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Prevalence of MTB/NTM Infection in Pulmonary and Extrapulmonary Samples among Tuberculosis Suspects in India

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Abstract: ***Tuberculosis is still one of the most wide-spread and serious infectious diseases globally. In addition to the Mycobacterium tuberculosis complex, Nontuberculous mycobacteria (NTM) plays a significant role as causative agents of opportunistic infections in immunocompromised patients. This study was conducted to evaluate the positivity rate of NTM and MTB in different type of sample with the help of real time PCR. In this study we analyzed that positivity rate for the MTB was highest in the sputum samples followed by the Bronchoalveolar lavage (BAL) and urine samples 84.61%, 35.71% and 35.38% respectively. While 65 semen samples showed no positivity.The prevalence of NTM was found less as compared to MTB infection with pulmonary samples, while in extra pulmonary samples NTM positivity was high in comparison to MTB.***

Keywords: MTB, NTM, Real time PCR, multiplex real time PCR

1. Introduction

Tuberculosis (TB) still one of the most prevalent and severe infectious diseases throughout the world (1). World Health Organization (WHO) has estimated in 2012, there were an approximately 8.7 million new cases of TB and 1.3 million died from the disease (2). Geographically, the load of TB is highest in Asia and Africa. India and China together account for approximately 40% of TB cases of the world (3). Rapid detection of active TB infection is significant for the timely detection of new cases, better disease management and to institute suitable antimycobacterial therapy (4). Mycobacterium tuberculosis (MTB) remains one of the leading causes of morbidity and mortality worldwide, but Mycobacterium species other than tuberculosis (MOTT) are increasingly important pathogens. The AFB (Acid Fast Bacilli) smear tests and cultures have low specificity, so there is a requirement of a laboratory test for specific detection of the M. tuberculosis complex as well as NTM that can be performed within a short period of time. In recent years, the development of molecular techniques has had a major influence on the diagnosis of mycobacterial infections (5). PCR-based assays for the detection of M. Tuberculosis and NTM approach the sensitivity of conventional cultures yet have the advantage of greater specificity and rapidity (3, 4, and 5). The multiplex PCR assay is a cost-effective diagnostic test because it allows for faster detection and a reduction in labor and reagent costs (7). Real-time PCR comprises amplification and fluorescence detection of an amplified DNA target in the same step. The purpose of this study was to evaluate the positivity of MTB and NTM in different type of samples with the help of real time PCR.

2. Materials and methods

This study was conducted at Oncquest Laboratories adj. Safdarjang hospital, 3 factory road New Delhi, India from July 2013 to July 2014 for detection of MTB/NTM, using 1146 clinical specimens. The clinical specimens contained both pulmonary and extrapulmonary specimens. Ethical approval was not needed for the current study as all samples were received for the clinical diagnosis and prior consent was taken from the patient that remaining sample can be used by oncquest laboratories for research purpose and identification of the subject will not be disclosed.

2.1 DNA extraction

Chromosomal DNA was extracted from the clinical samples by the DNA extraction method, as described in kit manual. Add 1ml of sterile water to the prepared sediment, centrifuge at 13,000 rpm for 5 minutes, and discards the supernatant with pipette. Add 100µL of DNA extraction solution to the sediment and vortex for 30 seconds. Locks the tube cap using a cap lock and boil for 20 minutes on heat block, centrifuge at 13,000 rpm for 5 minutes. Use 5µL of the supernatant as PCR template.

2.2 Real-time PCR

Real-time PCR amplification for MTB Complex and NTM was performed using the Anyplex™multiplex real time PCR kit (Seegene, Seoul, Korea), in accordance with the manufacturer's protocol in a Rotor gene Q (version 2.0) system, The Anyplex MTB/NTM Real time detection (V1.1) is a multiplex real-time PCR assay that permits the amplification of target DNA of Mycobacterium (NTM), Mycobacterium tuberculosis (MTB) and internal control. IS6110 gene and MPB64 gene were targeted for the detection of MTB and 16S rRNA gene was targeted for the detection of NTM. The cycling conditions were 15 min at 95°C and 45 cycles of 30 s at 95°C, 60 s at 60°C (single acquisition of fluorescence signals).

