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Cerebrospinal fluid Gene XPERT (CBNAAT) in children with tuberculous meningitis

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SUMMARY

Objectives: To observe the role of CSF Gene XPERT (CBNAAT) in diagnosis of tuberculous meningitis (TBM) and determine its sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).
Methods: A prospective study was done from October 2017 to March 2020. CSF samples of 55 children diagnosed as tuberculous meningitis as per defined clinical and imaging criteria, were subjected to routine CSF analysis, MGIT culture and CBNAAT. Children on prior anti-tuberculous therapy for more than one month were excluded from study.
Results: Of 55 children, meningeal signs were present in 54.5% children. Neurological deficits were present in 47.3%. Common CT brain findings were communicating hydrocephalus followed by infarct and basal exudates. CSF Gene XPERT (CBNAAT) were positive in 9 (16.4%), of which 6 was also culture positive and 3; negative. Two children were rifampicin resistant. Fifteen (27.3%) children had positive CSF culture. Gene XPERT showed sensitivity, specificity, PPV, NPV and diagnostic accuracy of 40%, 92.5%, 66.7%, 80.4% and 78.2% respectively as compared to culture.
Conclusion: Although sensitivity of CSF CBNAAT is low i.e. 40% but positive result not only confirm bacteriological diagnosis of tuberculous meningitis but also reveal about rifampicin sensitivity and resistance for plan of therapy.

1. Introduction

Mycobacterium tuberculosis is presently a leading cause of death worldwide alongside HIV. Among all tuberculosis patients, 25% have extrapulmonary site involvement namely, lymph node, meninges, kidney, spine and growing end of bones [1–3]. Tuberculous meningitis is the most severe consequence of *Mycobacterium tuberculosis* infections. There is high mortality and morbidity with severe neurological sequelae among survivals [4,5]. In India, pediatric tuberculosis has gained major attention in last two decades as health problem. Tuberculous meningitis is second most common cause of extrapulmonary tuberculosis after lymph node involvement [1].

The diagnosis of extrapulmonary tuberculosis is more difficult than pulmonary as direct smear microscopy and Cartridge Based Nucleic Acid Amplification Test (CBNAAT), which provide rapid and confirm bacteriological diagnosis have low yield [6]. The diagnosis of tuberculous meningitis (TBM) in young children is difficult due to the paucibacillary nature of disease in cerebrospinal fluid (CSF) [7]. Other tests for extrapulmonary tuberculosis i.e. tuberculin test and polymerase chain reactions (PCR) assays have been reported to have variable sensitivity and specificity [7]. So, there is need of rapid diagnois of TBM, which is essential for treatment initiation and improvement of outcome [8–10]. Cerebral imaging also contributes for diagnosis of probable or possible TBM like clinical and laboratory findings. However, discrimination between TBM and another cerebral disease is frequently very difficult. The findings in CT /MRI Brain are hydrocephalus, basal meningeal enhancement, tuberculoma and vasculitis [11–13].

WHO has reported high sensitivity (59–84%) and specificity (73–89%) of Xpert for CSF than conventional tools. Accuracy of nucleic acid-based amplification (NAA) tests, though better than that of conventional microscopic methods, was not considered completely satisfactory for many years because their low sensitivity and specificity in diagnosis of tuberculous meningitis [14,15]. There are few studies on CSF Gene Xpert for detection of Mycobacterium tuberculosis in children.

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Therefore this study was done to observe the sensitivity, specificity, positive predictive value and negative predictive value of CSF CBNAAT for diagnosis of tuberculous meningitis in children.

2. Methods

This study was carried out in the Department of Pediatrics and Microbiology on admitted children from October 2017 to March 2020. This prospective observational study was approved by Ethical committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The informed consent was taken from parent or legal guardian of patients before inclusion of the patient for the study.

2.1. Inclusion criteria

Children less than or equal to 18 years diagnosed as tuberculous meningitis based on history, clinical features, CSF laboratory findings and brain imaging were included in the study. Staging of tubercular meningitis was done as per British Medical Council Staging System [16].

