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Latent TB Detection and Isolation of MDR TB Bacteria in, MP, India

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Abstract

Aim: Tuberculosis which is an infectious disease caused by *M. tuberculosis* causing pulmonary and extra pulmonary tuberculosis in clinical suspects in Indore and region around central state of India by IGRA methods. In India, TB which is declared to be 'notifiable disease of the nation' by the RNTCP since 2012. We wanted to analyze present state of existence of the same in our city Indore and region around. We wanted to study the present strata of IGRA detected in population of Indore in MP correlating with the *Mycobacterium* isolates in our laboratory, though the protocol of DOTS have been followed in the country.

Experimental design: In present study, we tested clinical suspects using microbiology biology cultivation method and ELISA method for IGRA in Indore for *Mycobacterium tuberculosis* in the clinical suspects in our Indore lab, MP, India.

Place and duration: The study was one in Central lab-Oncquest India Ltd. in Indore between period 2012 to 2014.

Methodologies: The present study included 135 patients, including 49 male, and 86 female patients. We used developed TB-TMA method (Oncquest, Ltd.) to detect infection of clinical suspects and utilized culture susceptibility test to detect drug resistance in infecting *Mycobacterium* causing tuberculosis, tested at Central lab-Oncquest ltd. in India using microbiological methods. The method of drug resistance in Mycobacteria was performed using microbiology methods of drug resistance as described by Songara P, 2015.

Results: We found 53% samples to be positive from male group compared to 29% from female group of patients. We could isolate *Mycobacterium* sp from various clinical suspects using basic microbiology and cultivation methods. Were found 41.6% *Mycobacterium* to be sensitive to sensitive to INH 36.65 to RIF, 23.3 to PYRA, 305 to ETHM, 25% to STREPTO isolated from various samples from clinical suspects.

Conclusion: We were able to detect *M. tuberculosis* and determine their drug resistance in *Mycobacterium* method by MDR sure method.

Keywords: Tuberculosis; Drug-resistance

Abbreviation

TB: Tuberculosis; MTB: *Mycobacterium tuberculosis*; ATT: Antituberculosis Treatment; INH: Isoniazid; RIF: Rifampicin; PYRA: Pyrazinamide; ETHAM: Ethambutol; STREPTO: Streptomycin. BAL: Broncho Alveolar Levage; MDR sure: Molecular test for Multi Drug resistance Test for 1st line of drugs against Mycobacteria TB; TMA: Transcription Mediated Amplification

Introduction

Tuberculosis (TB) is a chronic infectious disease, caused by *Mycobacterium*, having high morbidity and serious health implications in infected individuals (World health Organization, 2013). TB is mainly a pulmonary disease, which may also spread out of respiratory system into the bloodstream, establishing itself in extra-pulmonary organs and becoming deadly [1]. It may further lead to its establishment in body as latent state in infected person persisting for years; latent dormant bacteria are capable of revoking later in life. The occurrence of TB disease is as much as one in five registered TB patients [2]. This disease occurs mainly in people, having impaired immunity and is found to commonly co-occur among patients infected with HIV infection. In women, the TB is found to be associated, during pregnancy and often contributes to infertility and maternal mortality [3,4]. TB disease not only impairs health, but also the socioeconomic status and the development of life, perpetuating the poverty cycle. The prevalence of TB in India was studied by the Indian Council of Medical Research, establishing the national program of 'directly observed therapy short course (DOTS) strategy, in India, approved by the World Health Organization (WHO) and the revised national TB control program revised national tuberculosis control program (RNTCP) [5]. In India, since 2012, TB is now declared to be 'notifiable disease of the nation' by the RNTCP [6]. The infection still remains one of the deadliest diseases in the country, and makes it worst with the development of resistance in the strains. Apart from just an increase in occurrence of this disease, there is also increase in spread of multi-drug resistant tuberculosis bacteria (MDR-TB), in the both

newly diagnosed patients, and previously treated cases. *Mycobacterium* can establish as latent tuberculosis, a condition, where bacteria stays in the body for a long time asymptomatically, which may revoke later in life causing tuberculosis disease again. Latent TB is normally is non-infectious but the active TB *Mycobacterium* can get passed to another person, asymptomatically, establishing itself as a chronic lymphatic infection. The bacteria, which can further develop hematogenous spread in lymphatics or other visceral organs as reported earlier [7]. Skeletal tuberculosis are complicated with psoas abscess which are mainly diagnosed using positron emission tomography-computed tomography [8], while the abdominal tuberculosis can also be identified using MDCT enterography [9]. The extra-pulmonary manifestation was also reported in pericardial fluid [10], as osteoarticular TB, the important forms of extra pulmonary TB, have a significant consequence if not recognized early and treated. Involvement of weight bearing joints and spine is also known. In high prevalence areas, young adults are more commonly affected. A high degree of clinical suspicion along with the radiological, microbiologic and biopsy findings are important for diagnosis and starting ATT, is main strategy [11]. New method of fine-needle aspiration cytology (FNAC) and fluid cytology are also demonstrated to be important in detection of extra-pulmonary tuberculosis as described earlier [12-18]. WHO has declared latent tuberculosis as too expensive and unaffordable for patients [19].

