Evaluation of Polymerase Chain Reaction and Cobas TaqMan Real Time PCR in the Diagnosis of Tuberculosis: Indian Prospective

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Abstract Background & Objectives: Globally, tuberculosis (TB) still remains a major public health problem. India is a high TB burden country contributing to 26 per cent of global TB burden. Pulmonary tuberculosis (PTB) cases are more common (~ 90% of cases) while extra pulmonary tuberculosis (EPTB) constitutes around 10 to 20% of all tuberculosis cases in India. The diagnosis of the EPTB cases is difficult because of few bacilli and consequently is associated with low sensitivity of Zhiel-Neelson (ZN) smear and culture on LJ media. The present study evaluates the utility of PCR for the detection of *M. tuberculosis* in paucibacillary extra pulmonary and pulmonary tuberculosis samples. Methods: A total of 561 samples (553 EPTB & 8 PTB cases) were collected from the extra pulmonary and pulmonary tuberculosis patients which were processed for ZN smear, culture on LJ media and conventional PCR using two gene targets (IS6110 and MPB64). Results: The PCR positivity of IS6110 and MPB64 gene targets was found to be 91.3% (N=63/69) and 89.9% (N= 62/69) in majority of smear negative & culture positive (as a gold standard) extra pulmonary cases, respectively. However the PCR positivity was observed 100% in smear positive, culture positive Line probe assay tested MDR PTB cases (true positive controls; N=34). Further the PCR specificity was determined >95% (true negative healthy controls; N=26). The positivity of *M. tuberculosis* by IS6110 & MPB 64 gene targets was found to be range of 88% to 100% in various clinical paucibacillary extra pulmonary samples i.e. pleural fluid, ascitic fluid, lymph node, pus, CSF and others. Our data on 64 samples (non respiratory, n=63 & respiratory samples, n=1) revealed 40.6% positivity by Cobas TaqMan Real Time PCR (utilizing 16S rRNA probe; Roche, USA). Interpretation & Conclusion: Our data revealed that utility

of both PCR and Real Time PCR in rapid diagnosis of *M. tuberculosis* in paucibacillary extra pulmonary tuberculosis samples in Indian scenario.

Keywords Culture, *M.Tuberculosis*, In-House PCR Assays, Extra Pulmonary Tuberculosis

1. Introduction

Tuberculosis (TB) remains a major global health problem, despite the availability of highly efficacious treatment for decades. India has the world's largest burden of tuberculosis (TB), accounting for one-fifth (21%) of the global TB incidence. The global annual incidence estimate is 8.7 million, most of them children (especially in endemic areas), and it leads to approximately 1.4 million deaths annually [1].

Pulmonary (PTB) are more common (in about 90% of cases) [2] while extra pulmonary tuberculosis (EPTB) constitutes around 10 to 20% of all tuberculosis cases in India (which affects mainly the lymph nodes, meningitis, kidney, spine, and growing ends of the bones), with a 25 to 50% case mortality rate within months. The most common form of extra pulmonary tuberculosis (EPTB) is tuberculous pleural effusion & lymphadenopathy. However, the disease in extra pulmonary tuberculosis cases most often remains undiagnosed and, even worse, untreated. Conventional methods like smear and culture on Lowenstein-Jenson (LJ) Media are of limited use in the diagnosis of extra pulmonary tuberculosis cases which is associated with low sensitivity because of few bacillary load, smear microscopy has both the problems of sensitivity and specificity [3 -5].

Several investigators reported 0.4-37% smear positivity & 12-45% culture positivity in variable proportional in different biological samples [6-10].

For the rapid and accurate diagnosis of infectious diseases, polymerase chain reaction (PCR) technique & TaqMan Real time PCR have attracted considerable interest because of high degree of sensitivity and specificity over the conventional methods, particularly with the hope of shortening the time required to detect and identify *Mycobacterium tuberculosis* in respiratory and non-respiratory samples. These molecular tools and methods can be used for the confirmation of identity of isolates, direct detection of gene sequences from the clinical specimen and also molecular detection of drug resistance [4, 11-13].

