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Evaluation of Pcr as Diagnostic Tool in Pediatrics Pulmonary Tuberculosis

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ABSTRACT

Introduction: *Tuberculosis even today, remains major health problem among children in the world especially in India. Amplification of M. tuberculosis specific DNA sequences (IS 6110) in clinical samples is the most sensitive and rapid method of detection available 56677.* ***Aim:*** *Evaluation of PCR as a diagnostic tool in Pulmonary Tuberculosis in children*

Methods: *Observational diagnostic study was done in children diagnosed with pulmonary tuberculosis. These specimens were subjected for microbiological studies and PCR studies. M. tuberculosis culture was done using LJ medium.* ***Results:*** *32 children were included in the study with pulmonary tuberculosis. Microbiological method has shown negative to all clinically confirmed cases. PCR shown 25 cases positive among 32 cases.* ***Conclusion:*** *Polymerase chain reaction in diagnosing pediatric pulmonary tuberculosis cases is superior to conventional microbiological method.*

KEYWORDS : Pediatrics, PCR, TB, Culture

Introduction

Tuberculosis is a chronic bacterial infection caused by Mycobacterium tuberculosis that is characterized by the formation of granulomas in infected tissues and by cell mediated hypersensitivity. Tuberculosis still ranks as "Captain of the Men of Death" the most important infectious disease in the world in terms of morbidity and mortality. Mycobacterium tuberculosis infects one third of the world's population and there are more than 6 million cases worldwide. Tuberculosis is the leading cause of death world due to any single infectious agent[1]. One of the major obstacles of diagnosing tuberculosis in children is the absence of a sensitive, specific and rapid method of diagnosis. Clinical signs and symptoms are less helpful, as more than 50% show no symptoms at all, at the time of presentation[2]. The "Gold standard" of diagnosis of tuberculosis in adults is isolation of mycobacterium tuberculosis, which unfortunately may not be applicable to children. Acid fast smear of a sputum sample to be positive, 5000 to 10,000 organisms to be present[3]. This amount of bacillary burden is rare in children and sputum cannot be obtained from children younger than about 10 years of age. The yield of three consecutive morning gastric aspirates for M. tuberculosis in children is only 30 to 50%[4]. Although yield from infants can be as high as 70%[5]. The yield of M. tuberculosis from bronchoscopy has been lower than from properly obtained gastric aspirates[6]. Even then, the delay in positive culture results currently prohibits their use in rapid diagnosis of tuberculosis[7]. Isolation of M. tuberculosis on solid medium often takes 3 to 6 weeks; where as a liquid medium (BACTEC) takes 9-16 days[8]. PCR is defined as a method to make many copies of discrete fragments of DNA present in a clinical specimen, thus facilitating detection of nucleic acids present initially only in very small (i.e., Picogram) quantities. A technical definition of the technique would be successive incubation steps at- different temperatures using a heat-stable DNA- dependent DNA polymerase. Essentially, PCR is a way to make millions of identical copies of a specific sequence, which is unique to a particular microbe. Fewer than 10 input molecules of target DNA can lead to positive signal. PCR can be performed within 8 hours[9].

Aim

Evaluation of PCR as a diagnostic tool in Pulmonary Tuberculosis in children

Material and Methods

Observational diagnostic study was conducted in Institute of Child Health and Hospital for Children, Chennai. Institutional Ethics Committee approval and Informed consent from children parents were obtained. Children from 6 months to 12 years suspected to have tuberculosis based on symptoms; contact history, clinical presentation and investigations were included. Complete physical examination including nutritional status, external markers for tuberculosis and detailed system examination were done. These children were subjected for relevant investigations like complete hemogram, Mantoux test, X ray chest, Resting gastric juice (Gastric lavage) and / or Broncho alveolar lavage collected. These specimens were tested in Tuberculosis Research Center, Chennai. These specimens were subjected for microbiological studies and PCR studies. M. tuberculosis culture was done using LJ medium.

