After oral administration, CYC is readily absorbed from the gastro intestinal tract, with peak blood levels attained in 4–8 h.[4]

Resistance to second-line drugs is associated with worse treatment outcomes since an inadequate or poorly

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administered second line treatment regimen allows a drug-resistant strain to become dominant in a patient infected with MDR-TB. Therefore monitoring CYC levels in plasma could be useful in the clinical management of patients with MDR-TB.

Several analytical methods have been developed for the determination of CYC in plasma, which includes high performance liquid chromatographic (HPLC) methods and liquid chromatographic mass spectrometric (LCMS/MS) methods. The HPLC methods reported were based on derivatisation technique and requires special columns and large volume of samples.[3,5–7] Several LCMS/MS methods for the quantification of CYC in plasma have been reported.[8–10] However, these expensive techniques are not affordable in developing countries and for resource poor settings. Moreover none of these methods have included second-line anti-TB and anti-retroviral drugs in their specificity experiments. Since CYC is used in combination with these drugs in TB patients with and without HIV, it is essential to rule out the interference of these drugs in the development of method for the determination of CYC in plasma. We developed and validated a simple, selective and sensitive HPLC method for the determination of CYC.

2. Materials and methods

2.1. Chemicals

Pure CYC powder was obtained from Sigma–Aldrich Chemical Company, St. Louis, MO, USA. MCX (1 cc/30 mg) cartridges from Waters India, acetonitrile (HPLC grade), isopropyl alcohol (IPA), formic acid, sodium dihydrogen orthophosphate (NaH2PO4) and disodium hydrogen orthophosphate were purchased from Qualigens (India). Ammonia solution was obtained from SD Fine Chemicals Limited. Deionized water was processed through a water purification system (Siemens, Germany). Pooled human plasma was obtained from a Blood Bank, Chennai, India.

2.2. Chromatographic system

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of two pumps (LC-10ATvp), Photo diode array detector (SPD-M10Avp) and auto sampler (SIL-HTA) with built in system controller. Class VP-LC workstation was used for data collection and acquisition. The analytical column used was Atlantis T3, 150 mm × 4.6 mm ID, 3 μm particle size (Waters, Ireland) protected by a compatible guard column.

An isocratic mobile phase consisted of a mixture of 10 mM phosphate buffer (sodium dihydrogen phosphate NaH_2PO_4 – 3.19 g and disodium hydrogen orthophosphate, Na_2HPO_4 – 10.99 g in 1000 ml water) and acetonitrile:IPA (90:10) in the ratio of 95:5 (v/v), was used to separate the analyte from the endogenous components. Prior to preparation of the mobile phase, the solvents were degassed separately using a Millipore vacuum pump. The PDA detector was set at a wavelength of 240 nm. The chromatogram was run for 15 min at a flow rate of 0.4 ml/min at 30 °C. Unknown concentrations were derived from linear regression analysis vs. concentration curve. The linearity was verified using estimates of correlation coefficient (*r*).

2.3. Preparation of standard solution

A stock standard (1 mg/ml) was prepared by dissolving CYC in water. The working standards of CYC in concentrations ranging from 5.0 to 50.0 μg/ml were prepared in pooled plasma.

2.4. Sample preparation

To 200 μl each of calibration standards and test samples, 200 μl of 1% formic acid was added and the contents were vortexed vigorously, centrifuged at 10,000 rpm for 10 min. The analyte was extracted through solid phase extraction cartridges. The eluted solution was evaporated to dryness. The dried residue was reconstituted in 200 μl of diluent (90% water:10% of acetonitrile:IPA; 90:10) and 50 μl was injected into the HPLC column.

2.5. Method validation

2.5.1. Accuracy and linearity

The accuracy and linearity of CYC standards were evaluated by analysing a set of standards ranging from 5.0 to 50.0 μg/ml. The within day and between day variations were determined by processing each standard concentration in duplicate for six consecutive days.

2.5.2. Precision

In order to evaluate the precision of the method, plasma samples containing varying concentrations of CYC were analyzed in duplicate on three consecutive days.

2.5.3. Recovery

Known concentrations of CYC (2.5, 7.5 and 10.0 μg/ml) were prepared in pooled human plasma and were spiked with lower and higher concentrations of standards. The percentage of drug recovery from plasma samples was calculated by dividing the difference in CYC concentrations by the added concentration. Recovery experiments were carried out on three different occasions.

2.5.4. Specificity

Interference from endogenous compounds was investigated by analysing blank plasma samples. Interference from certain anti-tuberculosis drugs such as rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin, ethionamide, levofloxacin and certain antiretroviral drugs, namely, nevirapine, efavirenz, zidovudine, didanosine, stavudine, lamivudine, saquinavir, lopinavir, ritonavir and indinavir at a concentration of 10 μg/ml was also evaluated.

2.5.5. Limits of detection (LOD) and quantitation (LOQ)

These values were estimated mathematically from the standard curve equations.[11] LOD was calculated using the formula $3.3 \times \sigma/S$, where σ is the standard deviation of Y-axis intercepts and S is the slope of the calibration curve. LOQ was calculated using the formula $10.0 \times \sigma/S$, where σ is the standard deviation of Y-axis intercepts and S is the slope of the calibration curve.

3. Results

Under the chromatographic conditions described above, CYC was well separated and seen as a discrete peak in the representative chromatograms of extracted CYC plasma standards 50.0 and 5.0 μg/ml and an extracted plasma sample from MDR-TB patient (Fig. 1a–c) with the retention time of 5.1 min. Blank plasma sample did not give any peak at the retention time of CYC (Fig. 1d).

In view of its potent antimycobacterial activity, CYC is used in the treatment of MDR-TB along with other second line anti-tuberculosis drugs. It is therefore becomes necessary to rule out the interference of other second line anti-TB drugs in

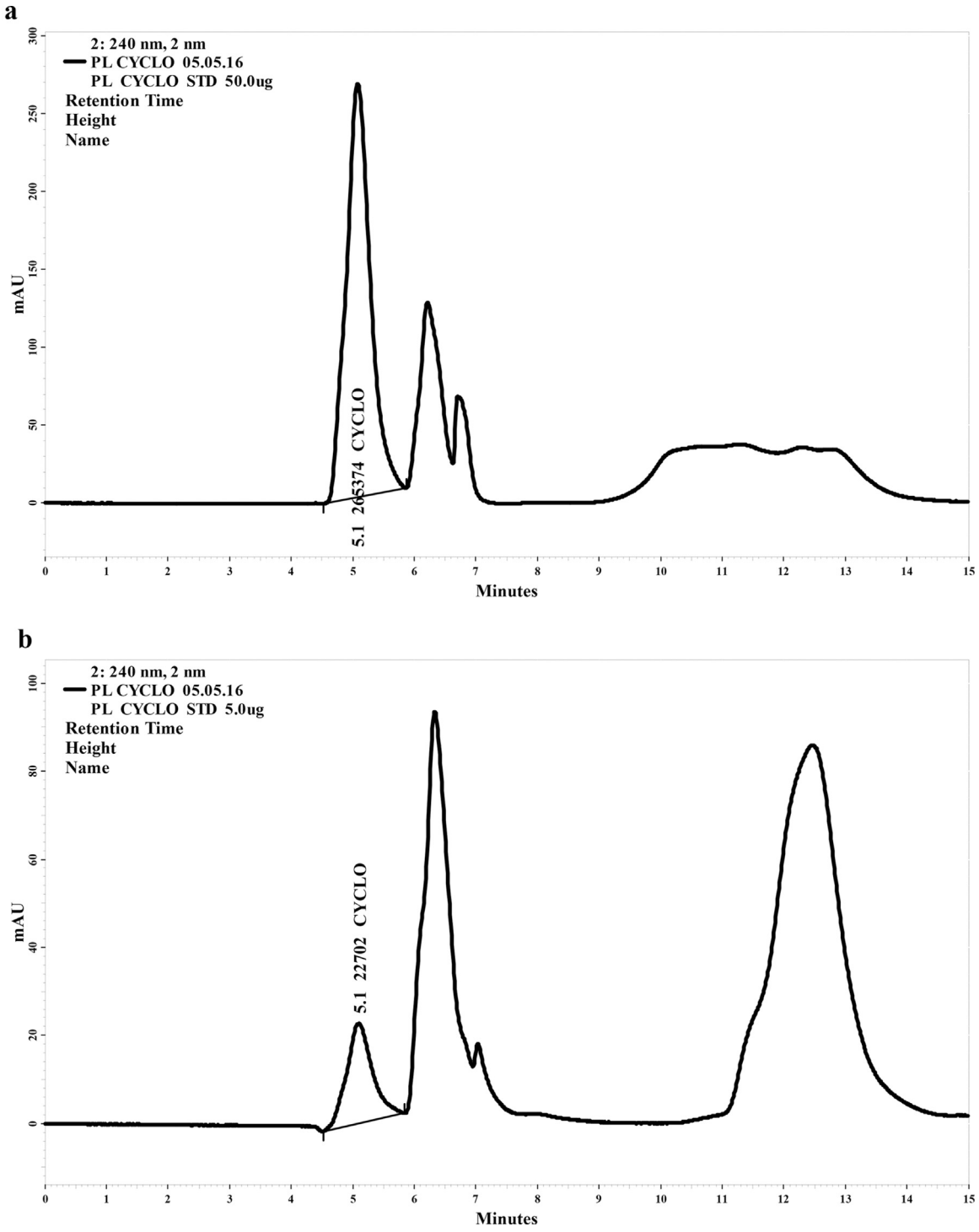

Fig. 1 – (a) Representative chromatogram of extracted cycloserine plasma standard 50.0 μg/ml. (b) Representative chromatogram of extracted cycloserine plasma standard 5.0 μg/ml. (c) Representative chromatogram of extracted plasma sample from MDR-TB patient. (d) Representative chromatogram of extracted blank plasma.

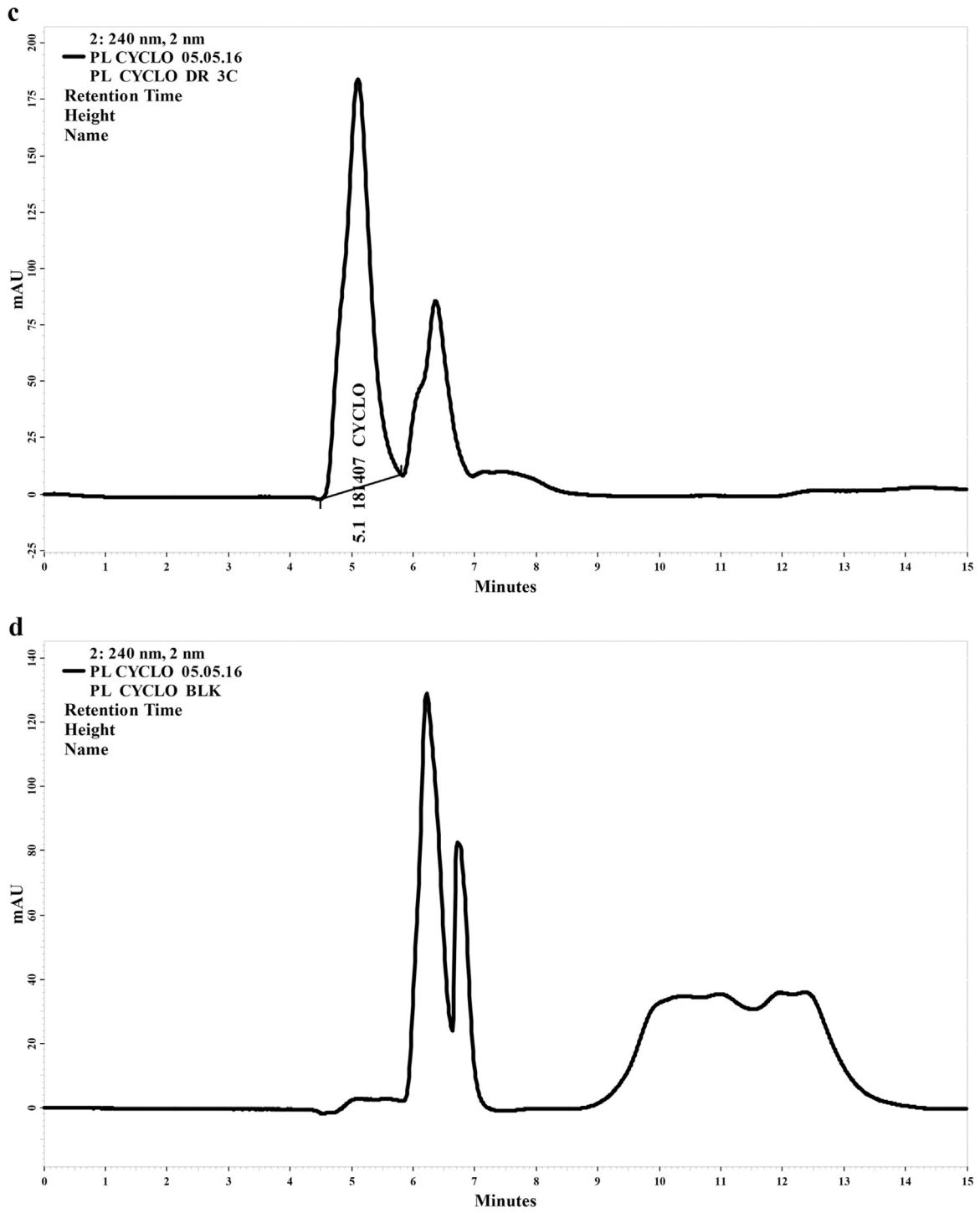

Fig. 1. (Continued).

the assay of CYC and establish the specificity of the method. No endogenous substances, first-line anti-TB drugs such as rifampicin, isoniazid, pyrazinamide, ofloxacin, moxifloxacin, second line anti-TB drugs such as kanamycin, ethionamide, levofloxacin, ethambutol or anti-retroviral drugs like efavirenz, nevirapine, lamivudine, stavudine, zidovudine, didanosine, saquinavir, lopinavir, ritonavir and indinavir interfered with the CYC chromatogram.

In the present method, CYC concentrations ranging from 5.0 to 50 μg/ml were checked for linearity. These concentrations span the range of clinical interest. The calibration curve parameters of CYC from six individual experiments for standard concentrations ranging from 5.0 to 50.0 μg/ml showed a linear relationship (Fig. 2). The mean correlation coefficient (R), coefficient of determinants (R^2), slope and intercept values were 0.997, 0.999, 4980 and 4626 respectively.

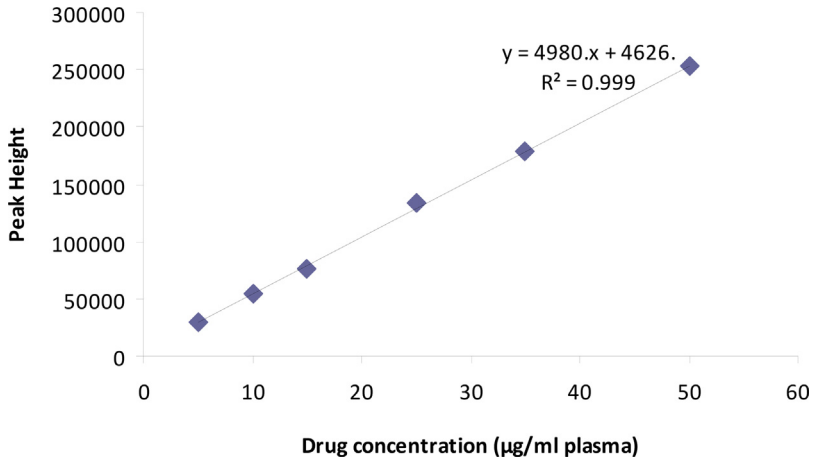

Fig. 2 – Calibration graph for plasma cycloserine standard concentrations (5.0–50.0 µg/ml).

The linearity and reproducibility of the various standards used for constructing calibration graphs for plasma CYC are given in Table 1. The within- and between-day relative standard deviation (RSD) for standards containing 5.0–50.0 µg/ml ranged from 0.88% to 4.18% and 1.72% to 4.96% respectively. The accuracy of plasma CYC concentrations ranged from 95.8% to 104.1%.

The precision of the method was further evaluated by analysing two plasma samples containing different concentrations of CYC (Table 2). The RSD for these samples were 8.4% and 8.2% respectively. The LOD and LOQ estimated mathematically from the standard curve equation[11] were 0.3 and 1.2 µg/ml respectively. The method reliably eliminated interfering materials from plasma, yielding a recovery for CYC that ranged from 102% to 109% (Table 3). The mean CYC concentrations measured on day 1 and 12, in two plasma samples were 31.25 and 29.38 µg/ml respectively. No degradation (<10%) of CYC in human plasma occurred up to 12 days when stored at −20 °C.

Table 1 – Linearity and reproducibility of plasma cycloserine standards.

Concentrations (µg/ml)	Mean height ± SD (RSD %)	
	Intraday (n = 6)	Inter day (n = 6)
50	266,678 ± 4332 (1.7)	265,110 ± 4692 (1.7)
35	210,201 ± 8805 (4.2)	210,201 ± 8805 (4.1)
25	148,074 ± 1529 (1.0)	145,840 ± 5354 (3.6)
15	83,063 ± 1252 (1.5)	83,470 ± 1185 (1.4)
10	55,279 ± 483 (0.8)	55,214 ± 537 (1.0)
5	27,247 ± 945 (3.4)	27,485 ± 828 (3.0)

Table 2 – Precision of plasma cycloserine concentrations.

	Sample 1 (µg/ml)	Sample 2 (µg/ml)
	28.80	25.94
	30.88	29.45
	35.03	29.97
	30.70	31.62
Mean	31.35	29.24
SD	2.63	2.39
RSD %	8.4	8.2

Table 3 – Recovery.

Base	Added (µg/ml)	Actual (µg/ml)	Obtained (µg/ml)	Recovery (%)
5	2.5	7.5	8.17	109
15	7.5	22.5	23.4	104
25	10	35	35.81	102

The method described was applied for the determination of CYC concentration in plasma from 6 patients who received a single oral dose of 1000 mg CYC (Fig. 3). Serial blood samples were collected at different time points after drug administration. The mean plasma peak concentration of CYC was 37.86 µg/ml and the mean time to attain the peak concentration was 4 h. This is in accordance with published literature.[12] The assay spans the concentration range of clinical interest.

4. Discussion

Several methods have been described to measure CYC levels in plasma for pharmacokinetic studies and therapeutic drug monitoring. A review of these methods revealed that some of these methods involved complex mobile phases, gradient mobile phase, or fluorescence detection using derivatisation techniques. Although LCMS/MS methods are available for the quantification of CYC in plasma, these expensive techniques are out of reach for several laboratories with resource poor settings. None of the HPLC or LCMS/MS methods included second-line anti-TB or anti-retroviral drugs in their specificity experiments. Treatment of MDR-TB is always a combination of second-line anti-TB drugs.

A sensitive and specific method for the quantification of CYC in plasma is described in this paper. Method validation was performed as per FDA guidelines and the results were within the acceptable limits. The therapeutic range for CYC is 20–35 µg/ml.[12] Although many methods are available that can quantitate plasma CYC by HPLC and LCMS, each method has

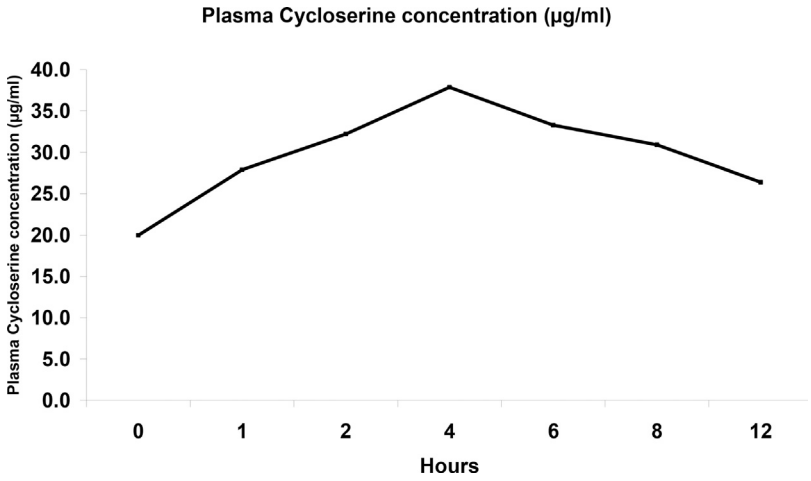

Fig. 3 – Plasma cycloserine concentrations in MDR-TB patients. The above values are mean plasma cycloserine concentrations obtained from 6 patients at different time points who were administered with an oral dose of 1000 mg cycloserine.

its own limitations. In conclusion, we have described a method for estimation of CYC in plasma, that has the sensitivity to quantitate CYC from 5 μg/ml which is sufficiently enough for pharmacokinetic studies and therapeutic drug monitoring. This method is reproducible and specific for the determination of CYC in human plasma, yielding satisfactory recovery from human plasma. Stability experiments showed that CYC was stable up to 12 days when stored at −20 °C. This could be used in pharmacokinetic studies and routine therapeutic drug monitoring of CYC.

Conflicts of interest

The authors have none to declare.

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Original article

Knowledge of private practitioners of Bangalore city in diagnosis, treatment of pulmonary tuberculosis and compliance with case notification

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ABSTRACT

One hundred and twenty-nine qualified private practitioners (PPs) were assessed on their knowledge in diagnosis of pulmonary tuberculosis (PTB), treatment of a new drug sensitive PTB case and practices of case notification, using semi-structured questionnaire. About 20% had adequate knowledge of diagnosis, 29% of treatment regimen, 54% the need for Direct Observation Treatment and 57% about role of sputum smear examination in monitoring treatment response. Of 85 (68%) PPs who had diagnosed any TB case during last two years, 54 (64%) had practised notification. These findings suggest the need for upgrading knowledge of PPs in TB diagnosis, treatment and notification.

1. Introduction

India has the highest burden of tuberculosis (TB) with an estimated 2.8 million incident cases and 480,000 deaths related to TB in the year 2015.[1] The first point of approach for majority of TB patients, is a private practitioner (PP).[2,3] However, studies in different parts of the country have revealed unsatisfactory knowledge and practices of PPs in diagnosis and treatment of TB.[3–11] Bangalore, a burgeoning metropolis in South India is divided into two Revised National Tuberculosis Control programme (RNTCP) districts – Bangalore city and Bangalore Urban covering populations of 7.8 million and 2.3 million respectively in 2015 with the total population of Bangalore urban conglomerate being more than 10 million.[12,13] Bangalore city has a large private health care sector and a good number of patients from surrounding districts also seek health care in the city. There are 9 tuberculosis units (TUs) in the city, each responsible for implementation of RNTCP as well as collaboration with the private sector in the respective areas. We undertook a cross sectional study among PPs practising within the geographical jurisdiction of one TU, to find out the proportions of PPs having adequate knowledge in diagnosis and treatment of a new drug sensitive pulmonary TB (PTB) case and their practices in TB case notification.

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2. Materials and methods

The study was carried out among PPs qualified in allopathic or indigenous systems of medicines. All such private health care facilities in geographical jurisdiction of Dasappa TU were mapped with the support of RNTCP staff, professional medical associations and chemists in the area. There were a total of 86 private health care facilities (nursing homes – 10, standalone clinics – 62, laboratories – 14) and the number of PPs practicing in these facilities was 133. All these PPs were invited to Continuing Medical Education (CME) programmes on Standards of TB care in India (STCI). A total of six CMEs were held during September to November 2015 at National Tuberculosis Institute, Bangalore (NTI). Invitations were extended through written communication as well as personal visits by field staff of NTI. Prior to initiation of CME, PPs were asked to fill up their responses in a semi-structured questionnaire (Appendix A) after obtaining informed written consent, in order to elicit their knowledge on tools for diagnosis of pulmonary TB in adults, treatment regimen and drug dosage for treating a new drug sensitive TB case, method of ensuring treatment adherence, monitoring treatment response, common adverse reactions to anti-TB drugs and their practice of TB case notification. Later, the NTI Medical Officer coded the responses for adequate knowledge (Annexure); a participant was considered to have adequate knowledge in a given aspect if the responses met the criteria as under:

Diagnosis:	Symptoms of PTB included at least persistent cough for ≥2 weeks, mentioned sputum examination as a diagnostic tool and knew that the X-ray based diagnosis was reliable only after a course of broad spectrum antibiotics for at least 10 days
Treatment regimen:	Mentioned at least 2 months of isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z) and four months of HR or HRE, in accepted doses as per STCI
Method of ensuring treatment adherence:	Knew about the role of Direct Observation of Treatment (DOT)
Monitoring treatment response:	Knew about the role of follow-up sputum examinations
Adverse reactions:	Was aware of at least four of the common side effects viz. nausea, vomiting, skin rash, tingling or numbness in hands and feet, joint pains, impaired vision, impaired hearing, dizziness, jaundice

Each participant was queried whether she/he had diagnosed/treated any TB case in last two years and if yes, whether notified any case to the Nodal Officer for TB Case Notification.

PPs who attended the CMEs were granted with Credit hours by the Karnataka Medical Council.

Data was digitised and analysed using Epi-info statistical package, version 3.5.4.

3. Results

Of 129 PPs who attended the CMEs and participated in the study; 121 (93.8%) had allopathic qualification; 62 (48%) had more than 15 years of professional experience, 38 (30%) between 5 and 15 years, 12 (9%) less than 5 years; the remaining 17 did not provide the information (Table 1). One hundred and thirteen (88%) knew about the role of sputum examination, however, only 25 (20%) mentioned all the three criteria for being considered as having adequate knowledge in diagnosis. The proportions of PPs having adequate knowledge on different aspects are given in Table 2.

Eighty-Five (68%) PPs had diagnosed one or more TB cases during last two years. Of them, 54 (64%) had notified at least one case in last 2 years; others were unaware that TB is a notifiable disease.

Table 1 – Profile of PPs (N = 129) including in the study.

	Frequency
By qualification	
BAMS	6 (4.7)
MBBS	59 (45.7)
P.G.	62 (48.1)
Not known	2 (1.6)
By years of experience	
<5 year	12 (9.3%)
5–15 year	38 (29.5%)
>15	62 (48.1%)
Not known	17 (13.2%)

Figures in parenthesis denote percentages.

Table 2 – Proportions of PPs having adequate knowledge.

	Number of PPs
Diagnosis	25 (20%)
Treatment regimen	37 (29%)
Ensuring treatment adherence	69 (54%)
Monitoring treatment response	73 (57%)
Adverse reactions	30 (23%)

Figures in parenthesis denote percentages.

