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Original Article

Paediatrics Section

Cartridge Based Nucleic Acid Amplification Test: Utility as Diagnostic Modality in Clinically Diagnosed Childhood Tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB) remains one of the deadliest communicable diseases worldwide. The introduction of early and rapid diagnostic test such as Cartridge Based Nucleic Acid Amplification Test (CBNAAT) creates opportunities for improvement in early and fast detection of TB and drug-resistance.

Aim: To determine the prevalence of Tubercular cases in clinically diagnosed TB patients and to determine the prevalence of Rifampicin resistance by CBNAAT.

Materials and Methods: This prospective observational study involved a total of 107 children from January 2018 to June 2019, fulfilling the inclusion criteria who were clinically diagnosed and found positive in conventional tests for TB. CBNAAT was used to confirm *Mycobacterium tuberculosis* (MTB) for the diagnosis of childhood TB and compare with other conventional methods. Chi-square test for the proportion was applied and p-value <0.05 taken as significant.

Results: Of the 107 patients studied, 44.9% were female and 55.1% were male. The age range was 3 months to 12 years. The cases were confirmed by CBNAAT in clinically suspected case of TB was found to be 17.8% (19 cases out of 107 patients). CBNAAT was able to detect MTB in 14% (15 cases out of 107 patients) of the gastric aspirate/sputum sample. Out of 107 patients, 69 children were clinically diagnosed as Neurotuberculosis and 13% (9 out of 69 cases) cases were confirmed by CBNAAT in Cerebrospinal Fluid (CSF). OF which 29 patients had CSF analysis suggestive of Tubercular Meningitis (TBM). Out of 29 patients, 10 (34.5%) cases were confirmed by CBNAAT. This test showed a significant association with positive Mantoux test (p-value 0.020) and CSF analysis (p-value 0.021) suggestive of TBM.

Conclusion: CBNAAT should be used in preference to conventional methods as the initial diagnostic test for patients suspected of having TB. CBNAAT positivity shows significant association with positive Mantoux test and CSF analysis suggestive of TBM.

Keywords: Mantoux test, *Mycobacterium tuberculosis*, Rifampicin

INTRODUCTION

TB is one of the leading causes of death from a single infectious agent [1]. With an estimated 2.2 lacs children affected per year, India shares 22% of global tubercular burden. Pulmonary TB is the most common form in children but the Extra-Pulmonary Tuberculosis (EPTB) forms a larger proportion of cases than in adults [2]. Globally, the case detection rate is 64%, which means 36% of incident TB cases are not detected. This leaves a gap of approximately 3.6 million people worldwide with TB who were "missed", either because they were not diagnosed or because they were diagnosed but not reported. The inability to clearly and timely diagnose TB among children is the reason why its true extent as causing mortality and morbidity is attributed to comorbid conditions [3].

Diagnosis of TB in children however is difficult because the routine sputum smear microscopy rarely identifies TB in children, Mantoux test may be negative if the child is malnourished. Since cavitary lesions due to pulmonary TB are rare in childhood, Chest-X Rays (CXR) is not always helpful except in adolescents. Lack of standard definitions, absence of simple/reliable diagnostic tests and more EPTB cases add to lack of data on paediatric TB, Conventional techniques, such as detection of acid fast bacilli by Ziehl-Neelsen staining are very economical, yet have a very low sensitivity [4]. Isolation of mycobacteria by culture on Lowenstein Jensen media, considered to be the gold standard, is not only time consuming but has a low sensitivity, especially in EPTB [5]. The World Health Organisation (WHO) has endorsed the use of CBNAAT to diagnose pulmonary TB and EPTB and rifampicin resistance. CBNAAT testing has been a part of Revised National Tuberculosis Control Program (RNTCP) since 2009 but it became available all over India, only 2015 onwards. So the data regarding sensitivity and drug resistance are still lacking.

In the study, CBNAAT was used to detect MTB for the diagnosis of childhood TB and compare with other conventional methods like Mantoux test, CXR, CSF analysis and Computed Tomography (CT) scan for TBM etc. This study also determined the prevalence of CBNAAT positive TB cases in clinically diagnosed TB patients and determined the prevalence of rifampicin resistance by CBNAAT. Early detection and management will improve outcomes in children with TB and reduce disease prevalence in society.