The treatment of infected patient with *Mycobacterium* include, the treatment with first line drugs, including, Isoniazid (INH), Streptomycin (STREP), Rifampicin (RIF), Ethambutol (ETHAM), Parazinamide (PYRA). The infection with *Mycobacterium tuberculosis* causes TB disease, which is mainly treated, using the regimen of Isoniazid (INH) and Rifampicin (RMP), drugs while non-tuberculosis *Mycobacterium*, using various combinations of drugs depending on infecting organism and its drug sensitivity. The typical histopathology view of TBC synovitis include caseous granulomas, surrounded by epithelioid histiocytes and multinucleated giant cells. The tissue infected by *Mycobacterium* (typical or atypical TBC) usually does not give a positive reaction with Ziehl-Nielsen stain. TB organism was found in the patients with the kidney transplant described earlier [13].The TB of prostate is less common when compared with vesiculo-seminal and epididymal TB [14], while the cases of ocular and extra ocular TB and 45 cases of isolated ocular TB were earlier identified in Italy. The cases of *Mycobacterium* induced uvitis also reported [15]. The clinical pictures were of active, bacteria leading to vision threatening cataract were reported [16], while genital tuberculosis leading to infertility in women have been known. Multidrug-resistant tuberculosis (MDR-TB) is tuberculosis disease caused by organisms, which show high-level resistance to drug Isoniazid and Rifampicin, although the bacteria may or may not show resistance to other anti-TB drugs. There is a constant increase in MDR-TB around the world, both among new cases, as well as in the previously treated ones as reported earlier by WHO [4,18]. The development of multi drug resistant bacteria (MDR) occurs due to interrupted, usage of antibiotics, by patient, who halts the medicine ragmen upon feeling better or due to lack of affordable drugs sufficient to kill 100% bacteria. Such organism there by, become resistant, even to the two most powerful anti-*Mycobacterium* drugs like INH and RMP. The first-line treatment anti-TB drugs may result in further spread of MDR bacteria in the Population [19]. Earlier, we had reported utilization of modern molecular biology methods in conjugation with classical methods for identification of *M. tuberculosis* infection to assist with the judgment for prescription of right therapy for the *Mycobacterium* infected

individual [20], The prevalence of pulmonary tuberculosis in district Jabalpur were also reported [21]. Since the modern molecular biological methods are still very expensive for the patients, in India. We present here with the prevalence detected of the same in this study. India is among 27 MDR-TB countries, we studied prevalence of MDR-TB existence in central state of India. India has huge burden of MDR-TB and is included among 27 countries, holding high MDR-TB [1,19]. The diagnosis of tuberculosis is not still affordable for the general people in India. We used IGRA test to detect test the serum of clinical suspects for release of Interferon upon exposure to *Mycobacterium*. Diagnosis of latent TB is though difficult, but the treatment of it is required to reduce the global stress due to tuberculosis. Earlier we had demonstrated our findings of *M. tuberculosis* utilizing combination of more cost effective classical microbiology and biochemistry methods, monitoring the growth and cultivation of *Mycobacterium*. Occurring in tuberculosis in central state of India [20]. Sensitivity for diagnosis and recording the prevalence of *Mycobacterium*, there drug susceptibility testing in the population infected with *Mycobacterium* sp. in central state of India, done in our Indore lab. In this work, we utilized interferon gamma release assay (IGRA) by *Mycobacterium* for detection of *Mycobacterium* in clinical suspects in year 2014-2015.

Material and Methods

Study settings

This study was done at Central Lab Onquest, Indore (MP.), which is a private NABL accredited lab, in Indore, MP. The data presented here with are tested in clinical suspects in population of city and around Indore in January 2014 to June 2015, where we were able to test.

Processing of specimens samples were obtained from the hospitals of Indore, in the laboratory in a cold box and were processed on the same day or were kept at +4°C in refrigerator, until their processing was done. The tissue samples were first decontaminated using N-acetyl-l-cysteine, 2% sodium hydroxide and sodium citrate, PBS (pH 6.8). As described earlier by Deva R et al. 2014, in short, we tested a total of samples from TB suspects in Indore and its surrounding region is presented, herewith in this report, in parallel we tested the serum samples for IGRA using ELISA. Patient demographic data like age, gender, address were obtained. We tested serum from clinical suspects were initially to screen them for tuberculosis infection by IGRA method. We tested the samples in our lab for detection of causative agent using the method developed by us as described earlier [21-32]. The tests were performed, the samples used included Sputum, BAL, pus, peritoneal fluid, pleural fluid, lymph node from clinical suspects using microbiological cultivation method and IGRA analysis of serum from clinical suspects done with the consent of patient under Medical supervision, according to guidelines for good clinical laboratory practice (GCLP) in our NABL (National board for accreditation for testing and calibration for laboratory.

