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Original Research Article

The Utility of CSF GeneXpert in Diagnosis of Tubercular Meningitis in Children Aged 2 Months to 12 Years

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Abstract

Background: CNS tuberculosis is the most devastating form of extra pulmonary tuberculosis in which TB meningitis is the most common form affecting 1-2 percent of untreated tuberculosis. Early treatment is critical to reduce TBM related mortality and morbidity. Diagnosing TBM based on smear microscopy has very low sensitivity and using CSF culture and sensitivity takes long time. Hence rapid and accurate detection methods are essential for initiation of early treatment especially in vulnerable pediatric age group.

Methods: It is a hospital based observational study conducted in Department of Paediatrics BMIMS, Pawapuri. All children with clinically suspected tuberculous meningitis were enrolled into the study and underwent detailed history taking, clinical examination, blood investigation, CT scan brain and Lumbar Puncture. CSF samples were sent for CSF Xpert MTB/RIF.

Conclusion: CSF gen expert was considered superior to smear for AFB in isolating TB bacilli in case of suspected TB meningitis. The sensitivity of CSF gen expert against clinical scoring was 46.15% and specificity was 100%.

Keywords: CSF, Xpert MTB/RIF, TBM, Clinical score, Children.

Introduction

Tuberculosis has been existed for million years and remains one of the most prevalent infections worldwide. The burden is actually higher than previously estimated, reflecting new surveillance and survey data from India. According to the new Global Tuberculosis Report 2016 released by the World Health Organisation on October 13 2016, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9million (56%) were among men, 3.5million (34%) were among women and 1million (10%) among children**.** [1] An estimated 1.8 million people died of TB in 2015. Six countries accounted for 60% of the total burden, with India bearing the brunt, followed by Indonesia, China, Nigeria, Pakistan and South Africa. India accounts for one fourth (23%) of the global TB burden. In 2015, an estimated 28lakh cases occurred and 4.8lakh people died due to TB. India bears second highest number of estimated HIV associated TB in the world. [2] Tuberculosis is the most common opportunistic infection in people living with HIV and it is the leading cause of death. The chance of developing TB in HIV patients are 20 times more in those without HIV [3]. People living with HIV constitutes about 11% of all new TB cases as per WHO 2016 global report. Tubercular meningitis is the most severe form of tuberculosis and

causes substantial morbidity and mortality in adults and children. It represents 1% of all tuberculosis cases. The often insidious and nonspecific presentation of TB meningitis makes diagnosis a challenge. Clinicians face substantial challenges in the diagnosis and management of tubercular meningitis. [4] Diagnosis usually relies on clinical evidence, which combines clinical, laboratory and radiological findings. Approximately a third of patients die soon after presenting to hospital, and many of those surviving are left with severe neurological sequela. [5] Hence it is recommended that all children under the age of 5 yrs with significant contact history should be started on chemoprophylaxis to prevent meningeal TB or other disseminated tuberculosis [6]. Good prognosis of TB meningitis depends on early detection and treatment. [7] A systemic meta-analysis by Denkinger and colleagues showed that the sensitivity of genexpert for extra pulmonary TB varied widely across different sample types in which the detection rate of TBM was moderate. In another study by Sagarika et al [8] from AIIMS Delhi showed that the sensitivity and specificity of detection of Mycobacterium tuberculosis DNA in CSF using PCR in diagnosing TBM was 87.6% and 92% respectively. In Vietnam Nhu and colleagues found 59% sensitivity for Xpert used on CSF v/s clinical TBM case definition. [9] A prospective study conducted by Vinod B et al from South Africa regarding the diagnostic accuracy of quantitative PCR in tubercular meningitis showed 67% sensitivity for microbiologically proven TBM but only 36% sensitivity against a clinical case definition and had specificity of 95%. In a study conducted by Nguyen et al [9] from Vietnam the sensitivity and specificity of CSF Xpert was 59.3% and 99.5% respectively. The various above mentioned studies showed genexpert sensitivity varies from 55%-80% and there is a paucity of literature in India. Hence this study was undertaken to assess the diagnostic utility of CSF GeneXpert in detecting Mycobacterium tuberculosis in TBM in children.

Objective

The utility of CSF gen expert in diagnosis of tubercular meningitis in children in the age group 2 months to 12 years.

Materials and methods

Hospital based prospective observational study, Children in the age group 2 months -12 years who were suspected case of tubercular meningitis.