Results

32 pediatric pulmonary tuberculosis cases were included in the study. Of the Pulmonary Cases, 71.8% of the cases contributed by persistent pneumonitis, with cavitary tuberculosis 9.3% in the second category and bronchiectasis and pleural effusion 6.25% each. Collapse – consolidation, and hilar node each contributing 3.1% (Table1)

TABLE 1 – TYPES OF PULMONARY CASES

Sl.No	Types	n	%
1.	Persistent Pneumonia	23	(71.8%)
2.	Cavitary Tuberculosis	3	(9.3%)
3.	Bronchiectasis	2	(6.25%)
4.	Pleural Effusion	2	(6.25%)
5.	Collapse Consolidation	1	(3.1%)
6.	Hilar Node	1	(3.1%)
	Total	32	100%

TABLE -2 DIAGNOSITC INDICATORS OF TYBERCULOSIS IN PULMONARY CASES

Indicators - n	Contact n (%)		Mantoux n (%)		CXR n (%)	
	+	-	+	-	+	-
Persistent Pneumonitis - 23	6 (26%)	17 (74%)	22 (95.7%	1 (4.3%)	23 (100%)	-
Cavitary Tuberculosis - 3	1 (33.3%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	3 (100%)	-
Collapse Consolidation - 1	1 (100%)	-	-	1 (100%)	-	-
Pleural Effusion -2	2 (100%)	-	-	2(100%)	2(100%)	-
Hilar Node -1	-	1 (100%)	1 (100%)	-	1(100%)	-

In the persistent pneumonia group 26% has positive contact history, 95.7% were positive Mantoux test and 73% had BCG scar. In the cavitary TB 33% had contact history, 66.7% were positive Mantoux and 66.7% had BCG scar. All had radiological evidence for tuberculosis. (Table 2)

TABLE 3 – PCR RESULTS IN THE PULMONARY DISEASES

Cases	PCR +	PCR -
Persistent Pneumonitis (23)	18	5
Cavitary Tuberculosis (3)	2	1
Bronchiectasis (2)	2	-
Collapse Consolidation (1)	1	-
Pleural Effusion (2)	2	-
Hilar Node (1)	-	1
Total	25	7

In the Pulmonary tuberculosis patients 25 were positive for PCR and 7 were being negative. The PCR positive patients were followed up for 3 months and found to be asymptomatic. (Table 3). Pulmonary tuberculosis cases (32) proven by Mantoux test, contact with adult case of tuberculosis, clinical presentation, X-ray evidence. All the cases yielded negative microbiological evidence for M. tuberculosis but PCR picked up in these categories.

Discussion

In Sunilkumar Jatna etal[10] studied the diagnostic efficacy of PCR in pulmonary cases. Out of 10 cases which satisfied the clinical criteria of pulmonary tuberculosis, 2 were positive for PCR and AFB culture. The sensitivity was 20%. Our study none of the 32 cases studied, yielded positive microbiological proof. But 25 cases had shown positive in PCR. Shawar JM etal[11] evaluated PCR from 76 clinical specimens. Compared with the culture. PCR had 55% and 98% sensitivity and specificity respectively in agarose gel electrophoresis. Despite this low sensitivity a rapid positive PCR result was accurate and clinically useful, was their conclusion. Brinon - Noel A etal.[12] attributed the false negative results could have resulted from Presence of inhibitor not detected by the control amplification Clanidge etal,[13] reported the presence of inhibitors in 8% of clinical specimens. Scientists have reported inhibitors in clinical specimens can go as high as 20%. Non-homogenous distribution of bacteria in the specimen no that traction tested does not contain mycobacteria and Low number of Mai in the specimens. Kumar D etal[14] showed that nucleotide sequence to 136110 is not always present within strains of M. tuberculosis causing disease in India. Primers with different nucleotide sequence are now being used in India by a few laboratories to ensure that all the cases are detected by PCR amplification.

Conclusion

Polymerase chain reaction in diagnosing pediatric pulmonary tuberculosis cases is superior to conventional microbiological method. A negative PCR never eliminates tuberculosis as a diagnostic possibility.

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