3. Results

A total of 1146 clinical samples were collected: 122 menstrual blood, 623 endometrium biopsy, 65 urine sample, 28 pus, 26 sputum samples, 63 csf, 29 ascitic fluid, 29 broncho aspirates, 66 pleural fluid samples, 24 peritoneal fluid, 65 semen, 21 synovial fluid 14 bal samples. In this

study we analyzed that highest positivity rate for the MTB were in the sputum samples followed by the BAL (bronchial alveolar lavage) and urine samples 84.61%, 35.71% and 35.38% respectively. While in 65 semen samples no sample was found positive for the MTB (table: 1).

Table 1: Positivity rate of the MTB In the samples

Sample type	*No of samples*	*NTM Positive*	*%Positivity*
Menstrual Blood	122	14	11.47 %
Endometrium	623	144	23.11%
Urine	65	23	35.38%
Pus	28	6	21.42%
Sputum	26	22	84.61%
C.S.F	63	14	22.22%
Ascitic Fluid	29	4	13.79%
Pleural Fluid	66	16	24.24%
Peritoneal Fluid	24	2	8.33%
Semen	65	0	0.00%
Synovial Fluid	21	1	4.76%
BAL	14	5	35.71%
Total	1146	251	21.90%

The infection of the NTM is less as compared to the MTB in the pulmonary samples and in the extrapulmonary samples the positivity rate of NTM is very high. Highest positivity was observed in menstrual blood samples followed by the urine (table 2).While the male patients were observed to higher positivity for MTB infection and NTM infection was observed more in female patients (table 3,4).

Table 2: Positivity rate of the NTM In the samples

Sample type	*No of samples*	*NTM Positive*	*%Positivity*
Menstrual Blood	122	28	22.95%
Endometrium	623	34	5.47%
Urine	65	7	10.76%
Pus	28	0	0.00%
Sputum	26	1	3.84%
C.S.F	63	0	0.00%
Ascitic Fluid	29	0	0.00%
Pleural Fluid	66	0	0.00%
Peritoneal Fluid	24	0	0.00%
Semen	65	1	1.53%
Synovial Fluid	21	0	0.00%
BAL	14	0	0.00%
Total	1146	71	6.19%

Table 3: Positivity rate (%) of MTB gender wise (n= 1146)

Positivity rate (%) of MTB gender wise (n= 1146)			
	Female(845)	Male (301)	Total
Positive	168	83	251
Negative	677	218	895
% Positivity	19.88	27.57	21.9

Table 4: Positivity rate (%) of NTM gender wise (n= 1146)

Positivity rate (%) of NTM gender wise (n= 1146)			
	Male (301)	Female(845)	Total
Positive	8	63	71
Negative	293	783	1076
% Positivity	2.65	7.45	6.19

4. Discussion

Mycobacteria are responsible for variety public health implications in the world. The differentiation of Mycobacterium tuberculosis from nontuberculous mycobacteria (NTM) is of prime significance for disease control and choice of antimicrobial therapy. Although progress in molecular diagnostics, the ability to fast diagnose M. tuberculosis infections by PCR is still insufficient, largely because of the possibility of false-negative reactions. Real-time PCR has been used to differentiate members of the M. tuberculosis complex from Non tuberculous mycobacteria (8, 9, 10). The positivity rate of the Mycobacterium and Non tuberculosis bacterium varies in different type of samples. We confined our study regarding the prevalence of MTB and NTM in different type of samples. During this study we find that the sputum samples have the maximum positivity followed by BAL and Urine samples. While menstrual blood sample have highest positivity for Non Tuberculosis Mycobacterium. In this study we also find that no sample was positive for MTB in all semen samples and in case of NTM infection Pus, C.S.F, Ascitic Fluid, Pleural Fluid, Peritoneal Fluid, Synovial Fluid, and BAL Fluid.

5. Conclusion

The above study indicated that real time PCR can be a method of choice in quick and correct diagnosis of MTB and NTM in pulmonary and extra-pulmonary samples. Early diagnosis is of great significance because of the high morbidity in developing country like India. The increased incidence of NTM emphasizes the need of correct species identification. This data also support the use real-time PCR assay with dual targets to improve MTB diagnosis in clinical samples. Moreover, our study reflects the prevalence of MTB and NTM in different type of samples in Indian scenario.

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