Inclusion criteria

Children between 2 months -12 years of age with clinically suspected TB meningitis

This is a hospital based observational study conducted at Department of Paediatrics, Bhagwan Mahavir Institute of Medical Science, Pawapuri. Study duration of One year. Children in whom TB meningitis was clinically suspected were enrolled into the study. Clinical suspicion is based on clinical evidence with one or more than one of the following laboratory evidence(s). Clinical evidences include fever more than 5 days, headache, vomiting, convulsionsand neck stiffness.

CSF study (WBC>10 cells/mm^3 with >50% lymphocytes, protein> 100mg/dl or glucose < 45mg/dl or CSF /blood glucose <50 %) CT findings (basal exudates, hydrocephalus, or focal brain abnormalities such as infarctions or intracranial tuberculoma) Tuberculosis outside the CNS or positive PPD skin test.

All enrolled children underwent detailed history taking and clinical examination after taking written consent from parents. All children were subjected to following list of investigations.

*Complete hemogram with ESR, HIV antibody test, Mantoux test, Chest X ray, Gastric lavage for AFB and genexpert, CSF cytology.

Mantoux test

2 TU units of PPD RT 23 containing Tween-80 as a stabilizer was given as intradermal injection in the middle third of flexor aspect

of left forearm. Before giving PPD, the area was cleaned with spirit and allowed to dry. Skin was stretched slightly and needle was inserted into the superficial layer of dermis, which results in formation of small wheal of 8-10 mm diameter. Test evaluated 48-72 hours after administration of PPD. Only induration was considered while interpreting the test and not the surrounding erythema. The diameter of induration was measured in millimetres transversely along the axis of forearm with the help of measuring tape. Induration >10mm was considered positive and in immunocompromised induration 5-9mm was considered positive.

Two early morning gastric lavage samples were taken after an overnight fasting of minimum of 4 hours with the help of nasogastric tube. Samples were sent immediately in sterile tubes to TB laboratory for analysis. Two gastric lavage samples were sent for AFB smear and one sent for genexpert. Child was positioned in lateral recumbent position with hips, knee and neck flexed. Shoulders and hips were kept aligned to avoid rotation of the spine. Care was taken not to compromise small infant's cardio respiratory status with positioning. 2 ml of CSF was taken for the genexpert testing. Double volume of reagent was added to each sample directly in the collection container. After 5-15 minutes of incubation, 2 ml of the sample was transferred to the cartridge. The cartridge was loaded and positioned on the Xpert machine (CEPHELD) and test was started. The report of the test was retrieved after 2 hours. The result contains two parts, the first part explains the presence of Mycobacterium tuberculosis complex (MTBC) and if positive for MTBC the second part explains the rifampicin sensitivity.

Results

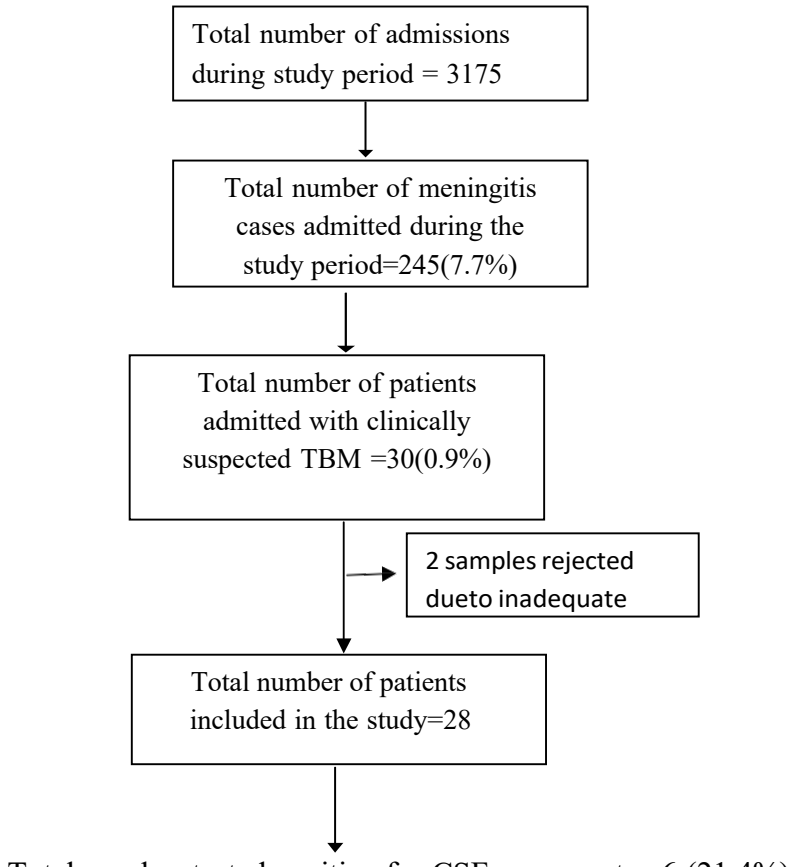

Figure 1: Flow chart of the study

Table 1: Distribution of suspected TBM cases according to age (n=28)