4. Discussion

In the present study, only about 20% of PPs had adequate knowledge of diagnosis. Majority were aware of the role of sputum examination but most did not know about the role of antibiotic treatment before X-ray based diagnosis. Similar observations had earlier been made in a PPM project in Hyderabad city.[5] Studies elsewhere in India revealed low awareness of symptoms suggestive of TB though there was an improvement in awareness about the role of sputum microscopy in recent studies.[8,9]

In our study, only about 30% had adequate knowledge of treatment regimen for drug sensitive TB. Similar observations were made in Delhi in a study carried out about 20 years back.[12] No improvement was observed in the knowledge of standard treatment regimen in Mumbai over a period of two decades.[3,6] Similarly, poor knowledge of treatment regimen and guidelines has been observed in other studies in India and some high burden countries though providers in South Africa had adequate knowledge.[14–19]

A little more than half of PPs in our study were aware of the role of DOT, in our study compared to about 70% observed in

North Gujarat.[20] These differences really reflect the efforts made by the local RNTCP official in sensitising PPs regarding notification.

About 57% of our study participants knew about role of sputum examination for monitoring response to treatment which was encouraging, as compared to 25% in West Bengal.[11]

Only a minority (23%) of our participants knew about the common side effects of anti-TB drugs. This is worrisome as side effects are a known risk factor for default and their inadequate management further accentuates the problem.[21]

About 32% of PPs in our study had not diagnosed any TB case in last 2 years. This was not unusual as also observed in other studies; many PPs either might not suspect TB or refer presumptive TB patients to RNTCP.[22,23] Of the PPs who had diagnosed any TB case in last 2 years, 54% reported having notified any TB case. However, this data being self-assessed may not be a true representation of the practice.

The differences in knowledge between allopathic doctors and those with qualifications in indigenous systems of medicine was not analysed as very few of the latter practiced in the area. Similarly, the differences by years of qualification was also not analysed as that was not the objective and the study was not powered to find that out. Studies conducted in other high TB settings have revealed no association of age, sex, level of qualification or years of practice with recognition of TB symptoms while association of level of qualification was observed with respect to treatment guidelines.[9,24–27]

Subsequent to data collection, we trained the study participants in STCI, diagnostic tools including molecular methods, standard treatment regimen for drug susceptible as well as drug resistant TB, patient support systems and different modes of case notification.

Though the study was carried out in limited geographical area and may not be generalisable to entire city or other parts of the country, the study results confirm the findings in other studies that not much has changed over the years as far knowledge of PPs regarding TB is concerned except a greater level of awareness about role of sputum examination. This is further substantiated by a recent study which reported poor adherence to standards of TB care by PPs in Andhra Pradesh.[28] Indeed, much intensified efforts need to be made by the public health authorities to reach out to PPs and impart the necessary knowledge and skills in order to achieve universal access to quality TB care.

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Conflicts of interest

The authors have none to declare.

Appendix A

Sl.No. _____

PPM project: Questionnaire

Qualification: _____ Years of experience _____

1. The most common presenting symptom of Pulmonary TB (PTB) _____
2. The most reliable test for diagnosis of PTB is _____
3. List two conditions that must be fulfilled before considering Chest X ray picture as basis of diagnosis of PTB?

   a_____

   b._____

4. (i) Write a prescription for a full course of anti-TB treatment for a 30 year old male patient weighing 50 Kg diagnosed as a new case of smear positive PTB not having any history of contact with a TB case, in the format as below:

Phase of treatment	Name of drugs prescribed (commercial / generic name)	Dose	Frequency (e.g. once daily / twice daily / thrice a week)	Full duration in weeks for which prescribed

(ii) How can the health providers ensure that this patient swallows all doses on scheduled dates?

_____

(iii) How will you monitor the response to treatment in this patient?

_____

_____

(iv) Mention common likely adverse reactions that the above patient may encounter during treatment?

a. _____ b. _____

b. _____ d. _____

5. (i) Have you diagnosed / initiated any TB patient on Treatment (ATT) in last two years? Yes ☐ No ☐

(ii) If yes, did you notify this case to the nodal officer of BBMP for TB case notification? Yes ☐ No ☐

(iii) If no, write the main reasons / constraints for not notifying

a. _____ b. _____

6 (i) Have you attended any workshop / CME on TB? Yes ☐ No ☐

(ii) If yes, state the year in which last attended _____

(iii) Which was the organizing agency RNTCP ☐ Professional Medical Association ☐ Others ☐

Sl.No._____

PPM project: Coding of questionnaire

Adequate knowledge for diagnosis of PTB Yes ☐ No ☐

Adequate knowledge of regimen for treatment of drug sensitive PTB Yes ☐ No ☐

Adequate knowledge of mode of treatment adherence Yes ☐ No ☐

Adequate knowledge of monitoring response to treatment Yes ☐ No ☐

Adequate knowledge of adverse reactions of anti TB drugs Yes ☐ No ☐

Complying with case notification Yes ☐ No ☐

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Original Article

Prevalence of pulmonary tuberculosis among adults in selected slums of Delhi city

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ABSTRACT

Background: A survey was carried out to estimate the point prevalence of bacteriologically positive pulmonary tuberculosis (PTB) among persons ≥15 years of age residing in Jhuggi–Jhopri (JJ) colonies – urban slums in Delhi, India implementing Directly Observed Treatment strategy since 1998.

Methods: Among 12 JJ colonies selected by simple random sampling, persons having persistent cough for ≥2 weeks at the time of the survey or cough of any duration along with history of contact/currently on ant-TB treatment/known HIV positive were subjected to sputum examination – 2 specimens, by smear microscopy for Acid Fast Bacilli and culture for *Mycobacterium tuberculosis*. Persons with at least one specimen positive were labelled as bacteriologically confirmed PTB. Prevalence was estimated after imputing missing values to correct bias introduced by incompleteness of data and corrected for non-screening by X-ray by a multiplication factor derived from recently conducted surveys.

Results: Of 40,756 persons registered, 40,529 (99.4%) were screened. Of them, 691 (2%) were eligible for sputum examination. Spot specimens were collected from 659 (99.2%) and early morning sputum specimens from 647 (98.1%).

Using screening by interview alone, prevalence of bacteriologically positive PTB in persons ≥15 years of age was estimated at 160.4 (123.7–197.1) per 100,000 populations and210.0 (CI: 162.5–258.2) after correcting for non-screening by X-ray.

Conclusion: Observed prevalence suggests further strengthening of TB control program in urban slums.

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1. Introduction

Tuberculosis (TB) has been known to be a major scourge in India since times immemorial. The exact magnitude of the problem was revealed by a nationwide survey during 1955–1958 when the national level prevalence of bacteriologically positive pulmonary tuberculosis (PTB) was found to be 400 per 100,000 population.[1] An estimated 1.5 million prevalent infectious cases at that point of time underlined the need for implementation of a cost effective operational strategy for controlling TB. Thus the National Tuberculosis Programme (NTP) was implemented in the entire country from 1962.

Subsequent sub-national surveys in different parts of the country revealed that the prevalence of TB continued to be high.[2]

In an attempt to plug in the gaps of NTP, the Directly Observed Treatment (DOTS), an internationally recommended strategy for TB control, Revised National Tuberculosis Control Programme (RNTCP) was launched in a phased manner from 1997.[3] It covered the whole nation by 2006. Although, implementation of RNTCP has led to improvements in case detection and high treatment success rates in most parts of the country, TB continues to be a major public health problem such that there occur about 2 million incident cases and 500,000 TB deaths each year.[4] Recently carried out sub-national surveys have revealed wide variation in the prevalence of TB across different parts of the country.[5–9] This diversity in magnitude of TB burden could be related to the background pool of already infected people, opportunities of transmission of infection, presence of risk factors for breakdown of TB infection and efficiency of early case detection and treatment activities. In this regard, the city slums are one such kind of place for propagating TB epidemic owing to poor living conditions facilitating transmission of infection and factors like poverty and malnutrition facilitating breakdown of infection to disease. The proportion of population residing in slums has been increasing in recent years especially in metropolitan cities like Mumbai and Delhi.[10,11] However, the magnitude of TB burden in city slum populations is largely unknown.

Therefore, we undertook a house-to-house survey in selected slums of Delhi, the capital of India to estimate the prevalence of bacteriological positive pulmonary TB disease. The RNTCP is being implemented in Delhi since 1998.

2. Materials and methods

2.1. Study site and setting

National Institute of Tuberculosis & Respiratory Diseases (NITRD), located in southern part of Delhi is a designated chest clinic under RNTCP and implements TB control activities in a population of about 0.8 million since 1998. There are 31 notified slums also called Jhuggi–Jhopri (JJ) colonies distributed amongst this population, majority of which belong to the low income group and work on daily wages. A JJ colony is an illegal settlement consisting of badly built houses – small structures made of mud, wood or metal having a thatch or tin roof covering.[12] The houses are overcrowded being shared by many people migrating from same native place or employed in the same workplace.

2.2. Study population

Persons ≥15 years of age residing in JJ colonies in the areas where NITRD has the responsibility of implementing RNTCP and residing in the area for ≥6 months.

2.3. Sample size

Sample size was calculated at 31,872 to estimate the prevalence within 25% of the true value at 5% level of significance, a design effect of 2 and the expected prevalence of bacteriologically positive PTB (positive for AFB on microscopy and/or culture) using interview as a screening tool at 400 per 100,000 populations. This expected prevalence was based upon the estimated ARTI in a recently conducted survey in the study area,[13] relationship between ARTI and prevalence of smear positive disease,[14] additional smear negative culture positive cases and the proportion of bacteriologically positive prevalent cases that would be missed out due to non-screening by X-ray.

2.4. Sampling

Using simple random sampling (SRS) method, all the 31 JJ colonies were arranged in the order of selection. Since the exact count of adult population in each JJ colony was not known, the survey was started in the first JJ colony as per the order of selection covering all eligible persons and proceeded to subsequent JJ colony in the list till the required sample size was achieved.

2.5. Field procedures

Field work was carried out during June 2013–May 2014.

The JJ colony where the survey was to be undertaken was identified, and a planning visit was made to familiarize the officials, elders and the community with the purpose and procedures of the survey and seek their cooperation. A rough sketch of lanes was drawn, after going around the colony so that no household was missed. The enumerators went to each household and recorded the demographic details (age, sex, occupation, resident status) of each person. Each eligible person (15 years or more in age and residing for ≥6 months in the household) was registered into a pre-designed individual card. Subsequently, a symptom elicitor queried each registered person for presence of cough and its duration, history of contact with a TB case in the household, whether known to be HIV positive and whether currently on ant-TB treatment (ATT). The project-in charge and the investigators randomly re-interviewed the registered persons for consistency of data as a quality control mechanism.

Persons having persistent cough for ≥2 at the time of the survey or cough of any duration along with history of contact/currently on ATT for pulmonary or extra-pulmonary TB/known HIV positive were considered eligible for sputum collection. Each such eligible person was briefed in the method of bringing out a good quality sputum specimen and given a pre-numbered sterile screw capped sputum container into

which an early morning specimen was given. He was visited by the laboratory technician (LT) next morning when a spot specimen was collected under direct supervision of the LT. Both the sputum containers were transported in a cold box on the day of collection to the RNTCP certified laboratory of the institute along with the patient details for further processing.

2.6. Laboratory procedures

Sputum specimens were subjected to smear microscopy for Acid Fast Bacilli (AFB) and culture for *Mycobacterium tuberculosis* following standard laboratory procedures, at the National Reference Laboratory (NRL) located at NITRD.[15]

Two direct smears were made from each specimen on new labelled slides under aseptic conditions in a bio–safety cabinet. Each smear was stained by the Zeihl–Neelson method and examined by 40× magnification. After the smears were made the remaining sputum specimen was homogenized and transferred to a McCartney bottle. The specimen was decontaminated by adding 4% sodium hydroxide in a volume twice that of sputum specimen (modified Petroff's method) and incubated in a shaker for 20 min. Sterile distilled water was then added up to the neck of the bottle and centrifuged at 3000 rpm (revolutions per minute) for 15 min. The supernatant was decanted, and the deposit was inoculated onto 2 slopes of Lowenstein–Jensen (LJ) medium. Cultures were incubated at 37 °C and examined for the presence of mycobacterial colonies every week for 8 weeks. Any growth was subjected to Niacin test and incubation on LJ medium containing p-nitro benzoic acid (PNB) in a concentration of 500 mg/ml. It was labelled as positive for *M. tuberculosis*, if Niacin test was positive, and no growth was observed on PNB containing medium. The quality assurance of sputum microscopy and culture was ascertained as per the existing RNTCP guidelines.

2.7. Ethical considerations

Survey was approved by the Institutional Ethics Committee of NITRD. Written consent for participation was sought from each person, after explaining procedures of the survey and its benefits to the person and community by field staff. No one was compelled to participate. The participating persons were informed of the findings of their sputum results and made aware regarding symptoms of TB and availability of quality services under RNTCP. In case of a positive smear and/or culture report, the patient was given the report and advised to contact the nearest public health facility providing RNTCP services for initiating ATT. A list of all the patients diagnosed during the survey was given to the TB unit under RNTCP for follow up. Persons with symptoms but not having TB were advised to seek health care at NITRD.

2.8. Definitions

Smear positive case: A person with at least one sputum specimen found to be positive for AFB on smear microscopy, irrespective of culture result.

Culture positive case: A person with at least one sputum specimen found to be positive for *M. tuberculosis* on culture, irrespective of smear result.

Bacteriologically positive case: A person with at least one sputum specimen found to be positive for AFB on microscopy and/or positive for *M. tuberculosis* on culture.

2.9. Statistical methods

Data were digitalized by the same data entry operator on two different occasions into two different files using FOXPRO version 2.5 and validated by using Epi Info (TM) version 3.5, matched and rectified.

Crude prevalence was estimated on dividing the total number of smear, culture or bacteriologically positive PTB cases detected during the survey by the total number (n) of persons screened by interview and whose results of microscopy as well culture of both sputum specimen were available. Standard error (SE) was estimated as standard deviation $(SD)/\sqrt{n}$ where $SD = \sqrt{P}(1-P)$. Confidence intervals (95%) were calculated as mean of the binomial exact ±2SE.

Individual level analysis was done using logistic regression model with robust standard error, to correct for bias due to missing data.[16] To include all registered persons in analysis, missing value imputation was undertaken for persons not interviewed and/or symptoms present but the result of one or both sputum specimen was not available either on smear microscopy and/or culture. It accounted for clustering in survey design, variation in number of persons registered in each cluster, between-cluster variability and uncertainty in estimating SE, under the assumption that data are missing at random within groups of individuals belonging to same age-group, sex and whether or not having sputum eligibility criteria as above. For missing value imputation of each variable, starting values were assigned to missing data, which in turn was obtained from a random sample of values from persons with available data. Model was fitted with this particular variable as outcome variable and other variables as explanatory variables. This was done sequentially in the order of proportion of data that were missing starting with variables with smallest amount of missing data. Finally, a logistic regression model with smear/culture/bacteriologically positive TB as the outcome variable and sex, age-group and TB sputum eligibility criteria as the explanatory variables were fitted. Newly imputed values were used as starting values for subsequent iteration of the process which was undertaken in ten cycles, to obtain one imputed data set. Five such data sets were imputed, and the average of their prevalence was taken as final prevalence.

Overall individual level prevalence of bacteriologically positive PTB in each district was corrected for non-screening by X-ray, applying a multiplication factor of 1.31 as derived from recently conducted surveys in five other parts of the country (unpublished data).

3. Results

In 14 (45%) JJ colonies surveyed, 12,229 households were identified, of which 11,646 (95%) participated in the survey, and 583 (5%) were found locked on repeated visits. A total of 40,756 persons ≥15 years of age were registered from these

households, of which 40,529 (99%) – males: 22190 (54.5%), females: 18462 (45.5%) were screened by interview, the remaining 227 (1%) not being available on repeated visits.

Of 40,529 persons interviewed, 691 (2%) were eligible for sputum examination. Of them, 482 (70%) were eligible on the basis of presence of cough alone ≥2 weeks, 97 (28%) had cough (any duration) while they were still on ATT from various sources and additional 12 (2%) had cough with history of contact but were not currently on ATT.

Of 691 persons eligible for sputum examination, spot specimens were collected from 654 (95%), of which 26 (4%) were positive for AFB on smear microscopy and 37 (6%) on culture. Overnight specimens were collected from 647 (94%), of which 33 (5%) were smear positive and 36 (6%) were culture positive. Contamination was seen in 2% and 3% of the spot and overnight specimens respectively. There were a total of 56 bacteriologically positive patients – smear +ve culture +ve: 27 (48%), smear +ve culture −ve: 7 (12.5%), culture +ve smear −ve: 22 (39.3%).

The crude prevalence of bacteriologically positive cases based on screening by interview followed by sputum examination was estimated at 138.2 (CI: 102.01–174.34) and the individual level prevalence was 160.4 (123.7–197.1) per 100,000 population. Prevalence after correcting the individual level prevalence for non-screening by X-ray was 210.0 (CI: 162.5–258.2).

The crude prevalence of smear positive cases based on screening by interview was 83.9 (CI: 55.7–112.1), and the individual level prevalence was 91.3 (69.3–113.2) per 100,000 population. Prevalence after correcting the individual level prevalence for non-screening by X-ray was 119.6 (CI: 90.8–148.3).

4. Discussion

In the present survey, prevalence of bacteriologically positive pulmonary TB in person ≥15 years of age, after correcting for non-screening by X-ray was estimated at 210 per 100,000 population, considering the individual level prevalence as the best estimate. Prevalence of smear positive pulmonary TB was estimated at 119.6 per 100,000 populations.

Based on symptom screening by interview alone, the prevalence (estimated by individual level method) in the present study population was 160 per 100,000 populations. In the recently carried out surveys during 2008–10 at five sites in other parts of the country where also only screening by interview was used, the prevalence of bacteriologically positive TB corrected for non-screening by X-ray varied from 129 (CI: 92–165) to 399 (CI: 325–469) per 100,000 populations as revealed by published data from two sites[7,8] and unpublished data from three other sites. However, in the present survey, the proportion of persons with symptoms was found to be lower as compared to most other areas where surveys have been carried out recently. We did not screen eligible persons for haemoptysis, chest pain, fever ≥2 weeks and previous history of ATT. Therefore, our prevalence could have been underestimated to a certain extent. Even with this limitation, the estimated prevalence is quite high to warrant more intensified efforts in slum areas towards early TB case finding and prompt and effective treatment.

Further, of 56 bacteriologically positive cases detected during the survey, 31 (55%) were currently on ATT. Of all 197 patients found to be currently on ATT, only 49 (24%) were on treatment under RNTCP as observed through cross checking with RNTCP TB registers. The rest were on treatment outside the RNTCP. Similarly, of 25 new cases detected during the survey and referred to RNTCP for ATT, only 7 sought treatments at RNTCP centres. The reasons for such low utilization of RNTCP services which could also be one of the contributing factors to high prevalence of TB need to be investigated so that necessary steps could be undertaken to raise the utilization level of RNTCP services by slum dwellers.

Conflicts of interest

The authors have none to declare.

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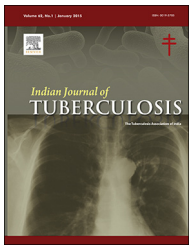

Original article

Factors predicting treatment success in multi-drug resistant tuberculosis patients treated under programmatic conditions

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ABSTRACT

Background: Treatment success in multi-drug resistant tuberculosis under programmatic conditions has been far from satisfactory. Knowledge of the factors predicting treatment outcome can guide us to take appropriate corrective measures for better results. However, there is a scarcity of data on these predictors in Indian patients. The present study was sought to evaluate association of different patient and disease specific factors with treatment outcome in MDR-TB patients.

Methods: It was a retrospective study that involved evaluation of data of MDR-TB patients who were started on Cat-IV treatment between January 2012 and December 2014. Medical records of 256 patients were scrutinized and necessary information on possible predicting factors like age, gender, body mass index, co-morbidities, previous TB treatment, blood investigations, treatment adherence, culture conversion time, etc. was retrieved. These factors were analyzed for their possible association with treatment outcome.

Results: Of the 256 patients, 132 (51.6%) achieved successful outcome after Cat-IV anti-TB regimen. On multivariate logistic regression analysis age (adjusted OR = 0.95; 95% CI 0.91–0.98; $p = 0.01$), serum albumin level (adjusted OR = 3.71; 95% CI: 1.22–11.3; $p = 0.02$) and treatment adherence (adjusted OR = 4.52; 95% CI: 1.2–16.6; $p = 0.02$) were independently associated with treatment success. Co-morbidities like diabetes and alcoholism and previous anti-TB treatment didn't affect the treatment end result significantly.

Conclusion: The treatment outcome in MDR-TB has not significantly improved since the inception of DOTS-Plus strategy. Interventions to improve nutrition and treatment adherence might help to improve the success rate in MDR-TB treatment.

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1. Introduction

The emergence of drug resistance in tuberculosis (TB), particularly multi-drug resistant TB (MDR-TB) is a significant public health problem worldwide. Globally, there were an estimated 480,000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100,000 people with rifampicin-resistant TB (RR-TB) in 2015, which were eligible for MDR-TB treatment.[1,2] As per WHO 2016 data, India has around 80,000 registered MDR-TB cases which is among the highest in the world.[1] To control this rising problem, Revised National TB Control Program (RNTCP) launched programmatic management of drug resistant TB (PMDT) services in 2007 in India for the management of multi-drug resistant & extensively drug resistant tuberculosis.

DOTS-Plus program follows a standardized regimen of treatment (labeled as Cat-IV) which has shown feasibility and effectiveness in many countries. However, the picture has been dismal in India with 46% treatment success rate recently reported by WHO.[1] It is evident that there is a great scope of improvement in the program. MDR-TB treatment is associated with certain modifiable and non-modifiable factors which can predict/determine treatment outcome. Knowledge of these can guide us about the corrective measures which can improve MDR-TB outcome. However, there is scarcity of published data on determinants of outcome in Indian patients.[3–5] Different studies from other parts of the world have documented varied results which can't be extrapolated to the Indian settings due to differences in study design and treatment strategies.[2,6,7]

Chandigarh is amongst the first few union territory of India where PMDT services were started in 2012. It has a designated drug resistant TB (DRTB) centre at Govt. Medical College & Hospital (GMCH) which caters to the population of Chandigarh as well as adjoining 5 districts of Haryana. No study has been undertaken in this region to evaluate factors predicting treatment success in MDR-TB patients. Hence, the present study was undertaken to generate some useful data on these outcome predictors that might help the TB control program to achieve better cure rates in MDR-TB.

2. Materials and methods

It is a retrospective study conducted in the department of Pulmonary medicine, GMCH, Chandigarh. All MDR-TB patients (residents of Chandigarh and 5 districts of Haryana) who were registered and initiated on treatment between January 2012 and December 2014 and had achieved an outcome were recruited in the study. The study was approved by institutional ethics committee of GMCH. Informed consent was not required as whole data was retrieved from the medical records of patients who had already completed treatment and/or had achieved some outcome.

2.1. Operational definition

Multidrug resistant TB is defined as tuberculosis resistant to both isoniazid and rifampicin, first line anti-TB drugs. Different outcomes to Cat IV treatment viz. cure, treatment default, treatment completed, death and treatment failure were defined as per RNTCP-PMDT guidelines.[8] For the purpose of analysis, treatment outcome in the study was divided into 2 groups: treatment success and poor outcome. Treatment success was represented by cure or successful completion of treatment. Poor outcome was defined in the event of default, death, failure or switched to Category V treatment.[6] A patient was labeled as non-adherent to Cat IV therapy if he had missed ≥10% of the total prescribed dose of ATT.[9] This non-adherence was applied only to those drug interruptions that occurred without doctor's advice and were short of default.

2.2. Methodology

The diagnosis of MDR-TB was done at RNTCP accredited Intermediate Reference laboratory (IRL) using liquid culture, line probe assay or cartridge based nucleic acid amplification test (CBNAAT) according to the standard guidelines.[8] Thereafter, information on demographic and clinical profile of patients including smoking and alcohol abuse, co-morbidities (diabetes and HIV Status), history of previous anti-tuberculosis treatment (ATT) was recorded. Routine investigations done as a part of pre-treatment evaluation were extracted from the treatment card and patient's record at the DRTB centre and office of State TB officer. Further details on the current course of Cat IV ATT particularly treatment adherence, time of culture conversion, adverse drug reactions, final outcome, weight gain etc. were also retrieved. Treatment outcome was calculated as numbers and percentages. Different patients and disease related factors selected from literature review as well as from our previous knowledge were analyzed for their possible association with the treatment outcome.

2.3. Statistical analysis

Quantitative variable were summarized as mean ± SD and qualitative variable as percentages. Unpaired *T*-Test and Chi-square test were used to compare continuous and nominal variables respectively between patients in 2 outcome arms. We used binary logistic regression analysis with forward inclusion approach to find association between potential predictors and treatment outcome. Univariate and multivariate models were used to measure crude and adjusted odds ratios (OR) respectively, with their 95% confidence intervals (CI), for different factors predicting outcome. *p*-Value was considered significant at <0.05.

3. Results

Total 301 of MDR-TB TB patients from the defined territory were registered at the DRTB centre, GMCH during the specified time period. Out of them, 256 had a declared outcome and hence were considered for final analysis.

3.1. Baseline characteristics

Mean age of the patients was 35.3 ± 14.9 years (range 12–71 years). The study cohort included 2 children (both aged 12 years) with 64% of patients belonging to the age group of less

than 40 years. Males outnumbered females in the ratio of 2:1. Majority of patients were undernourished with low mean BMI of 16.0 ± 3.5 kg/m^2 and serum albumin level of 3.5 ± 0.77 g/dl. Table 1 shows demographic and clinical characteristics of patients between 2 treatment outcome groups. As compared to patients with poor outcome, subjects with treatment success were younger in age ($p = 0.002$) and better nourished in terms of BMI ($p \leq 0.001$) and serum albumin level ($p = 0.001$) (Table 1). Fifty five patients had a BMI above the lower cutoff value of 18.5 kg/m^2 out of which 64% had successful outcome. Whereas, 50% of the patients with low BMI ($n = 182$) achieved treatment success in MDR-TB.

3.2. *Treatment outcome*

Out of 256 patients, 103 patients were declared cured and 29 treatment completed with a treatment success rate of 51.6%. (Fig. 1) Treatment adherence was seen in 65.8% patients, whereas 67 patients had history of missing ≥10% of the total prescribed dose of ATT. There was a statistically significant weight gain in successfully treated patients as compared to unsuccessful group (weight gain (kg) in patients with treatment success v/s poor outcome 7.3 ± 5.8 v/s 2.9 ± 3.7; $p \leq 0.001$).

