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Clinical Study On Utility Of Cartridge-Based Nucleic Acid Amplification Test(Cbnaat) In Diagnosis Of Tubercular Meningitis

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Abstract.
Background: *The aim was to determine utility of Cartridge based nucleic acid amplification test (CBNAAT) in diagnosis of mycobacterium tuberculosis in children with neurotuberculosis diagnosed on the basis of clinical evaluation, CSF findings and neuroimaging.*
Methods: *A hospital based cross sectional study was conducted in Pediatric Department of RIMS , Ranchi from April 2020 to December 2020. Total 30 children of age group of 2 months to 12 years with the diagnosis of tubercular meningoencephalitis (TBME) on the basis of clinical evaluation, CSF examination and neuroimaging were included in the study.*
Results: *A total 30 children were enrolled. Maximum number of cases admitted with TBME were among 1-5 years of age group (60.91%). CSF and gastric aspirate were examined by CBNAAT for MTB. 2 (6.6%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 6 (20%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate.*
Conclusions: *TBME is a major health problem in children below 5 years. Gene Xpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens. CBNAAT for mycobacterium tuberculosis was positive in 2 (6.6%) children from CSF and 6 (20%) from gastric aspirate. Negative CBNAAT should not prevent any patient with suspected features of TBME from starting anti tubercular treatment (ATT) as sensitivity of this test remains low. Final judgement to start ATT should be based on clinical, biochemical and radiological profile especially in CNS tuberculosis.*

Keywords: *CBNAAT, Cererospinal fluid, Gastric aspirate, Tubercular meningoencephalitis.*

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I. Introduction

Tuberculosis has caused human disease for more than 4,000 yr and is one of the mos timportant infectious disease worldwide. There are five closely related mycobacteria in the mycobacterium tuberculosis complex: M. tuberculosis ,M. bovis ,M. africanum ,M.microti ,M. canetti. M. tuberculosis is the most important cause of tuberculosis disease in humans. The tubercular bacilli are non-spore forming , non-motile , pleomorphic ,weakly gram-positive curved rods 1-5μm long ,slender ,and slightly bent. Tuberculosis of the central nervous system is the most serious complication in children and is fatal without prompt and appropriate treatment. Tubercular meningitis usually arises from the formation of a metastatic

caseous lesion in the cerebral cortex or meninges that develops during the lymphohematogenous dissemination of the primary infection. Tubercular meningitis complicates approximately 0.3% of untreated tubercular infections in children. It is most common in children 6months to 4 years old. Occasionally ,tuberculous meningitis occurs many years after the infection ,when rupture of one or more of the subependymal tubercles discharges tubercle bacilli into the subarachnoid space. The clinical progression of tubercular meningitis may be rapid or gradual. Rapid progression tends to occur more often in infants

and young children ,who can experience symptoms for only several days before the onset of acute hydrocephalus ,seizures ,and cerebral edema. There is 3 stages in tubercular meningitis. The 1st stage typically lasts for 1-2 wk characterized by non-specific symptoms of fever ,headache ,irritability ,drowsiness and malaise. The 2nd stage usually begins more abruptly. The most common features are lethargy ,nuchal rigidity ,seizures ,positive kernigs and brudzinski signs , hypertonia ,vomiting ,cranial nerve palsies ,and other neurologic signs. The 3rd stage is marked by coma ,hemiplegia or paraplegia ,hypertension ,decerebrate

posturing ,deterioration of vital signs ,and eventually death. The diagnosis of tubercular meningitis can be difficult in its course ,requiring a high degree of suspicion on the part of the clinician. The TST is non reactive in upto 50%cases ,and upto 20-30%children have normal chest radiography. The most important laboratory test for the diagnosis of tubercular meningitis is examination and culture of the lumbar CSF. Cartridge based nucleic acid amplification test (CBNAAT) OR GENE-XPERT has now emerged as potential important tool for diagnosing tubercular meningitis. The main nucleic acid amplification test (NAAT) studied in children with tuberculosis is PCR ,which uses specific DNA sequences as markers for microorganisms. Gene – Xpert MTB/RIF is a real time PCR(polymerase chain reaction) assay for the M. tuberculosis that simultaneously detects rifampicin resistance ,which is often used as a proxy for MDR tuberculosis. This assay uses a self-contained cartridge system ,which yields results from direct specimen in 2hrs and is less operator dependant than traditional PCR detection methods. Sensitivity and specificity of Xpert have averaged PCR detection methods72-77% and 99% in acid-fast bacilli (AFB) sputum smear-negative adults and 99-100% in AFB sputum smear-positive adults ,respectively. Xpert improved the sensitivity of detecting pediatric TB cases by 36-44%. Xperts sensitivity and specificity to detect rifampin resistance in sputum samples from adults with tuberculosis was 86%and 98%,

respectively. Although cartridges for the Xpert are expensive ,it offers advantages in rapid detection of MDR-TB and is especially useful in settings lacking laboratory infrastructure.So, the present study is interested to know the utility of CBNAAT for detection of tubercular meningitis aiming for its detection earlier and management.