MATERIALS AND METHODS

This observational prospective cross-sectional study was carried out in the Paediatric Department of UPUMS Saifai, Etawah from January 2018 to June 2019. Children of age group 3 months to 12 years who were admitted in Department of Paediatrics with clinical diagnosis of TB and fulfilling the inclusion criteria were enrolled in the study. Ethical clearance was obtained from Ethical Committee of the University (Letter no. 795/UPUMS/Dean/2019/EC No. 27/2018). Written and informed consent was taken from guardian of each patient.

Inclusion criteria: A clinically diagnosed TB case refers to a pulmonary or extra-pulmonary TB patient who has been diagnosed with active TB by a clinician on the basis of clinical findings or having clinical features suggestive of TB anywhere in the body with one or more below mentioned findings:

1. Persistent fever/cough of more than two weeks and loss of weight/no weight gain.

2. Any patient in contact with active TB case.
3. Positive Mantoux test.
4. Headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness or lethargy.
5. CSF cytological or biochemical analysis suggestive of TBM.
6. Radiological lesions consistent with TB on CXR or CT scan of head.
7. Ultrasonography (USG) of abdomen suggestive of abdominal TB.
8. Progressive enlargement of lymph node >2 weeks, size >2 cm or sinus formation.
9. Patients having ascites with hepatosplenomegaly.

Exclusion criteria: Patient already diagnosed to have TB or already taking Anti-Tubercular Drugs (ATT) patient having Human Immunodeficiency Virus (HIV) infection or whose guardian did not give consent for enrolling in study were excluded from the study.

During the study period, 156 children were presented to the Department with clinical TB out of which 49 were excluded from the study and finally 107 children were studied and analysed. All the children who were included in the study were evaluated for relevant clinical history, family history and history of contact with tubercular patient, dietary history and immunisation status. The socio-demographic data obtained and clinical examination was performed on each of participants with emphasis on anthropometry, general physical examination and systemic examination.

Investigations

Complete blood count with Erythrocyte Sedimentation Rate (ESR) and relevant investigations were sent to diagnose TB. ESR >15 mm in 1 hour considered as raised or high [6].

The chest radiograph was done in all patients suspected to have TB. Patients having radiological signs like hilar lymphadenopathy, effusion, miliary pattern, cavity, consolidation, calcification, fibrosis, collapse were considered suggestive of TB [7]. Mantoux test was done in all patients with 5 TU PPD RT23 with standard method. The result was interpreted after 72 hours of injection. The induration was measured in horizontal direction. Induration of 10 mm or more is considered positive and less than 10 mm considered negative [8]. Sputum/gastric aspirate specimens were collected for detection of MTB by CBNAAT from all subjects suffering from TB, diagnosed by one of the conventional methods. A single morning sputum specimen was collected. Those who could not produce sputum, 10 mL gastric aspirate sample were collected by standard procedure after at least eight hours, of fasting. CSF study and neuroimaging in the form of Contrast-Enhanced Computed Tomography (CECT) was performed only in selected subjects under clinical suspicion of TBM.

On CSF analysis, cell count of 10-500 predominantly lymphocytes in CSF with sugar <50 mg/dL and protein 100-3000 mg/dL was considered positive for TBM [9]. A 2 mL CSF was properly stored for CBNAAT analysis.

Patients having CECT head findings like basal exudates, Lepto-meningeal enhancement, hydrocephalus, cerebral infarction, tuberculoma, vasculitis, diffuse brain oedema were considered to have TBM [10].

USG abdomen was done in suspected gastrointestinal TB patients and findings like mesenteric lymph nodes >15 mm, fluid in abdomen, hepatosplenomegaly, ileocecal TB, were considered to have gastrointestinal TB [11].

CBNAAT was performed as per the manufacturer's instructions. In brief, 1-2 mL of sample (CSF/sputum/gastric aspirate) was collected in the conical tube and sample reagent (sodium hydroxide and isopropanolol) was added in 1:2 proportion and shaken 10-20 times vigorously. A 2 mL of this mixture was transferred into cartridge. This loaded cartridge was inserted into MTB-RiF platform where sample automatically filtered and washed. Ultrasonic lysis of filter captured organism occurs to release DNA molecules mixed with dry Polymerase Chain Reaction (PCR) reagents than semi-nested real time amplification and detection in integrated reaction tube with printed result in 2 hours [12].

