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RESEARCH ARTICLE

ROLE OF PCR AS A DIAGNOSTIC SCREENING PROCEDURE FOR TUBERCULOUS MENINGITIS IN PEDIATRIC POPULATION

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ABSTRACT

Introduction: Diagnosis of tuberculous meningitis in children is generally based on clinical symptoms, CSF findings, CT scans and response to anti tubercular drugs since conventional methods like microscopy and culture are unsatisfactory. Therefore the present study was undertaken to evaluate the efficacy of polymerase chain reaction for diagnosis of tuberculous meningitis in children.

Materials and Methods: The CSF samples referred to the pathology laboratory of a tertiary care center from children >6 months of age and with a high index of clinical suspicion of tuberculous meningitis were considered as cases and those with non tuberculous meningitis as controls. CSF examination was done in both the groups including gross appearance, lymphocytic count, protein, sugar, AFB staining and PCR for *M. tuberculosis* using IS6110 as the target gene.

Results: A total of 126 CSF samples from pediatric patients were included in the study of which 77 were cases and 49 were controls. Presence of cobweb and predominant lymphocytosis were significantly higher in the tuberculous meningitis group than the control group. AFB and TB PCR were positive in 7 (9.1%) and 18 (23.4%) cases respectively and in none and one controls respectively (p value= 0.04 for AFB positivity and 0.0007 for TB PCR positivity). TB PCR (23.4%) was 2.5 times more sensitive than AFB stain (9.1%) for diagnosing *M. tuberculosis*.

Conclusions: Though PCR is a good tool for confirming tuberculous meningitis in children, better gene targets should be looked for, to make it an ideal screening test.

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INTRODUCTION

Tuberculous meningitis, one of the most severe forms of extra pulmonary tuberculosis, occurs in 7-12 percent of tuberculosis patients in developing countries (Michael *et al*., 2002). It is common among children and is often a post primary manifestation that develops 2-12 months after primary infection. Rapid detection of the causative organism is of paramount importance in tuberculous meningitis as the disease can be fatal and clinical outcome depends heavily on the stage at which treatment is initiated (Leonard and Des Prez, 1990). Diagnosis of tuberculous meningitis is based on clinical symptoms, neurological signs, CSF findings, CT scans and the response to anti tubercular drugs. Conventional methods like microscopy and culture, although considered as gold standards, are quite inadequate in children because of lower *M. tuberculosis* counts and poor tussive force in the CSF sample combined with difficult adequate specimen collection in this age group. Acid fast staining requires a large number of organisms (>10^4 cells/ml) and it has been reported that the positivity of acid fast smears in children with tuberculosis is low and in tubercular meningitis in particular it is only 8 to 10 percent (Davis *et al*., 1993). Culture requires 6-8 weeks due to slow growth of *M. tuberculosis* and is often negative. Newer methods including adenosine deaminase assay have decreased the time of diagnosis but the sensitivity and often the specificity reported is insufficient and needs improvement (Kulkarni *et al*., 2005). Use of PCR for detection of M. tuberculosis has been well established in developed countries, though experience in developing countries is limited (Jain *et al*., 2002). Therefore the present study was undertaken to evaluate the efficacy of polymerase chain reaction for diagnosis of tuberculous meningitis, in especially the pediatric age group, using cerebrospinal fluid as the clinical specimen.

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MATERIALS AND METHODS

The present study was conducted at the Department of Pathology, King George's Medical University (KGMU), Lucknow from October 2014 to September 2015. The cases included in the study were children greater than 6 months of age whose CSF samples were referred to the department of pathology and were admitted in the department of pediatrics,

KGMU with possible tuberculous meningitis, as assessed by clinical presentation, medical history, radiological findings, response to treatment, CSF biochemistry and AFB microscopy. The control group included admitted pediatric cases with non tuberculous meningitis. All the CSF samples received were subjected to physical examination including colour, appearance and cobweb formation; total and differential counts; and protein and sugar assessment. For establishing the microbiological diagnosis of TBM, the CSF samples were subjected to Ziehl-Neelsen staining of the centrifuged deposits and to conventional PCR targeting the IS6110 target gene as per a previous protocol (Jain *et al*., 2002). Briefly, the primers used were forward primer: 5'CCTGCGAGCGTAGGCGTCGG 3' and reverse primer: 5'CTCGTCCAGCGCCGCTTCGG 3' yielding a product size of 123 bp. Amplification reaction was performed in a final volume of 20 µl containing 2 µl of the extracted DNA. The PCR cycle consisted of following steps: denaturation at 94^0C for 1 min, annealing at 60^0Cfor 1 min, polymerization at 72^0C for 1 min followed by a final extension at 72^0C for 7 min.