II. Aims And Objectives:

1.To study the utility of Cartridge Based Nucleic-Acid Amplification Test in diagnosis of Tubercular Meningitis in children aged 2months to 12 years in patient admitted in RIMS ,Ranchi.

III. Materials And Methods

The present study will be conducted in the Department of Paediatrics ,Rajendra Institute of Medical Sciences, RIMS examined thoroughly and investigated.

Study design: Hospital based cross sectional study.

Sample size: Case: 30 suspected meningitis children admitted in department of Paediatrics ,RIMS ,Ranchi

Study period: April 2020 to December 2020

Study tools: Lumbar puncture needle, plain vials ,cotton swab ,betadine ,spirit ,gloves ,leucoplast.

Inclusion Criteria:

- Children between the age group of 2 months to 12 years.
- Children presenting with fever >2weeks duration ,with headache ,signs of meningeal irritation ,altered consciousness level and focal neurologic deficits and CSF finding (lymphocytic pleocytosis ,moderately elevated protein levels ,low glucose) with or without favourable finding on cranial imaging (hydrocephalus ,basal meningeal enhancement ,tuberculoma ,vasculitis leading to infarcts.
- Children whose parents giving written informed consent.

Exclusion Criteria :

- Children below 2months of age or above 12 years of age.
- Children having fever duration <2weeks.
- Children with already diagnosed other causes of meningitis like viral ,fungal or autoimmune , etc.
- Children whose parents are not consenting for participation in study.

IV. Methodology:

All children who fulfil the inclusion criteria during the study period were recruited in the study. Written informed consent was taken from parents of all children who fulfilled the inclusion criteria. After detailed history including clinical ,demographic profile ,socioeconomic status , contact history with pulmonary tuberculosis ,past history of tuberculosis ,vaccination history and general physical and systemic examination, lumbar puncture was done under strict aseptic condition and in case of papilledema, a guarded procedure was done or procedure was delayed to settle down the papilledema. Around 2-3ml of csf was collected in 4 containers. Three of the containers was sent for CSF routine examination including sugar estimation ,protein estimation ,total count ,differential count ,RBC count ,AFB staining ,conventional culture ,and for ADA. Apart from CSF analysis other blood test is done like complete blood count ,ESR , LFT,RFT and electrolytes.

In case of altered sensorium or seizure ,brain imaging (CT scan /MRI) was done along with chest Xray. The following criteria were used as composite gold standard to diagnose TBM.

 CLINICAL CRITERIA: Fever >2weeks ,headache ,neck stiffness ,neck rigidity ,neck pain ,altered sensorium ,convulsion ,cranial nerve palsies ,photophobia ,weight loss ,dysarthria ,loss of apetite.

 SUPPORTIVE CRITERIA : chest Xray ,CSF and neuroimaging (CT/MRI brain) ,tuberculin skin test .

Laboratory Investigation:

1. CSF for routine examination ,microbiological examination ,ADA and CBNAAT.
2. Blood for CBC ,RFT ,LFT ,ESR and electrolytes.

All the information was recorded in predesigned proforma formed in Microsoft excel for final analysis.

Statistical analysis

Data will be analysed with appropriate statistical tests and methods to determine the significance and power of study.

Statistical test: Anova test and Student's T Test will be applied to compare our findings.

Data analysis: Data will be analysed using SPSS 21.0 Software version.

V. Results

Thirty children between 2 months to 12years of age with the diagnosis of tubercular meningoencephalitis on the basis of clinical evaluation, CSF examination and neuroimaging were included in the study during the study period of one year (July 2017 to June 2018). 18 children (60.9%) admitted with tubercular meningoencephalitis were in the 1-5 years of age group (60.91%). CSF was examined in all children with tubercular meningoencephalitis. Majority of children (56.36%) cell counts were in the range of 101-500 cells/µl (>50% lymphocytic pleocytosis presented in all cases). Mean CSF cell count was 198.09±177.86 per µl. 81.82% children CSF protein level was in range of 40-400 mg/dl. Mean CSF protein level was 230.98±167.73 mg/dl. CSF was analysed for glucose levels. In majority of patients (40%) CSF glucose level was in range of 20-40 mg/dl followed by 31.82% was in range of <20 mg/dl. Mean CSF sugar level was 33.86±18.22 mg/dl. None of the CSF sample and gastric aspirate sample among the study group demonstrated AFB on Ziehl-Neelsen staining.

Table 1: Distribution of cases according Ziehl-Neelsen Staining of CSF and gastric aspirate sample.

