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 Leavage; 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
The treatment of infected patient with *Mycobacterium* include, the treatment with first line drugs, including, Isoniazid (INH), Streptomycin (STREP), Rifampicin (RIF), Ethambutol (ETHAM), Paraazinamide (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 pulmonary 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 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*.
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                          | 2                      | 2                      |
| 15-24      | 7                         | 5                    | 17                   | 8                          | 4                      | 4                      |
| 25-39      | 8                         | 4                    | 37                   | 5                          | 4                      | 5                      |
| 40-54      | 6                         | 7                    | 1                    | 1                          | 4                      | 5                      |
| 50-60+     | 1                         | 5                    | 4                    | 5                          | 4                      | 5                      |
| Not known  | 1                         | 2                    | 0                    | 1                          | 1                      | 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 155 (30.4%) | Sputum             | 68           |
| 2      | Pulmonary            | Broncho 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 Mycobacterium 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

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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, 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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