The role of PCR in the diagnosis of extra pulmonary tuberculosis cases has been evaluated extensively as an alternative diagnostic tool and has yielded variable results, with sensitivities ranging between 42 and 100% and specificities ranging between 85 and 100% using various PCR targets such as IS6110, MPB64 (MPt64), TRC4, GCRS, etc. However, due to variability in the sensitivity rates in different studies, the role of PCR remains controversial [3, 9, 11, 14-16].

Highly conserved insertion sequences, IS6110, is most commonly used in the detection of *M. tuberculosis*. The range of IS6110 copies among isolates varied from 0-19 in the *M. tuberculosis* genome [16, 17]. However, the sensitivity and specificity of IS6110 sequence in the diagnosis of tuberculosis remains uncertain, and needs to be include other various PCR targets reliable screening test for tuberculosis in clinical specimen such as devR, TRC4, GCRS, MPB64 (MPt64), etc. [5, 11, 14,15]. MPB64 is secretory protein, it has been implicated in the virulence and pathogenesis of *M.tuberculosis* [18]. These assays targeting various gene segments, have abbreviated the turn around time for definitive mycobacteriological detection in the laboratory to 1–2 days, besides being more sensitive than conventional methods. A prompt diagnosis is indispensable for initiating appropriate treatment. The Cobas Amplicor *M.TUBERCULOSIS* assay for direct detection of *M.tuberculosis* complex (*M.TUBERCULOSISC*) in pulmonary tuberculosis samples have been used in many studies [12, 19, 20, 21]. The Amplicor assay was approved by the FDA for testing on smear-positive respiratory samples. The Cobas Amplicor *M.TUBERCULOSIS* assay is based on amplification of a segment of the 16S rRNA gene, followed by colorimetric detection of the PCR product by probe hybridization. The present study evaluates the utility of PCR (IS6110 and MPB64 gene targets) & TaqMan Real Time-PCR in the detection of *M.tuberculosis* in majority of smear negative paucibacillary extra pulmonary samples in Indian scenario.

2. Material & Methods

2.1 Study Subject

From 2011 to 2013 year, we conducted this study in National Institute of Tuberculosis and Respiratory Diseases, New Delhi, India. Different categories of samples (sputum for pulmonary tuberculosis and body fluids for extra-pulmonary cases) were collected from all study population. Current study was approved by research & ethics committee of National Institute of Tuberculosis and Respiratory Diseases in 2011.

2.2. Laboratory Methods

2.2.1 Collection of the Clinical samples

A total of 561 clinical samples [(423 male; age mean 38.5) and (138 female; age mean 31.2) of suspected tuberculosis cases were collected in sterile container from the outdoor and indoor Department of National Institute of Tuberculosis and Respiratory Diseases, Delhi for this pilot study during the period of 2011-2013 year. The clinical samples were from Delhi (N=157), Delhi NCR (N=322), Bihar (N=9), Haryana (N=25), Rajasthan (N=1), Uttar Pradesh (N=41), Uttaranchal (N=3), Tamilnadu (N=1) and West Bengal (N=2).

A total 561 clinical samples included, 416 pleural fluid, 31 pus, 47 ascitic fluid, 34 lymph node aspirate, 5 cerebrospinal fluid (CSF), 4 endometrial fluid, 6 peritoneal fluid, 6 sputum, 2 bronchial wash and 10 others were processed for the investigation of smear, culture on Lowenstein Jenson (LJ) media and for conventional & Real Time PCR to detect *M.tuberculosis* identification in clinical samples (Figure 1). Majority of the samples were pleural fluid. A detailed clinical history, sex, and age were also collected from the requisition form that accompanied with samples.

On the other hand healthy controls (N=26) TB negative and TB positive (smear positive, culture positive Line probe assay tested in MDR PTB; N=34) were also recruited as negative and positive controls H37Rv, *M.tuberculosis*, respectively (Figure 2 and Figure 3).

Inclusion criteria: The inclusion criteria of the study was recruitment of tuberculosis samples which included pleural fluid, ascitic fluids, CSF, lymph nodes aspirates, pus, endometrial fluid, peritoneal fluid, sputum, bronchial wash and others samples) with required sufficient volume to process the paucibacillary samples.