Age in year	Total	Percentage
< 1 yr	6	21.4
1-5 yr	15	53.6
5-12 years	7	25.0
Total	28	100

The most common age group of suspected TBM is between 1-5 years accounting for53.6% of the total cases. Median age in our study population was 3 years.

Table 2: Distribution of suspected TBM cases according to sex (n=28):

Sex	Total	Percentage
Male	17	60.7
Female	11	39.3
Total	28	100

Out of 28 children 17 (60.7%) were male and 11 (39.3%) were female. Male to female ratio in the study population was 1.5:1.

Table 3: Laboratory features of Definite TBM (n=6)

Features	Definite TBM
ESR >50	3(50%)
HIV positive	1(16.67%)
Mantoux test positive	3(50%)
Gastric lavage for smear for AFB positive	1(16.67%)
Gastric lavage for Genexpert positive	1(16.67%)

Table 4: CSF analysis of Definite TBM cases (n=6)

CSF findings	Definite TBM (percentage)
CSF >10 cells	5(83.33%)
CSF >50% lymphocytes	6(100%)
CSF Protein >100mg/dl	4(66.67%)
CSF Glucose <50%	2(33.33%)
CSF ZN stain	Nil

Table 5: CT Brain findings in Definite TBM (n=6)

Features	Definite TBM (percentage)
Hydrocephalus	5 (83.33%)
Basal exudates	4(66.67%)
Granuloma	2(33.33%)
Infarct	2(33.33%)

Table 6: Comparison between age of presentation and CSF genexpert positivity(n=6)

Age	CSF Genexpert		Total
	Positive	Negative	
<1 yr	3 (50%)	3	6
1-5 yr	2(33.33%)	13	15
>5 yr	1(16.67%)	6	7
Total	6	22	28

Fisher's exact test p value= 0.196, p-value is not significant

Table 7: Comparison between CSF genexpert positivity and contact history

CSF Genexpert	Contact h/o TB		Total
	Present	Absent	
Positive	4	2	6
Negative	6	16	22
Total	10	18	28

Fisher's exact test p value=0.079, p value is not significant

Table 8: Comparison between Gastric lavage genexpert and CSF genexpertpositivity:

Gastric lavageGeneXpert	CSF GeneXpert		Total
	Positive	Negative	
Positive	1	1	2
Negative	5	21	26
Total	6	22	28

Fisher's exact test p value=0.352, p value is not significant

Table 9: Comparison between CSF genexpert positivity and CT findings

CT Findings	CSF Genexpert		Total	Fisher's exacttest p value
	Positive	Negative		
Hydrocephalus	5	7	12	0.021
Tuberculoma	2	0	2	0.009
Infarct	2	3	5	0.292
Basal exudates	4	4	8	0.025

p value is significant for hydrocephalus, tuberculoma and basal exudate

Table 10: Comparison between CSF genexpert positivity and CSF findings

CSF Findings	CSF GeneXpert		Total	Fisher's exact test pvalue
	Positive	Negative		
CSF >10 cells	5	10	15	0.086
CSF >50% lymphocytes	6	10	16	0.005
CSF Protein >100mg/dl	4	4	8	0.025
CSF Glucose <50%	2	4	6	0.440
CSF ZN stain	Nil	Nil	Nil	Nil

p-value is significant in terms of CSF > 50 %lymphocytes and protein >100mg/dl

Discussion

Diagnosis of Tuberculous meningitis in children is often challenging, due to protean clinical manifestations and paucibacillary nature, which pose great difficulty in isolation of Mycobacterium tuberculosis from CSF. In 1-2 percent of untreated cases of tuberculosis develop TB meningitis, which has very high morbidity and mortality. Permanent neurological damage is seen in one third cases of TBM and one third cases die soon after presenting to hospital. A quick and reliable test is required to diagnose TBM in early stage to prevent morbidity and mortality. Hence WHO has recommended CSF genexpert for early diagnosis of TBM. In our study out of 28 children admitted with suspected tuberculous meningitis, 6 (21.4%) were positive for CSF GeneXpert. Various studies conducted by Gerardo A U et al [10], Nguyen T Q et al [9] and Rakesh et al have shown CSF genexpert positivity ranging from 24% to 38% which is similar to our study. We have compared CSF genexpert positivity and TBM clinical scoring as per International consensus for case definition. In our knowledge this was the first study where CSF genexpert positivity was compared with clinical