3.3. *Factors predicting treatment outcome*

Different demographic, clinical and treatment related variables were evaluated for their possible association with treatment success. On univariate analysis age, nutritional parameters (hemoglobin, body mass index (BMI) & serum albumin), outcome in previous anti-TB therapy, number of previous anti-TB courses and treatment adherence during Cat-IV anti-TB therapy had statistically significant association with treatment outcome (Table 2).

After applying forward stepwise (likelihood) binary logistic regression analysis on the multivariate data, 3 variables viz.

Table 1 – Baseline characteristics of MDR-TB patient in 2 treatment outcome groups.

S. no.	Parameter	Total	Successful outcome	Poor outcome	*p*-Value
1	Age (years)	35.3 (14.9)	32.4 (13.6)	38.3(15.6)	0.002
2	Gender (M/F)	170/86 (2:1)	85/47	85/39	0.51
3	BMI (kg/m^2)	16.0 (3.5)	16.7 (3.41)	15.1(3.4)	<0.001
4	Diabetes	26 (10.2)	9/122	17/109	0.06
5	Smoker	70 (27.3)	34/109	36/100	0.46
6	Alcoholic	54 (21.1)	26/106	28/97	0.52
7	Hemoglobin (g/dl)	11.25 (2.09)	11.68(1.87)	10.76(2.21)	0.001
8	Serum albumin (g/dl)	3.5 (0.77)	3.7 (0.70)	3.25 (0.79)	0.001

BMI: body mass index; quantitative data is indicated as mean ± SD and qualitative data as number (%).

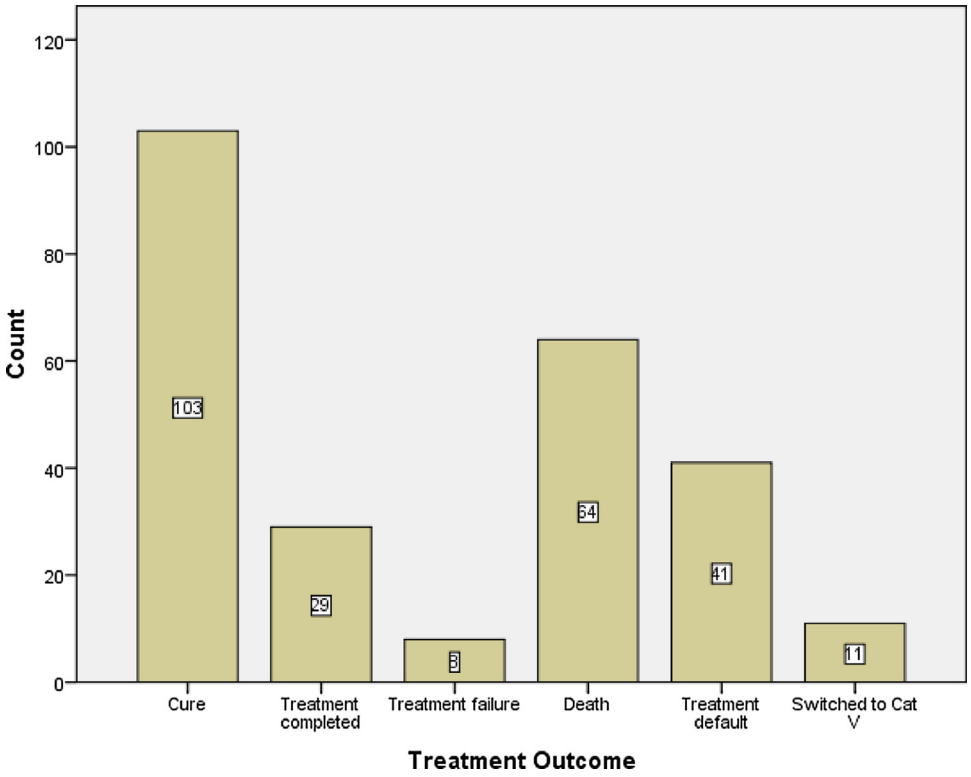

Fig. 1 – Distribution of patients in each of the treatment outcome arms.

Table 2 – Logistic regression analysis of the independent variables with the treatment outcome in MDR-TB.

	Parameter	Univariate analysis			Multivariate analysis		
		Unadjusted odds ratio	95% CI	P value	Adjusted odds ratio	95% CI	P value
1	Age	0.97	0.95–0.99	0.002	0.95	0.91–0.98	0.01
2	Female gender	1.26	0.75–2.12	0.38			
3	BMI	1.15	1.06–1.24	0.001			
4	Diabetes	0.43	0.18–1.01	0.053			
5	Smoking	0.80	0.45–1.43	0.46			
6	Alcoholic	0.81	0.43–1.49	0.48			
7	Successful outcome in previous ATT	2.08	1.17–3.67	0.01			
8	No of previous ATT course ≤ 1	2.05	1.15–3.67	0.01			
9	Hemoglobin	1.25	1.09–1.43	0.001			
10	S. albumin	2.26	1.34–3.80	0.002	3.71	1.22–11.3	0.02
11	3 month Sputum culture conversion	1.79	0.85–3.78	0.12			
12	Treatment adherence	3.74	2.04–6.84	<0.001	4.52	1.21–16.6	0.02
13	Adverse drug reaction	0.56	0.28–1.11	0.98			

CI: confidence interval; ATT: anti-TB treatment; BMI: body mass index.

age, serum albumin and treatment adherence were found to have statistically significant association with treatment outcome (Table 2).

4. Discussion

The study was conducted to find out factors that predict treatment success in MDR-TB patients treated under programmatic management of drug resistant tuberculosis (PMDT). The results showed that treatment adherence and baseline serum albumin level had positive association with treatment success whereas age was associated with poor outcome in MDR-TB patients.

Under DOTS-Plus program, patients receive a standardized regimen comprising of six drugs for first 6–9 months of intensive phase and four drugs for 18 months of continuation phase. Our study achieved a treatment success rate of 51.6% which is significantly less than the figures reported in recently published meta-analysis.[2,7,10] On the other hand, 2 Indian studies with similar design also showed a lower success rate ranging from 38 to 45%.[4,5] It is important to note that the result of these Indian studies, including ours, are far below the WHO target of 75–90% success rate.[11] This very fact questions the superiority of programmatic over individualized treatment of MDR TB which has also been negated in a recent meta-analysis.[10] Nevertheless, there is an urgent need to find out the factors that determine poor outcome and to take corrective measures accordingly.

In our study, age (taken as continuous variable) was found as an independent predictor of treatment outcome in MDR-TB (adjusted OR for successful outcome 0.95; 95% CI: 0.91–0.98) which is comparable to a previous study.[12] The affect of age on treatment outcome was independent of co-morbidities like diabetes and smoking that are also potential determinants of adverse outcome. Adherence to anti-TB drugs is a key component of any TB control program that plays its role in ensuring treatment success. The fact has been strengthened by the independent association of treatment adherence with treatment success in our study. To best of our knowledge, treatment adherence as an outcome predictor in MDR-TB per se has not been evaluated previously in a similarly designed study. Instead, education level has been validated as an important determinant of treatment success which, in fact, may be a surrogate marker of treatment adherence.[5]

Nutritional management as a part MDR-TB treatment is often ignored in our daily practice. One of the key findings of the present study was the positive association between nutritional status and MDR-TB treatment success. All nutritional parameters viz. hemoglobin, BMI and serum albumin level were significantly associated with treatment outcome on univariate analysis. However, serum albumin level was found to be the best parameter that remained statistically significant in multivariate logistic regression model. The association of low BMI with adverse outcome in MDR-TB has been validated in previous studies.[2,12] Similarly, severe anemia has been found to increase the chances of treatment failure in drug resistant TB.[13] However, albumin level as a predictor of treatment outcome has been scarcely evaluated in previous studies.[6] Nevertheless, the positive association between nutrition and treatment success reinforces the importance of nutritional management in the MDR-TB treatment and highlights the need to execute it stringently in programmatic management of drug resistant TB.

In the present study, 64% of patients had past history of taken more than 1 course of anti-TB treatment. Previous anti-TB treatment has been associated with poor outcome in previous studies.[14,15] In contrast, neither the number of previous courses nor their outcome affected the outcome to current Cat IV treatment in our study. It seems that previous TB episodes should not affect current outcome provided anti-TB drugs used in the Cat IV regimen are sensitive and there are no major sequalae to previous TB treatment.

Alcohol addiction has been associated with poor outcome including default and death in previous studies,[2,16,17] however the relation could not be validated in our study probably due to lesser number of subjects with alcohol dependence. Contrary to previous study[6] and meta-analysis,[2] treatment outcome had no predisposition to male gender in our study. Diabetes has been known to affect treatment outcome in both drug susceptible[18] and drug resistant TB cases.[19] In contrast, a recent meta-analysis ruled out any effect of diabetes on

treatment outcome in MDR-TB. The present study also yielded similar result, however, a low sample size of 26 diabetics might have limited its validity.

Baseline resistance to fluororquinolones[2,12] and kanamycin[15] has been evaluated in few studies as a predictor of adverse outcome in MDR-TB. However baseline DST to 2nd line anti-TB drugs was not routinely performed under program during the study period, as a result, these could not be evaluated in our study. However, only 11 patients in the present study had to be switched to Cat V regimen, which indicate presence of a low level of baseline resistance to fluororquinolones. HIV infection is an evident risk factor for poor outcome in MDR-TB,[12,14] however, presence of only 4 HIV reactive cases in the present cohort limited the power to detect its effect on the treatment outcome.

To best of our knowledge, this is the first Indian study to comprehensively evaluate a multitude of patient and disease related factors in a multivariate logistic regression model, predicting treatment success in MDR-TB patients under programmatic conditions. A sample size of over 250 patients ensured the validity of results. In view of uniformity in management of MDR-TB under the program, the results may also be extrapolated to other parts of the country. However, the study had few limitations. It was a retrospective study involving the scrutiny of patients' records. Incomplete data on certain parameters notably adverse drug reaction, previous ATT and radiological features might have affected some of the results.

Our study revealed an unsatisfactory treatment outcome in MDR-TB under programmatic conditions which needs attention of health care providers. Nutrition and treatment adherence are 2 key modifiable parameters that might determine treatment success in MDR-TB. Hence interventions to improve these by ensuring DOT, imparting education and awareness to the patients and providing comprehensive nutritional management might help to achieve WHO targets of treatment success in MDR-TB.

Conflicts of interest

The authors have none to declare.

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Original Article

Socio-demographic profile and outcome of TB patients registered at DTC Rewa of Central India

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ABSTRACT

Background: Tuberculosis is a specific infectious disease caused by Mycobacterium tuberculosis. The disease is usually chronic with cardinal features such as persistent cough with or without expectoration, intermittent fever, and loss of appetite, weight loss, chest pain and hemoptysis.

Objective: (1) To assess the socio-demographic profile of the patients attending DOTS Center. (2) To assess outcome of treatment under DOTS Center.

Methodology: This is a Prospective Longitudinal study conducted among the patients attending DOTS center of DTC located at S.G.M.H. campus Rewa Madhya Pradesh Central India, during the last quarter of 2014, Study Duration: One year and two months i.e. starting from 1st September 2014 to 31st October 2015; total study sample size consisted of 137 patients who were newly registered during the last quarter of 2014 (from 1st October to 31st December 2014) at DOTS Center of DTC. After applying inclusion and exclusion criteria, a total of 133 newly registered patients were enrolled that can be considered as total sample size in the present study.

Result: Study population comprises a total of 133 patients; out of which 84 (63.15%) were male and 49 (36.84%) were female. In both most common age group are 21–30 year were 41 (30.82%) patients and least common was pediatric TB in age group <10 year were 10 (7.51%) patients, lower socio-economic class (class-V) 53.38% followed by class-IV or Lower middle class 29.32%, only 1.5% were from upper class. 96 (72.18%) patients were of category-I patient and 37 (27.81%) were category-II patient, 51 (38.34%) patients were cured, 70 (52.63%) had their treatment completed, so overall treatment success rate was 90.97%; in that, 2 cases were (1.50%) failure, 4 (3%) defaulters, 2 (1.50%) died during treatment and 4 (3%) were transferred out.

Conclusion: Study concluded that most of the patients belonged to lower socioeconomic status and in productive age group so it will increase the economic burden over the family; therefore, after increasing the living standard the outcome of disease becomes favorable.

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1. Introduction

Tuberculosis is a specific infectious disease caused by Mycobacterium tuberculosis. The disease primarily affects lungs and causes Pulmonary TB (PTB). It can also affect intestine, meninges, bones and joints, lymph glands, skin and other tissues of the body which is known as extra-pulmonary TB. The disease is usually chronic with cardinal features such as persistent cough with or without expectoration, intermittent fever, and loss of appetite, weight loss, chest pain and haemoptysis.[1] TB has co-evolved with humans for many thousands of years, and perhaps for several million years.[2] The oldest known human remains showing signs of tuberculosis infection are 9000 years old.[3] Phthisis is a Greek term for tuberculosis, around 460 BC, Hippocrates identified phthisis as the most widespread disease of the times involving coughing up blood and fever, which was almost always fatal.[4] It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory TB disease. TB is also called Koch's disease, after the scientist Koch. The bacillus causing TB, Mycobacterium tuberculosis, was identified and described on 24 March 1882 by Robert Koch.[5] The first genuine success in immunizing against tuberculosis was developed from attenuated bovine-strain tuberculosis by Albert Calmette and Camille Guerin in 1906 it was called ''BCG''.[6]

Tuberculosis is one of the three primary diseases of poverty along with AIDS and malaria.[7] A third of the world's population is thought to be infected with M. tuberculosis, and new infections occur at a rate of about one per second.[8] It is a disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world. Left untreated, each person with active TB disease will infect on average between 10 and 15 people every year and this continues the TB transmission. Overall 5–10% of people who are infected with TB bacilli become sick or infectious at some time during their life. People with HIV and TB infection are much more likely to develop TB. The risk for developing TB disease is also higher in persons with diabetes, other chronic debilitating disease leading to immune-compromise, poor living conditions, tobacco smokers, etc. The WHO declared TB a global health emergency in 1993, because of its toll on the health of individuals and the wider social and economic impact on overall development of country. And the Stop TB Partnership developed a Global Plan to Stop Tuberculosis that aims to save 14 million lives between 2006 and 2015.[9]

Tuberculosis is treatable with a course of antibiotics. The most successful strategy to treat TB patients is DOTS. By keeping this entire thing in mind the present study was conducted at DTC cum DOTS center in Rewa M.P. central India, by considering the objective that to assess the socio-demographic profile of the patients attending DOTS Center and to assess outcome of treatment under DOTS Center.

Methodology: This was a prospective longitudinal study conducted among the patients attending DOTS center of DTC, during the last quarter of 2014, Study Duration: One year and two months (i.e. 1st September 2014 to 31st October 2015), *Study population*: Only newly registered patients during the last quarter of 2014 (from 1st October to 31st December 2014.) and were receiving DOTS therapy at this DOTS center were included in this study; a total of 137 new patients were registered during the last quarter of 2014, out of 137 patients, 4 were MDR patients, so these patients were excluded from the study population because treatment out-come of these MDR patients was not supposed to be completed till the completion of the present study. After applying inclusion and exclusion criteria, a total of 133 newly registered patients were enrolled as total sample size in the present study, so the present Study comprised of 133 patients of TB who were newly Registered in the last Quarter of 2014 which also included 1 transferred in patient from the other TU. The data were collected by pre-designed and pre-tested questionnaire, after obtaining informed verbal consent from patient. *Data entry and analysis*: All the data were collected on pretested questionnaires. The collected data were scrutinized for completeness and consistency collected and the data were analyzed by using MS excel; instat Graph pad and Epi-cal info 2000 were used to apply appropriate statistical tests.

2. Results

In the present study, Table 1 we distributed the patients according to their age and sex; we observed that study population comprises a total of 133 patients; out of which 84 (63.15%) were male and 49 (36.84%) were female. In both male and female, most common age group are 21–30 year were 41 (30.82%) patients and least common was pediatric TB in age group <10 year were 10 (7.51%) patients. Age-wise distribution was more varying in extreme of age, i.e. this age and sex-wise distribution of patients was found to be statistically not significant ($P \geq 0.05$).

In the present study, out of a total of 133 patients in the study, majority of patients 30.82% belonged to ST category, and least ones were from GN category 16.54%. According to education 38.34% of study population were ill-literate and 61.66% were literate. In their socio-economic status, majority of cases were from lower socio-economic class (class-V) 53.38%, but only 1.5% were from upper class or class-I. Considering the type of family-wise distribution of patients, 53.38% patients belonged to joint families and 39.84% were from nuclear families but the least ones (6.76%) were from extended family. According to occupation, majority (50.37%)

Table 1 – Age and sex-wise distribution of patients.

S.N.	Age in years	Male (84)	Female (49)	Total (133)
1	<10	5	5	10
2	11–20	10	9	19
3	21–30	29	12	41
4	31–40	14	10	24
5	41–50	8	7	15
6	51–60	10	3	13
7	>60	8	3	11

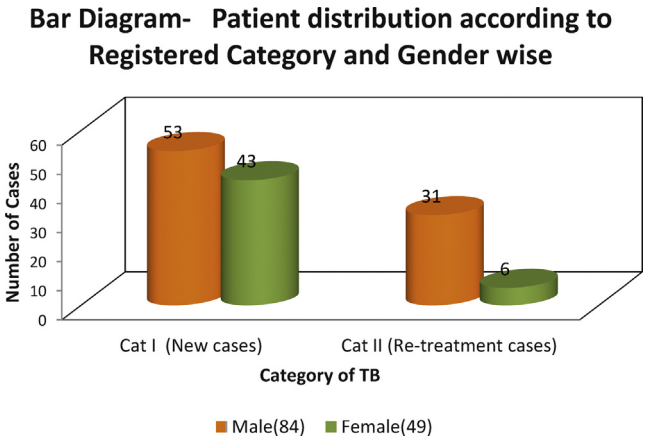
Chi square = 5.013, d.f. = 6, p value = 0.5421.

Table 2 – Distribution of the study population by socio demographic characteristics (N = 133).

S.N.	Socio-demographic profile	No. of participants (133)	Percentage (%)
1.	**Caste**		
	General (GN)	22	16.54
	Other Backward Caste (OBC)	33	24.81
	Schedule Caste (SC)	37	27.81
	Schedule Tribe (ST)	41	30.82
2.	**Education**		
	Illiterate	51	38.34%
	Primary School	29	21.80%
	Middle School	27	20.30%
	High School	12	9.02%
	High-Secondary School	10	7.51%
	Graduate	3	2.25%
	Post-Graduate	1	00.75%
3.	**Socioeconomic status**		
	(a) Class-I	2	1.5
	(b) Class-II	8	6.0
	(c) Class-III	13	9.77
	(d) Class-IV	39	29.32
	(e) Class-V	71	53.38
4.	**Family status**		
	Nuclear	53	39.84
	Joint	71	53.38
	Extended	9	6.76
5.	**Occupation**		
	Unemployed	5	3.75%
	Laborer Unskilled	67	50.37%
	Laborer Skilled	16	12.03%
	House-wife	21	15.78%
	Govt. Service	5	3.75%
	Businessman	6	4.51%
	Farmer	7	5.26%
	Other	6	4.51%

were unskilled laborers and least ones were from Govt. services and unemployed patients were 3.75%.

As shown in Bar diagram, in the present study it was found that 96 (72.18%) patients were of category-I patient and 37 (27.81%) belonged to category-II patient, whereas in case of sex-wise distribution of TB category, 63.09% males were of category-I and 36.90% were of category-II, and in females 87.75% were of category-I and 12.24% were of category-II. The overall sex-wise distribution of different category of TB patients was found to be statistically significant ($p = 0.002$) (Table 2).



As shown in below Table 3. Out of 133 patients 30 (22.56%) patients were new sputum smear positive, out of them 26 (86.66%) was declared as cured, 2 (6.66%) patients completed their treatment, yet no failure has been noticed, 1 (3.33%) was transferred out of patients and remaining 1 (3.33%) were defaulted. Thus treatment success rate (cure rate plus treatment completion rate) of new sputum positive patients was 93.32%. Out of 28 (21.05%) retreatment cases 18 (64.28%) were declared cured, and 4 (14.28%) patients completed their treatment, so treatment success rate for retreatment cases was found to be 78.56%; 1 (3.57%) died during treatment, 2 (7.14%) were defaulted during treatment, 2 (7.14%) were failure and remaining 1 (3.57%) patient was transferred. Among 35 (26.31%) new smear negative patients, 7 (20.00%) were declared cured, 27 (77.14%) completed their treatment so treatment success rate for new smear negative patients was 97.14%, and 1 (2.85%) was defaulted during treatment. So in the total 93 Pulmonary TB cases, 51 (54.83%) were cured, 8 (8.60%) and completed their treatment. Thus treatment success rate (cure rate plus treatment completion rate) of pulmonary TB cases was 84 (90.32%); 1 (1.07%) died, 2 (2.15%) were failures, 3 (3.22%) were defaulted and remaining 3 (3.22%) were transferred. Out of 40 (30.07%) extra-pulmonary patients 37 (92.50%) completed their treatment, 1 (2.50%) patient died, 1 (2.50%) was defaulted and 1 (2.50%) was transferred out in extra-pulmonary cases. Among the total of 133 cases 51 (38.34%) were cured, 70 (51.87%) were treatment completed, 4 (3%) defaulted, 4 (3%) transferred out, 2 (1.50%) were died and 2 (1.50%) were

Table 3 – Treatment outcome in patients according to clinical type.

Type of patients		Cured	Treatment completed	Died	Failure	Defaulted	Transferred out	Total
Pulmonary	New smear positive	26 (86.66%)	2 (6.66%)	00 (00%)	(00%)	1 (3.33%)	1 (3.33%)	30 (22.56%)
	Retreatment	18 (64.28%)	4 (14.28%)	1 (3.57%)	2 (7.14%)	2 (7.14%)	1 (3.57%)	28 (21.05%)
	New smear negative	7 (20.00%)	27 (77.14%)	00 (00%)	00 (00%)	00 (00%)	1 (2.85%)	35 (26.31%)
Extra-pulmonary		00(00%)	37 (92.50%)	1 (2.50%)	00 (00%)	1 (2.50%)	1 (2.50%)	40 (30.07%)
Total		51 (38.34%)	70 (52.63%)	2 (1.50%)	2 (1.50%)	4 (3.0%)	4 (3.0%)	133 (100%)

treatment failure and later on these failure cases were enrolled as MDR.

3. Discussion

The present study was undertaken at DTC cum DOTS Centre of Rewa District, Madhya Pradesh. Period of study was selected for one year two months i.e. 1st September 2014 to 31st October 2015, from the 1st October of the Last quarter of 2014 to 31st December 2014 data were collected, and then as per the RNTCP Guidelines follow of these patients was done and by keeping in the mind regarding time period of completion of their treatment to show the outcome of treatment. After applying exclusion and inclusion criteria, a total of 133 patients included in the present study who were newly registered in the last quarter of 2014 and were receiving drugs at the selected DTC cum DOT Center. After analyzing data, the discussion of the present study is discussed as per following heads-In the present study, 63.15% were males and 36.84% were females. Most of the patients nearly 3/4th belonged to the economically most productive age group i.e. 21–50 years. Similarly age-wise distribution of TB patients was also found in the study conducted by Kaur et al.[10] found that the maximum patients, that is 55.9% of patients belong to 15–30 yrs. but in case of sex-wise distribution more were female patients in comparison of present study. Similarly a study conducted by Bisoi et al.[11] that the maximum 79.1% patients belonged to 15–54 years of age group and 64% were males and 36% were females. Hence such age- and sex-wise distribution is also supported by many other studies like Kolappan et al.[12] in their study in Tiruvallur district in Tamil Nadu was also found that majority 47.4% patients were 15–34 Years of old although in their study 51.2% were female in comparison of 48.8% males may be because of more number of female were included in their study.

In the present study, majority of patients were Schedule Tribe (ST) 30.82% followed by 27.81% were SC, 24.81% were OBC, and least one were from GN category 16.54%. This may be due to fact that the patients were from Govt. Health facility and were availing free health services so that they mainly belong to lower caste but in comparison of Rewa District, the schedule cast population is 16.22%, and ST population is 13.19% according to Census of India.[13] Similarly in the study conducted by Goel et al.[14] was found SC/ST patient were 32.7%, OBC 21.4% and GEN were 45.9% it is controversial to this study it may due to by chance or may be due to that in their study area most of the residents were belong to higher caste 1.28%, OBC cases were 1.09% and remaining were other patients.

In the present study 34.34% patients were illiterate and 61.66% were literate. Similarly Jaggarajamma et al.[15] had also found that 35.71% patients were illiterate, Manmeet Kaur et al.[10] had also found that 24.1% males and 21.8% females were illiterate and 66.7% males and 70.9% females had educated up to high school.

In the present study, majority 50.37% were unskilled laborer followed by house wife 15.78%, least one were belonged to the Govt. Services and unemployed patients were 3.75%. So disease was more common in laborers group. Similarly a study conducted by Pandit et al.[16] at Anand district of Gujarat also found that 47.9% TB patients were laborers followed by housewife 31.7%. Similarly George et al.[17] also showed in U. P. that 44% patients were unemployed and 40% were unemployed in Karnataka and 27% and 43% were unskilled workers in respective state.

In the present study nearly 3/4 of patients belong to lower socio-economic status. This type of distribution of socio economic class of the present study may be due to that all are from Rewa District where average per capita income is less in comparison of other areas of India. This distribution can be occurred due to all patients were taking health services from Govt sector where mostly lower economic people prefer to come in contact. Similarly Pandit et al.[16] in a study carried out at Anand district of Gujarat found that 81% of patients were from lower socio-economic class. Similarly Goel et al.[14] study showed that 87.8% patients were from lower socio-economic status, so all these studies was support the finding of present study.

In the present study, it was found that 96 (72.18%) patients were of category-I patients and 37 (27.81%) belonged to category-II; in case of sex-wise distribution of TB category, 63.09% males were of category-I and 36.90% were of category-II, and in females 87.75% were of category-I and 12.24% were of category-II. The overall sex-wise distribution of different category of TB patients was found to be statistically significant ($p = 0.002$).

Similarly a study conducted by Chandrasekaran et al.[18] from May 1999 through December 2004, 49.6% patients were started on Category I treatment, 15.28% on Category II, 35.09% on Category III and 2.11% on non-DOTS. Similarly a study conducted by Vasantha et al.[19] was also found that in their study out of 3818 patients treated, 1944 (51%) were under category I, 449 (12%) under category II and 1425 (37%) under category III. This study was supporting the finding of present study.