2.2. Exclusion criteria

Children who had received prior antituberculous therapy for one month or more were excluded from study.

All demographic details and clinical manifestations were recorded in standard pretested proforma. Complete blood count, renal and liver function test, tuberculin test, ELISA for HIV, chest radiographs and cranial tomography or MRI of brain were done in all patients. Lumbar puncture was done after excluding raised intracranial pressure. The collected cerebrospinal fluid was subjected to routine cytochemical study, Gram's and AFB staining and aerobic culture. Two mL CSF was collected in two different sterile container and was transported to microbiology laboratory for the Gene Xpert (CBNAAT) and MGIT culture of *Mycobacterium tuberculosis*. On the basis of clinical features, imaging, cerebrospinal fluid and imaging study children of tuberculous were categorized into three groups [11].

1. Definite TBM: AFB seen on CSF microscopy, positive CSF *M. tuberculosis* culture, or positive CSF *M. tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis.
2. Probable TBM: total score of ≥12 when neuroimaging available or total score of ≥10 when neuroimaging unavailable.
3. Possible TBM: total score of 6–11 when neuroimaging available or total score of 6–9 when neuroimaging unavailable.

2.3. Procedure of CBNAAT

The collected 2 ml of CSF was centrifuged at 3000g for 15 min. Supernatant is poured off and pellet is resuspended in 0.7 ml of phosphate buffered saline. 1.3 ml of sample reagent (2:1 sample reagent to sample ratio) is added and incubated for 15 min followed by vortex during this time. Than 2 ml is added into the cartridge and loaded in Gene Xpert equipment for analyzing the sample. Results were reported as positive or negative for *Mycobacterium tuberculosis.* Susceptibility to rifampicin was also reported as resistant or sensitive.

2.3.1. Statistical analysis

The data was analyzed using the SPSS software application (version 23.0: SPSS, Chicago, IL, USA).

3. Results

The present study included 55 cases of tuberculous meningitis admitted in ward based on clinical findings, CSF and imaging study. Out of 55 children with tuberculous meningitis, 14 (25.4%) were of less than 5 years of age, 28 (50.9%) of 5 to 10 years of age; and 13 (23.7%) in 10 to 18 years age group. The most common symptom was fever (for more than15 days) in 43 (78.1%) followed by headache (67.2%) and seizures (67.2%). Neck rigidity and kernig's sign was present in 30 (54.5%) and Brudzinski sign; 26 (47.3%). Eleven (20%) children had cranial nerve palsy and 26 (47.3%) had motor deficits (Table 1).

In this study, 28 (50.9%) children were in stage III, followed by stage II in 22 (40%). Only 5 (9.1%) children were in stage I. Seven (12.8%) children were included in **definite** TBM, 24 (43.6%) children were in **probable** category, whereas same number of children in **possible** category (Fig. 1).

Tuberculin test was positive in 22 (40%) children and 31 (56.3%) had chest radiograph suggestive of tuberculosis (milliary tuberculosis, cavity and hilar/paratracheal lymph nodes). The most common CT brain findings were communicating hydrocephalus in 23 (41.8%) followed by infarct; 16 (21.9) and basal exudates; 10 (18.2%). Tuberculoma was observed in 4 children only.

CSF routine cytochemical study of CSF showed lymphocytic predominance with low glucose along with high protein counts. Nine (16.4%) out of 55 cases showed positive CSF CBNAAT for *Mycobacterial tuberculosis,* of which 2 were rifampicin resistant (Table 2). MGIT culture was positive in 15 (27.3%) children. Six children, who are positive for CBNAAT, were also positive in CSF culture. Gastric aspirates were positive for *Mycobacterial tuberculosis* in 11 children. CSF CBNAAT was positive in 3,5 and one cases of definite, probable and possible category of tuberculous meningitis.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of CBNAAT are 40% (CI:22.9–52.2), 92.5%(CI:86.1–97.1), 66.7%(CI:38.2–87), 80.4% (CI:74.9–84.4), and 78.2%(CI68.9–84.8) as compared to CSF culture for *Mycobactium tuberculosis* (Table 3).