Statistical analysis: Done with the help of GraphPad Prism v. 5.

controls. The male to female ratio of the study population was 1.2:1 and maximum children (40.2%) were less than five years of age. The findings of the gross CSF appearance, increased CSF protein levels and decreased CSF sugar levels were not significantly different among the cases and controls though presence of cobweb and predominant lymphocytosis were significantly higher in the tuberculous meningitis group than the non tuberculous meningitis group (Table 1). AFB and TB PCR were positive in 7 (9.1%) and 18 (23.4%) cases respectively though in the control group TB PCR was positive only in one case and AFB was positive in none (p value= 0.04 for AFB positivity and 0.0007 for TB PCR positivity) (Table 2). TB PCR positivity was highest among the 5-10 year age group (n=9, 11.7%) though the positivity was not significantly higher as compared to the other age groups (Table 3). Among the 18 suspicious tuberculous meningitis cases whose samples tested positive by PCR analyses, 14 (77.8%) patients showed a positive response to ATT treatment, 1 (5.6%) patient died, 2 (11.1%) patients were discharged on request, while 1 (5.6%) patient left against medical advice, hence the response to ATT treatment could not be assessed in these 3 (16.6%) patients. For establishing an accurate diagnosis of tuberculosis, the performance characteristics of the two diagnostic tests

Table 1. Comparison of CSF findings among cases (tuberculous meningitis suspects) and controls (non tuberculous meningitis)

CSF Finding		Positive in Cases (%) (n=77)	Positive in Controls (%) (n=49)	p- value (Odd's ratio)
Gross Appearance	Clear	38 (49.4)	16 (32.7)	0.11 (4.36)
	Turbid	31 (40.3)	23 (46.9)	
	Xanthochromic	8 (10.3)	10 (20.4)	
	Presence of cobweb	12 (15.6)	1 (2.1)	0.02 (1.28-2.0)
Level of lymphocytoses	≥90 percent	46 (60)	2 (4.1)	<0.0001 (58.4)
	75-89 percent	14 (18)	7 (14.3)	
	60-74 percent	13 (17)	11 (22.4)	
	<60 percent	4 (5)	29 (59.2)	
CSF Biochemistry	Increased protein levels (>50 mg/dl)	70 (90.9)	45 (91.8)	1.0 (0.59-1.5)
	Decreased sugar levels (<30 mg/dl)	68 (88.3)	43 (87.8)	1.0 (0.65-1.6)

Table 2. Comparison of AFB and TB positivity in cases and control groups

Study group	AFB positives (%)	TB PCR positives (%)
Cases (n=77)	7 (9.1)	18 (23.4)
Controls (n=49)	0 (0)	1 (2.1)
p- value (95% CI)	0.04 (1.46-1.97)	0.0007 (1.41-2.10)

Table 3. Age and sex distribution of the tuberculous meningitis suspects and their TB PCR positivity

Age group	Males (n=42)		Females (n=35)		Total (n=77)		p-value (Chi square)
	Number	PCR positive (%)	Number	PCR positive (%)	Number	PCR positive (%)	
<5 years	16 (38.1)	2 (4.7)	15 (42.9)	2 (5.7)	31 (40.2)	4 (5.2)	
5-10 years	20 (42.6)	6 (14.3)	9 (25.7)	3 (8.6)	29 (37.6)	9 (11.7)	
11-14 years	6 (14.3)	2 (4.8)	11 (31.4)	3 (8.6)	17 (22.1)	5 (6.5)	0.20 (3.19)
Total	42	10 (23.8)	35	8 (22.9)	77	18 (23.3)	
p- value (95% CI)	1.00 (0.63- 1.64)						

Table 4. Comparison of performance characteristics of TB PCR and AFB staining for diagnosing *M. tuberculosis*

Performance characteristic	TB PCR	AFB staining
Sensitivity	23.4%	9.1%
Specificity	97.8%	100%
Positive predictive value (PPV)	94.73%	100%
Negative productive value (NPV)	44.9%	41.2%
Diagnostic accuracy (DA)	52.4%	44.4%

RESULTS

A total of 126 CSF samples from pediatric patients were included in the study of which 77 were cases and 49 were

(TB PCR and AFB staining) were compared, considering clinical findings of tuberculosis as the diagnostic gold standard. TB PCR was 2.5 times more sensitive than AFB stain for diagnosing *M. tuberculosis*, though the specificity, positive

predictive value, negative productive value and diagnostic accuracy of both the tests were similar (Table 4).