In the discussion of outcome of the present study out of 133 patients 30 (22.56%) patients were new sputum smear positive, out of them 26 (86.66%) were declared as cured, 2 (6.66%) patients were completed their treatment, no failure have been noticed, 1 (3.33%) were transferred out patients and remaining 1 (3.33%) were defaulted. Thus treatment success rate (cure rate plus treatment completion rate) of new sputum positive patients was 93.32%. Out of 28 (21.05%) retreatment cases 18 (64.28%) were declared cured, 4 (14.28%) patients were completed their treatment, so treatment success rate for retreatment cases was found to be 78.56%.

In contrast to present study a study conducted by Bisoi et al.[11] they found that all the 286 patients put on DOTS were analyzed for treatment outcome. In new sputum-positive pulmonary TB cases, cure rate was 53.8% and cure rate out of all smear-positive cases (new smear-positive + re-treatment smear-positive) was 56.63% out of 113. Fifty-two percent of total patients, 9.7% of new sputum smear-positive and 78.8% of new sputum smear-negative completed the treatment. Altogether 16.4% patients defaulted from treatment. Default patients among the new smear-positive cases were 24.7%, among smear positive relapse 16.7%, among smear-positive failure 33.3% and among other cases treated with Cat II regimen were 14.2%. Percentage of death was 2.6% among new

smear positive cases, 5.1% among new smear-negative, 9.1% among smear-positive treatment after default cases, and total death rate was 3.1%. Failure percentage among new smear-positive cases was 8.6% and out of all cases it was 4.2%. Total transferred-out cases were 4 out of 286, i.e., 1.4% which are more or less similar to the findings of the present study.

Similarly Pardeshi et al.[20] found that a study was conducted to compare and quantify the treatment outcome in re-treatment cases as compared to the new smear positive cases of Tuberculosis under Revised National Tuberculosis Control Program in District Tuberculosis Center, Yavatmal district, Maharashtra in 2003. The cure rates were 68% and 84% in the new smear positive and the re-treatment group respectively. Favorable outcomes were significantly less in the re-treatment group (66.47%) as compared to the new smear positive cases (84.28%).

4. Conclusion

As per the guidelines of RNTCP the cure rate should be more than 85% for new sputum positive patients, so we can says that the goal RNTCP program was full-filled by that DTC cum DOTS center in S.G.M.H. Campus Out of 133 patients 30 (22.56%) patients were new sputum smear positive, out of them 26 (86.66%) were declared as cured, 2 (6.66%) patients were completed their treatment, no failure has been noticed among of them, 1 (3.33%) patient was transferred out and remaining 1 (3.33%) was defaulted. Thus treatment success rate (cure rate plus treatment completion rate) of new sputum positive patients was found 93.32%. At the end of treatment as per DOTS schedules sputum smear examination is mandatory to know the exact treatment cure rate but still as per findings of the present study it has been observed that there is lack of follow up for sputum smear examination of patients at the end of completion of treatment. Such attempts regarding sputum smear examination at the end of treatment can stop further spread of MDR/XDR and can also help to detect treatment failure.

Conflicts of interest

The authors have none to declare.

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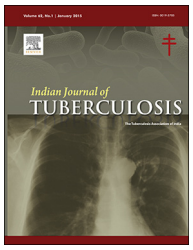

Original article

Tuberculosis related stigma and its effect on the delay for sputum examination under the Revised National Tuberculosis Control Program in India

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ABSTRACT

Background: One major barrier to achieve goal of tuberculosis (TB) control program globally, is the stigma attached to the disease. Perceived stigma can delay sputum test in time. Delay will lead to spread of infection in the community. There is no scientific information available in India exactly looking into the association between delay in sputum examination and stigma.
Aim: We conducted a study in rural West Bengal among persons with cough for 2 weeks or more to assess their level of stigma, its influence on delay for sputum test and identify factors those shape the level of stigma.
Methods: A community based cross sectional survey was conducted from February to June 2015 in West Bengal, India. We interviewed 135 persons of 15–60 years. Data were collected using a pretested structured questionnaire. Chi-square and logistic regression analysis were done using SPSS 23.0 statistical software.
Results: Among the 'lower stigma' group (score 4–24), 'delay' (14–25 days) is found among 46.2% respondents and 'much delay' (26–120 days) among 53.8%. Among the 'higher stigma' (score 25–36) group, 'delay' is found among 20.5% respondents and 'much delay' among 79.5%. Persons with lower stigma are 0.17 times likely to delay than persons with higher stigma [adjusted odds ratio (AOR): 0.17 (0.044–0.668), $p = 0.011$)]. Important influencers of stigma are caste [AOR: 5.90 (1.66–20.90), $p = 0.006$], number of family members [AOR: 3.46 (1.08–11.06), $p = 0.009$] and residence in urban or rural [AOR: 3.97 (1.03–15.27), $p = 0.045$].
Conclusion: Revised National Tuberculosis Control Program in India should de-stigmatize the community giving priorities to lower castes, big families and rural areas.

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1. Introduction

Population of India is second highest in the world and whereas, annually 25% of the global burden of tuberculosis (TB) incidences is from India which comes around 2.2 millions in 2015. TB incidence per 100,000 population has declined to 167 in 2016 from 216 in 1990.[1,2] According to the Revised National Tuberculosis Control Program (RNTCP), the control of TB lies mostly at three levels: (i) TB suspects are referred to the sputum testing facility under RNTCP, (ii) diagnosed cases are put on treatment and (iii) those who are put on treatment, they remain adherent.[3] First and foremost important step is that TB suspects are referred to the facility for sputum test, otherwise they continue to spread infections.[3] TB suspect referral in India is 157 per 0.1 million population compared to 151 per 0.1 million population in West Bengal, when the expected rate is 203 per 0.1 million.[4–6] As per the RNTCP guidelines, a person with cough for two weeks or more should do sputum examination to diagnose whether TB infection has occurred to avoid further spread of the infection within the community.[3] Around 26.3% patients did not turn out to facilities for sputum examination after having cough for many days in Bangladesh.[7] However, it is found that various caste and vulnerable groups lack the knowledge about TB and its services.[8] To comply with the objective of RNTCP fully; the program has to mobilize community to public facilities for sputum test as first and foremost strategy.[9] RNTCP which is implemented through public health system is yet to pay adequate attention to social, psycho-social, cultural and political factors that make TB endemic among poor and excluded population. Many socially excluded patients are at risk of delayed presentation, poor adherence, and loss to follow up.[10,11] Among many factors those influence delay in presenting them to facility for sputum examination for diagnosis of TB; factors like caste, poverty, residence (urban or rural), accessibility and knowledge about TB and psycho-social issues are important. One of the most important barriers is stigma attached to TB which remains very much under-researched in the context of India. Association between delay in seeking diagnostic sputum examination services and level of stigma is highly significant (odds ratio (OR): 5.9 and $p = 0.01$).[12] Stigma is a deeply rooted socio-cultural vague concept and cannot be easily assessed. TB stigma is an under researched area. Studies in diverse contexts are needed so that stigma will be considered as a priority in the organization of care for people affected by TB.[13] Stigma associated with TB has been identified as a major barrier to health care access and to quality of life in TB management.[14] One of the most important barriers to achieve success in TB control program globally is the stigma attached to TB in most societies. Because of fear of infection, most of the community members want to isolate the infected person from social functions, crowd or even with restriction within the household if the infected person belongs to the family.[15] But there is very little information available from Indian context on how stigma influences access to sputum examination services. Research will be relevant to find out level of stigma, effects of stigma in delay of seeking sputum examination services and influencers of stigma in the context of TB diagnosis. This research study aims to explore the level of stigma perceived by people about TB, influence of stigma in the delay of diagnosis by sputum examination and different influencers those shape the form and extent of stigma.

2. Methods

2.1. Study design and setting

We have conducted a cross sectional epidemiological study. In West Bengal there are 20 districts having 3 subdivisions – Jalpaiguri, Presidency and Bardhaman. From each sub-division, one district has been randomly selected. Thus, Birbhum, Jalpaiguri and North 24 Parganas have been selected those geo-ethnographically represent the state. As per RNTCP one Tuberculosis Unit (TU) caters 0.5 million population and one Designated Microscopy Centre (DMC) caters 0.1 million population. From each district 4 TUs have been selected using stratified sampling technique. From each TU, 3 DMCs have been selected using same technique. Thus total 36 DMCs have been selected. One DOT provider has been selected randomly from each DMC.

2.2. Sample size

Taking 26.3% as proportion of delayed access for sputum examination,[12] sample size has been calculated to be 111 [$n = (1.96)^2 \times 0.26 \times (1–0.26)/(0.10)^2 \times 1.5 = 111$] at 95% confidence limit, allowable error 10% and design effect 1.5. We have got 135 persons with cough for two weeks or more in our study as respondents.

2.3. Selection of respondent

From the selected DOT provider of the respective DMC, we have requested them to take to 3–4 persons with cough for two weeks or more. Thus we interviewed 135 such respondents. We included respondents of the group from 16 to 60 years, with cough for two weeks or more but and given consent for the study. We excluded them who have not done sputum examination test anywhere else.

2.4. Data collection

Stigma has been assessed based on 9 question items adopted as per the tool of Moya used in 2010. Cronbach's alphas for the scales were 0.911 for the community perspectives toward TB scale.[14] It has 1–4 options as per Likert scale. For 1, the respondent has strong disagreement; for 2, the respondent has disagreement; for 3, the respondent has agreement and for 4, the respondent has strong agreement. The tool and the statements for stigma measurement has been field tested and changed as per feedback received.

2.5. Statistical analysis

We have used profile of the participants and their stigma level as two important research variables in our study. Out of

9 stigma items, for each item of the stigma, we have calculated number and percentages. Assessment of stigma has been estimated in 1–4 scale (1 – very little stigma, 2 – little stigma, 3 – moderate level of stigma, 4 – very high stigma). Using 11 item questionnaires, therefore, total score could be 9–36. Mean score is 22.9 and median is 24. Therefore, it is assumed that within 9–34 range, the low stigma level lies and in 25–36 range the high stigma level lies.

We have classified delay in seeking sputum examination services in two groups – delay (14–25 days) and much delay (26–120 days). Here we got mean 25.5, median 25, SD 14.04, minimum 14 and maximum 120 in days.

Composite knowledge score about TB has been estimated. Knowledge score on symptoms of TB has been done based on six itemized questions. Maximum possible score is 6 for those who could correctly answer all 6 questions and minimum is 0 for those who could not correctly respond to any question. Mean score was 1.92 and therefore median is 2, two knowledge groups are made in 0–2 and 3–6 knowledge groups are made to define high and low knowledge score.

SPSS statistics software version 23.00 has been used for statistical analysis. Apart from basic descriptive statistics Chi-square analysis has been done to find out associations between level of stigma and different covariates. Then multiple logistic regression model has been used to calculate OR having its upper bound and lower bounds of limits at 95% confidence limit.

2.6. *Ethical consideration*

Standard ethical permission for human studies was obtained from all concerned authorities before commencement of the study. The Department of Health and Family Welfare, Government of West Bengal gave formal permission (vide memo HTB/31-2008/1054, dated 25th August, 212) to conduct such study within the community.

3. Results

3.1. *Characteristics of the participants*

The study population has a total size of 135, out of which Birbhum has 36 (26.7%), Jalpaiguri 42 (31.1%) and North 24 Parganas 57 (42.2%). Out of 135 respondents from three districts, 36 (26.7%) respondents are within 16–20 years, 45 (31.1%) respondents are within 20–40 years and 61 (42.2%) respondents are within 40–60 years of age group. Around 95 (70.4%) respondents are male and 40 (29.6%) are female. Among the respondents 103 (89.7%) respondents are married, 27 (17.2%) are never married and 5 (3.2%) respondents belong to other marital groups like separated, divorced or widow. Among all respondents, 49 (31.2%) respondents are illiterate, 49 (36.3%) completed primary education, 16 (10.19%) completed upper primary level, 12 (7.6%) completed secondary level and rest 9 (5.7%) completed above secondary level of education. General caste counts 29 (24.8%), scheduled caste (SC) counts 40 (25.5%), scheduled tribe (ST) counts 35 (22.3%) and other backward class (OBC) counts 21 (15.5%) of the total study population. Hindu religion counts 106 (78.5%), Muslim counts 21 (13.4%) and other religion counts 8 (5.1%) of the study population. Only 11 (8.1%) respondents are employed at Government or private, 82 (52.2%) are daily wage labor, 2 (1.5%) are domestic servant, and adequately 40 (25.5%) respondents belong to unorganized sectors. Total 73 respondents (46.5%) belong to below poverty line (BPL) and 62 respondents (39.5%) belong to above poverty line (APL). Total 43 (27.4%) respondents reside in urban area compared to 92 (58.6%) respondents reside in rural area. Among the respondents, 40 (25.5%) are slum dwellers and 28 (17.8%) reported to be in-migrant from Bangladesh, our neighboring country. When probed about personal histories, 86 (54.8%) respondents reported to use any kind of substances those may include guthka, tobacco, brown sugar, alcohol and others.

3.2. *Form and extent of stigma attached to TB*

In Table 1, we have provided parentages of stigma related statements. It is found that stigma related statements have been agreed by respondents with range from 38.5% to 50.4%. Strong agreement on the statements ranges from 0% to 18.5%.

Total stigma score can be from minimum 9 to maximum up to 36. In the study group, mean stigma score is 22.9 as median is 24. Therefore, we have grouped level of stigma as lower stigma level (9–24) and higher stigma level (25–36). Among 135 respondents, 52 (38.5%) lie within the lower stigma group and 83 (61.5%) respondents lie within the higher stigma group. Stigma score has been divided in three groups. Score group from 11 (minimum) to 22 counts 74 (54.8%), score group 23–34

Table 1 – Stigma issues attached to tuberculosis (showing adequate prevalence of the stigma in magnitude).

#	Stigma issues attached to TB	Strongly disagree (%)	Disagree (%)	Agree (%)	Strongly agree (%)
1	I do not prefer a person with TB living in our community	36.3	23.0	38.5	2.2
2	I like to keep distance from people with TB	15.6	32.6	45.2	6.7
3	A person with TB is disgusting	20.0	28.9	49.6	1.5
4	I feel uncomfortable about being near those with TB	12.6	35.6	45.2	6.7
5	I do not want those with TB playing with our children	4.4	26.7	50.4	18.5
6	I do not want to talk to others with TB	11.1	37.8	41.5	9.6
7	If a person has TB, I as part of the community will behave differently toward that person for the rest of his/her life	14.8	41.5	41.5	2.2
8	I may not want to eat or drink with friends who have TB	7.4	33.3	45.2	14.1
9	I usually try not to touch others with TB	16.3	41.5	42.2	0.0

counts 60 (44.4%), score group 35–44 (maximum) counts 1 (0.7%), which has been presented in Fig. 1.

3.3. *Level of stigma and delay in sputum examination*

We have divided delay in sputum examination in six groups. They are delay group 14–28 days counting 91 (67.4%), group 29–43 days counting 39 (28.9%), group 44–58 days counting 2 (1.5%), group 59–73 days counting 2 (1.5%) and group 74 days or its above counting 1(0.7%). It is presented in Fig. 2.

We have further classified delay in seeking sputum examination services in two groups – 'delay' (14–25 days) and 'much delay' (26–120 days). Among the lower stigma group, delay is among 24 respondents (46.2%) compared to much delay among 52 persons (53.8%). Whereas, among the higher stigma group, 'delay' is among 17 persons (10.5%) compared to 'much delay' is among 66 persons (79.5%). Using Chi-square analysis, we have seen the association to be highly significant ($p < 0.001$) (Table 2).

Persons with lower stigma are almost 5.8 times less likely to delay than persons with higher stigma [adjusted odds ratio (AOR): 0.17 (0.044–0.668), $p = 0.011$)]. We have plotted stigma score and delay in scatter plot (Fig. 3) to see that majority of the respondents are distributed within 18–40 days of delay (range 14–120 days) and 15–30 stigma score (range 11–44).

3.4. *Influencers of stigma attached to TB*

Because stigma has been a strong force to influence the delay, we have explored what are those factors actually shaping

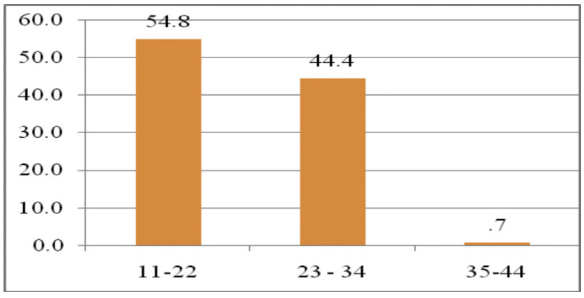

Fig. 1 – Distribution of stigma score in four groups (11–22, 23–34 and 35–44).

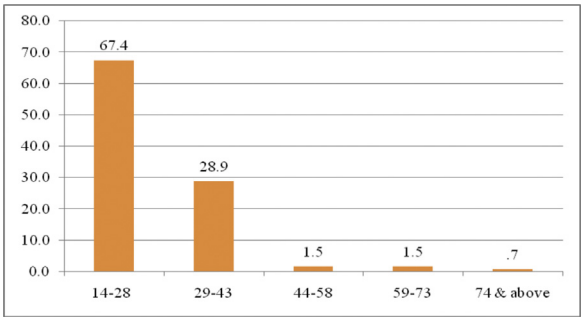

Fig. 2 – Delay in sputum examination in days (six groups: 14–28, 29–43, 4–58, 59–73, 74–88, 89 and above).

Table 2 – Level of stigma and delay in seeking of sputum examination.

Stigma level	Delay *n* (%)	Much delay *n* (%)	Total N (%)	*p* value
Low level of stigma	24 (46.2)	28 (53.8)	52 (100.0)	0.001
High level of stigma	17 (20.5)	66 (79.5)	83 (100.0)	
Total	90 (66.6)	45 (33.4)	135 (100.0)	

the stigma. As shown in Table 3 using Chi-square analysis we have seen that stigma is significantly associated with literacy ($p = 0.023$), caste ($p = 0.002$), poverty ($p = 0.001$), number of family members ($p = 0.009$), staying in urban or rural ($p = 0.001$), and knowledge level ($p = 0.036$). On multiple logistic regression important influencers of stigma those have been revealed out are caste [OR 5.90 (1.66–20.90), $p = 0.006$], number of family members [OR 3.46 (1.08–11.06), $p = 0.009$] and residence in urban or rural [OR 3.97 (1.03–15.27), $p = 0.045$].

4. Discussion

As per our review goes, our study is an attempt for the first in India to measure the form and extent of stigma attached to TB and its effect on delay in sputum test among the TB suspects. We also explored different factors those shape the form and extent of stigma. Stigma attached to TB remains poorly researched. However, its consequences are huge in terms of mortality, morbidity and additional costs involved.[13] In our study 40.7% respondents do not like to live with a person with TB. Around 68.9% respondents do not prefer that their children would play with a person having TB. In Ethiopia, around 30.3% respondents considered that people would avoid them if they had TB and 15.1% wanted not to disclose TB to others. Around 20% respondents thought that TB would have affect their marriage, having sex with partner and were afraid from social isolation.[16] In a study in Karnataka, India around 51.2% respondents had perception about some form of stigma. In Karnataka, India around 55.0% respondents wanted to keep TB diagnosis confidential out of which 61% had problems with their spouses and 58% with family members.[17] It is therefore, depicted that form and extent of stigma in our study is never the less than several examples cited, but if not more. The form and extent of stigma attached to TB must have isolated infected persons and moved several suspects or diagnosed o be infected cases, hidden leading to spread of infection to others. This is a major setback in the achievement of target for early and adequate referral of TB suspects from the community to the RNTCP designated facilities.

In our study, among the high level of stigma group (score 25–36), 17 persons (20.5%) had delayed seeking (14–25 days) and 66 persons (79.5%) had much delayed seeking (26–120 days) of sputum examination. Adverse consequences due to spread of infection from hidden TB has large public health implication. Factors those influence stigma are gender, income, occupation, family history, and marital status were found to be not significantly associated with stigmatization.[17] In our study factors those influence stigma are caste, family

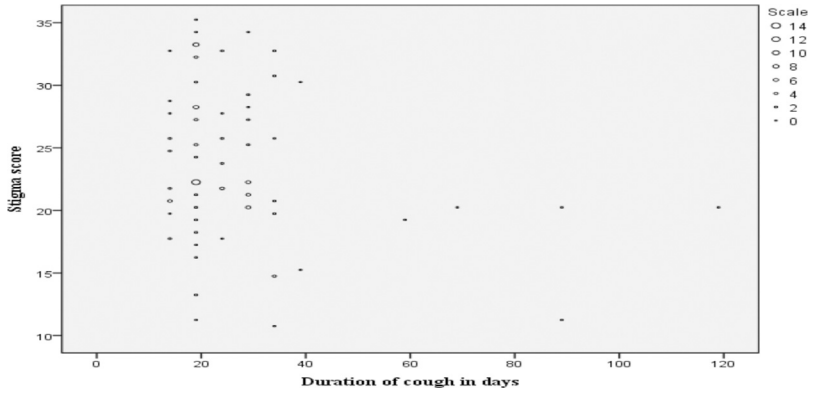

Fig. 3 – Delay in sputum test in days (six groups: 14–28, 29–43, 4–58, 59–73, 74–88, 89 and above).

Table 3 – Factors influencing stigma level among the respondents.

	Bivariate analysis (Chi-square)			Multivariate analysis (logistic regression)			
	Low stigma	High stigma	*p*	*p*	AOR	LL of 95% CI of AOR	UL of 95% CI of AOR
Male	38	67	0.365	0.482	0.639	0.183	2.231
Female	14	16					
<20 years	5	10	0.369	0.120	0.174	0.019	1.582
20–40 years	28	36		0.543	0.686	0.204	2.312
40–60 years	19	37					
Married	36	67	0.311	0.330	3.409	0.289	40.153
Unmarried	14	13		0.078	13.613	0.749	247.473
Others	2	3					
Literate	13	36	0.023	0.294	0.536	0.167	1.721
Illiterate	39	47					
General	23	16	0.002	0.006	5.902	1.666	20.907
Others	29	67					
Hindu	39	67	0.282	0.994	1.005	0.277	3.646
Others	13	16					
APL	33	29	0.001	0.126	2.293	0.792	6.634
BPL	19	54					
0–4 member family	19	16	0.009	0.036	3.467	1.087	11.064
>4 member	33	67					
Urban	27	16	0.001	0.045	3.970	1.032	15.272
Rural	25	67					
Slum	22	18	0.011	0.678	1.267	0.414	3.876
Not slum	30	65					
In-migrant	12	16	0.665	0.486	1.771	0.355	8.846
No	40	67					
Any substance use	1	53	0.634	0.101	2.731	0.823	9.060
No	19	30					
Government	3	8	0.422	0.579	0.583	0.087	3.918
Private	35	39		0.773	1.195	0.357	4.001
Others	14	26					
Visited physician	19	26	0.331	0.227	0.482	0.147	1.575
No visit	33	57					
Knowledge score (0–2)	41	52	0.036	0.174	2.337	0.688	7.942
Knowledge score (3–5)	11	31					

size and residence (urban or rural). Social isolation of TB patients was also described in Ghana. In Ghana the community felt that TB patients should not sell their items in the market.[15] In Nepal, there was a general believe that you should not meet with people who have TB and not visit a home where there is a household member with TB.[18] Some demographic variables like never being married was influencer of more stigma in India and Malawi, but not in Bangladesh.[19] They are not found to be influencer in our study. Even though stigma attached to TB has been explored to certain extent, but not specifically attempt has been made to associate level of stigma with delay in sputum examination which is first and foremost strategy for success in RNTCP. The association between stigma and delay is highly significant ($p = 0.001$). Persons with lower stigma are almost 5.8 times less likely to delay than persons with higher stigma [AOR: 0.17 (0.044–0.668), $p = 0.011$)]. Our study thus is a unique attempt to establish the effect of stigma on delay in sputum test and thus puts a question mark on the preparedness of RNTCP in India. Manpower of RNTCP and the community at large are yet to be prepared to de-stigmatize the community so that early referral is made.[20,21] Level of stigma between two types of respondents, those who have done sputum test and those who have no done, could have been compared to get a better picture of differences between the compliant and not complain group. This could not be done due to resource constraints as the study was done by a PhD scholar. However, difference between delay and much delay in sputum test has been a good procedure because we presume that much delay group has more stigma than the delay group. In the higher stigma level group, in our study 79.5% respondents have much delay and 20.5% have delay in sputum test.

As on date and our knowledge goes, there is no concrete and comprehensive communication strategy that exists for the RNTCP in India neither in West Bengal as a state nor in India for de-stigmatizing the community with an aim for improving early referral.[20,21] There is a need that separate communication strategy may be developed. Our study findings are suggestive that stigma reduction strategies may be helpful that takes special care for areas like caste, large families and urban rural differences. This can gear up community mobilization toward government facilities for early sputum examination services which is actually are first and foremost step of RNTCP toward achieving program goals. Continuous health education on TB aimed at raising awareness and correcting misconceptions is also needed.[22] More examples would be required to say how different strategies can work to reduce stigma in the context of TB diagnosis and its treatment adherence.[23–25] It was found TB stigma did not differ much in population of TB infected individuals in south India after completion of a DOTS program through health education.[26] It is found in a few studies that stigma reduction has been successful through TB clubs, particularly in Africa. These clubs consist of health workers and TB patients. They used to meet weekly to offer social support TB infected persons through transportation, counseling for treatment adherence and also monitor treatment side effects. Individuals enrolled in TB clubs had less TB stigma than those who are not.[27,28] In a study in Pakistan, TB-positive persons had a 13% reduction of DOTS default than patients who did not receive counseling. The results were better for women.[29] Additional studies in our region where TB stigma is common should be conducted to estimate the impact of TB clubs, counseling or such strategies to bring down stigma and improve TB diagnosis, treatment adherence and TB morbidity and mortality.[30]

5. Conclusion

Our study emphasizes the need of looking into stigma as a hindrance for achieving RNTCP goals especially the target of suspect referral. The form and extent of stigma level among the community is adequate to influence delay in seeking timely sputum examination. Considering its greater public health consequences, manpower in the community and community at large should be prepared to de-stigmatize the environment for enhanced and early suspect referrals. At present under RNTCP there is no specific plan for this at national level even not at West Bengal where the study has been conducted. However, it is evident from our study that specific plan for better program outcome may be prescribed.

Conflicts of interest

The authors have none to declare.