4. Discussion

Childhood tuberculosis comprises 10%–20% of all cases in India and contributes about 8%–20% of deaths related to TB [1]. Tuberculous meningitis (TBM) is a major cause of mortality and morbidity due to diagnostic delay in children [5]. There is also lack of available tests with good sensitivity and specificity. So, there is need of test to diagnose tuberculous meningitis earlier with good accuracy. The present study was conducted to observe the role of CBNAAT in diagnosis of TBM. Out of 55 cases, only 9 (16.4%) were Gene Xpert positive, of which two were rifampicin resistant. Maximum numbers of children were in 5–10 years of age group i.e. 28 (50.9%) as against Ratageri *et al.* [17] *and* Solomons *etal* [18].

On physical examination, Neck rigidity and kernig's sign was present in 30(54.5%) and Brudzinski sign; 26(47.3%). Eleven (20%) children

Table 1
Basic characteristics of children with Tuberculous meningitis.

Characteristics	No. of children (n = 55)	%
Age		
<5yrs	14	25.4
5–10 yrs	28	50.9
10–18 yrs	13	23.7
Fever	43	78.1
Headache	37	67.2
Seizure	37	67.2
Abnormal chest X-ray	31	56.3
Positive Neck rigidity and Kernig's sign	30	54.5
Brudzinski sign	26	47.3
Vomiting	28	50.9
Papilledema	26	47.3
Cranial nerve palsy	11	20
Motor deficit	26	47.3
Hemiplegia	16	29
Paraplegia	3	5.4
Quadriplegia	7	9.8

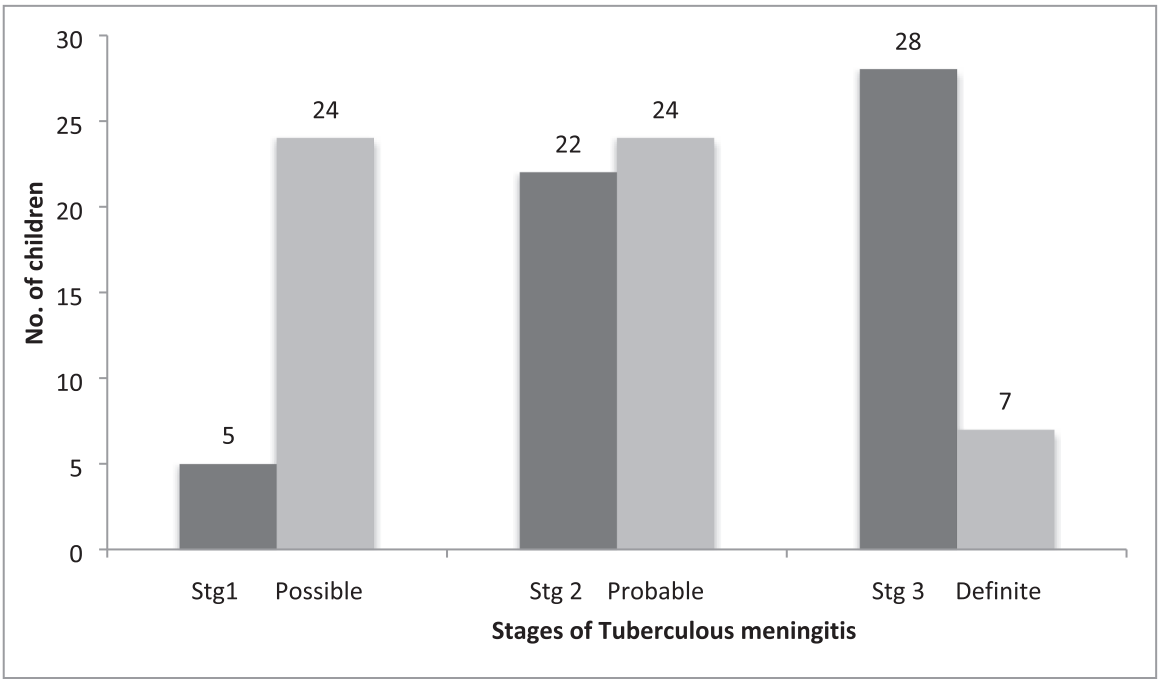

Fig. 1.