scoring based on International Consensus for Case Definition of TBM. The sensitivity and specificity of CSF genexpert against clinical scoring (≥ 10) was 46.15% and 100% respectively. In another study conducted by Nhu and colleagues in Vietnam found 59% sensitivity for Xpert used on CSF v/s clinical TBM case definition.[9] Study by Vinod B et al from South Africa regarding the diagnostic accuracy of quantitative PCR in tubercular meningitis showed 36% sensitivity against a clinical case definition. Children from lower socioeconomic status constituted 83.33% as per Kuppuswamy classification, similar findings were seen in study conducted by Israni et al(39) ,where 85% of cases were from lower socioeconomic status. Incidence of Tuberculous meningitis is more in lower socioeconomic status because of overcrowding, poor hygienic practises , poor health care seeking and high illiteracy rate. [12] Severly malnourished children in our study constituted 50% (3) of cases according to WHO classification. In another study conducted by Israni et al [11] from North India,76% of children were malnourished according to IAP classification, while in another study conducted by Yaramis et al from Turkey, 29% had malnutrition. In another study from Agra by Dayal R et al malnourishment was found in 67.8% of cases. This variation in the association of malnutrition is mainly due to the regional variation in the nutritional status of the children and also indicates the more incidence of TBM among malnourished. In our study clinical examination findings revealed motor deficits present in 4 out of 6 positive cases (66.67%), meningeal signs present in 3 out of 6 positive cases (50%), cranial nerve palsy was there for one case (16.67%). None of them had optic nerve atrophy. In the study conducted by Nyugen et al the signs of meningeal irritation was present in 81% of cases, motor deficit was present in 47% of cases and cranial nerve palsy was present in 25% of cases. This difference may be because of i) more cases of HIV co-infection in other studies where fever, headache and meningeal signs were rare manifestations [13] and ii) sample size difference. The other reason could be due to more number of cases in current study were in infancy, where signs of meningeal irritation are usually difficult to elicit. BCG vaccination protects against severe form of tuberculosis, lower coverage of vaccination may be cause of prevalence of TBM. Studies have proven that BCG vaccination has got certain effect in reducing the severity and mortality associated with TBM [14]. In our study TBM was more in BCG immunised children. Out of 6 positive cases, 5 (83.33%) cases were immunised with BCG. In a study conducted by Dayal R et al 27 % of cases were immunised with BCG. In our study more number of BCG vaccinated children are having TBM probably because of the fact that malnourished or underweight or lower socioeconomic status of study population have less protective effect from BCG [15]. There was a significant relation between CSF genexpert positivity and mantoux positivity. Out of 6 positive cases 50% (3) had positive mantoux test. Similar findings were noticed in study conducted by Rock R B et al [14] and Nicolette N B et al [16] where Mantoux positivity was noticed in 55% of cases. In a retrospective study conducted by Egidia et al and another study conducted by Yaramis a et al, 36% and 30% of cases respectively had shown positive reaction to tuberculin injection. The difference in observation may be due to many factors like improper storage of PPD, faulty technique in giving injection, immune suppression or recent vaccination with BCG. Abnormal chest Xray findings were seen in 2 cases, of which one (16.67%) had miliary picture and one (16.67%) had consolidation. In a study conducted by Egidia et al [11] Chest Xray features were of miliary pattern in 16% of cases and consolidation in 8% of

cases. In another study conducted by Solomons et al [17] chest Xray suggestive of PTB is found in 47%. [18]

Conclusion

CSF genexpert is considered superior to smear for AFB in isolating TB bacilli in cases of suspecting TB meningitis . The sensitivity of CSF genexpert against clinical scoring is 46.15% and specificity of 100%. There is significant association between Mantoux positivity and CSF genexpert positivity. There is statistically significant association between CSF genexpert positivitywith

*CSF protein >100mg/dl

*CSF lymphocytes >50%

*Hydrocephalus in CT brain

*Basal exudates in CT brain

*Granulomas in CT brain

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