DISCUSSION

A total of 126 CSF samples from pediatric patients were assessed by polymerase chain reaction during the study period. Children younger than 6 months of age were not included in the study as tuberculous meningitis is reported to be uncommon under the age of 6 months (Gulati *et al*., 2006). The target sequence for the detection of *M. tuberculosis* in the CSF samples was chosen to be IS-6110 considering the fact that most strains of mycobacteria have between 8 and 15 copies of IS-6110 sequence (Van Soolingen *et al*., 1993). In the present study a combination of raised W.B.C. count with more than 90% lymphocytosis, elevated protein levels (more than 50gm/dl) and decreased sugar levels was found to have a moderate sensitivity and specificity for diagnosing tuberculous meningitis, which is comparable to sophisticated techniques like PCR. A previous study points out that this combination of CSF examination is even better than the AFB smear or TB culture, the microbiologic gold standard for *M. tuberculosis* diagnosis (Pasco, 2012). Though, these findings should be interpreted judiciously since CSF sugar levels are not always decreased as most of the patients had already received dextrose or glucose infusion at the time of admission as part of the preliminary treatment. Thus CSF glucose level should always be assessed in parallel with the blood glucose level (Caws *et al*., 2000). The AFB positivity in our study was found to be comparable with most of the previous studies, ranging from 8 to 10% (Davis *et al*., 1993; Newton, 1994). Microscopy using the Ziehl Neelson staining procedure has been the main means of rapidly confirming mycobacterial etiology in the acutely ill patient. But this method has a low sensitivity as the detection limit of microscopy is 10^4 mycobacteria per milliliter, whereas the majority of patients with tuberculous meningitis have fewer mycobacteria in their CSF samples (Michael *et al*., 2002). The sensitivity of PCR in diagnosis of tuberculous meningitis in the present study was 23.37 percent where as previous studies using clinical criterion as the gold standard for evaluating the efficacy of PCR, have reported PCR sensitivity ranging from 32 to 94 percent (Nyugen *et al*., 1996; Portillo Gomez *et al*, 2000; Rafi and Naghily, 2003). This may probably be explained by the fact that the previous studies included both the pediatric and adult age group whereas the present study was done exclusively in children and therefore the average amount of CSF samples received ranged between 0.4 to 0.8 ml. The volume of the CSF sample have an important impact on the quality of results obtained and use of at least 2 ml CSF is imperative. Another possible reason for lower sensitivity of PCR in the present study might be use of IS-6110 as the target sequence. It has been shown that a large number of clinical isolates of *M. tuberculosis* originating from India have either a single copy or no copy of IS-6110 (Van Soolingen *et al*., 1993; Das *et al*., 1995; Narayanan *et al*., 2001).

The specificity of PCR in the present study was 97.9% which is comparable to that of the previous studies ranging from 93 to 100% (Kulkarni *et al*., 2005; Seth *et al*., 1996). The only false positive case in the present study was a 5 years old female presenting with fever, loss of appetite and headache for 5 days. On physical examination, cervical lymph node was found to be enlarged which was labeled as benign reactive hyperplasia on FNAC. There were no signs of meningeal irritation and CSF biochemistry was absolutely normal. Radiological examination also revealed no abnormality. She was started on a 7 day course of amoxicillin and discharged, with the advice to follow up, but the patient did not return. The only possible reason for a positive PCR in this case seems to be that patient might have presented in too early stages of tuberculous meningitis. Lack of specific signs and symptoms and a normal CSF biochemistry report would have led to missing the diagnosis as tuberculous meningitis. 77.8% of the PCR positive tuberculous meningitis cases in the present study showed a good response to ATT therapy which was marked by gradual loss of signs and symptoms such as fever, headache, neck rigidity, positive Kernig's sign etc. The patient who died had tubercular lymphadenitis and thalamic infarcts on CT scan as a supportive evidence for tuberculous meningitis. Rest 3 patients, in whom the response to ATT could not be assessed, also had supportive evidences in favor of tuberculous meningitis viz, positive contact history, tuberculin test positivity, evidence of extraneural tuberculosis or radiological evidence. Thus it becomes apparent that PCR do deserves a place in laboratory diagnosis of tuberculous meningitis though low sensitivity could question the utility of PCR in diagnosis of tuberculous meningitis as a screening procedure.

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