Data of clinically presentations, radiological and other laboratory examinations for diagnosis of extra-pulmonary tuberculosis (included smears and culture and other cyto-pathological reports wherever available etc) were also collected from OPD and ward for these patients.

2.2.2. Processing of the samples

All 561 samples were decontaminated by standard protocol i.e. *N*-acetyl-L-cysteine (NALC)–sodium hydroxide (NaOH) procedure which included 2% NaOH, 2.9% trisodium citrate, 0.5% NALC [22]. The processed samples were used for ZN smear, culture on LJ medium (bacteriological identification as a gold standard) and for

DNA extraction from all body fluids as well as sputum by QIAamp DNA Mini Kit (Qiagen, Germany) and by Amplicor Respiratory samples Preparation Kit according to manufacturer's instructions and protocols (Roche, USA). Equal volume (100µl) of decontaminated samples was used in DNA extraction for conventional PCR and Real Time PCR method. Eluted DNA was stored at -20°C. To avoid contamination during DNA extraction and amplification, strict precautions were taken, including separate areas for DNA extraction, reagent preparation, amplification and product detection and regular meticulous cleaning of surface with 10% hypochlorite were also applied to maintain the standard molecular laboratory procedures.

2.2.3. DNA Amplification for *Mycobacterium tuberculosis* Detection

Amplification of bacterial DNA was performed by using In-House PCR (IS6110 and MPB64 gene targets) in extracted DNA of all clinical samples as per the below mentioned protocols.

2.2.3.1 In-house PCR

Total 25µl of PCR reaction volume was containing 1x

PCR buffer, 0.2 mM dNTPs, 50ng of each primer (IS6110: Forward 5'- CCT GCG AGC GTA GGC GTC GG-3' and Reverse 5'-CTC GTC CAG CGC CGC TTC GG-3') & MPB64: Forward 5'-TCC GCT GCC AGT CGT CTT CC-3' & Reverse 5'-GTC CTC GCG AGT CTA GGC CA-3' [14, 23], 1 unit of *Taq* polymerase (Banglore Genei, India) and 10µl of tested sample DNA along with positive control (*M.tuberculosis* H₃₇Rv) and negative control lacking DNA (nuclease free water in place of template DNA).

The amplification was accomplished on a thermal cycler (9700 Applied Biosystem, thermal cycler, USA) with the following cycling parameters : 5min. at 94°C then one cycle of 30sec at 94 °C, 30sec at 68°C , 30sec for 72 °C , followed by 40 cycles , and a final extension of 10min. at 72°C. The PCR products were resolved by 2% agarose gel electrophoresis for 60mins. The product size of IS6110 (123bp) & MPB64 (240bp) were visualized on 2% agarose gel electrophoresis for identification of these single gene targets amplicons (Figure 2, 3 and 4). DNA from PCR negative samples was spiked with *M.tuberculosis* DNA (H₃₇Rv strain) and reamplified to check for amplification inhibitors giving false negative results.

Figure 1. Graphical representation of clinical tuberculosis samples (N=561) recruited for the study during 2011-2013 period. Further extra pulmonary samples were categorized as pleural fluid (N=416), ascitic fluid (N=47), pus (N=31), Cerebrospinal fluid (CSF, N=5), lymph node (N=34), endometrial fluid (N=4), peritoneal fluid (N=6), others (N=10). PTB samples were sputum (N=6) and bronchial wash (N=2).

Figure 2. IS6110 gene targeted In-house PCR for detection of *M. tuberculosis*. Electrophoretic separation of the amplicon into 2% agarose gel is documented across Lanes 1-14. Lanes-1 represents the negative control (nuclease free water), Lane-2 to 12 represent clinical healthy control samples, Lanes-13 (PC-1 diluted), 14 (PC-2 concentrated) was positive control (*M. tuberculosis* culture H₃₇Rv). The presence of a 123 bp amplicon in the lanes 13 and 14 indicated the presence of the target.

