Prospective study to find out the role of cerebrospinal fluid examination by cartridge-based nucleic acid amplification test and culture and sensitivity as a diagnostic method in childhood central nervous system tuberculosis

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ABSTRACT

Introduction: Central nervous system (CNS) tuberculosis (TB), particularly tuberculous meningitis, is the severest form of *Mycobacterium tuberculosis* (MTB) infection causing severe neurological defects or even death. The recent introduction of cartridge-based nucleic acid amplification test (CBNAAT) has significantly transformed the diagnostics of TB in adults, but its application for the diagnosis of pediatric TB is under evaluation. **Objective:** This study was conducted by for cerebrospinal fluid (CSF) examination in the detection of MTB by the culture and sensitivity and CBNAAT in the diagnosis of childhood CNS TB. **Methods:** A prospective hospital-based study was conducted from July 2017 to June 2018 in the pediatric department of a tertiary care unit. A total of 65 randomly selected patients, suspected of CNS TB, were included in this study. CSF was tested for CBNAAT and culture and sensitivity other routine investigations such as specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for both CBNAAT and culture and sensitivity. Mantoux test was also performed, and the statistical analysis was performed using Chi-square test. $p=0.05$ was considered to be statistically significant. **Results:** Culture positive TB was found in 26 out of the 65 children. The sensitivity, specificity, PPV, and NPV for CBNAAT and culture and sensitivity were 65.71%, 90.00%, 88.46%, and 69.23%, respectively. CBNAAT was detected to be more in 12 TB cases and was more sensitive than culture and sensitivity. Positive history of contact ($p=0.04$), reactive Mantoux test ($p<0.005$), presence of Bacillus Calmette Guerin (BCG) scar ($p=0.02$), and low socioeconomic status were independently associated with a positive CBNAAT result. **Conclusion:** Analysis of CSF sample with CBNAAT is a sensitive and specific method for rapid diagnosis of CNS TB in children. Compared to culture and sensitivity, CBNAAT offers better sensitivity and its scale-up will improve access to CNS TB diagnostics in children. Although a negative CBNAAT does not rule out TB!

Key words: *Cartridge-based nucleic acid amplification test, Cerebrospinal fluid, Comparative study, Culture and sensitivity, Pediatric central nervous system tuberculosis*

In spite of ongoing tuberculosis (TB) control activities from more than 50 years now, TB still continues to be India's most severe health crisis and kills an estimated 480,000 Indians every year and more than around 1400 every day [1]. Central nervous system (CNS) TB, particularly tuberculous meningitis (TBM), is the most severe form of *Mycobacterium tuberculosis* (MTB) infection, causing severe neurological defects or even death in more than half of those affected. Manifestations of CNS TB are (1) intracranial – TBM, tuberculous encephalopathy, tuberculous vasculopathy, CNS tuberculoma (single or multiple), and tuberculous brain abscess and (2) spinal – Pott's spine and Pott's paraplegia, non-osseous spinal tuberculoma, and spinal meningitis [2,3].

At present, the diagnosis of CNS TB remains a complex issue because the most widely used conventional "gold standard" based on bacteriological detection methods such as direct smear and culture identification, cannot rapidly detect MTB bacilli in cerebrospinal fluid (CSF) specimens with sufficient sensitivity in the acute phase of TBM [2,3]. Smear microscopy and culture are often negative in TBM, and a combination of consistent CSF findings, clinical and radiological criteria are required to optimize the diagnosis [4,5]. The recent introduction of cartridge-based nucleic acid amplification test (CBNAAT) has been bliss, as this assay is rapid and provides results within 2 h.It is a nucleic acid amplification test which simultaneously detects DNA of MTB complex and resistance to rifampicin (RIF) in <2 h [6]. Therefore, a planned study was conducted on the role of CSF examination in the detection of MTB by CBNAAT and culture and sensitivity in the diagnosis of childhood CNS TB.