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Original Article

Expression profile of CXCL12 chemokine during *M. tuberculosis* infection with different therapeutic interventions in guinea pig

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ABSTRACT

Mycobacterium indicus pranii (MIP) already established as an immune-modulator in mycobacterial infections generates immune response by acting on CXC chemokines. In the present study, the immunomodulatory effect of MIP in conjunction with chemotherapy against M.tb infection was evaluated by colony forming units (CFUs) following aerosol infection to guinea pig and by measuring CXCL12 chemokine expression using q-PCR and in situ RT-PCR. Different experimental groups included, infection (Rv), immunoprophylaxis (RvMw), chemotherapy (RvCh) and combination of immunoprophylaxis + chemotherapy (RvChMw) group and normal healthy (NH) group. In the combination of immunoprophylaxis + chemotherapy (RvChMw) group, the CFU counts reduced significantly ($p < 0.001$) at 4th week of infection as compared to other treated groups (RvMw and RvCh group). The expression of CXCL12 was recorded in all the treated groups of animals. The study demonstrated suppressed expression of CXCL 12 in both immunoprophylaxis as well as chemotherapy groups (6th and 8th week) that become elevated in immunoprophylaxis plus chemotherapy group (10th week), at which time point no CFUs were detected in RvCh and RvChMw group. The findings indicate that the expression of CXCL12 is associated with good response to anti – tubercular treatment. Thus, prior immunization with MIP appears to show good immunomodulatory effect to release CXCL12 chemokine during infection and also correlates with enhanced effect to chemotherapy.

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1. Introduction

Tuberculosis is an infectious diseases caused by *Mycobacterium tuberculosis*, (MTB) affecting 30% population of the world and resulting 1.8 million deaths per year.[1] To control tuberculosis infection, efforts have continued globally for effective vaccination alternates and chemotherapy.[2,3] Several effective vaccine candidates are being tested against tuberculosis infection with or without chemotherapy using animal models like guinea pig considered to be a good model of human diseases due to their susceptibility against tuberculosis and immunological phenomena similar with human disease pathology.[2,4] The host immunity play important role in control of infection via granuloma formation at the site of infection, and this process possibly depend on the activation of T lymphocytes, migration of monocyte and macrophages.[5,6] The granuloma contains the mycobacterial infection and averts dissemination within and beyond the tissue. The granuloma development is directed by cytokines and chemokines, and fashioned by infiltrating leucocytes and restricted tissue cells.[7–9] Chemokines are tiny molecular peptide molecules (8–14 kDa) that adjust inflammatory and immunological responses at cellular level and have important role in localization of various cell types including, monocytes, T-lymphocytes and macrophages at the site of *M. tuberculosis* (MTB) infection.[10] Various type of chemokines have been studied by various workers in different animals with MTB infection.[11–13] In addition, IFN-γ and other cytokines and chemokines that have been documented as bio-markers for diagnosis of MTB infection. For example, IL-10, IL-6, IL-4, CXCL12, CXCL10, CXCL8, and CCL8 levels are possibly associated with tuberculosis.[14,15] CXCL10, CXCL11 and other chemokines of CXC family are actively involved in accelerating the immune responses with vaccines like BCG, MIP and other vaccine candidates, and the levels of chemokines reduced with chemotherapy.[16–18] CXCL12 may have a role in preventing and replication of HIV-1 via R5 and R4 blocking, while other CC chemokines supports HIV-replication.[38,39] More than fifty various cytokines and chemokines analyzed and induced in Shame and BCG vaccinated non human primate lungs including CXCL12.[41] In addition, CXCL10, CXCL11 and CXCL12 chemokines and other molecules detected by Yu et al. in plasma which participated in MTB infection.[40]

Mycobacterium indicus pranii (MIP), a saprophytic cultivable mycobacterium, is an established immunomodulator, employed to generate immune response against mycobacterial infections such as tuberculosis and leprosy.[22–24] MIP has ability to enhance bacterial clearance when used as a immunotherapeutic and immunoprophylactic tool and augments chemotherapy effect against mycobacterial infection in humans as well as in animal models,[21,25,26] and induces chemokine expression in MTB infected guinea pig as previously reported.[16] However, its influence on CXCL12 remains undefined.

CXCL12 chemokine is associated with pulmonary granuloma formation, organized lymphoid structure and T lymphocyte and neutrophil prevalence at the late stage of infection and may be a potential biological marker for tuberculosis diagnosis.[15,19] The expression level of CXCL12 possibly help in Th_{17} immune response during treatment as previously reported that Th_{17} and Th_1 cells were implicated in immunopathogenesis of tuberculosis uveitis.[20] Over the years good immunomodulators like *Mycobacterium indicus pranii* having immunotherapeutic[25,26] and immunoprophylactic[16] potential against tuberculosis have been reported. In previous reports the various cytokine and CXC chemokines have been found to induced following BCG vaccination in animals, Shame-vaccinations in non-human primate and MIP vaccination in Guinea pig during M.tb infection.[16,17,25,41] In our previous study we uncover the expression profile of CXCL10 and CXCL11 following MIP immunization in guinea pig during infection[16] and more work needed to understand expression profile of other protein molecules of CXC family. Due to limitation of reagents and chemicals we are able to study CXCL12 chemokine which was not studied during tuberculosis infection with special reference to MIP as a immunoprophylactic tool. Therefore, the study attempted to discriminate the immunomodulatory effect of MIP on CXCL12 expression in different treatment groups.

However, the expression profile of CXCL12 chemokine during MTB infection, with prior immunoprophylaxis, with or without chemotherapy at early and late stage of infection remains unknown. The present study investigates the effect of prior immunoprophylaxis with or without chemotherapy on CXCL12 chemokine in guinea pigs experimentally infected with MTB. The information about the expression profile of CXCL 12 may through light on its possible role in terms of protection or hypersensitivity.

2. Material and methods

2.1. Mycobacterial strains

M. tuberculosis reference strain, H37Rv (obtained from the Mycobacterial Repository Centre of our Institute) was used for aerosol infection of guinea pigs. For immunoprophylaxis of infected animals, the saprophytic non-pathogenic, heat killed, *Mycobacterium indicus pranii*, was obtained from Cadilla Pharmaceuticals Ltd, Ahmadabad. Prior to aerosol infection, H37Rv was cultured in Middlebrook 7H9 broth (Difco Laboratories) supplemented with oleic acid-albumin dextrose-catalase (OADC). The log phase culture was harvested and CFUs were estimated before storing the aliquots at −70 °C. Working stocks of H37Rv were diluted to $\sim 4 \times 10^7$ CFU/ml in sterile distilled water for animal exposure.[16]

2.2. Animal model

Healthy out-bred Hartley strain guinea pigs were procured from CCS Haryana Agriculture University, Hisar, India. All the animals were maintained at our Animal Experimentation Facility under optimum conditions and used in experimentation. All the experiments were performed after taking the approval of the Institutional Animal Ethical Committee.

2.3. Animal grouping and aerosol infection

The study incorporated five groups of three animals each at every time point and all the experiments were performed as

replicates. All the experimental group of animals were infected (except control group) with approximately 200–250 bacilli/lung to attain earlier establishment and development of the infection, as described previously as well as to evaluate the efficacy of candidate vaccines against infection.[21] Study includes, the first group (NH), uninfected healthy animals were used as control group. A second group of animals (Rv), was infected with *M. tuberculosis* H37Rv (with 4.37×10^7 bacilli) by aerosol route using Inhalation Exposure System (Glas-Col, USA). These guinea pigs were not given any treatment. In The third group, immunoprophylaxis (RvMw) was given, two doses (one month of gap) of heat killed MIP (Each dose consists of 5×10^8 bacilli in 0.1 ml volume) subcutaneously, before aerosol infection. The fourth group, chemotherapy (RvCh) group, was infected with H37Rv and from the second day of infection standard chemotherapy was started orally. The fifth group, Immunoprophylaxis + Infection + chemotherapy, (RvChMw) was given two doses of heat killed MIP before H37Rv as described above for 3rd group and chemotherapy instituted post-infection.

Drugs, namely RIF (10 mg/kg), ETH (15 mg/kg), INH (5 mg/kg), PZA (25 mg/kg) was prepared as per average body weight (~400 g) of guinea pigs and 0.5 ml of this drug solution was given orally by gavages before feeding, for five days in a week for the complete study period.

Four weeks after challenge, the animals were sacrificed at pre-decided time points (1st day, 4th, 6th, 8th and 10th week after infection) and different assays were performed. Right lung of each guinea pig was homogenized in normal saline. 100 μl from different dilutions (1:10, 1:100, 1:1000) of the homogenate was plated onto nutrient Middlebrook 7H11 agar medium (BD Difco) as per manufacturer's instructions and incubated up-to 28 days at 37 °C for CFUs counts.

2.4. RNA isolation and RT-PCR

RNA was extracted using Tri-reagent (Invitrogen, Germany) from guinea pig lung tissue and preserved in RNA*later*. (Ambion, USA). RT-PCR was carried out with 10 μg of total RNA using cDNA synthesis kit (Fermantas, Germany). For CXCL12, guinea pig specific small nucleotide primer sequences (forward, 5′-CCTGCCGCTTCTTTGAGA-3′; reverse, 5′-CCTGGATCCACTTCAGCTTC-3′) and GAPDH (forward, 5′-ATGATTCTACCCACGGCAAG-3′; reverse, 5′-GATCTCG-CTCCTGGAAGATG-3′) were designed and checked via Primer3 software (www.frodo.ei.mit.edu/primer3). The RNA samples were free from DNA contamination as checked by performing PCR. The validity of primers was checked by performing PCR prior to qPCR in 25 μl reaction volume using 2.5 mM MgCl2, 2.5 U Taq DNA polymerase (Fermantas), 1× Taq assay buffer, 0.2 mM each dNTP, 4 μM of each primers and cDNA (500 ng) by a profile of 94 °C for 5 min, 94 °C for 1′, 62° C for 1′ and 72 °C for 1′, 72 °C for 7 min for 35 cycles. The PCR products were analyzed on 1.5% TAE-agarose gel.

2.5. Quantitative PCR

Approximately 500 ng of total RNA from guinea pig lung tissue of each group was converted into cDNA using first strand cDNA synthesis kit. The cDNA was used to performed qPCR with primers for CXCL12 and GAPDH as stated above using SYBR Green-I PCR reagents on Light Cycler 480 System (Roche, Germany). The generated data was quantified by Real Time RT-PCR and normalized to GAPDH value derived from control and relative expression level of gene, was calculated by the $2^{-\Delta Ct}$ method.[37]

2.6. Histopathology and in situ RT-PCR

The right lung lobes were removed aseptically, sliced in to equal pieces and placed in 4% buffered formaldehyde for 1 h at 4 °C and then preserved into 70% alcohol till further use. Lung tissues were processed after twenty-four hours and paraffin-embedded and 4–5 μm sections were cut by microtome (Lieca, Germany) and instantly fixed onto glass slides coated with 2% silane (Sigma–Aldrich, India). These sections fixed slides were used for haematoxylin and eosin (H&E) and Fite-Faraco staining. Microscopic examination was carried out using Olympus BX51 (Olympus, Japan) microscope. Tissue sections prepared from guinea pig lungs were used for in situ amplification for CXCL12 and GAPDH genes using the gene specific primers as per standardized protocol reported earlier.[16]

2.7. Statistical analysis

Graph pad prism5 with ANOVA was applied to analyze the data obtain from guinea pig lung tissue. A comparison between the groups was made of the CFU data and qPCR analysis. $p < 0.05$ values were considered statistically significant.

3. Results

3.1. CFU counts

A homogenous infection was recorded by CFU at day one and bacterial counts at the 4th week time point in all the treated groups (RvMw, RvCh and RvChMw) had significantly ($p < 0.001$) lower counts as compared to the remaining Rv group. CFU counts were significantly lowest ($p < 0.001$) at 4th week of infection in RvChMw group followed by Rv, RvMw and RvCh groups and no CFU were detected in RvCh and RvChMw groups at 6th, 8th and 10th week of infection (Table 1), indicating clearance of bacteria from the lung tissue. The lowest count at 4th week in RvChMw group indicates that immunoprophylaxis (MIP) improved the efficacy of chemotherapy which is visible earlier than chemotherapy alone.

3.2. Histopathological observations and in situ expression of CXCL12 chemokine

The lung tissue sections of infected animals (Rv group) stained with H and E, illustrated 750 μ mean diameter of the structure of granuloma with necrosis. Infection reflected an infiltration fraction with wide range from 5 to 65% (mean = 32.5), lesions varied from 2 to 9 (mean = 4.5) and acid fast bacilli (AFB) were observed in lung tissue sections of infected animals. The infected and immunoprophylaxis group showed the infiltration of

Table 1 – Bacterial counts (log_{10} CFUs) in lungs of drug treated guinea pigs infected with *M. tuberculosis* (H37Rv).

Groups	n[a]	log_{10} CFU at different time points				
		1st day ($p > 0.05$)	4th week ($p < 0.0001$)	6th week ($p < 0.0001$)	8th week ($p > 0.05$)	10th week ($p > 0.05$)
Rv	6/6	2.45 (±0.21)	5.95 (±0.24)	5.72 (±0.29)	4.93 (±0.43)	5.06 (±0.17)
RvMw	6/6	2.43 (±0.28)	5.75 (±0.23)	5.02 (±0.26)	4.67 (±0.09)	4.95 (±0.36)
RvCh	6/6	2.45 (±0.11)	4.08 (±0.42)	0	0	0
RvChMw	6/6	2.43 (±0.19)	2.47 (±0.22)	0	0	0
NH (control)	6/6	0	0	0	0	0

[a] *n*, number of guinea pig from different chemotherapeutic groups showing viable bacteria (log_{10} CFU mean ± SD of *M. tuberculosis* H37Rv) at each time point of a sacrifice/total number of animals tested. The treatment (5 day per week) duration was 10 weeks for all experiments.

mononuclear cells with a mean of 35% and 65%, respectively, while, 20% and 5% of infiltration were found in the chemotherapy and combination of immunoprophylaxis + chemotherapy groups (Fig. 1). The presence of CXCL12 chemokine expression was confirmed by the findings of in situ RT PCR inside the lung tissue of guinea pig that was studied by qPCR and found positive. CXCL12 was reflected as isolated signals in all the studied groups at different times of post-infection (Table 2 and Fig. 2).

3.3. *Chemokine CXL12 mRNA expression analyses*

The mRNA expression level of CXCL12 was found differentially expressed in different treated groups at different time periods. In infected (Rv) group, CXCL12 mRNA expression level was found elevated at 4th week of study time point post-infection followed by decrease in the succeeding time-points. In immunoprophylaxis (RvMw) group, at 4th week time point of post-infection the expression level of CXCL12 mRNA was found up-regulated, followed by decline at 6th and 8th week of post-infection, in turn followed by elevation was at 10th week post-infection. In chemotherapy (RvCh) and combination (RvChMw) groups, the expression level of CXCL12 chemokine was up-regulated at 4th week time point of post-infection and followed by decreased at 6th week of post infection. The mRNA expression level of CXCL12 was found once again elevated both at 8th and 10th week of post-infection and this response is higher (<0.05, significantly) than immunoprophylaxis alone group. CXCL 12 levels in combination with chemotherapy (RvChMw) group were again higher (<0.05) than chemotherapy alone group (Fig. 3).

4. Discussion

The protective immune response to MTB is guided by cytokines and chemokines at cellular level and these have a pivotal role in determining the course of infection with MTB.[27,28] CXC chemokines such as CXCL8, CXCL10 and CXCL11 induces memory T cells and control the immune reactions via polarization of Th1 cells involved in granuloma development.[29–31] CXC chemokines participate in active immunity by activating and recruitment of T cells during MTB infection.[32,33] The guinea pig is considered an appropriate animal model for screening the protective efficacy of drug compounds and vaccine candidates against MTB infection,[34,35] and provide the platform to investigate the biological phenomena at in situ and molecular level following vaccination.[2,36]

The present study, attempts to investigate the profiles of CXCL12 chemokine expression with prior two doses of MIP immunization during the period of MTB infection in this experimental animal model. The CFUs counts and histopathological observation like infiltration fractions (mean = 32.5) and lesion numbers (mean = 4.5) and the presence of AFB in

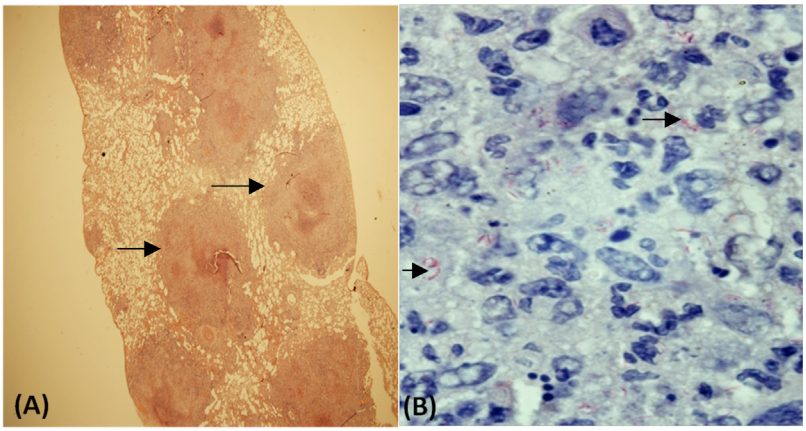

Fig. 1 – (A) Haematoxylin and eosin stained section of guinea pig lung showing granuloma with central necrosis viewed under low magnification 20×. (B) Fite–Faraco stained section of guinea pig lung tissue showing cells of granuloma, positive for acid fast bacilli (AFB) 60×.

Table 2 – In situ RT-PCR analysis of chemokines inside mycobacteria infected lung tissue of guinea pig in different groups at different time points of post infection.

Chemokine	Animal groups															
	Rv				RvMw				RvCh				RvChMw			
	Time point (weeks)				Time point (weeks)				Time point (weeks)				Time point (weeks)			
	4	6	8	10	4	6	8	10	4	6	8	10	4	6	8	10
CXCL12	++	–	–	–	+	–	–	+	–	–	–	+	–	–	+	+

– no signals, + few signals, ++ moderate signals.
Rv – animals infected with H37Rv.
RvMw – prior immunization with MIP than infected.
RvCh – infection + treated with standard chemotherapy.
RvChMw – prior immunization with MIP than infected + treated with standard chemotherapy.

tissue sections provide evidence regarding the establishment of infection/disease in different study groups of animals.

In our study we showed for the first time that CXCL12 expression levels increased in infected group of animals at early stage of infection and consistently decreased in infected (Rv) group as disease progressed. Similarly, CXCL12 increased in treated groups (RvMw and RvCh, RvChMw) at early stage of infection later on the expression of CXCL12 was found

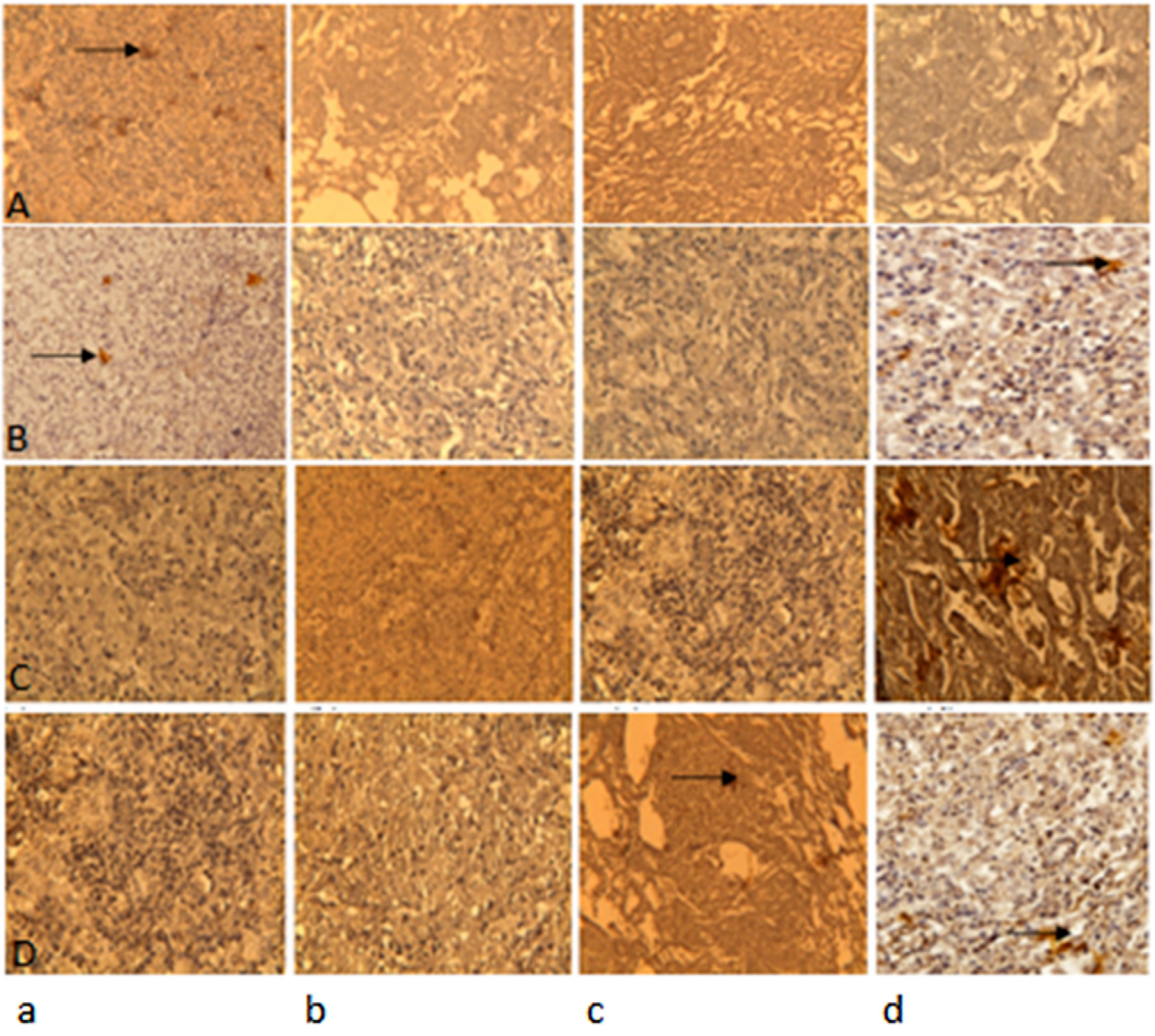

Fig. 2 – Representative photomicrograph showing moderate level of CXCL12 expression in lung of guinea pig demonstrated by in situ RT-PCR, (A) in infected (Rv), (B) immunoprophylaxis (RvMw), (C) chemotherapy, (D) immunoprophylaxis + chemotherapy (RvChMw) group at 4th, 6th, 8th, and 10th week represented by a, b, c and d, respectively. End product = yellow brown, counter stain Haematoxylin (original magnification 60×).

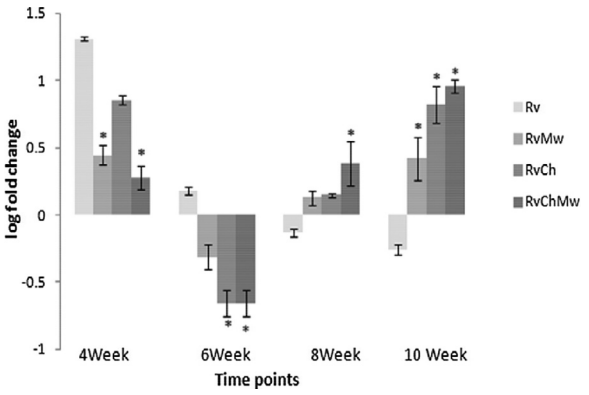

Fig. 3 – Expression levels of mRNA of CXCL12 in different therapeutic groups with an elevation at different time points. The data was quantified by RealTime RT-PCR and normalized to GAPDH values from healthy controls. The expression levels of CXCL-12 at 4th, 8th and 10th week time points was found significant ($p < 0.05$) (marked *) in all the groups (RvMw, RvCh, RvChMw), as compared to (Rv) at same time point.

decreased, as previously reported that response to chemotherapy correlates with chemokines expression.[18] The mRNA level of CXCL12 again were found to be increased in all the treated groups (RvMw, RvCh and RvChMw) at late stage of infection except infected (Rv) group as colony forming unit (CFU) reduced or eliminated from lungs. Thus, finding indicated that CXCL12 expression correlated with attempt to oppose the infection at early stage, but it was consistently reduced while disease progressed and immunity failed in infected group of animals. However, the expression level of CXCL12 increased in treated (RvMw, RvCh and RvChMw) groups. The highest expression levels were observed in combination of immunoprophylaxis and chemotherapy (RvChMw) as compared to other groups (RvMw, RvCh) of treatment, indicated that the combination of therapeutic agents – i.e. chemotherapy and prophylaxis with MIP induced better CXCL12 during infection and the expression changes with time.

Earlier studies addressed the tradition of MIP as a prophylactic vaccine against pulmonary tuberculosis and were observed to provide protection from infection in humans and animals.[21,26] Most of the candidate for immunotherapy act through modulation of cellular immune response; Th_1 response against *M. tuberculosis* was increased and the immunosuppressive response was down-regulated.[25] Previously it has been reported that MIP is an effective inducer which generates strong Th_1 response.[25]

As previously reported, MIP as prophylactic tool was found more effective when used combined with standard chemotherapy to control the disease.[16] In the present study, the expression of CXCL12 chemokines at early and late phases of infections illustrates that MIP is proficient in inducing chemokines that may facilitate protection against infection and the combination of MIP and standard chemotherapy reduces the infection effectively and early.

5. Conclusion

To conclude, MIP alone provided partial protection against the infection, but even when infection progressed prior immunoprophylaxis with MIP appears to enhance the effect of chemotherapy and early treatment response. Additionally, CXCL12 chemokine expression have been shown that MIP may be a better immunomodulator to release CXCL12 chemokines at the site of infection and correlates with the protection. However, detailed mechanisms and further studies are needed to see the effect of MIP as an immunoprophylactic agent on the expression of other cytokines and chemokines that may be contributing to progress of infection specially with chemotherapy.

Conflicts of interest

The authors have none to declare.

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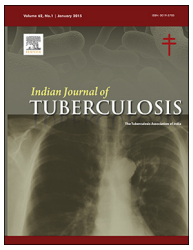

Original article

Quality of life of diabetic patients with smear positive PTB in southeastern Iran: A cross-sectional study in a poor region of Iran

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ABSTRACT

Background: The quality of life is an important indicator of quality of care in chronic diseases such as diabetes and TB. The present research is conducted with an aim to assess the Quality of Life of Diabetic Patients with Smear Positive PTB.

Methods: This cross-sectional study was conducted on 62 diabetic patients with smear positive PTB from January to May 2016 in a diabetes clinic in Zahedan city (southeast of Iran). A simple random sampling method was used in this study. Instrument for data collection was quality of life (SF-36) questioner.

