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Original Research Article

Diagnostic value of polymerase chain reaction targeting insertion sequence IS1081 for the diagnosis of pediatric tuberculosis

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ABSTRACT

Background: Aim of this study was to evaluate the efficacy of PCR targeting IS1081in diagnosis of pediatric tuberculosis and compare the results with MGIT culture.
Methods: This prospective study was conducted in the department of pediatrics, S.N. medical college, Agra. 100 subjects (28 pulmonary 72 extra pulmonary) were registered in study. The specimens obtained from these cases were subjected to Ziehl–Neelsen staining (ZN), MGIT 960 TB culture and PCR targeting insertion sequence IS1081. Sensitivity, specificity, PPV and NPV of PCR were calculated in pulmonary and extra pulmonary specimens. The results of PCR IS1081 were compared to MGIT culture.
Results: Microscopy with ZN staining was positive in 12 (12%) samples. MGIT culture was positive in 44% samples with maximum positivity in sputum (70%). PCR IS1081 has shown 93.3% sensitivity in pulmonary tuberculosis, while PCR IS1081 has shown 93.1% sensitivity in extra pulmonary tuberculosis. In diagnosis of childhood tuberculosis PCR IS1081 was found to be statistically significant (p value <0.05) as compared with MGIT culture. Result was statistically significant (p value <0.05) in CSF samples only.
Conclusions: The study concluded that the PCR targeting sequence IS1081 technique is the most sensitive technique for a quick identification of MTB in pulmonary and extra pulmonary tuberculosis.

Keywords: MGIT, PCR IS1081, Pediatric tuberculosis, ZN straining

INTRODUCTION

Tuberculosis (TB) is a communicable disease that is one of the leading cause of death from a single infectious agent (ranking above HIV/AIDS). It is caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB). Global Tuberculosis report 2019 of World Health Organization (WHO) estimates show that globally there are 10 million incident cases of TB of which 80% are in Developing countries, with India ranked as the highest burdened country, accounting for one-fifth of the global TB incidence. Out of these, one fourth number of cases occur in pediatrics age group.[1]

Pediatric TB diagnosis is impeded by the difficulty of obtaining sputum samples from children and the paucibacillary nature of their disease that often necessitates invasive procedures such as gastric aspiration or bronchoscopy.[2]

The diagnosis of pediatrics tuberculosis largely depends on unreliable methods such as ZN microscopy, L-J culture, and Tuberculin test.[3] Direct AFB (acid fast bacilli) smear and L-J (Lowenstein-Jensen) culture lack sensitivity for diagnosis of tuberculosis and culture takes at least six to eight weeks. Smear for AFB is reported to be positive in 10 to 37 percent of patients and L-J culture is reported to be positive in variable proportion (12 to

80%) in different body fluids.[4] The existing methodologies remain ineffective due to less number of mycobacteria and/or because of time consuming procedures. Accurate and early diagnosis of TB is crucial for its effective management and timely treatment. At present, nucleic acid amplification based assays are the most suitable choices for the identification of *Mycobacterium tuberculosis* with high degree of sensitivity and specificity.[5,6] Several studies have been performed to detect *M. tuberculosis* in pulmonary and extra pulmonary clinical samples using PCR targeting different DNA sequences (IS6110, MBP64, IS1081) of *M. tuberculosis*.[7]

The purpose of this study was to evaluate the effectiveness of PCR in diagnosis of pediatrics tuberculosis, and to assess the performance of insertion sequence IS1081 based PCR assay as compared to conventional culture by MGIT method for the diagnosis of pediatrics tuberculosis.

METHODS

This prospective study was conducted in the Department of Pediatrics, S.N. Medical College, Agra and National JALMA Institute for Leprosy and Other Mycobacterial Diseases (I.C.M.R.), Agra from May 2011 to October 2013. Subjects less than 18 years diagnosed with TB using national guidelines on pediatric TB, 2012 were included in the study.[8] Those already receiving antitubercular therapy and those with other coexisting medical and surgical illnesses were excluded from the study. A written informed consent was taken from guardians. The study was approved by the Ethical Committee of the Institute.

Clinical details were recorded for all the study subjects. Complete blood count, Chest X Ray and tuberculin test with 2TU of tuberculin purified protein derivative were done in all subjects. CT scan head/MRI, USG abdomen/CT abdomen were done where required. In LN tuberculosis, FNAC was done. At least 5ml of various body fluids (CSF, gastric aspirate, sputum, pleural fluid, ascitic fluid and lymph node aspirate) were subjected to microscopy, cytological and biochemical examination. MGIT culture and PCR targeting IS1081 were performed in all the specimens.