ZIEHL-NEELSEN STAINING	ZN STAINING IN CSF		ZN STAINING IN GASTRIC ASPIRATE	
	NO OF CASES	PERCENTAGE	NO OF CASES	PERCENTAGE
NEGATIVE	30	100	30	100
POSITIVE	0	0	0	0
TOTAL	30	100	30	100

None of the CSF and gastric aspirate sample among the study group demonstrated AFB on Ziehl-Neelsen staining Table 1.

CSF and gastric aspirate were examined by CBNAAT for MTB. 5 (4.55%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 16 (14.55%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate. CBNAAT detected MTB three times more often in gastric aspirate than CSF in diagnosed cases of tubercular meningoencephalitis (Table 2).

TABLE 2: CBNAAT IN CSF AND GASTRIC ASPIRATE SAMPLE OF THE STUDY POPULATION

TEST RESULT	CBNAAT IN CSF		CBNAAT IN GASTRIC ASPIRATE	
	NO OF CASES	PERCENTAGE	NO OF CASES	PERCENTAGE
POSITIVE	2	6.6	6	20
NEGATIVE	28	93.7	24	80
TOTAL	30	100	30	100

VI. Discussion

Thirty children between 2 months to 12 years of age with the diagnosis of tubercular meningoencephalitis on the basis of clinical evaluation, CSF examination and neuroimaging were included. CSF was examined in all children with tubercular meningoencephalitis. None of the CSF sample among the study group demonstrated AFB on Ziehl-Neelsen staining Table 1. Singh R et al, in their study on clinical profile of pediatric neurotuberculosis.9 CSF analysis was done in 37 cases out of 46. CSF smear microscopy for acid-fast bacilli were negative in all the cases. CSF and gastric aspirate were examined by CBNAAT for MTB. Only 2 (6.6%) children had CBNAAT positivity in CSF. Gastric aspirate was positive among 6 (20%) children. None of the patient had CBNAAT positive result both in CSF and gastric aspirate. Out of 30 children, this test was positive in 8 (26%) patients. Table 2. Avashia S et al, conducted study on 300 extra pulmonary samples, which

included 103 pleural fluids, 81 pus, 45 CSF, 35 Lymph node tissue, 20 ascitic fluids and 16 synovial fluid.10 out of these 37% (111) patients were Gene Xpert MTB/RIF Assay positive and 36% (40 out of 111) were ZN smear positive. 71 were ZN smear negative but came out to be positive with Gene Xpert assay test, 189 cases were negative for both ZN smear and Gene Xpert MTB/RIF assay. M. tuberculosis was detected in 56.7% (46/81) pus samples, 23.3% (24/103) pleural fluid samples, 54.2% (19/35) lymph node samples, 33.3% (15/45) CSF samples, 20% (4/20) ascitic fluid samples and 18.7% (3/16) synovial fluid samples. The result of the study revealed a maximum positivity rate by Gene Xpert which indicated that it is a more sensitive technique as compared to conventional methods. Moure R et al, conducted study of 149 smear negative extrapulmonary samples, out of these 108 was culture positive for MTB.11 Gene xpert detected DNA of MTBC in 63 of the 108 clinical extrapulmonary specimens with MTBC-positive cultures. None of the culture negative sample (41) showed positive result with Gene xpert. Among the 149 specimens studied, 108 specimens had a positive culture of MTBC: (i) 43 liquid specimens (37 sterile fluids, 3 gastric aspirates, and 3 urine specimens) and (ii) 65 nonliquid specimens (34 lymph nodes, 17 abscess aspirates, 12 tissue samples, and 2 stool specimens). Out of 8 gastric aspirate samples 3 were positive for culture, the sensitivity of the Xpert assay was 66.67% (2/3) in culture positive cases and 2/14 CSF samples were culture positive both these culture positive samples were also positive for Gene Xpert assay.

Singh A et al, in 2018 conducted a study to know the role of CBNAAT in diagnosis of tubercular meningitis.12 62 patients were included in the study who had features suggestive of tubercular meningitis. According to the universal case definition, the patients were divided into probable, possible and definitive TBM. Out of 62 patients included in the study, 6 (4%) were Definite TBM, 33 (58%) were probable TB, 17 (30%) were possible TBM and 5 (8%) were not TBM. Total 22 patients had M. TB detected in their CSF on CBNAAT, out of a total of 57 TBM patients. The sensitivity of CBNAAT in present study was 38.6%. Neurotuberculosis is a pauci bacillary disease, number of bacteria are scanty and difficult to demonstrate. Most clinical and neurological manifestation or complication in neurotuberculosis are because of inflammatory immuneresponse rather than direct damage because of mycobacterium tuberculosis.13

VII. Conclusion

Gene Xpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative body fluid specimens at both hospitals as well as point-of-care settings in regions with high TB burden. Negative CBNAAT should not prevent any patient with suspected features of TBME from starting ATT as sensitivity of this test remains low. Final judgement to start ATT should be based on clinical, biochemical and radiological profile especially in CNS tuberculosis.

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