Figure 3. IS6110 PCR (123bp) in PTB cases. Lane 1- Negative control (NC), Lane 2 to 9- clinical sputum samples (smear positive, culture positive and LPA positive), Lane 10- positive control (PC) *H₃₇Rv M.tuberculosis* and Lane 11- 100bp marker(M). Lane 2, 3 & 4-10 indicated the presence of amplified gene target.

Figure 4. PCR targeted MPB 64 gene for the detection of *M. tuberculosis*. Electrophoresis separation of the amplicon into 2% agarose gel is documented across Lanes 1-4. Lane-1 represents the negative control (nuclease free water), Lane-2 represents clinical EPTB samples, Lane-4 was positive control (*M. tuberculosis* culture H37Rv). The presence of a 240 bp amplicon in the lanes 2 and 3 indicated the presence of the target.

2.2.3.2 Detection of *M.tuberculosis* by Real Time PCR

Qualitative detection of *Mycobacterium tuberculosis* (*M.TUBERCULOSIS*) complex DNA were carried out using COBAS TaqMan *M.TUBERCULOSIS* Test (Roche Diagnostic Systems, USA) in suspected tuberculosis samples. This procedure targets 16S rRNA genes of mycobacterium genus and detects amplified products with specific probe and colorimetric assay. The Cobas taqMan *M.TUBERCULOSIS* test utilizes the TaqMan 48 analyzer for automated amplification and detection. The test includes two major steps: (i) preparation of DNA (ii) real time PCR. The assay permits the detection of amplified *M.TUBERCULOSIS* amplicon and internal control DNA, which is amplified and detected simultaneously with the samples. The internal control DNA (non-infectious plasmid DNA) was used to detect polymerase inhibitor that might be present in samples.

A total of 64 samples (2 smear positive, 7 culture positive and 55 smear negative and culture negative samples) were performed for Real Time PCR.

2.2.3.2.1 DNA extraction

The Amplicor Kit (having its own positive and negative controls for the test) was used for DNA extraction of the samples and controls i.e. MYCO (-) & MYCO (+) using manufacturer's protocol.

Briefly, a 100 µl aliquot of the liquefied, decontaminated and concentrated respiratory and non-respiratory samples was used for *M.TUBERCULOSIS* detection. 500µl wash solution (RW) was added to each 100 µl sample tube, vortexed & centrifuged at 12,500x g for 10 minutes. 100 µl lysis reagent (RL) for each samples and 200 µl RL for 50µl controls [MYCO (-) & MYCO (+)] were added to the cell pellet, vortexed & incubated at 60°C±2°C for 45 minutes.

100 µl neutralization reagent (RN) was added to each specimen and control tube. Samples were vortexed for 5 seconds at half speed and stored at 2-8°C before addition into working master mix.

2.2.3.2.2 Amplification and detection

For 6 reactions PCR master mix (µl MYCO Mg²⁺, 50 µl MYCO IC and 460 µl *M.TUBERCULOSIS* MMX) was prepared according to manufacturer's protocol. 50 µl template DNA of samples or controls were added into each K-tube containing 50µl and loaded in Real Time PCR. The TaqMan 48 Analyzer determined the cycle threshold value (C_T) for the DNA of *M.TUBERCULOSIS* and checked whether the C_T values of the internal control DNA, the *M.TUBERCULOSIS* (+) Control, and the MYCO (-) Control were within the normal ranges. The *M.TUBERCULOSIS* (+) Control contains approximately 20 Copies/test of *M.tuberculosis* plasmid DNA sequence, for MYCO (-) Control the C_T values for *M.TUBERCULOSIS* DNA was

above the limit for the assay or no C_T value for *M.TUBERCULOSIS* DNA was obtained, but valid C_T value was obtained for the Mycobacterium Internal Control DNA.

3. Results

The present study determined the significance of conventional and Real Time PCR for the early detection of *M.tuberculosis* in clinical samples in smear negative, culture positive paucibacillary tuberculosis cases. Out of 561 clinical suspected tuberculosis samples, 72 were found to be culture positive (data represented in Table-1) subjected to smear microscopy after ZN staining, culture on LJ media (as gold standard) to analyzed the mycobacterium growth and further conventional PCR (IS6110 and MPB64) and Real Time TaqMan PCR (16SrRNA).