Samples were decontaminated by N-Acetyl L-Cysteine (NALC) or sodium hydroxide (NaOH) for 15-20 min and centrifuged at 4000 rpm for 15 min. Processed sediment was transferred to a MGIT 960 tube containing Modified Middlebrooks 7H9 broth previously inoculated with 0.8 ml of the PANTA-growth supplement mixture.[9] DNA was extracted according to the CTAB-phenol chloroform extraction method. The extracted DNA template was used for the amplification using IS1081 insertion sequence primer specific to Mycobacterium tuberculosis complex. The forward and reverse primers used were MTC IS1081 F 5`-CTC TCG ACG TTCA TCG CCG-3` and R 5`-TGG CGG TAG CCG TTG CGC-3`.[10] The amplified product was electrophoresed into 1% agarose gel. The gels were stained with ethidium bromide and visualized in a UV-trans illuminator.

Statistical analysis

Data were analyzed using SPSS version 21.0. Sensitivity, Specificity, Negative predictive value (NPV) and Positive predictive value (PPV) of the tests were calculated. Chi square test was applied to compare the results of various tests. For all tests, p value <0.05 was considered statistically significant.

RESULTS

During the study period, 100 children of both sexes with a clinical manifestation of TB were enrolled in the study. 38 (38%) were females and 62 (62%) males giving an average sex ratio of 1:1.63. The mean age of patients was 5.34±1.5 years. Out of 100 samples, 28(28%) were pulmonary {sputum 10 (10%), gastric aspirate 18 (18%)} and 72 (72%) were of extra pulmonary tuberculosis {CSF 45 (45%), ascitic fluid 6 (6%), pleural fluid 9 (9%) and LN aspirate 12 (12%)}. History of tubercular contact was reported for 34 children (34%).

Table 1: Clinical parameters of study subjects.

Parameter		Number (%)
Age	1 month - <4 years	22(22%)
	4 - <8 years	38(38%)
	8 - <12 years	29(29%)
	>12 years	11(11%)
Sex	Male	62(62%)
	Female	38(38%)
Tubercular contact history	Positive	34(34%)
	Negative	66(66%)
Nutritional status	Normal	22(22%)
	Weight for age or BMI <-3rd SD	35(35%)
	Weight for age or BMI between -2nd SD to -3rd SD	43(43%)
Tuberculin skin Test	Positive	41(41%)
	Negative	59(59%)
Type of tuberculosis	Pulmonary TB	28(28%)
	Extra pulmonary TB	72(72%)

About 22 children have normal nutritional status, 35 children were severely malnourished (weight for age or BMI <-3rd SD), 43 children were moderately malnourished (weight for age or BMI between -2nd SD to -3rd SD). Tuberculin Skin Test was positive in 41 (41%) children (Table 1). Chest Xray was suggestive of tuberculosis (cavity, consolidation, miliary shadow) in 33 subjects. USG / CT Abdomen was suggestive of tuberculosis {mesenteric lymphadenopathy (>15mm in

size or central necrosis), ascites, thickened omentum} in 9 cases. Neuroimaging (CT/ MRI) was suggestive of tuberculosis (tuberculoma, infracts, hydrocephalus, basal exudates) in 26 cases. CSF was suggestive of tuberculosis (lymphocytosis, protein >80mg/dl, sugar <40mg/dl) in 25 cases. Pleural fluid and Ascitic fluid examination was suggestive of tuberculosis (exudative, lymphocytosis) in 10 cases. LN Aspirate examination was suggestive of granulomatous inflammation in 7 cases.

Microscopy with ZN straining was positive in 12 (12%) samples. ZN straining was positive in 7 sputum, 3 gastric aspirate and 2 CSF samples. MGIT culture was taken as a gold standard method to diagnose pediatric tuberculosis. It was positive in 44% samples with maximum positivity in sputum (70%).

Table 2: Performance of PCR (IS1081) in diagnosis of pulmonary tuberculosis.

Parameters	Sputum (n =10)		Gastric aspirate (n=18)	
	MGIT (+)	MGIT (-)	MGIT (+)	MGIT (-)
PCR (+)	6	2	8	5
PCR (-)	1	1	0	5
Sensitivity (%)	85.7		100	
Specificity (%)	33.3		50	
PPV (%)	75		61.5	
NPV (%)	50		100	

Table 3: Performance of PCR (IS1081) in diagnosis of extra pulmonary tuberculosis.