Results: Total quality of life score was 48 that showed an average level of quality of life. Sixty-five patients with diabetes and affected by smear positive pulmonary tuberculosis (PTB) with the average age of 51.30 ± 10.84 years participated in this research. Four patients (0.06%) suffered from type 1 diabetes and 58 (94%) from type 2 diabetes, and all of them were smear positive PTB patients. Study of their quality of life revealed that, in general, the average scores for quality of life in the two main subgroups of physical health and mental health were lower than the average and, among the eight studied dimensions, the highest scores were those for physical activity (60 ± 14.23) and the lowest (31.42 ± 12.14) for general health in the subgroup of physical health.

Conclusion: Results indicated that the patients had a low quality of life although they received the care and treatments that are effective in patients with diabetes and suffering from smear-positive PTB.

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1. Introduction

Tuberculosis (TB) is one of the important contagious diseases in the world and has been one of the global health challenges, especially in the developing countries.[1] About one-third of the world population is infected with TB and at risk of developing TB. The latest statistics published by the World Health Organization (WHO) has shown that 14.5 million people are affected by TB, to which 10.4 million new cases were added in 2015.[2]

More than 90% of deaths caused by TB occur in developing countries; incidence of TB in Central Asia in 2010 was estimated to be 15.2 million, and it was predicted that this figure would increase three-fold by 2030.[3] Moreover, the increase in the number of patients with diabetes mellitus (DM) to 250 million, the prediction that this figure will double during the next 20 years, and the relationship between these two diseases, have made the WHO worried about TB and pessimistic of being able to control it.[4]

According to the WHO report, the Eastern Mediterranean Region accounts for more than seven percent of the global TB prevalence, and there were about 670,000 people (109 per 100,000) affected by TB in this region in 2013.[1] Iran is one of the developing countries at the risk of TB prevalence. According to WHO statistics, its TB incidence rate was 34 per 100,000 people in 2000 but declined to 17 per 100,000 in 2015. Nevertheless, TB is still considered one of the health problems in Iran.[5]

Studies show that the Sistan and Baluchestan Province in southeastern Iran had the highest smear positive cases in the country (about 76 per 100,000 people), while the incidence rate for the entire country was 17 per 100,000 people in 2015.[6]

The relationship between TB and (type 1) diabetes was reported in many studies before 1950. In the 1960s, and results of numerous studies by researchers on the concurrence of these two diseases (with the emphasis on type 2 diabetes) were reported, and once again diabetes was referred to in the literature as a factor threatening the development of TB.[7] The mechanism related to the concurrence of these two diseases is not completely clear, but researchers believe that diabetes activate latent Mycobacterium TB infection and causes progression of TB through suppressing immune responses and by influencing leukocyte bactericidal activity.[8] In 2008, a meta-analysis showed that patients with diabetes were 3.11 times more likely to develop TB than people without diabetes.[3]

The risk ratio of TB in patients with diabetes is higher in developing countries that face a high prevalence of TB. In a study in Ethiopia, it was reported that incidence rates of TB were 26 and 7 times higher in patients with insulin-dependent diabetes and insulin-independent diabetes, respectively, compared to the general population.[9] These two diseases are of different nature, but their long course of treatment and care is not limited to their early and late complications. Like other chronic diseases, patients with these two diseases are faced with many challenges that reduce their quality of life including pressures resulting from their control and treatment, following long-term, complicated, and expensive treatment-care programs, numerous complications and potentially toxic medications, social stigmas resulting from being affected by the diseases, the need for repeated visits to doctors and to undergo various and numerous tests, concern about the outcomes of the diseases and about transmission of the diseases to the children, disorders in social and familial relationships, disordered social and family relationships, sexual problems, problems at work, etc.[10] One of the most important complaints these patients make is their impaired quality of life. Quality of life is a multi-dimensional concept defined by the WHO as the individual's perception of life, values, goals, standards, and personal interests.[11]

Quality of life is often used synonymously with concepts such as health and satisfaction with life. In medical sciences, quality of life is used in two contexts: general quality of life, which studies general factors, and quality of life related to heath, which addresses the effects of various diseases in the physical, mental, and social dimensions,[12] and which is influenced by the individual's opinions, experiences, expectations and understanding.[13] At present, this concept has attracted special interest and importance in relation to persons with chronic illnesses, and it is used as an index to study the effects of diseases and of treatments and care that the patients receive. Moreover, quality of life has been recognized as the outcome of health kits (health packs) in medical treatment and as the main issue in taking care of patients in recent years.[14]

Quality of life studies with the purpose of determining the efficiency and effectiveness of treatment-care programs have received special attention. Moreover, quality of life plays an important role in accepting treatment and care programs, in accepting an illness and in coping effectively with problems resulting from it. Since TB and diabetes are of considerable prevalence in southeastern Iran for various geographical, social, and cultural reasons, the present research intended to identify the effects the concurrence of these two diseases of different nature has on quality of life in patients affected by them.

2. Material and methods

2.1. *Participants*

This cross-sectional study was conducted on 62 diabetic patients with smear positive PTB from January to May 2016 in a diabetes clinic in Zahedan city (Southeast of Iran). Simple random sampling method used in this study. We use Cochrane formula for calculate sample size with confidence interval 0.95%. The number of patents in this center was 143. Sample size was 60. Inclusion criteria were having diabetes and smear positive which approved by physician and lab tests as The International Classification of Diseases-10 (ICD-10) standards.[15] Exclusion criteria were having gestational diabetes, having history of kidney and cardiac diseases.

2.2. *Instrument*

For data collection, we used The SF-36 Quality-of-Life scale. Reality and validity of SF-36 scale were approved in different local[16,17] and international[18,19] studies. This questioner included two parts: (1) demographic characteristics, (2) SF-36

items. Demographic characteristics were age, gender, marital status, and education level and employment status.

SF-36 questionnaire included 8 sub-scales in 36 items. 8 sub-scales included: physical functioning (10 items), physical role functioning (4 items), pain (2 items), general health (5 items), energy/fatigue (4 items), social functioning (2 items), emotional role functioning (3 items) and mental health (5 items). Scoring range was from 0 to 100. The score of zero would show lowest quality of life and 100 would show best quality of life.

2.3. Procedure

Questionnaires were distributed among patients, and most patients completed in 10 min or less. There was no time limit for the completion of the questionnaire. In case of illiterate patients, their questionnaires were filled by a researcher assistant during interview.

2.4. Ethical consideration

The study was approved by the Institutional Review Board of Zahedan University of Medical Sciences with written consent prior to data collection.

2.5. Statistical analysis

Descriptive and interpretative tests of the frequency, mean, and standard deviation (SD) were used to describe sample demographics. Due to lack normal distribution of data nonparametric tests (Spearman's correlation coefficient, Mann–Whitney *U* test and Kruskal–Wallis *H* test) were used to interpret the relationship of variables.

3. Results

Sixty-two patients with the average age of 51.30 ± 10.84 years (range 18–70 years) who were affected by diabetes and smear positive PTB participated in the present research. Table 1 shows the gender, marital status, education level, and occupation of these participants.

Four (0.06%) of the patients suffered from type 1 diabetes and fifty eight (94%) from type 2 diabetes, and they were all affected by smear positive PTB. Of the 62 patients, 52.5% were females and 78% were married. More than 50% of the participants finished elementary school education or were illiterate, half of the females were housewives, and retired individuals formed the largest category of occupations (12.8%). Forty eight percent of the patients had a history of diabetes in their first-degree relatives, 22% injected insulin, and 78% took pills as treatment for diabetes. Seven percent of the participants suffered from various types of diabetic wounds (4% first-degree diabetic ulcers and 8% diabetic retinopathy). Total score of quality of life was 49 that showed an average level of quality of life among patients in present study. Study of the participants' quality of life revealed that the average scores for quality of life in the two main subgroups of physical health and mental health were lower than the average, and the highest and lowest scores for the eight studied dimensions of quality of life belonged to the subgroup of physical activity (60 ± 14.23) and to the subgroup of general health (31.42 ± 12.14), respectively. The independent *t*-test and ANOVA were used to study the relationship between the variables (gender, education level, marital status, and occupation) and the scores received for the quality of life dimensions. Results indicated that the gender variable was related to the average scores for all the quality of life dimensions except for social functioning ($p > 0.05$), and the average scores received by men were higher compared to females (Table 2).

Table 1 – Personal characteristics of the patients.

Variables	N	%
Gender		
Male	29	47.4
Female	33	52.3
Marital status		
Bachelor	2	3
Married	48	78
Divorced	2	3
Widow	10	16
Occupation		
Employed	2	3
Housewives	32	51
Retired	28	46
Education		
Illiterate	32	51
Guidance	22	35
High school	5	8
University	4	6

4. Discussion

The scores for the physical health and mental health dimensions of the quality of life in the present research were

Table 2 – Comparison of the means (±SD) of the scores received for the quality of life dimensions in the studied male and female patients.

Quality of life subscales	Female (N = 33)	Male (N = 29)	*p*-Value
Physical functioning	63/52 ± 21/43	77/38 ± 22/54	0.001
Physical role functioning	40/33 ± 37/78	62/51 ± 37/50	0.001
Pain	35/41 ± 26/59	72/54 ± 26/15	0.001
General health	37/29 ± 20/49	47/36 ± 24/65	0.001
Energy/fatigue	47/28 ± 21/43	27/06 ± 23/61	0.001
Social functioning	68/25 ± 25/71	57/74 ± 42/63	0.05
Emotional role functioning	38/25 ± 42/27	55/74 ± 20/06	0.001
Mental health	50/78 ± 22/03	61/24 ± 21/76	0.001

57.76% and 55.27%, respectively, while the corresponding average scores for American patients with diabetes and suffering from smear positive PTB were 39.30% and 41.12%, respectively.[20] These differences could be due to the attitudes of the American patients toward their health and lifestyle. According to the findings of the present research, the scores of the male patients for quality of life were higher compared to the female patients, and this result has also been confirmed in several other studies. The better quality of life in the male patients may be due to the fact that most of them were employed while only eight percent of the female patients had occupations. This could create differences in the lives and social roles of the female patients, thereby, influencing their quality of life. Quality of life studies based on age confirmed that average scores made for the quality of life declined with increases in age. Since increases in age are accompanied by physiological and psychological changes and by reduced abilities, this factor could reduce the quality of life of the patients and exacerbate their inability in controlling the disease and in adapting themselves to it.[21]

In the present research, married patients enjoyed better quality of life in its various dimensions. Different studies obtained contradictory results in this respect.[22] It seems that in the Iranian society marriage is a positive factor in the various aspects of life and the family, as a source of support, can positively affect the various quality of life dimensions. Background variables such as family history and presence of foot ulcers had no significant relationship with any of the quality of life dimensions. Although other studies also confirm these results,[23] yet the present study, as various other ones, showed that weight reduction had negative effects on quality of life in relation to health.[24]

In the present study, a significant relationship was also observed between quality of life and the body mass index, which may be due to the cultural viewpoint that being fat is equivalent to being healthy and/or fat people have a positive attitude toward themselves. Weakness and fatigue, chest pain, cough, pain in the hands and feet, dizziness, and numbness and tingling sensations in the hands and feet are among the common problems in patients with TB. It seems that concurrence of diabetes with TB affects all quality of life dimensions in the patients, further lowers their quality of life dimensions, and delays their response to treatment and improvement compared to patients only affected by diabetes. This concurrence affects the dimensions of quality of life including energy and vivacity, performance of physical role and physical performance, and especially physical health (which is influenced by the other quality of life dimensions and indicates the long-term effects of a disease). Among the laboratory variables, only hemoglobin A1C, which is one of the standard and important scales in predicting complications resulting from disease and quality of life in patients with diabetes suffering from smear positive PTB, had a significant inverse relationship with the dimensions of physical health, bodily limitations, and general health. Other studies have also reported a weak relationship between quality of life and laboratory variables.[25] Physicians must be aware that quality of life does not necessarily agree with controlling laboratory criteria and these criteria cannot indicate tangible bodily sensations and only serve as a criterion for the quality of disease treatment. Excessive attempts at improving the A1C hemoglobin status and other laboratory values sometimes even result in severe physical and mental burden for the patients because they try to take special care of themselves, which will result in changes in their various quality of life dimensions.

5. Conclusion

Results of the present study indicate that despite the effective treatments and care provided for patients with diabetes who are also affected by smear positive PTB, these patients have a low quality of life. Considering the differences in common influential factors including education level made in the present research, special attention must be paid not only to policy making and treatment but also in providing care programs for the patients with diabetes who also suffer from smear positive PTB in order to maintain and upgrade their quality of life and feeling of well-being.

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Conflicts of interest

The authors have none to declare.

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Original Article

Clinical features vary by the aetiology of meningitis in HIV seropositive patients: A four-year study from a tertiary hospital in India

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ABSTRACT

Meningitis is a serious infection of the nervous system associated with high mortality in Human Immunodeficiency Virus (HIV) seropositive individuals. Asian clinical studies describing meningitis in people living with HIV are scarce. We describe the clinical features of meningitis in 116 HIV seropositive patients from a tertiary hospital in India as a cross-sectional observational study. The mean age of the patients in our study was 35 ± 9 years with 70.6% of them being men. Eighty-five percent of the patients had an altered sensorium during the illness. Tuberculous meningitis [82.6%] was the most common cause. Clinical features varied by aetiology. Cranial nerve deficits [40%] were common in Cryptococcal meningitis. Hydrocephalus [3%], infarcts [15.9%] and IntraCranial Space Occupying Lesions (ICSOLs) [39.1%] were common in tuberculous meningitis.

1. Introduction

Meningitis is an inflammation of the meningeal layers covering the brain.[1] In patients with weakened immunity such as HIV seropositive individuals, the inflammation rapidly involves the brain.[2] It is one of the most deadly afflictions that kill millions of patients living with HIV [PLWH].[3]

Clinical studies have described in detail, the risk factors,[4] manifestations and prognosis of meningitis[5] in PLWH. These have contributed to better understanding and management of these patients. But most of the data describe western[6] and African patients.[7] An accurate portrait of the disease from Asian countries is lacking.[8] It is a surprising lacuna, considering that there are about 5 million PLWH in Asia alone.[9]

Infectious diseases are immensely influenced by the socio-demographic and climatic conditions of the local milieu. Their profile and presentation differ from one region to another.[10] A systematic description of meningitis in PLWH from different regions of the world is needed to understand the disease across its spectrum. This knowledge can guide in making diagnostic algorithms and treatment protocols for an informed and empirical evidence based patient management. This is of paramount importance as delays in the critical hours may be fatal to the patients or result in devastating permanent disabilities. Hence a depiction of the patients with meningitis in HIV seropositive from geographically varied locations is thus essential.

We present the clinical profile of HIV seropositive patients with meningitis from an Indian tertiary care hospital.

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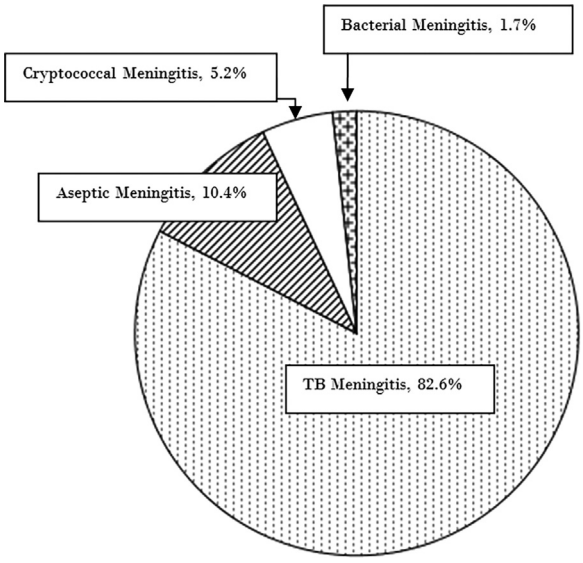

Fig. 1 – Etiological diagnoses of meningitis in PLWH.

2. Methods

Setting: Guntur Medical College Hospital, Guntur – a 1200 bedded Govt. tertiary referral hospital in South India with a catchment population of 20 million.

Period: January 2012 to May 2016-07-27.

Patients: 116 adult [>14 years] PLWH with meningitis[11–14] diagnosed by clinical examination, CerebroSpinal Fluid (CSF) analysis and Computerized Tomography (CT) imaging of the brain.

Study: Cross sectional, observational.

Ethical clearance: Written informed consent was obtained from all patients, and the study was approved by the Institutional Ethical Committee at Guntur Medical College, Guntur.

Statistics: Data were tabulated in MS Excel 2010 and analysed by using SPSS software version 21. Summary of the data is presented with mean and standard deviation. Statistical significance with two sided CI and $p < 0.05$ was tested by χ^2 test for qualitative data and one way Analysis of Variance (ANOVA) for quantitative data for comparison of parameters between the various etiological groups. All missing data were disregarded from analysis.

3. Results

116 seropositive meningitis patients studied at GGH, Guntur were of the mean age of 35 ± 9 years with 70.6% men. Eighty-three percent of the patients were diagnosed with Tuberculosis (TB) meningitis (Fig. 1). Eighty-five percent of the patients had altered sensorium, 11.9% had cranial nerve dysfunction, and 28.2% had other focal neurological deficits.

Certain distinctive clinical features presented in meningitis due to the varied aetiologies. Cranial nerve deficits were most common in Cryptococcal meningitis while other focal neurological deficits were higher in TB meningitis (Table 1).

CSF was less clear in appearance in TB meningitis than in other meningitides. Inflammatory cell count was low in Cryptococcal meningitis. A high level of CSF protein and Adenosine DeAminase (ADA) was seen in TB meningitis (Table 2).

Neuroimaging showed that hydrocephalus, infarcts and ICSOLs were more common in TB meningitis than that in others. However patients with aseptic meningitis showed basal meningeal enhancement on contrast in relatively more number of cases (Table 3).

4. Discussion

Meningitis in PLWH in our cohort was most commonly due to TB, followed by aseptic meningitis. The clinical features varied across the different etiological diagnoses.

Many other studies[3,15–17] have reported Cryptococcus as the chief cause of meningitis in PLWH. Only Georgia[18] in Europe reports higher TB meningitis than others in PLWH.

As the state of Andhra Pradesh has a high prevalence of TB,[19] this infection may be more common than Cryptococcus in our region. The immuno-suppressed individuals are susceptible to the prevalent infection, hence the TB predominance in our study.

Table 1 – Clinical features of meningitis in PLWH of different aetiologies.

Characteristic	TB meningitis [95]	Aseptic meningitis [12]	Cryptococcal meningitis [6]	Bacterial meningitis [3]	*p* value
Focal neurological deficit	33.5%	10%	0	0	0.2
Cranial nerve deficits	11.8%	0	40%	0	0.2
Altered sensorium	85.3%	80.0%	83.3%	100%	0.9

Table 2 – CSF analysis of meningitis in PLWH of different aetiologies.

CSF characteristic	TB meningitis [95]	Aseptic meningitis [12]	Cryptococcal meningitis [6]	Bacterial meningitis [3]	*p* value
Clear CSF	26.3%	40%	50%	100%	0.3
Cell count	154 [100]	184 [247]	41 [45]	90 [16]	0.4
Lymphocytes%	81 [17]	73 [31]	65 [44]	35 [14]	0.3
Protein	221 [128]	108 [88]	87 [10]	170 [52]	0.1
Glucose	49 [28]	35 [17]	47 [23]	21 [10]	0.6
ADA	15 [8]	6 [4]	7 [5]	2 [2]	0.01

Table 3 – Neuroimaging features of meningitis in PLWH of different aetiologies.

CT imaging characteristic	TB meningitis [95]	Aseptic meningitis [12]	Cryptococcal meningitis [6]	Bacterial meningitis [3]	*p* value
Hydrocephalus	3%	1%	0	0	0.7
Basal meningeal enhancement	3%	11.1%	0	0	0.7
ICSOL	15.9%	0	0	0	0.05
Infarcts	39.1%	11.1%	16.7%	0	0.2
Pre-contrast basal hyperdensities	0	0	0	0	

The high percentage of patients with altered sensorium in our study may be due to many factors. In general PLWH are more prone to metabolic[20] disturbances especially in this critical illness background. Their weakened immunity also results in a virulent expansion of the pathogens with rapid involvement of the brain itself. This also results in cranial nerve involvement and focal neurological deficits. Ganiem et al., report 46.5% patients with altered consciousness, 25.5% with cranial nerve involvement and 25.5% with focal neurological weakness in their series. A clinical study from Uganda[21] showed confused behaviour in 35% of patients.

The unchallenged invasion of the brain by the pathogenic organisms in HIV infected patients may lead to cranial nerve deficits and other focal neurological deficits. In our study we found that such deficits in almost one-third of the patients. Forty percent of the patients with Cryptococcal meningitis had cranial nerve deficits. The expanding growth of the opportunistic fungus in the absence of immunity especially in the basal regions of the brain may result in the high cranial nerve deficits.[22]

In contrast to standard criteria of a clear CSF in TB meningitis, we found that the CSF was less clear in appearance in TB meningitis than in other meningitides.[23] This might be due to higher virulence of the bacteria in HIV patients. This might also account for the high level of CSF protein and ADA seen in TB meningitis. Other studies[5] in regions with high HIV and TB prevalence also found evidence of a high degree of inflammation in the CSF. This flagrance of the tuberculous bacilli might account for the high [33.5%] proportion of focal neurological deficits in the patients.[24]

As expected in an immunocompromised state, the inflammatory cell count was low in Cryptococcal meningitis. Similar picture of weak CSF inflammatory state in cryptococcal meningitis was reported in studies[3,5] across the world.

Typically[23] neuroimaging showed that hydrocephalus, infarcts and ICSOLs were more common in TB meningitis than in others. Inexplicably a high number of patients with aseptic meningitis showed basal meningeal enhancement.

In summary, our study of the clinical profile of meningitis in HIV seropositive individuals showed distinct features based on the etiological cause. Contrary to many Indian studies, we found that Mycobacterium tuberculosis was the most common organism causing meningitis in HIV seropositive patients. Physicians should be aware of this possibility and prompt investigation in this direction should be performed for accurate diagnosis and early intervention.

Conflicts of interest

The authors have none to declare.

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Original article

Awareness of health care workers, patients and visitors regarding air borne infection control – A descriptive study from a Tertiary Care Centre in Kerala, southern India

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ABSTRACT

Airborne infections are major public health concern especially in hospitals and public spaces in a highly populated country like India. Generating awareness about good infection control practices among common man and health care workers are important steps in curtailing transmission of air borne infections. In this study we were trying to assess the awareness of airborne infection control measures among patients, bystanders and healthcare workers in a tertiary care hospital at Kochi, Kerala. Self-administered questionnaire which included 10 questions for health care staff and 12 questions for lay men prepared on the basis of NAIC and NCDC guideline were given to the study participants. 143 health care staff and 332 laymen were participated in the study. In both groups majority of the responses were correct. However, only a small proportion of health care staff correctly answered fast tracking of a patient with TB (14.7%) and minimum air exchanges in air-conditioned settings (15.4%). Among laymen only a few correctly identified ideal place for sputum collection (43.3%) and role of hand washing in preventing flu (36.4%). Overall more intervention needed in improving awareness about good infection control practices among both health care staff and laymen.

1. Background

Airborne transmission of infectious disease is a major public health concern. Exposure of human beings to different airborne pathogens has resulted in the emergence of epidemics of respiratory infections. Most of the microorganisms released from infectious patients can disperse in a wide geographical area by air currents and finally can be inhaled by susceptible individuals who have had no direct contact with the primary source. Increasing transmission of TB among household contacts and global spread of Influenza A H1N1 has highlighted the need for air borne infection control precautions at all levels from health care setting to households.

This airborne transmission becomes more prevalent in healthcare settings because of overburdened hospitals and the presence of immune suppressed patients.[1,2] All health facilities are visited by patients with TB and other air borne diseases

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Table 1 – Characteristics of the health workers participated in the study.

Characteristics	Categories	Number	Percentage
Category of staff	Doctors	22	15.38%
	Nurses	66	46.15%
	Nursing attenders	14	9.79%
	Laboratory staff	21	14.68%
	RT/MRT technicians	09	6.29%
	Others/not specified	11	7.69%
Years of experience	Less than one year	16	11.18%
	1–3 years	28	19.58%
	3–5 years	38	26.57%
	5–10 years	34	23.77%
	More than 10 years	27	18.88%
Department	Pulmonology	19	13.29%
	General medicine	24	16.78%
	Microbiology	18	12.59%
	ENT	11	7.69%
	Obstetrics & gynaecology	10	6.99%
	Medical ICU	13	9.09%
	Infection control	10	6.99%
	Surgical specialties	17	11.89%
	Others	21	14.69%

for diagnosis and cure. Inadequate or absence of infection-control guidelines in hospitals has resulted in acquisition of infections among health care workers, nosocomial transmission of infections among patients admitted for some other reasons and their bystanders, especially immune compromised patients.[3–5]

National Guidelines on Airborne Infection Control in Health Care and other settings in India (NAIC) was released in 2010 to reduce the risk of airborne infections in health care facilities.[6] Lack of awareness and inconsistent application of infection control guidelines contribute to the risk of transmission of air borne infections in hospitals. Also generating awareness among common man is an important step in curtailing transmission of air borne infections in hospitals and community.

Amrita Institute of Medical Sciences (AIMS), Kochi, Kerala is a tertiary care teaching hospital with 2500 beds. AIMS has received many awards for strengthening patient safety through an effective Antibiotic Stewardship Program, infection prevention and control practices.[7] Air Borne Infection Control guidelines has also been implemented and monitored regularly. Hospital Infection Control committee meets every monthly and recently a checklist for AIC has also been incorporated. Risk assessments help identify strengths, weaknesses, and opportunities for improvement. The current study was done with the objectives to identify the gaps in awareness among health care workers, patients and bystanders regarding air bone infection control in AIMS.

2. Materials and methods

Two questionnaires were developed-one for health workers and another for patients and bystanders. The questionnaire was developed based on NAIC guidelines. The questionnaire for health workers included 10 questions including six hypothetical scenarios. The questionnaire for patients and bystanders included 10 questions including four hypothetical scenarios and four agree/disagree questions based on NAIC and National Centre for Disease Control guidelines in this regard. Content validity for the questionnaire was checked by two experts in the field. The questionnaires were also translated to regional language and back translated to check for consistence. Questionnaires in both languages were pilot tested before use. Sample questions included in the questionnaires were given in Box 1.

The unlinked anonymous and self-administered questionnaire in both languages was distributed among the hospital staff, patients and bystanders of selected departments with a request to fill and return. It took approximately 10–15 min to fill the questionnaire.

Ethical clearance for this study was obtained from Institutional Ethics Committee. The data was entered in Microsoft Excel. Descriptive analysis was done calculating frequencies and percentages.