Table 2
Cerebrospinal fluid analysis of children with Tuberculous meningitis.

CSF routine microscopy	Mean ± Standard deviation
TLC (/micro liter)	149.78 ± 26.5
Neutrophils (%)	33 ± 10.8
Lymphocytes (%)	55.11 ± 10.00
Glucose (mg/dL)	57.22 ± 14.7
Protein (mg/dL)	106.33 ± 28.4
CBNAAT positive	9(16.4%)*
Rifampicin sensitive	7(77.8%)
Rifampicin resistant	2(22.2%)
CBNAAT negative	46(83.7%)
MGIT culture positive	15(27.3%)

* CSF CBNAAT was positive in 3, 5 and one cases of definite, probable and possible category of tuberculous meningitis.

Table 3
CSF CBNAAT for diagnosis of Tuberculous meningitis in children.

	Sensitivity %	Specificity %	PPV %	NPV %	Diagnostic accuracy%
Present Study	40	92.5	66.78	80.4	78.2
Vadvai et al. [19]	29	–	–	–	–
Bhatia et al. [20]	38.24	–	–	–	–
Ratageri et al. [17]	46.15	100	—	–	–
Solomon et al. [18]	26	100	100	53	–
Banker et al. [22]	84.9	86.7	33.09	98.6	86.6
Xiaocui et al. [21]	34	96	98.6	15.4	–

had cranial nerve palsy and 26(47.3%) had motor deficits in form of hemiplegia, quadriplegia and paraplegia.

As there is no standard diagnostic criteria for TBM, we classified suspected TBM patients into definite (12.8%), probable (43.6%) and possible (43.6%) as per consensus case definition. Gene Xpert was endorsed by WHO in 2013 for extrapulmonary specimens. In our study sensitivity is 40%. This result is comparable to study of Vadvai *et al.* who reported sensitivity for CSF of 29% [19]. Bhatia *et al.,* 2016 has also reported sensitivity of 29% [20] but Nguyen *et al.* [14], (2014) in adult has observed higher sensitivity i.e.59.3% of CBNAAT in cerebrospinal fluid in diagnosis of tuberculous meningitis (Table 3).

In present study, sensitivity, specificity , positive predictive value, negative predictive value and diagnostic accuracy of CBNAAT is 40%, 92.5%, 66.7%, 80.4% and 78.2% respectively as reported by Ratageri *et al.* [17] and Xiaocui et al. [21] There is higher positivity of CBNAAT of CSF in definite criteria. Nguyen *et al.,* in adults reported sensitivity of 59.3%, specificity of 99.5%, negative predictive value is 72.5% and positive predictive value is 99.1% [14].

An outcome of children with tuberculous meningitis depends on many factors such as GCS, stage of TBM, raised intra cranial pressure. In our study, 14 children got successfully discharged without morbidity, 29 (52.7%) with morbidity (paresis, cranial nerve palsy, deafness, visual disturbances, cognitive defects) and 12 (21.8%) had died.

Although CSF detection by CBNAAT is low (16.4%) in present study with small sample size of children, positive result not only confirm diagnosis of tuberculous meningitis but also reveal about rifampicin sensitivity and resistance. Therefore CBNAAT also helps in planning of therapy.

The small sample size, small volume i.e. only 2 ml of cerebrospinal fluid used for CBNAAT and paucibacillary nature of the disease are limitations of the present study.

CRediT authorship contribution statement

Annapurna Rai: Data curation, Formal analysis. **Rajniti Prasad:** Conceptualization, Supervision. **B.K. Das:** Conceptualization, Supervision. **Shampa Anupurba:** Investigation. **Utpal Kant Singh:** Methodology.

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