METHODS

A prospective hospital-based study was conducted from July 2017 to June 2018 in the department of pediatrics, in a tertiary care

center. The study group comprised 65 randomly selected patients ≤12 years of age of either gender who met the following criteria:

a. Children who were clinically presented with vague ill health lasting 2–8 weeks before the development of meningeal irritation. These non-specific symptoms included malaise, anorexia, fatigue, fever, myalgia, and headache.
b. Children who presented with stiffness of neck along with: Focal neurological deficits, behavioral changes and alteration in consciousness; fever with weight loss or no weight gain; history of contact in last 2 years; tuberculin skin test positivity; coexistence of precipitating illnesses; ultrasonograpic (USG) of the abdomen suggestive of TB; CSF findings consistent with tubercular meningitis; cranial computed tomography (CT) suggestive of tuberculoma; and history of antiretroviral treatment.
c. Children who are not treated with antitubercular drugs.

Subjects who were already on anti-tubercular therapy (ATT) were excluded from the study. All the selected patients were subjected to detailed history, general physical examination, systemic examination, hematological investigations (level of hemoglobin, total leukocyte count, and differential leukocyte count, and erythrocyte sedimentation rate), radiological investigations (chest X-ray, USG cranium, cranial CT, and magnetic resonance imaging), tuberculin sensitivity test (performed by intradermal injection of 0.1 ml purified protein derivative [PPD] containing 5 tuberculin units [PPD-5] into the volar [ventral] surface of forearm by a disposable plastic tuberculin syringe), and CSF for culture and sensitivity (gold standard) and for CBNAAT (with use of quality controls). Other relevant investigations were carried out wherever necessary to support the diagnosis.

All the details of the examination were recorded on a specially designed pro forma with pre-tested information of study. Sensitivity, specificity, PPV, and NPV were calculated for both CBNAAT and culture and sensitivity (gold standard). The data were coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of percentages. Chi-square test was used for qualitative data whenever two or more than two groups were compared. Level of significance was set at $p \leq 0.05$.

RESULTS

Majority of the cases, included in the present study, were in the age group of 0–4 years (56.9%) followed by age group of 8–12 years and least were in 4–8 years (20%). Among the total study population, 55.4% were males and 44.6% were females. Maximum number of cases belonged to lower middle class (Class III) (38.5%), and upper lower class (Class IV) (33.8%), and only 3.1% cases were of upper class (Class I) as per the Kuppuswamy scale for socioeconomic status [7]. Tubercular meningitis was the most common type of CNS TB included in this study. History of contact was present in 60% cases while it was absent in 40% of cases. Majority of cases (69.2%) were having BCG scar 55.4% of cases had reactive Mantoux test while those with non-reactive Mantoux test were 44.6%. Fever, altered sensorium, meningeal irritation, and seizures were the most common presenting complaints.

As shown in Table 1, history of contact with TB was positive in 46.7% cases while 53.3% had no history of contact and CBNAAT detected *rpoB* gene 25 cases having a history of contact (p=0.04). A total number of children with positive Mantoux test were 36 while with negative Mantoux test were 19 and *rpoB* gene was detected by CBNAAT in 25 children with positive Mantoux test and 11 children with negative Mantoux test (p<0.005).

Table 2 shows that culture was positive in 65.4% of children with negative history of contact and 56.4% of children with a positive history of contact (p=0.046). Furthermore, culture was positive in 19 children with positive Mantoux test and 22 children with negative Mantoux test (p=0.01). There was a statistically significant association between results of culture and sensitivity for MTB and CBNAAT (p=0.003).

As shown in Table 3, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of culture and sensitivity for MTB were 52.78%, 75.86%, 73.08%, and 56.41%, respectively, as compared to the Mantoux test. The sensitivity, specificity, PPV, and NPV of CBNAAT were 65.71%, 90.00%, 88.46%, and 69.23%, respectively, as compared to culture and sensitivity (gold standard).