Table 1. PCR positivity in culture positive (Gold standard) suspected TB samples

Samples		Smear	Positive PCR for	
Type	No.		IS6110 N (%)	MPB64 N (%)
Ascitic fluid	1	Pos (n=0)		
		Neg (n= 1)	1 (100)	1(100)
		Total No. (%)	1 (100)	1(100)
Lymph node	5	Pos (n=0)		
		Neg (n=5)	5 (100)	5 (100)
		Total No. (%)	5 (100)	5 (100)
CSF	1	Pos (n=0)		
		Neg (n=1)	1 (100)	1 (100)
		Total No. (%)	1 (100)	1(100)
Pleural Fluid	48	Pos (n=7)	7 (100)	7 (100)
		Neg (n=41)	36 (87.8)	35 (85.4)
		Total No. (%)	43 (89.6)	42 (87.5)
Pus	9	Pos (n=0)		
		Neg (n=9)	8 (88.9)	8 (88.9)
		Total No. (%)	8 (88.9)	8 (88.9)
Others	5	Pos (n=2)	2 (100)	2 (100)
		Neg (n=3)	3 (100)	3 (100)
		Total No. (%)	5 (100)	5(100)
Total No. of EPTB	69	Pos (n=9)	9 (100)	9 (100)
		Neg (n=60)	54 (90)	53 (88.3)
		Total No. (%)	63 (91.3)	62 (89.9)
Sputum	3	Pos (n=2)	2 (100)	2 (100)
		Neg (n=1)	0	0
		Total No. (%)	2 (66.7)	2 (66.7)
Total No. of PTB cases	3	Pos (n=2)	2 (100)	2 (100)
		Neg (n=1)	0	0
		Total No. (%)	2 (66.7)	2 (66.7)
Total EPTB + PTB cases (%)	72		65 (90.3)	64 (88.9)

Table 2. PCR positivity in culture negative suspected TB specimens

Specimens		Smear	Positive PCR for	
Type	No.		IS6110 N (%)	MPB 64(%)
Ascitic fluid	46	Pos (n=0)	-	-
		Neg (n= 46)	30 (65.3)	21 (45.7)
		Total No. (%)	30 (65.3)	21 (45.7)
Lymph node	29	Pos (n=2)	2 (100)	1 (50)
		Neg (n=27)	21 (77.8)	16 (59.3)
		Total No. (%)	23 (79.3)	17 (58.6)
CSF	4	Pos (n=0)	-	-
		Neg (n=4)	1 (25)	2 (50)
		Total No. (%)	1 (25)	2 (50)
Endometrial Blood	4	Pos (n=0)	-	-
		Neg (n=4)	3 (75)	3 (75)
		Total No. (%)	3 (75)	3 (75)
Pleural Fluid	368	Pos (n=6)	5(83.3)	5 (83.3)
		Neg (n=362)	210(58.1)	204 (56.4)
		Total No. (%)	215 (58.4)	209 (56.8)
Pus	22	Pos (n=3)	2 (66.7)	2 (66.7)
		Neg (n=19)	14 (73.7)	14 (73.7)
		Total No. (%)	16 (72.7)	16 (72.7)
Peritoneal fluid	6	Pos (n=0)	-	-
		Neg (n=6)	4 (66.7)	2 (33.3)
		Total No. (%)	4 (66.7)	2 (33.3)
Others (EPTB)	5	Pos (n=0)	-	-
		Neg (n=5)	5 (100)	4 (80)
		Total No. (%)	5 (100)	4 (80)
Total No. EPTB cases	484	Pos (n=9; 1.9%)	7 (77.8)	7 (77.8)
		Neg (n=475; 98.1%)	290 (61.1)	267 (56.2)
		Total No. (%)	297 (61.4)	274 (56.6)
Sputum	3	Pos (n=0)	-	-
		Neg (n=3)	2 (66.7)	1 (33.3)
		Total No. (%)	2 (66.7)	1 (33.3)
Bronchial wash	2	Pos (n=0)	-	-
		Neg (n=2)	2 (100)	2 (100)
		Total No. (%)	2 (100)	2 (100)
Total No. PTB	5	Pos (n=0)	-	-
		Neg (n=5)	4 (80)	3 (60)
		Total No. (%)	4 (80)	3 (60)
Total PTB + EPTB cases (%)	489		301 (61.6)	277 (56.6)

