Original Research Paper Medical Science

Evaluation of Polymerase Chain Reaction in Pediatric CNS Tuberculosis

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ABSTRACT

Introduction: The spectrum of tuberculosis disease caused by M. tuberculosis in children can range from asymptomatic primary infection to progressive severe form CNS tuberculosis.
Aim: Aim of this study is to evaluate PCR as a diagnostic tool in Central Nervous System Tuberculosis in children
Methods: Observational diagnostic study was done in children diagnosed with CNS tuberculosis. CSF specimens were subjected for microbiological studies and PCR studies. M. tuberculosis culture was done using LJ medium.
Results: 17 children were included in the study with CNS tuberculosis. Microbiological method has shown negative to all clinically confirmed cases. PCR had shown 11 cases positive among 17 cases.
Conclusion: Polymerase chain reaction in diagnosing pediatric CNS tuberculosis cases is superior to conventional microbiological method.

KEYWORDS Pediatrics, PCR, TB, CNS

Introduction

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] Adult tuberculosis is considered to be the fountainhead of pediatric tuberculosis.[2] This is because children with tuberculosis, rarely, if ever infect other children[3]. Extrathoracic disease is more common in children than in adults. Miliary tuberculosis is an early complication of primary infection as result of the release of large amount of bacillus into the circulation, leading to disease in two or more organs[4]. Tubercle bacilli are distributed by the blood stream in to all parts of the central nervous system during lymph hematogenous spread[5]. The tubercle bacilli can affect central nervous system in various ways, producing tuberculous meningitis[6], serous meningitis, tuberculoma[7], or mainly affect the spinal cord causing spinal tuberculous lepto menigitis[8]. Tuberculous meningitis arises from caseous foci. often very small one situated in brain or meninges (Rich Focus)[9]. The foci discharges tubercle bacilli into the subarachnoid space - thick gelatinous exudate has the prediction for the base of brain accounts for frequent involvement third, sixth and seventh nerves and optic chiasm. Tuberculous meningitis never seen in infants younger than 4 month of age, it is most common in children younger than 6 years of age. It usually appears 2 to 6 months after initial infection. For the clinical purpose TB meningitis has been divided into 3 stages[6]. Aids in the diagnosis of TB meningitis are history of contact with an adult with tuberculosis (however, this history is often negative because the incubation period of meningitis is short and the contagious adult has not been discovered yet[10]), positive tuberculin testing (only in 50% of cases, chest roentgenogram reveals changes in 43% of cases[11] and characteristic findings in spinal fluid. Spinal fluid will have increased pressure — with initial polymorphonuclear and later lymphocyte predominant response with progressive raise in the protein with formation of pellicle. Only about 50% of cases of tuberculous meningitis in children can be confirmed by spinal fluid culture. CT may show infarct or vasculitis and Hydrocephalus. Long term sequelae of TBM are numerous and include blindness, deafness; intracranial calcification, diabetes insipidus, obesity; paraplegia and mental retardation.[12] Tuberculoma occur most often in children younger than 10 years of age and are located at the base of brain around the cerebellum, in contrast to adults, where supratentorial location is common[13]. 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[14]. One of the most exciting developments in molecular biology in recent years has been the introduction of Polymerase chain reaction. Potentially, Polymerase chain reaction represents a direct application of biomolecular research technique from the bench to bedside and recognition of impact and promise of this technique, its developer, Kary Mullis, was awarded the Nobel prize in Chemistry for 1993[15,16].

Aim

Aim of this study is to evaluate PCR as a diagnostic tool in Central Nervous System Tuberculosis in children.

Materials 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 CNS 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 and other imaging studies like CT scan & USG and CSF analysis. CSF 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

17 pediatric CNS tuberculosis cases were included in the study. Out of 17 cases of TBM 29.4% had positive Mantoux test, 29.4% had positive contact, 88.3% had BC Scar, 52.9% had positive CSF findings and 9 had positive CT Findings. (Table 1)

TABLE – 1 DIAGNOSTIC INDICATOR OF TUBERCLULOSIS IN TBM CASES

	Positive n (%)
Mantoux	5(29.4%)
Contact	5(29.4%)
BCG Scar	15(88.3%)
CSF (Cells & Proteins)	9(52.9%)
CT Findings	Infract -2 Hydrocephalus
Total	17

TABLE 2 - EFFICACY OF PCR AGAINST CLINICAL GOLD STANDARD IN TBM

	TBM Cases
PCR Positive	11
PCR Negative	6
	17

In the CNS category totally 23 patients (17 cases diagnosed based on clinical, investigatory parameters and response to treatment) were studied. All the 17 cases were negative in CSF microbiological test. Out of the 17 cases 11 were picked by the PCR the sensitivity of 64.7%.

Discussion

Seth P etal[17] on from All India Institute of Medical Sciences analyzed the role of PCR in Diagnosing tuberculous meningitis. Out of 40 TBM cases (diagnosed based on defined clinical features supported by indirect evidences the CSF examination) none had bactoriologi.1 evidence for M. tuberculosis. Of the 40 cases 36 were positive by PCR - 85% sensitivity. Of the 49 control 3 were positive by PCR- Specificity 93.8%. Our study had also no bacteriological proof with the sensitivity of 64.7%. Prahalad etal[18] from KKCTH, evaluated the role of PCR in diagnosing TBM. Out of 28 IBM cases - defined based on clinical criteria, history of contact with TB, tuberculin test, radiology. CT Scan and out come of therapy with ATT. PCR was positive in 23 cases - sensitivity was 82.14%. Linuma Y. etal evaluated the diagnostic value of Amplicor PCR assay for initial diagnosis and assessment of treatment response for pulmonary tuberculosis. 208 specimens from 155 patients who were bacteriological and / or clinically diagnosed were evaluated by PCR found to be 69.2% sensitivity[19]. Seth P. Ahuja GK etal evaluated the role of PCR in the rapid diagnosis of clinically suspected TBM in children. They observed that the conventional bacteriological methods rarely detect M. tuberculosis in CSF and are of limited use in the diagnosis of TBM. Clinical features suggestive of TBM supported by indirect evidence such as CSF Examination and CT of head have been used for the early diagnosis of TBM. PCR was positive in 2/4, 19/20, 13/16, patients with highly probable, possible and TBM respectively. None of the CSF samples were positive by conventional bacteriological methods. They concluded that PCR along with the suggested clinical criteria offers a rapid and fairly accurate diagnosis of TBM[17]. Ceyhan etal evaluated the role of PCR in the diagnosis of childhood tuberculosis using IS 6110 of M. tuberculosis as a primer. They concluded that PCR is more sensitive than culture[20]. SunilKumar Jatana, etal evaluated the role of PCR in the diagnosis of pediatric tuberculosis. They found that PCR was 100% sensitive in pleural and CSF samples where as 20% in pulmonary. Samples specificity of PCR was 94%[21].

Conclusion

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

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