Statistical analysis

Comparision of IGRA test was carried out by using chi-square (χ^2) and probability P-value <0.05 was considered significant.

Results

In year 2014 to 2015 49 male samples were analyzed and 86 female samples blood samples were analyzed for IGRA out of them 535 males and 29% females showed positivity of IGRA test as shown in Table 1.

We were able to isolate *Mycobacterium* from 30.4% isolates from sputum samples, extra-pulmonary samples 14.8%, around 6% from fluids as shown in Table 2.

We found 53% samples to be positive from male group compared to 29% from female group of patients as shown in Table 1.

Age (Years)	Total Male Samples Tested	No. of Male Negative	No. of Male Positive	Total Female Samples Tested	No. of Female Negative	No. of Female Positive
1-14		3	0		2	2
15-24		7	5		17	8
25-39		8	4		37	5
40-54		6	7		1	4
50-60+		1	5		4	5
Not known		1	2		0	1
Total	49	26	23 (53%)	86	61	25 (29%)

Table 1: Age distribution of detected *Mycobacterium* test by IGRA method in patients' blood sample.

Number	Type of sample	Total no. (%)	Sample	Total number
1	Pulmonary	Pulmonary 115 (30.4%)	Sputum	68
2	Pulmonary		Broncheo Alveolar Levage	47
3	Extra pulmonary	20 (14.8%)	Pus	14
4	Extra pulmonary		Peritoneal fluid, Pleural fluid, Lymph node	6

Table 2: Isolation of *Mycobacterium* sp from various clinical suspects using microbiology methods.

We could detect *Mycobacterium* from different samples from clinical suspects. As shown in Table 2, 30.4% of cultures were associated with pulmonary tissue and 14.8 with extra pulmonary tissues, while pulmonary samples were analyzed using sputum as test sample. To clarify that the population was associated with infections with drug resistant *Mycobacterium* we checked the drug sensitivity of samples, which had come to our lab for diagnosis. From various samples we found the drug sensitivity pattern of *Mycobacterium* as shown in Table 3.

Drugs sensitivity	Total Number of Resistant Bacteria Isolated Form the Population of Indore
INH	25 (41.6%)
RIF	22 (36.66%)
PYRA	14 (23.3%
ETHAM	18 (30%)
STREPTO	15 (25%)

Table 3: Drug sensitivity pattern of MTB isolates to anti tubercular (ATT) drugs.

Discussion

We analyzed and quantified the interferon gamma release against *Mycobactrium* in serum samples of clinical suspects of tuberculosis. We found 46% of serum positive patients by IGRA method. The test also indicates suspects reactivity to *Mycobacterium tuberculosis*. We found about 29% of female patients had reacted positively when the serum was tested. IGRA is an indirect test for infection of *Mycobacterium* which might get un identified by classical microbiological methods IGRA test cannot be used for measurement of tuberculosis diagnostic infection. Being an endemic infection in country [33] the regimen of RIF with ISO was proposed for latent infection as this treatment can be hazardous. It is important to diagnose the patients still having latent infection and also the patients having MDR infection to be cured before they stop any further spread of bacteria in population with developed strains of *Mycobacterium*. As shown in Table 2 we found association 68 isolates form sputum, 47 from BAL, 15 from extra pulmonary samples, including 14 from pus, 6 from peritoneal fluid, pleural fluid, and lymph nodes as shown in Table

2 for analysis of IGRA results we correlated the occurrence of and their drug sensitivity in culture in presence of different antibiotics. We found 41.6 % bacteria sensitive to INH, 36.66% to RIF, 23.3% to PYRA, 30% ETHAM and 25% to STREPTO. The patients were needed to be under strict medical completely to hold the establishment of bacteria to dormant latent infectious disease establishment to eliminate tuberculosis completely the tuberculosis of latent infection should be encouraged, apart from encouraging patient if infected by resistant strain to complete regimen. Resazurin Tube Method: rapid, simple, and inexpensive method for detection of drug resistance in the clinical isolates of *Mycobacterium tuberculosis* [34], evidence based management of drug resistant tuberculosis was put forwarded [35] and intensive therapy for treatment of latent TB was suggested [36] which intrigued us to study the existence IGRA in clinical suspects in population of Indore and the region around presented in this publication.

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