Table 3. Comparative results of Cobas TaqMan Real Time PCR (16SrRNA gene) in TB samples

Smear/ culture (N, %)	smear +ve, culture +ve (a=0/64; 0%)	smear +ve, culture -ve (b=2/64; 3.1%)	smear -ve, culture +ve (c=7/64; 10.9%)	smear -ve, culture -ve (d=55/64; 85.9%)	Total PCR positive (N=64)
IS6110 PCR (%)	-	2 (100)	7 (100)	35 (63.6)	44 (68.7)
MPB 64 PCR (%)	-	1 (100)	7 (100)	32 (58.2)	40 (62.5)
Real Time PCR (16SrRNA; %)	-	2 (100)	5 (71.4)	19 (34.5)	26 (40.6)
Over all PCR positive (PCR & RT-PCR) (N=64)	-	2 (100)	7 (100)	42 (76.4)	51 (79.7)

PTB cases: N=1 ; EPTB cases: N=63; Total No = 64

Total Smear positives: n= 2(3.1%)

Total Culture positives: n=7(10.9%)

Total Smear and Culture positives: n=9(14.1%)

3.1. In-House Pcr Results

This study showed that the PCR positivity of IS6110 and MPB64 gene targets was found to be 91.3% (63/69) and 89.9% (62/69) in majority of smear negative & culture positive (as a gold standard) paucibacillary extra pulmonary cases, respectively. However the PCR positivity was observed 100% in smear positive, culture positive Line probe assay tested in MDR PTB cases (true positive controls; N=34 Figure 3). Further the PCR specificity was determined > 95% (true negative healthy controls or non-TB subjects; N=26; Figure 2). The PCR positivity of *M. tuberculosis* by IS6110 & MPB 64 gene targets was found to be 88% to 100% in various clinical samples paucibacillary smear negative extra pulmonary samples i.e. pleural fluid (88%), ascitic fluid (100%), lymph node (100%), pus (89%), CSF (100%) and others (100%; Table-1). On the other hand, our data in culture negative TB samples revealed 61.4% and 56.6% PCR positivity for IS6110 and MPB 64 gene targets, respectively (Table: 2)

3.2. Real Time PCR Results

Simultaneously, with conventional PCR (IS6110 and MPB64), only 64 samples were performed for COBAS TaqMan *M.TUBERCULOSIS* test Kit by Real Time PCR. Out of 64 samples, 68.7% (44/64) and 62.5% (40/64) samples were positive with both conventional PCR methods (IS6110 and MPB64, respectively) whereas *M.tuberculosis* was detected only in 40.6% (26/64) cases by TaqMan Real Time PCR technique. The over all PCR positivity (conventional and RT-PCR) was increased up to 79.7% (51/64; Table 3) to detect *M.tuberculosis* in tuberculosis samples.

4. Discussion

The major challenge in the diagnosis of extra pulmonary tuberculosis is detection of *M.tuberculosis*. Conventional methods including smear and culture have poor sensitivity

due to the paucibacillary load in the samples. Without an affirmative answers, clinicians could not start treatment due to the delayed diagnosis. Therefore, a high index of suspicion is necessary to make an early diagnosis, and quite often, more than one procedure is necessary for the confirmation of the diagnosis. With the development of novel & rapid molecular techniques, this delay in the accurate diagnosis of the disease is minimized but till date it could not replace traditional techniques in contrast to diagnostic modalities for other pathogens, like Chlamydia or Mycoplasma[5,7,9,10,12,24,25]. In order to identify tuberculosis in patients of suspected extra pulmonary tuberculosis for the detection of *M. tuberculosis* in clinical samples, two well established gene targets for conventional PCR (IS6110 & MPB64) were used. Current study showed the different PCR positive rates in various categories of body fluids. The PCR positivity of *M. tuberculosis* by IS6110 & MPB 64 gene targets was found to be 88% to 100% in various clinical samples paucibacillary smear negative extra pulmonary samples i.e. pleural fluid (88%), ascitic fluid (100%), lymph node (100%), pus (89%), CSF (100%) and others (100%). Our data revealed the significance importance of *M.tuberculosis* PCR by utilizing two gene targets in the diagnosis of paucibacillary smear negative cases in Indian scenario.