3. Results

A total of 143 questionnaires from healthcare workers were included in the final analysis. Characteristics of health care workers participated in the study are shown in Table 1. Majority of the participants (46.15%) were staff nurses. All but one of the study participants agreed that health workers are at increased risk of developing air borne infections. Majority of the responses were correct for questions like identifying place with lowest risk for air borne infection control (89.5%), precautions to be taken inside ICU for preventing H1N1 (88.8%), indications for wearing N95masks (79.7%) and advice on disposing sputum of a patient with TB (78.3%). However, it was evident that only a small proportion of workers know about fast tracking of a patient with TB (14.7%) and minimum air exchanges in air-conditioned settings (15.4%). The responses were presented as graph in Fig. 1.

A total of 332 questionnaires filled by patients and bystanders were analysed. 87% knew about cough etiquette, 83.4% answered proper method of sputum disposal, 78% agreed

Box 1. Examples of questions in the questionnaire

Questions to General Public

1. Imagine that you are affected with a flu. What precautions you will take to ensure that you will not transmit the disease to others?
   A. I will not take any precautions as it is a flu
   B. I will always cover my mouth and nose while coughing
   C. I will ask others to cover their mouth and nose while I am coughing
   D. I don't know
2. If any of your friends has doubtful Tuberculosis, which will be the best method to prevent transmission of the disease to you?
   A. I will never touch his belongings including mobile phones
   B. I will offer the person suspected with the disease a mask and advise him to wear
   C. I will take medicines to make sure that I won't get the disease
   D. I don't know

Questions to Health workers

1. Which among the following setting in hospital has **lowest risk** of transmission of an air borne infection?
   A. Rooms where windows and doors are open with cross ventilation
   B. Rooms with split A/C
   C. Rooms with ceiling fan and split A/C
   D. Rooms with A/C with three air current exchanges per hour
   E. Enclosed dark rooms with restricted air movement
2. You have been in a busy General Medicine OPD. You have seen referral letter for Mrs Rajamma where diagnosis was written as sputum positive TB. What will you do?
   A. Should ask her to wait at a corner and will call her only after seeing all other patients
   B. Shall allow her to jump the routine queue and be seen earlier than other patients
   C. She can come in her routine turn to visit the doctor
   D. I will wear a disposable single layer surgical mask before talking to her
   E. I don't know what to do
3. Mr Rajan, is admitted to ward with a lower respiratory tract infection. What will you do?
   A. Advise him to cover his mouth and nose with a tissue or kerchief when coughing or sneezing
   B. Schedule his X ray appointment at a non busy time
   C. Provide him a mask to wear and counsel him on its use
   D. Counsel him on how to dispose his sputum properly
   E. A and D
   F. All of the above

that frequency of visit to a respiratory patient in hospital to be reduced, 68.1% knew about segregation of infectious TB patient inside house, 59.9% knew about the importance of natural ventilation in preventing air borne diseases. However, only a few correctly identified ideal place for sputum collection (43.3%) and role of hand washing in preventing flu (36.4%). The responses were represented in Fig. 2.

4. Discussion

Recognising the impact of Tuberculosis on the society, it is important to promote implementation of air borne infection control guidelines both in hospital setting and in the community. It has been revealed that most of the countries where a significant reduction in the incidence of TB has been observed, airborne infection control practices have played a crucial role. However, in India, implementation of airborne infection control strategies is still in the early stages.

The suggested measures in NAIC guidelines have been broadly categorised into three major categories, namely administrative control or work practice, environmental control and personal respiratory protection measures. The physical separation of TB patients or people suspected of having TB requires rational design, construction or renovation, and use of buildings. Controls aimed at reducing TB transmission in health-care settings include triage, physical separation of TB patients or people suspected of having TB, cough etiquette, respiratory hygiene and minimise time spent in health care facilities.

Human Resource Development for infection control is an important step for implementing this. It has to be ensured that health workers at the different levels of the health system should have the professional competence necessary to successfully implement infection control measures. Even though the general awareness was satisfactory, we could identify gaps in some of the domains of awareness regarding air borne infection control guidelines and practices.

Deficient areas in knowledge among health workers in the current study were on fast tracking of TB patients and minimum air exchanges for an AC room. Fast tracking is a process which will allow patients with respiratory symptoms to jump the routine queue and be seen earlier than other patients.

Infection control practices require a system-wide approach, and health care workers at all levels should receive training and be engaged in improving their own and patient safety. It is going on at regular interval at our hospital. We have planned for periodic assessment of knowledge and also to emphasise on knowledge deficient areas during regular trainings.

Despite a very high literacy rate and a strong health system, awareness regarding precautions for preventing air borne infection control among people is not universal in Kerala. Advocacy, communication and social mobilisation have to be an essential component of the infection control plan. These activities should include civil society and community involvement, behavioural change campaigns and reinforcement of positive message for patients and visitors. Education on cough hygiene and sputum disposal can easily be imparted to patients at hospitals through posters and other means in the waiting area, as well as by actual discussion by a paramedical staff or volunteers while the patient is waiting for his turn.

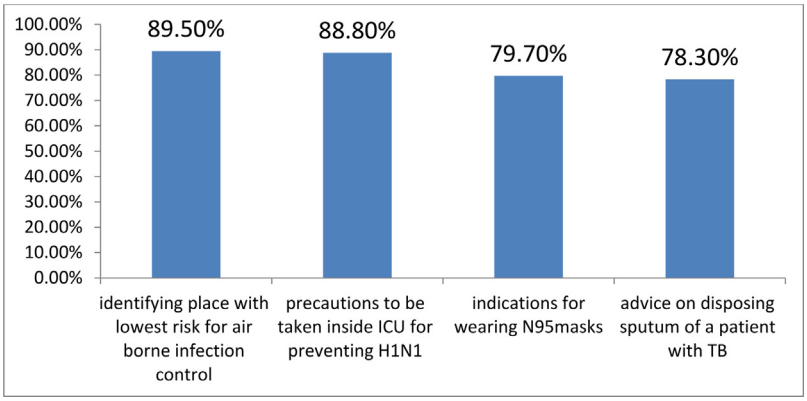

Fig. 1 – Awareness of health workers regarding air borne infection control (N = 143).

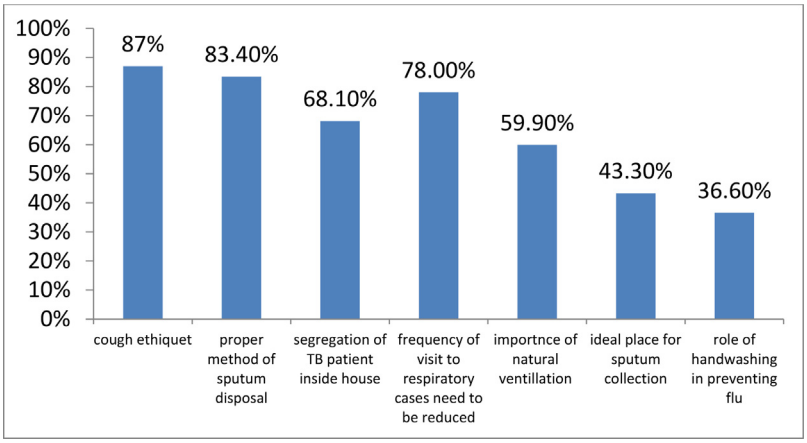

Fig. 2 – Awareness of patients and visitors regarding air borne infection control (N = 323).

The study is done only in one centre and may not be representative or applicable across all health care settings. We have assessed only knowledge, which might not necessarily reflect practises. Despite these limitations, the study has many public health implications. Directions for future research include validating the questionnaire used in this study. Similar studies may be done by all health care institutions to identify the gaps in knowledge for appropriate actions.

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Conflicts of interest

The authors have none to declare.

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Case Report

Localized hepatic tuberculosis presenting as severe hypercalcemia

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ABSTRACT

Hypercalcemia might present itself in association with granulomatous diseases such as tuberculosis. We report a rare case of a 62-year-old man with hypercalcemia due to hepatic tuberculosis. The diagnosis was based on laparoscopic and a histopathological examination. After treatment with anti-tuberculosis medication, the patient's serum calcium levels were within normal limits. Tuberculosis needs to be excluded as a diagnosis in any febrile patient with hypercalcemia, especially in countries where tuberculosis is endemic.

1. Introduction

Tuberculosis (TB) is a disease caused by the *Mycobacterium tuberculosis* and is the main cause of death worldwide from a curable infectious disease.[1] The most common clinical presentation is the pulmonary disease. Extrapulmonary forms are rare and found more often in immunocompromised patients.[2]

One of the main presentations of extrapulmonary tuberculosis is hepatic tuberculosis, which may occur alone or in association with pulmonary involvement.[3] Despite its rarity, hepatic tuberculosis does not have any typical clinical or radiological manifestations. Therefore, it constitutes a challenge in establishing an effective diagnose.[2]

A diagnosis can be obtained through a histopathological examination of the material, by obtaining it through autopsies, biopsies, and other surgical procedures.[4] Among several methods, a biopsy done through an image-guided aspiration is considered to be one of the best available methods. In order to confirm the diagnosis, it is necessary to visualize a caseous granuloma, a polymerase chain detection (PCR), or acid-fast bacilli for *M. tuberculosis* in the aspiration or in the biopsy material.[5]

2. Case report

A 62-year-old male, retired, heavy equipment operator from Concordia do Pará, Brazil, was hospitalized with complaint of intermittent fever, night sweats, nausea, and vomiting. He reported a weight loss of approximately 60 pounds which started one year before admission. As the fever began to occur

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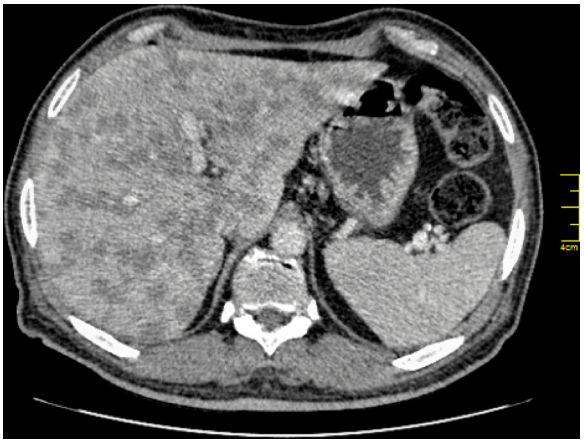

Fig. 1 – Abdomen tomography (venous phase) showing multiple hepatic nodulations.

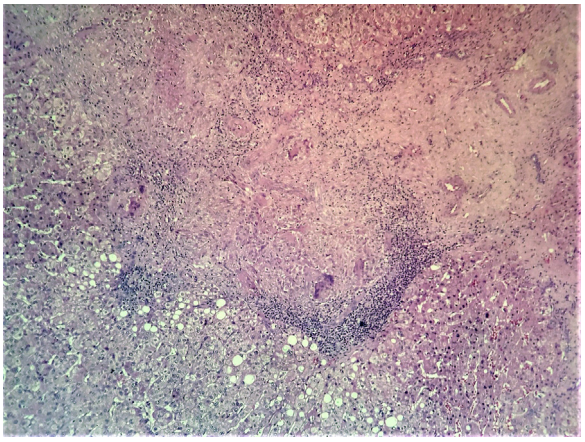

Fig. 2 – Biopsy of liver revealed chronic granulomatous inflammation with caseous necrosis and multinucleated giant cells.

on a daily basis, coupled with abdominal pain in the right hypochondriac region and epigastric region, he sought care at a local hospital where he was treated for 9 days with Ceftriaxone and Metronidazole, without any improvement in his condition. Consequently, the patient was transferred to Jean Bitar Hospital.

During the admission process, he was feverish and nauseous, with uncontrollable vomiting, cramps, loss of strength in the lower limbs, mental confusion, and constipation. At the physical evaluation, he was thin, without signs of jaundice, and had a scaphoid abdomen. His liver was palpable within 4 cm from the right costal border and it was painful, with a smooth edge, softened consistency, and had no nodulations. There were no other relevant data at the physical evaluation. He also suffered from untreated diabetes and hypertension and had a history of frequent intake of wild animal's meat.

The laboratory investigations revealed hypercalcemia and compromised renal function. Ionized calcium was 1.7 mmol/L, creatinine was 3.0 mg/dL, and urea was 57 mg/dL. The parathyroid hormone (PTH) was below normal limits; thyroid function was normal. Radiographic examination of the thorax was unaltered. Abdominal tomography revealed multiple hepatic nodulations of equal density, diffusely distributed by the parenchyma, suggesting neoplastic metastatic process (Fig. 1). Tumor markers were within the limits of normality. The serologies were non-reactive for hepatitis C, hepatitis B, HIV, histoplasmosis, paracoccidioidomycosis, cryptococcosis, and echinococcosis. He underwent gastrointestinal upper endoscopy and colonoscopy, but there were not any pathological findings.

Hepatic biopsy was performed by laparoscopy, and histopathological examination revealed a tuberculous granuloma (Fig. 2). The standard treatment for tuberculosis was introduced according to the guidelines under Brazilian Ministry of Health: a combination of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol for 2 months followed by Rifampicin and Isoniazid for 4 more months. Vigorous hydration, furosemide, and corticoid were administered in order to treat hypercalcemia (Fig. 2).

3. Discussion

Hypercalcemia occurs when calcium levels are above the upper limit of normality. These values are usually 10.5 mg/dL for total serum calcium and 1.32 mmol/L for serum ionized calcium.[6] Its clinical manifestations depend on serum calcium levels as well as on the speed of its installation. Polyuria, polydipsia, dehydration, mental confusion, proximal muscular weakness, hyporexia, nausea, and constipation are some of its consequences.[7]

The main causes of hypercalcemia are primary hyperparathyroidism in outpatients and cancer in hospitalized patients.[8] Other uncommon causes include granulomatous diseases (like tuberculosis and sarcoidosis), adrenal insufficiency, thyrotoxicosis, vitamin D intoxication, advanced chronic kidney disease, and medications such as lithium, antacids, and thiazide diuretics.[9]

In the case presented, the patient had a liver biopsy result compatible with tuberculosis and the granulomatous diseases are the main diagnostical hypothesis for hypercalcemia. Other comorbidities or use of medications associated with hypercalcemia were excluded. Although he has had altered renal function, suppressed levels of PTH excluded primary hyperparathyroidism or even hypercalcemia caused by renal failure as possibilities of diagnosis.

In patients with granulomatous diseases such as tuberculosis, extrarenal production of 1,25-dihydroxyvitamin D by macrophages leads to increased bone resorption and intestinal calcium absorption.[10] This patient had a normal level of 25-hydroxyvitamin D, but the evaluation of the serum level of 1,25 dihydroxyvitamin D would have been clinically useful, since it may be elevated in patients with granulomatous disease-related hypercalcemia.

Hypercalcemia in patients with tuberculosis is generally mild and asymptomatic, occurring in both pulmonary and extrapulmonary forms. Its development depends in part on dietary calcium and the efficacy of 25 (OH) vitamin D3 synthesized from 7-dehydrocholesterol on the skin. The active forms of tuberculosis are associated with severe hypercalcemia, and about to 85% of patients normalize their serum calcium levels after 18–24 months of anti-tuberculosis

medication.[11] The treatment for the patient was instituted as soon as the liver histopathological examination was obtained, leading to progressive improvement of symptoms and normalization of calcium levels.

Symptomatic hypercalcemia is treated with vigorous hydration associated with loop diuretics such as furosemide, glucocorticoids, bisphosphonates, calcitonin, and dialysis.[6,12] Antituberculous therapy, hydration, corticoid, and diuretics were satisfactory for the rapid normalization of serum calcium levels and improvement of the renal function of the patient.

In conclusion, academic literature discusses that there are reports of hypercalcemia associated with granulomatous diseases, such as pulmonary or extrapulmonary tuberculosis, in which hypercalcemia is understood to be a complication of active tuberculosis. Therefore, in patients with febrile symptoms, gastrointestinal complaints, hypercalcemia, and living in endemic areas, extrapulmonary forms should be considered as an etiology.

Conflicts of interest

The authors have none to declare.

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Case Report

Isoniazid induced early-onset of motor dominant neuropathy and treatment with high dose of pyridoxine

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neuropathy within 10 days after the initial administration of conventional doses of isoniazid. This atypical clinical course should be known in order to improve the outcome of adverse events due to anti-tuberculosis treatment.

1. Introduction

Isoniazid, also known as isonicotinyl hydrazide, is considered to be an ideal anti-microbial agent because of its low cost, excellent intracellular penetration, bioavailability, and a narrow spectrum of action.[1] Nervous system toxicity with current anti-tuberculosis pharmacotherapy is relatively uncommon, although the frequency of the usage of anti-tuberculosis therapy requires that physicians should be aware of such toxicity. Peripheral neuropathy is a rare adverse effect associated with isoniazid, and it occurs after the prolonged use of this drug.[2] This usually presents with paresthesias which can be accompanied by muscle aches, occasionally muscular weakness, and can progress to more severe symptoms such as ataxia.[2] Risk factors for developing neuropathy after isoniazid therapy include old age, slow acetylator status, diabetes, renal failure, alcoholism, malnutrition, human immunodeficiency virus (HIV) infection, chronic hepatic failure and pregnancy.[2]

Here we report a case of acute isoniazid induced peripheral neuropathy with predominant motor functional impairment associated with paraparesis. To our knowledge, there has been no report of a patient who developed severe peripheral

2. Case report

A 30 year old woman with a history of 20 days of cough, fever and weakness was admitted in a private hospital at Jodhpur. Patient was initially diagnosed as community acquired pneumonia and treated with intravenous antibiotics for 1 week. Patient got only partially relieved so she came to our department of pulmonary medicine in February 2016. The patient had no history of immunodeficiency, no diabetes, no renal failure, no hepatic failure, no HIV infection and she was a nonsmoker. Pulmonary tuberculosis was suspected and the investigation of sputum of acid-fast bacilli (AFB) was turned out to be positive. Diagnosis of pulmonary tuberculosis was made on bacteriological and on radiographic basis. Therapy was prescribed for the first 2 months of isoniazid, rifampicin, ethambutol and pyrazinamide. The regimen of Directly Observed Treatment Short-course (DOTS) under Revised National Tuberculosis Control Program (RNTCP) of anti-tuberculosis treatment is: isoniazid 5 mg/kg + rifampicin 10 mg/kg + ethambutol 15 mg/kg + pyrazinamide 25 mg/kg per and pyridoxine 10 mg.[3] 10 days after starting treatment, the patient complained of weakness of toes of both lower limbs which was proximal in progression, ultimately involved ankle and knee joint. The patient complained of difficulty in standing and rising from a chair. There was no burning, no pain and no numbness or tingling. On examination her body mass index (BMI) was 18 kg/m^2. Neurological examination revealed pure motor paraparesis

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with a muscle testing of 0/5 in the lower extremities, 4/5 in the shoulders and elbows and 4/5 in the wrists and hands. There were no deglutition or sphincter disorders or difficulty breathing. Achilles and patellar tendon reflexes were absent. Peripheral joints were free. There was no objective sensory finding and no cranial nerve lesions. Isoniazid was stopped and dose of pyridoxine was increased to 100 mg. After 20 days of isoniazid hold and 100 mg pyridoxine on neurological examination, there was no improvement in power of muscle and sensory involvement of both lower limbs was present. Patient had undergone series of laboratory investigations.

Laboratory test	Values
Complete blood count (CBC)	11,000/cmm
Haemoglobin (Hb)	10.6 g/dl
Erythrocyte sedimentation rate (ESR)	90 mm/h
Serum Bilirubin (total)	0.72 mg/dl
Renal function test	Normal
Antinuclear antibody (ANA)	Negative
Serum Calcium	8 meq/l
Serum vitamin B12	>1200 pg/ml
Cerebrospinal fluid (CSF)	Normal

Electromyography demonstrated bilateral sural and superficial peroneal sensory nerve action potentials were absent. Bilateral tibial and peroneal compound muscle action potentials were absent. Active degenerartion 3+ was noted in right tibialis anterior, tibialis posterior and medial gastrocnemius. Single small polyphasic potential was noted in right tibialis anterior.[4] The result of median, ulnar, radial sensory-motor conduction study was normal. There was no cord compression on magnetic resonance imaging of the thoracolumbar spine. Computed tomography brain scan was normal. On the basis of physical examination and laboratory investigations other etiologies of neuropathy were ruled out and diagnosis of isoniazid induced neuropathy was made. Patient was advised to continue the same treatment.

At the end of 4 months of DOTS regimen, i.e., May 2016, sputum for acid-fast bacilli (AFB) was positive. Patient was considered a case of failure. Category 2 under RNTCP was started with dosage of rifampicin 10 mg/kg + ethambutol 15 mg/kg + pyrizinamide 25 mg/kg + streptomycin (0.75 mg) and pyridoxine 100 mg with isoniazid under hold. Under RNTCP guidelines patient's sputum was sent for 1st line drug sensitivity testing (DST). Mycobacterium tuberculosis sensitive for rifampicin and isoniazid was found in the result of DST. After 2 months of isoniazid hold and 100 mg of pyridoxine, patient did not improve. Pyridoxine dose was doubled to 200 mg[5,6] and physiotherapy was started. The physiotherapy aimed to prevent complications of supine positioning and consisted of articular mobilization and actively assisted muscle strengthening and functional work. After 8 months of follow up, there was progressive knee (4/5) and pelvic muscle (4/5) recovery, the BMI of the patient increased to 22 kg/m^2, she recovered muscle strength in lower limbs to 4/5 with recovery of her ability to walk up and down stairs and she was able to independently undertake activities of daily living. Follow up sputum analysis for mycobacteria were negative.

3. Discussion

Isoniazid induced neuropathy is dose related. Symptoms after initiation of treatment in patients receiving conventional doses rarely appear before 6 months. Isoniazid induced neuropathy usually manifests as paresthesia that begins in the feet and can reach the hands and arms. The patient first complains of numbness or tingling of the feet, a burning sensation and pricking pain. There has been no report to our knowledge of a patient who developed severe peripheral neuropathy barely 2 weeks after the initial administration of conventional doses of isoniazid and in the absence of predisposing factors. Furthermore, our patient developed mainly motor neuropathy with dominant paraparesis with sensory characteristics, unlike the typical peripheral neuropathy related to isoniazid. Therefore, isoniazid should not be dismissed as a possible cause in the event of rapid development of peripheral neuropathy with predominant motor symptoms after starting antituberculosis drugs, even in the absence of predisposing factors. It has been reported that smoking induces vitamin deficiency, but our patient had no history of smoking. The only possible explanation of this onset was her low BMI. Clinicians should be aware of the rapid onset of this side effect. The rehabilitation is a part of the multidisciplinary management in this type of pathology. It aims to prevent decubitus complications, maintenance of cardiovascular endurance and recovery of muscle function, walking and autonomy.

4. Conclusion

Isoniazid can cause rapid onset of peripheral neuropathy with predominant motor symptoms and should be considered as a possible cause in cases presenting with such symptoms soon after starting the medication. Dose of pyridoxine for treatment of neuropathy can be increased up to 200 mg.

Conflicts of interest

The authors have none to declare.

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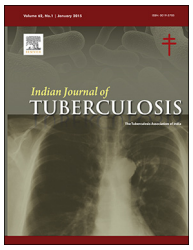

Case Report

Tubercular esophagocutaneous fistula: Rare case report

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ABSTRACT

Tubercular esophagocutaneous fistula is a rare entity with only about five cases reported so far. It can be as a result of primary involvement of esophagus by tuberculosis or due to spread of infection from adjacent structures like lungs or mediastinal lymph nodes. The fistula usually heals with initiation of antitubercular therapy and surgery is rarely required. Here we report a case of 65-year-old diabetic male who developed esophagocutaneous fistula secondary to caseation of mediastinal lymph nodes and was successfully treated with antitubercular treatment.

1. Introduction

Tuberculosis of the esophagus is exceedingly rare, occurring in less than 0.2% of all tuberculosis patients.[1–3] Most of the studies are reported from areas where tuberculosis is endemic but with the world-wide spread of human immunodeficiency virus infection which is often complicated by tuberculosis, there will be an upsurge in the reported cases of this infection from all over globe. It usually results from a spread of an adjacent focus, such as the lung or mediastinal nodes, spine, larynx or pharynx and tends to involve the proximal rather than the distal esophagus.[1–5] Rarely infection may spread from the lung, spine or via the blood stream. Esophagocutaneous fistula is a very rare complication of tuberculosis.[6]

2. Case report

A 65-year-old male presented with history of cough with mucoid expectoration, low-grade fever, loss of appetite and weight since 9 months. There was history of leakage of semisolid foods and liquids from a suprasternal fistula while swallowing since 4 days. Patient was operated 8 months back for a suprasternal abscess for which incision and drainage was done. Patient did not follow-up after that. There is no history of tuberculosis in past or any Kochs contact. Patient was recently diagnosed with diabetes mellitus. No other comorbidity.

On examination, patient was moderately built. Pulse was 96/min, blood pressure – 130/80 mmHg, RR – 24/min, SpO_2 was 91% on room air. On general physical examination, there was no pallor, icterus, clubbing, cyanosis, pedal edema or any lymphadenopathy. Examination of the neck showed a 3 cm × 3 cm wound just above the suprasternal notch in midline. There was no surrounding redness or tenderness or any pus discharge from the wound. When the patient was made to drink semisolid foods or liquids, it used to leak out from the wound. Respiratory system examination did not reveal any findings.

Laboratory investigations revealed Hb 9 g%, Total white blood cell counts of 9000, differential counts of N65/L30/E4/M1, FBSL 163 mg/dl, RFTs and LFTs were within normal limits.

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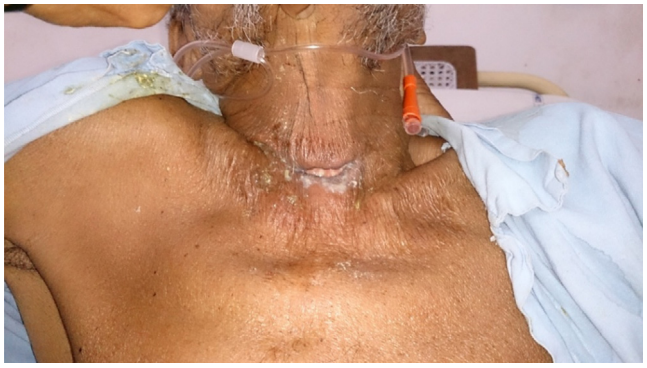

Fig. 1 – Fistula opening just above the sternal notch.