Table 1: Association between CBNAAT test and a history of contact and Mantoux test (n=65)

CBNAAT	History of contact		Mantoux test	
	No	Yes	Negative	Positive
Detected	10 (28.6%)	25 (71.4%)	10 (28.6%)	25 (71.4%)
Not detected	16 (53.3%)	14 (46.7%)	19 (%)	11 (%)
Total	26 (40.0%)	39 (60.0%)	29 (%)	36 (%)
p value	0.04		<0.005	

CBNAAT: Cartridge-based nucleic acid amplification test

Table 2: Distribution of culture and sensitivity and history of contact

Culture and sensitivity	History of contact		Mantoux test	
	No (%)	Yes (%)	Negative	Positive
Negative	17 (43.6)	22 (56.4)	19	7
Positive	9 (34.6)	17 (65.4)	17 (65.4%)	22
Total	26 (40.0)	39 (60.0)	36	29
p value	0.046		0.01	

Table 3: Accuracy of CBNAAT and culture and sensitivity

Culture and sensitivity	CBNAAT		Statistic	Value (%)
	Positive	Negative		
Positive	23	3	Sensitivity	65.71
Negative	12	27	Specificity	90.00
Total	35	30	PPV	88.46
p value	0.003(S)		NPV	69.23

PPV: Positive predictive value, NPV: Negative predictive value, CBNAAT: Cartridge-based nucleic acid amplification test

DISCUSSION

The present study showed a sensitivity of 88.46% and specificity of 69.23% for CBNAAT and culture on CSF samples of children suspected to be suffering from CNS TB. PPV and NPV were 65.71% and 90%, respectively. In a study by Kumari *et al*. [8]. GeneXpert MTB/RIF test showed a sensitivity of 60% and specificity of 97.9% for diagnosing TBM, the NPV was 95.9% and PPV was 75%. Singh *et al*. [9] showed that LJ culture had sensitivity, specificity, PPV and NPV of 8%, 100%, 100%, and 33.01%, respectively, and overall, nested polymerase chain reaction (PCR) had sensitivity, specificity, PPV, NPV, and diagnostic accuracy of 89.34%, 97.06%, 98.53%, 80.5%, and 91.74%, respectively. In a study by Solomons *et al.* [10], culture, GenoType and Xpert combined performed best with 56% sensitivity and 98% specificity. A study by Gupta *et al.* (2016) [11] revealed that the sensitivity and specificity of TB PCR for diagnosis of TBM were 77.78% and 100%, respectively, and PPV was 100% and the NPV was 78.95%.

It was observed that the positivity rate of CBNAAT in patients with a history of TB contact was significantly higher (p=0.04) than in those with no history of contact. Furthermore, the positivity of CBNAAT was higher in lower socioeconomic groups (Cass III and Class IV). Gupta *et al.* (2016) [11] obtained a significant association between positive history of contact and positive Xpert assay (p=0.03). Yin *et al*. (2014) [12] demonstrated positivity of Xpert associated significantly with a history of contact with TB in patients (p=0.010).

Positive CBNAAT results were obtained in 57.1% of cases which had BCG scar and in 42.9% of cases with absent BCG scar which shows the statistical difference (p=0.02). Yin *et al.* (2014) [12] demonstrated that positivity rate of Xpert MTB/RIF assay in patients with no BCG scar was significantly higher than in those with BCG scar (p=0.001).

Statistically significant association (p=0.05) was found between low socioeconomic status (57% positivity in Class III) and culture and sensitivity for MTB. Association between Mantoux test and also BCG scar with culture and sensitivity (p=0.01 and of <0.003, respectively) was statistically significant.

Association between CBNAAT and culture and sensitivity was statistically significant (p=0.005) and so was the association between Mantoux test and culture and sensitivity (p=0.01). The study had three key findings: (i) CBNAAT performs well in rapidly and accurately diagnosing childhood CNS TB; (ii) CBNAAT assay is way more sensitive than culture for the diagnosis of childhood CNS TB; (iii) and the assay detected an extra 12 cases of CNS TB compared to that with culture. As per the study, CBNAAT is useful for rapid, sensitive, and accurate diagnosis of CNS TB in a pediatric inpatient setting.

CONCLUSION

It was concluded that CBNAAT on CSF sample rapidly and correctly identified CNS TB. In a nutshell, CBNAAT is a highly specific test for the diagnosis of CNS TB in children and a positive CBNAAT should warrant commencement of the full course of ATT as per schedule!

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