Extra pulmonary TB is a significant health problem (10-20%) in both developing and developed countries. The reported prevalence of extra pulmonary tuberculosis in India varies between 8.3% and 13.1% in different districts according to cohort analysis [3].

Several studies reported low detection rate by smear in extra pulmonary tuberculosis cases are less than 10% in pleural fluid [6]; 0.4% [26] in endometrial samples, 13% in lymph node, skin and other body fluids [19], 5.4% in peritoneal fluids, CSF, cervical lymph node biopsies, tissue biopsy and pericardial fluid [10] , zero percentage in endometrial biopsy, gastric aspirate and pus [9] whereas culture positivity was 4% in peritoneal fluids, CSF, cervical lymph node biopsies, tissue biopsy and pericardial fluid [10]; 43.3 % in lymph node, skin, other body fluids &

sputum [19], 12 to 70 % in pleural fluid [6] and 4.3% in endometrial biopsy, gastric aspirate and pus [9]. In contrast to above reported studies, the current study showed a significant utility of PCR and Real time PCR in smear negative, paucibacillary respiratory & non respiratory TB samples.

Various sensitivity of PCR using IS6110 has been reported in extra pulmonary samples i.e. 74.1% in tissue [27], 83% in pleural fluid, pleural tissue, and lymph node [15], 69.1 % and 87.5% in lymph node [5,12], 75% in clinical samples [7], 40% in EPTB [9]. Chakarvorty et al [15] reported that IS6110 PCR efficiency was 90.9% for pleural tissue and 85.5% for pleural fluid and 68.4% lymph node samples. But in our findings by IS6110 & MPB64, positivity was increased in pleural fluid (89%) and lymph node (100%) smear negative & culture positive samples. These results further emphasized the significance of molecular diagnostic method for earlier diagnosis.

It has been reported that the sensitivity of PCR can be increased by using one more sets of primers in EPTB cases i.e. 77% [5]. Similarly in our study, positivity of the PCR to detect M.tuberculosis was also increased (i.e. combined results of conventional and real time PCR in 64 tuberculosis samples) 79.7% (51/64). M.tuberculosis detection rate by single PCR was low i.e. 68.7% for IS6110, 62.5% for MPB64 PCR and 40.6% by Real time PCR. The low positivity rate through Amplicor Real time Roche assay in smear negative extra-pulmonary tuberculosis samples in our study revealed its more significance in smear-positive respiratory samples compared to smear negative paucibacillary extrapulmonary cases. It has been reported earlier that the Amplicor Real time assay has high sensitivity rate to detect M.tuberculosis in smear-positive respiratory samples [20].

Boukaline et al.19 reported the testing of EPTB cases by Real Time PCR method for M.tuberculosis diagnosis and analyzed the 53.8% sensitivity. Rimek et al.12 reported 87.5% & 45.5% sensitivity, 100.0% & 91.3% specificity of In-house PCR and the COBAS M.tuberculosis assays, respectively. This pilot study suggested the significance of the molecular tools in different body fluids of the suspected EPTB cases, compared to conventional method like smear (3.1%) and culture (10.9%).

5. Conclusion

This study reveals rapid molecular test such as PCR with more than one gene targets & Real Time PCR methods have significant role for the early diagnosis (by decreasing time duration) of extra pulmonary tuberculosis cases, which is not diagnosed by conventional test (smear & culture). Further study needs to include more gene targets and to develop multiplex PCR assays for rapid diagnosis of M.tuberculosis in developing countries like India.

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