Initial chest X-ray revealed miliary infiltrates bilaterally with widening of the right paratracheal stripe. Sputum for AFB was negative on two separate occasions but came as 1+ later. A computed tomography scan of neck and thorax was done with oral contrast. It showed an air-lined tract extending from the right esophageal wall winding around the right tracheal wall to midline where it is seen to extend to cutaneous surface at the

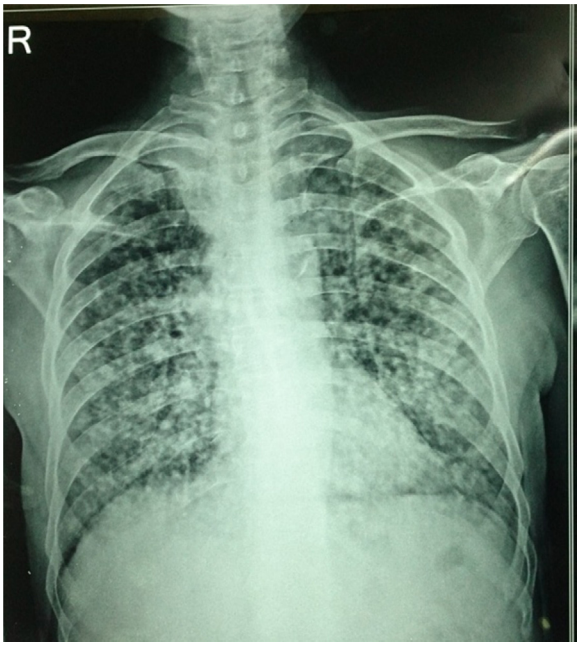

Fig. 2 – Chest X-ray showing miliary tuberculosis.

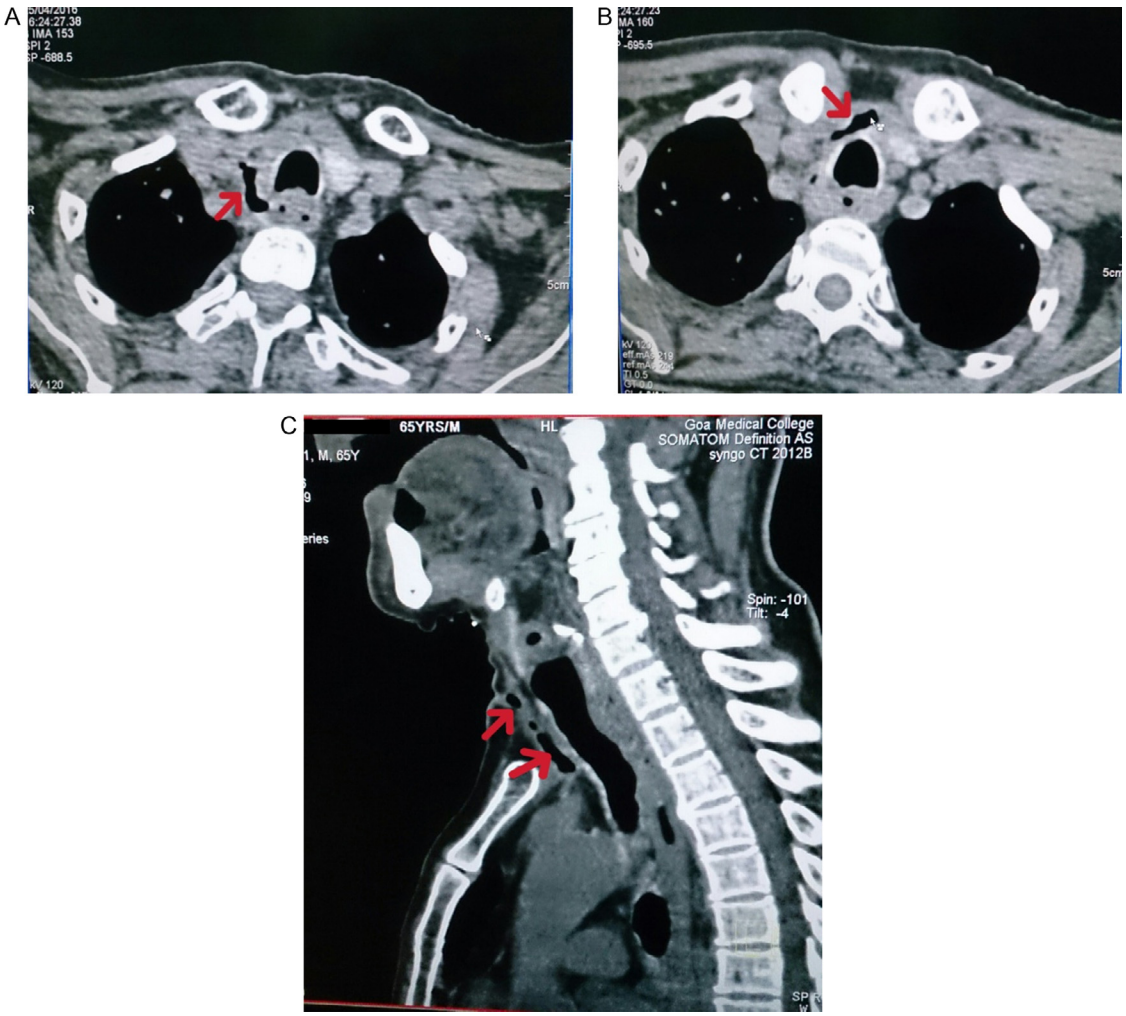

Fig. 3 – (A and B) CT thorax (axial view) showing fistulous tract originating from esophagus and curving around the trachea (red arrow). (C) CT thorax (sagittal view) showing fistulous tract anterior to trachea (red arrow).

level of thoracic inlet. Enlarged lymph nodes are noted along both jugular chains, in prevascular, paratracheal and subcarinal regions largest measuring 2.3 cm × 1.5 cm. Innumerable millimetric nodules are seen randomly scattered in both lungs suggestive of miliary TB. Upper GI endoscopy and biopsy could not be done due to financial constraints (Figs. 1–3).

A ryles tube was passed and patient was started on DOTS category 1 regimen containing isoniazide, rifampicin, pyrazinamide and ethambutol. Patient tolerated the doses and was discharged. At 4 weeks follow-up patient's general condition improved and his ryles tube was removed. There was no leakage from the wound.

3. Discussion

Esophageal tuberculosis can be primary or secondary. Usually it is secondary to tuberculous caseation involving the neighboring structures. In the setting of an esophageal stricture or ulceration, swallowed infected sputum can give rise to primary mucosal involvement of the esophagus.[7] In our case secondary mechanism appears more likely since patient had mediastinal lymph nodes which might have caseated and the necrotic material might have tracked through the neck muscles into the chest wall forming a cold abscess which was drained surgically 8 months back forming a sinus tract. These necrotic mediastinal lymph nodes over a period of 8 months might have eroded into the esophagus forming a fistula between esophagus and chest wall. Since the patient was immunocompromised due to diabetes, tuberculosis of lymph nodes might have disseminated via hematogenous route to lungs giving rise to miliary seeding.

The differential diagnosis of the fistulous tracts of the esophagus includes malignancy, Crohn's disease, radiation injury and trauma. The diagnosis can be confirmed by demonstration of the fistulous tract with radiocontrast studies. Computed tomographic scanning with oral and intravenous contrast is useful to delineate fistulous tracts and mediastinal adenopathy.[8] Pathologic and microbiologic confirmation can be obtained by biopsy and culture of sinus tract endoscopically or percutaneously. Since patient could not afford an endoscopy it was not done in our case. But sputum positivity and caseating lymphadenopathy supported diagnosis of tuberculosis. Esophageal tuberculosis is reported to respond well to medical treatment in 4–8 weeks.[8] Currently our patient is on AKT and responded well with closure of fistula within 1 month.

Conflicts of interest

The authors have none to declare.

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Correspondence

Intraventricular tuberculoma: An unusual presentation of brain tuberculosis

Dear Editors,

A 5-year-old child presented to us with complains of fever, headache for past 20 days and abnormal movement of right eye for last 10 days. Fever was low grade and it was associated with continuous headache. It was followed by abnormal horizontal movement of right eye. There was no history of rash, altered behavior, yellowish discoloration of eyes or urine, abnormal body movement, or bleeding from any site. History of contact with Koch was present. On examination patient was found to have features of raised intracranial tension, along with signs of meningeal irritation. Fundus was done which showed blurring of disc margins along with hyperemic disc. MRI was done which showed multiple tuberculomas of variable size scattered throughout the brain parenchyma. Apart from that well opacified granuloma was seen inside the frontal horn of lateral ventricle in sagittal section (Fig. 1). The diagnosis of tuberculomas was further confirmed by spectroscopy showing elevated lipid peaks. Chest X-ray was also suggestive of miliarly tuberculosis. Patient was put on anti-tubercular (ATT) drugs under strict supervision and shunt surgery was planned in case of any deterioration.

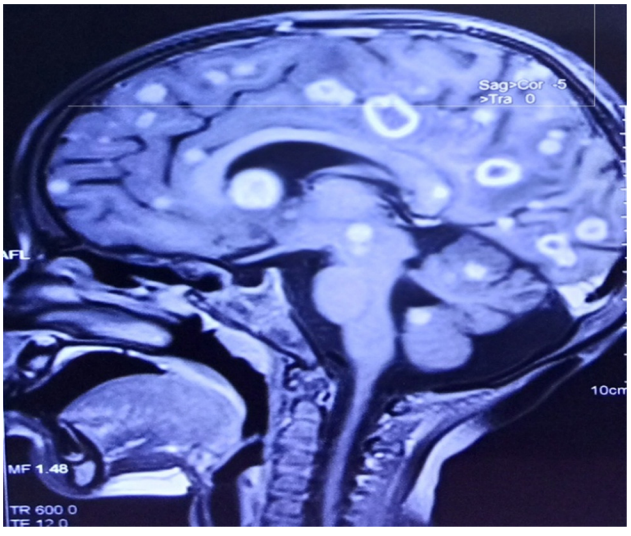

Fig. 1 – Sagittal view of MRI showing intraventricular tuberculoma.

Tuberculosis is still one of the leading causes of death among the infectious diseases. The World Health Organization (WHO) declared tuberculosis as a global emergency in 1993. In recent year's there are increased incidence of cases presenting with prolonged nonspecific symptoms like headache, generalized apathy and poor appetite which later on found to have multiple brain tuberculomas.[1] Tuberculomas constitute 33% of intracranial space-occupying lesions in patients in developing countries.[2] There is no data regarding incidence of intraventricular tuberculoma as it is a rare entity and there are only few descriptions in the literature. Its diagnosis is difficult because of its non-specific clinical features and poor microbiological yield in view of its paucibacillary nature. Therefore Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) findings are useful in establishing early diagnosis. The characteristic CT features of intraventricular tuberculoma are peripheral enhancement with central necrosis and disproportionately extensive cerebral edema and hydrocephalus.[3] MR imaging usually shows tuberculoma as peripherally isointense and centrally hypointense on T1-weighted images. The isointense region becomes hypointense on T2-weighted images with enhancement by gadolinium injection.[4] The MRI findings in the current case showed a well-defined and opacified tuberculoma inside the lateral ventricle along with multiple parenchymal tuberculomas. There are only few case reports of intraventricular tuberculomas.[4,5] Intraventricular tuberculomas are not commonly seen because of strong immune response of the ventricles. Presence of intraventricular tuberculoma and its reporting to the clinician is important because of its therapeutic implications. Initiation of ATT can leads to marked inflammatory response which will block the CSF outflow and patient might develop brainstem herniation in already compromised intracranial pressure. ATT should be started under strict supervision and shunt surgeries should be considered prior to initiation of ATT or in evolving andhydrocephalus.

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Abstracts

Prevalence of dysglycemia and clinical presentation of pulmonary tuberculosis in Western India

Mave V, Meshram S, Lokhande R, Kadam D, Dharmshale S, Bharadwaj R, Kagal A, Pradhan N, Deshmukh S, Atre S, Sahasrabudhe T, Barthwal M, Meshram S, Kakrani A, Kulkarni V, Raskar S, Suryavanshi N, Shivakoti R, Chon S, Selvin E, Gupte A, Gupta A, Gupte N, Golub JE. *International Journal of Tuberculosis and Lung Disease* 2017;**21(12)**:1280–1287. https://doi.org/10.5588/ijtld.17.0474

Setting: Pune, India.
Objectives: To estimate the prevalence and risk factors of pre-diabetes mellitus (DM) and DM, and its associations with the clinical presentation of tuberculosis (TB).
Design: Screening for DM was conducted among adults (age > 18 years) with confirmed TB between December 2013 and January 2017. We used multinomial regression to evaluate the risk factors for pre-DM (glycated hemoglobin [HbA1c] > 5.7–6.5% or fasting glucose 100–125 mg/dl) and DM (HbA1c > 6.5% or fasting glucose > 126 mg/dl or random blood glucose > 200 mg/dl or self-reported DM history/treatment) and the association of dysglycemia with the severity of TB disease.
Results: Among 1793 participants screened, 890 (50%) had microbiologically confirmed TB. Of these, 33% had pre-DM and 18% had DM; 41% were newly diagnosed. The median HbA1c level among newly diagnosed DM was 7.0% vs. 10.3% among known DM ($P < 0.001$). DM (adjusted OR [aOR] 4.94, 95% CI 2.33–10.48) and each per cent increase in HbA1c (aOR 1.42, 95%CI 1.01–2.01) was associated with >1 + smear grade or >9 days to TB detection.
Conclusion: Over half of newly diagnosed TB patients had DM or pre-DM. DM and increasing dysglycemia was associated with higher bacterial burden at TB diagnosis, potentially indicating a higher risk of TB transmission to close contacts.

Conflicts of interest

The authors have none to declare.

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Should sputum-negative presumptive TB patients be actively followed to identify missing cases in India?

Waikar S, Pathak A, Ghule V, Kapoor, A, Sagili, K, Babu, ER, Chadha, S. *Public Health Action* **7(4)**:289–293. https://doi.org/10.5588/pha.17.0035

Setting: Sputum smear microscopy, the primary diagnostic tool used for diagnosis of tuberculosis (TB) in India's Revised National TB Control Programme (RNTCP), has low sensitivity, resulting in a significant number of TB cases reported as sputum-negative. As the revised guidelines pose challenges in implementation, sputum-negative presumptive TB (SNPT) patients are subjected to 2 weeks of antibiotics, followed by chest X-ray (CXR), resulting in significant loss to care among these cases.
Objective: To determine whether reducing delays in CXR would yield additional TB cases and reduce initial loss to follow-up for diagnosis among SNPT cases.
Methods: In an ongoing intervention in five districts of Maharashtra, SNPT patients were offered upfront CXR.
Results: Of 119 male and 116 female SNPT patients with a mean age of 45 years who were tested by CXR, 32 (14%) were reported with CXR suggestive of TB. Administering upfront CXR in SNPT patients yielded twice as many additional cases, doubling the proportion of cases detected among all those tested as against administering CXR 2 weeks after smear examination.
Conclusion: Our interventional study showed that the yield of TB cases was significantly greater when upfront CXR examination was undertaken without waiting for a 2-week antibiotic trial.

Conflicts of interest

The authors have none to declare.

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Whole genome sequencing of clinical strains of *Mycobacterium tuberculosis* from Mumbai, India: A potential tool for determining drug-resistance and strain lineage

Chatterjee A, Nilgiriwala K, Saranath D, Rodrigues C, Mistry N. *Tuberculosis* 2017;**107(December)**. https://doi.org/10.1016/j.tube.2017.08.002

Amplification of drug resistance in Mycobacterium tuberculosis (*M.tb*) and its transmission are significant barriers in controlling tuberculosis (TB) globally. Diagnostic inaccuracies and delays impede appropriate drug administration, which exacerbates primary and secondary drug resistance. Increasing affordability of whole genome sequencing (WGS) and exhaustive cataloguing of drug resistance mutations is poised to revolutionise TB diagnostics and facilitate personalized drug therapy. However, application of WGS for diagnostics in high endemic areas is yet to be demonstrated. We report WGS of 74

clinical TB isolates from Mumbai, India, characterising genotypic drug resistance to first- and second-line anti-TB drugs. A concordance analysis between phenotypic and genotypic drug susceptibility of a subset of 29 isolates and the sensitivity of resistance prediction to the 4 drugs was calculated, viz. isoniazid-100%, rifampicin-100%, ethambutol-100% and streptomycin-85%. The whole genome based phylogeny showed almost equal proportion of East Asian (27/74) and Central Asian (25/74) strains. Interestingly we also found a clonal group of 9 isolates, of which 7 patients were found to be from the same geographical location and accessed the same health post. This provides the first evidence of epidemiological linkage for tracking TB transmission in India, an approach which has the potential to significantly improve chances of End-TB goals. Finally, the use of *Mykrobe Predictor*, as a standalone drug resistance and strain typing tool, requiring just few minutes to analyse raw WGS data into tabulated results, implies the rapid clinical applicability of WGS based TB diagnosis.

Conflicts of interest

The authors have none to declare.

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Prediction of smear positive TB cases at different types of designated microscopy centres, Karnataka, India

Nagaraja SB, Shastri S, Tripathy JP, Sharma G, Kunjathur SM, Singarajipur A, Chadha S. *Journal of Tuberculosis Research* Pub. Date: November 23, 2017. https://doi.org/10.4236/jtr.2017.54027

Background: Under the Revised National Tuberculosis control Programme (RNTCP) in India, the designated microscopy centres (DMCs) form the basic unit of smear positive TB case detection in a district. There is a need by the programme managers to estimate the mean and range of smear positive tuberculosis (TB) cases that can be detected at DMCs located in different type of health facilities to channelize their resources.
Methods: It is a cross-sectional study conducted in the state of Karnataka, India during January 2014 to December 2014 based on the compiled reports from past five years received from all the 30 districts of the state. The prediction was made based on the performance of these DMCs in the last five years using a modeling technique.
Results: The proportions of the DMCs located at health facilities are Primary Health Institutions/Centres (PHIs)—73%, Tuberculosis Units (TUs)—15%, Medical colleges (MC)—7%, District TB centres (DTC)—3% and Private Practitioners (PP)—2%. The maximum number of cases that can be detected at DTC is 3621 (SD 54), TU is 9224 (SD 90), PHI is 20,412 (SD 135), PP is 859 (SD 26) and MC is 8322 (SD 84).
Conclusion: The predicted values will essentially serve as a tool for the programme managers of Karnataka to plan, strategize and monitor the performance of DMCs in the state.

Conflicts of interest

The authors have none to declare.

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Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: A prospective cohort study

Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, Bridge SC, Bangdiwala AS, Meya DB, Denkinger CM, Muzoora C, Boulware DR on behalf of the ASTRO-CM Trial Team. *The Lancet Infectious Diseases* January 2018;**18(1)**:68–75. https://doi.org/10.1016/j.ijtb.2018.02.011

Background: WHO recommends Xpert MTB/RIF as initial diagnostic testing for tuberculous meningitis. However, diagnosis remains difficult, with Xpert sensitivity of about 50–70% and culture sensitivity of about 60%. We evaluated the diagnostic performance of the new Xpert MTB/RIF Ultra (Xpert Ultra) for tuberculous meningitis.
Methods: We prospectively obtained diagnostic cerebrospinal fluid (CSF) specimens during screening for a trial on the treatment of HIV-associated cryptococcal meningitis in Mbarara, Uganda. HIV-infected adults with suspected meningitis (eg, headache, nuchal rigidity, altered mental status) were screened consecutively at Mbarara Regional Referral Hospital. We centrifuged CSF, resuspended the pellet in 2 mL of CSF, and tested 0.5 mL with mycobacteria growth indicator tube culture, 1 mL with Xpert, and cryopreserved 0.5 mL, later tested with Xpert Ultra. We assessed diagnostic performance against uniform clinical case definition or a composite reference standard of any positive CSF tuberculous test.
Findings: From Feb 27, 2015 to Nov 7, 2016, we prospectively evaluated 129 HIV-infected adults with suspected meningitis for tuberculosis. 23 participants were classified as probable or definite tuberculous meningitis by uniform case definition, excluding Xpert Ultra results. Xpert Ultra sensitivity was 70% (95% CI 47–87; 16 of 23 cases) for probable or definite tuberculous meningitis compared with 43% (23–66; 10/23) for Xpert and 43% (23–66; 10/23) for culture. With composite standard, we detected tuberculous meningitis in 22 (17%) of 129 participants. Xpert Ultra had 95% sensitivity (95% CI 77–99; 21 of 22 cases) for tuberculous meningitis, which was higher than either Xpert (45% [24–68]; 10/22; $p = 0.0010$) or culture (45% [24–68]; 10/22; $p = 0.0034$). Of 21 participants positive by Xpert Ultra, 13 were positive by culture, Xpert, or both, and eight were only positive by Xpert Ultra. Of those eight, three were categorised as probable tuberculous meningitis, three as possible tuberculous meningitis, and two as not tuberculous meningitis. Testing 6 mL or more of CSF was associated with more frequent detection of tuberculosis than with less than 6 mL (26% *vs* 7%; $p = 0.014$).
Interpretation: Xpert Ultra detected significantly more tuberculous meningitis than did either Xpert or culture. WHO now recommends the use of Xpert Ultra as the initial diagnostic test for suspected tuberculous meningitis.
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Conflicts of interest

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Vitamin D deficiency in patients with tuberculous meningitis and its relationship with treatment outcome

Dangeti GV, Mailankody S, Neeradi C, Mandal J, Soundravally R, Joseph NM, Kamalanathan S, Swaminathan RP, Kadhiravan T. *International Journal of Tuberculosis and Lung Disease* 2017;**22 (1)**:93–99. https://doi.org/10.5588/ijtld.17.0304

Setting: Data on vitamin D deficiency in tuberculous meningitis (TBM) and its relationship with treatment outcomes are limited. Some of the beneficial effects of vitamin D might be mediated through interleukin-1β (IL-1β).

Objective: To assess the frequency of vitamin D deficiency among TBM patients, its association with treatment outcomes and correlation between vitamin D and IL-1β levels in cerebrospinal fluid (CSF).

Design: We prospectively studied a consecutive sample of human immunodeficiency virus-negative patients with TBM treated at a hospital in southern India. We defined good outcome as survival without severe neurological disability. Serum total 25-hydroxy vitamin D (25[OH]D) and IL-1β levels in CSF were estimated on pretreatment samples.

Results: We studied 40 patients with TBM; 22 (55%) patients had stage 3 disease. Treatment outcome was poor in 21 (53%) patients: 15 (38%) patients died and 6 (15%) had severe neurological disability. The overall mean serum 25(OH)D level was 32.30 ± 16.38 ng/ml. Ten (25%) patients had vitamin D deficiency (<20 ng/ml), and 12 (30%) patients had vitamin D insufficiency (20–30 ng/ml). However, pretreatment serum 25(OH)D levels did not differ significantly by outcome (good vs. poor outcome: 28.30 ± 14.96 vs. 35.92 ± 17.11 ng/ml, $P = 0.141$). Moreover, IL-1β levels in CSF did not correlate with serum 25(OH)D levels (Spearman's ρ 0.083, $P = 0.609$).

Conclusion: Vitamin D deficiency/insufficiency is common among patients with TBM. However, serum 25(OH)D levels are not associated with IL-1β levels in CSF or treatment outcome.

Conflicts of interest

The authors have none to declare.

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Detection of resistance to fluoroquinolones and injectable drugs among antituberculosis drugs by allele-specific primer extension on a microsphere-based platform

Kim K, Yang JS, Choi HB, Lee SH. *Journal of Microbiological Methods* 2018;**144(January)**:111–116. https://doi.org/10.1016/j.mimet.2017.11.007

Molecular drug susceptibility testing (DST) for antituberculosis drugs is important for improving the efficacy of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) treatment. In this study, we developed a molecular high-throughput assay system based on allele-specific primer extension (ASPE) and MagPlex-TAG microspheres, referred to here as TAG-ASPE, which can detect mutations related to resistance to injectable second-line drugs and fluoroquinolones. Target genes were amplified by multiplex PCR using DNA from H37Rv and 190 clinical *Mycobacterium tuberculosis* strains and extended by ASPE using 22 ASPE primers. ASPE products were then sorted on the TAG-ASPE array and detected using a Luminex 200 system. The performance of the TAG-ASPE method was compared with that of sequencing and phenotypic DST. Comparison of the TAG-ASPE method with sequencing showed that the sensitivity and specificity of the TAG-ASPE method were 100% [95% confidence interval (CI), 96.38–100%] and 100% (95% CI, 95.70–100%) for the *rrs* gene and 100% (95% CI, 96.90–100%) and 100% (95% CI, 95.07–100%) for the *gyrA* gene, respectively. Compared with phenotypic DST, the sensitivity and specificity of the TAG-ASPE method for detecting drug-resistance mutations against injectable second-line drugs were 92.52% (95% CI, 85.8–96.72%) and 98.7% (95% CI, 92.98–99.97%), respectively. Additionally, the sensitivity and specificity for fluoroquinolone-resistance detection were 85.4% (95% CI, 78.36–90.85%) and 100% (95% CI, 92.38–100%), respectively. The results of this study demonstrate that the TAG-ASPE method can effectively detect mutations conferring resistance to second-line antituberculosis drugs in numerous clinical specimens.

Conflicts of interest

The authors have none to declare.

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Low pre-diagnosis attrition but high pre-treatment attrition among patients with MDR-TB: An operational research from Chennai, India

Shewade HD, Nair D, Klinton JS, Parmar M, Lavanya J, Murali L, Gupta V, Tripathy JP, Swaminathan S, Kumar AMV. *Journal of Epidemiology and Global Health* 2017;**7(December (4))**:227–233. https://doi.org/10.1016/j.jegh.2017.07.001

Background: Worldwide, there's concern over high pre-diagnosis and pre-treatment attritions or delays in Multidrug resistant tuberculosis (MDR-TB) diagnosis and treatment pathway (DTP). We conducted this operational research among patients with presumptive MDR-TB in north and central Chennai, India to determine attrition and turnaround times (TAT) at various steps of DTP and factors associated with attrition.

Methods: Study was conducted in Revised National Tuberculosis Control Programme setting. It was a retrospective cohort study involving record review of all patients with presumptive MDR-TB (eligible for DST) in 2014.

Results: Of 628 eligible for DST, 557 (88%) underwent DST and 74 (13%) patients were diagnosed as having MDR-TB. Pre-diagnosis and pre-treatment attrition was 11% (71/628) and 38% (28/74) respectively. TAT [median (IQR)] to test from eligibility for DST and initiate DR-TB treatment from diagnosis were 14 (9.27) and 18 (13.36) days respectively. Patients with smear negative TB and detected in first quarter of 2014 were less likely to undergo DST. Patients in first quarter of 2014 had significantly lower risk of pre-treatment attrition.

Conclusion: There was high uptake of DST. However, urgent attention is required to reduce pre-treatment attrition, improve TAT to test from eligibility for DST and improve DST among patients with smear-negative TB.

Conflicts of interest

The authors have none to declare.

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