Parameters	CSF (n=45)		Ascitic fluid (n=6)		Pleural fluid (n=9)		LN aspirate (n=12)	
	MGIT (+)	MGIT (-)	MGIT (+)	MGIT (-)	MGIT (+)	MGIT (-)	MGIT (+)	MGIT (-)
PCR (+)	17	17	2	3	3	3	5	3
PCR (-)	1	10	0	1	0	3	1	3
Sensitivity (%)	94.4		100		100		83.3	
Specificity (%)	37.0		25		50		75	
PPV (%)	50		40		50		62.5	
NPV (%)	90.9		100		100		75	

PCR IS1081 has shown 93.3% sensitivity (with 95% confidential interval), 46.1% specificity (with 95% Confidential Interval), positive and negative predictive value of PCR IS1081 was observed as 66.6% (with 95% CI) and 85.7% (with 95% CI) in pulmonary tuberculosis (Table 2). While PCR IS1081 has shown 93.1% sensitivity (with 95% confidential interval), 39.5% specificity (with 95% Confidential Interval), positive and negative predictive value of PCR IS1081 was observed as 50.9% (with 95% CI) and 89.4% (with 95% CI) in extra pulmonary tuberculosis (Table 3). In diagnosis of childhood tuberculosis, PCR IS1081 was statistically significant (p value <0.05) as compared with MGIT culture. Result was statistically significant (p value <0.05) in CSF samples only (Table 4).

DISCUSSION

In this study neurotuberculosis cases (45%) were highest, which is most severe form of pediatric tuberculosis. This may be due to majority of cases were undernourished and data collected from a tertiary center. Tuberculin Skin Test was positive in 41 (41%) children which is lower then 52.3% reported by Udani et al, which may be due to false negative results due to higher incidence of under nutrition in our study.[11] Microscopy with ZN staining was positive in 12 (12%) samples, which is comparable as reported by Hemant et al.[12] MGIT culture was taken as a gold standard method to diagnose pediatric tuberculosis because it takes less time as compared to conventional LJ medium. It was positive in 44% samples with maximum positivity in sputum (70%) which is comparable as reported by Rodrigues C et al, and Rishi et al.[13,14]

PCR IS1081 has shown 93.1% sensitivity (with 95% confidential interval) which is similar as reported by Fidelis et al, and Fatolahzadeh B.[15,16] This study showed that MGIT culture was positive in 44(44%) specimens, whereas IS1081 PCR showed that 74 (74%) specimens were positive for Mycobacterium tuberculosis. The difference was found to be statistically significant (p <0.05). PCR IS1081 was statistically significant (p <0.05) test as compared to MGIT culture in diagnosing CNS TB. PCR IS1081 showed 75.5% positivity in CSF similar to reported by Siddiqui et al.[17] PCR IS1081 showed 75% positivity in pulmonary TB which is higher than 43.75% reported by Niyaz et al, which may be due to blood sample used in latter study and there was also a history of previous ATT intake in most of the patients.[18] IS1081 showed 67.9 % positivity in extra pulmonary TB which is lower than 84.6% reported by Bahador et al, which was because of small sample size and taking only pleural fluid in account in latter study.[19] The conventional

Mycobacterium tuberculosis detection techniques based on microscopic examination, culture of Mycobacterium tuberculosis on LJ medium and Tuberculin Skin Test are still in widespread use for diagnostic purposes, though they still fail to provide the required sensitivity and specificity. MGIT culture is better than conventional LJ medium as it has high yield and is less time taking. The PCR IS1081 would be particularly useful in the diagnosis of childhood TB as it is highly sensitive and rapid method test. Disadvantages of PCR IS1081 are high cost and low specificity due to high false positive results due to non-tubercular mycobacteria. Also, it does not differentiate between live and dead bacilli.

CONCLUSION

Diagnosis of pediatric tuberculosis is quite challenging due to inadequate sample and paucibacillary nature. Conventional methods like microscopy, LJ culture and serology are time consuming and have low yield. To prevent the dreadful complications of tuberculosis it is essential to diagnose early and start treatment. MGIT culture is better than conventional LJ medium as it has high yield and is less time taking. PCR IS1081 is highly sensitive and rapid method in the diagnosis of childhood TB. It is especially useful in diagnosis of extra pulmonary tuberculosis.

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