In the present study, the cases were enrolled on the basis of clinical suspicion of TB and further subjected to other conventional methods for the diagnosis of TB. These patients were tested for CBNAAT positivity and Rifampicin resistance. ZN staining was not performed in this study as it is time consuming, provide lesser yield and not advocated by RNTCP in children.

STATISTICAL ANALYSIS

All the data was collected, compiled, analysed and interpreted statistically through relevant statistical methods. Software used was SPSS (version 22) for Windows. A p-value of <0.05 considered statistically significant.

RESULTS

One hundred and seven children, between 3 months to 12 years, with clinical diagnosis of TB were investigated, out of which 59 (55.1%) were male. The mean age was 6.55±3.93 years. Out of 107 enrolled patients, 29 (27.1%) had pulmonary TB and 78 (72.9%) had extra-pulmonary TB. Only 35 (32.71%) patients had history of contact with TB patient. Various conventional investigations done to diagnose TB and their results are given in [Table/Fig-1].

Investigations (N=107)	Positive	Negative
Chest X-Ray (CXR)	51 (47.7%)	56 (52.3%)
Mantoux test	74 (69.2%)	33 (30.8)
Raised ESR	82 (76.6%)	25 (23.4%)
CSF examination (n=69)	29 (42.0%)	40 (58.0%)
CECT head (n=34)	29 (85.3%)	5 (14.7%)
Type of sample sent for CBNAAT		
Sputum/Gastric aspirate (N=107)	15 (14.0%)	92 (86.0%)
CSF (n=69)	9 (13.0%)	60 (87.0%)
Total 107 (Sputum/Gastric Aspirate + CSF)	19 (17.8%)	88 (82.2%)

[Table/Fig-1]: Investigations done to diagnose TB.
CBNAAT: Cartridge based nucleic acid amplification test; ESR: Erythrocyte sedimentation rate; CSF: Cerebrospinal fluid; CECT: Contrast-enhanced computed tomography

Total 107 sputum/gastric aspirate and 69 CSF samples were collected for CBNAAT analysis. CBNAAT was able to detect MTB in 15 (14%) of the gastric aspirate/ sputum sample and in 9 (13.04%) cases of CSF sample. In 5 cases, MTB was detected in both CSF as well as in gastric aspirate/sputum sample. Among the 19 CBNAAT positive cases none found rifampicin resistant.

Various conventional diagnostic methods done for the diagnosis of TB were compared with CBNAAT positivity and observations are given in [Table/Fig-2]. CBNAAT positivity showed a significant association with positive Mantoux test and CSF analysis suggestive of TBM.

DISCUSSION

In this study, the performance of CBNAAT in sputum/gastric aspirate and CSF of clinically diagnosed TB cases was evaluated. The MTB was detected in 19 (17.8%) patients.

Das PK et al., reported, 3% to 5% quarter-to-quarter period CBNAAT positivity in the paediatric age group and prevalence of

Variable	CBNAAT (N=107)		Total	p-value
	Positive (19)	Negative (88)		
X-ray chest suggestive of pulmonary TB	10	41	51	0.633
Positive mantoux test	9	65	74	**0.020**
Raised ESR	16	66	82	0.972
USG abdomen suggestive of abdominal TB	0	02	02	0.904
CECT head suggestive of neurotuberculosis	6	23	29	0.997
CSF suggestive of tubercular meningitis	10	19	29	**0.021**

[Table/Fig-2]: Comparison of CBNAAT with clinical diagnostic methods of Tuberculosis. p-value<0.05 to be considered significant

MTB detection was reported to be 3.88% [13]. One study from Institute of Medical Sciences, India, reported 11.1% CBNAAT positivity in children [14]. This might be due to selection of patients on the clinical basis only. Kasat S et al., found 15% positivity of CBNAAT in adult extra pulmonary TB cases, which is comparable to this study [15]. Khorgade RR and Bhise PR found significantly high (42.56%) CBNAAT positivity in pulmonary TB adult cases [16]. The high positivity of CBNAAT can be explained by the selection of adult pulmonary TB cases.

CXR was suggestive of TB in 47.7% of all cases while it was suggestive in 52.6% of CBNAAT positive cases. There was no association between CBNAAT positivity and chest CXR finding. Swaminathan S et al., found normal chest radiographs in more than half of the children with confirmed TB [17]. Pepper T et al., also reported high rate of normal CXR among persons with culture-confirmed pulmonary TB [18]. Chest radiography is a relatively insensitive tool apart from its known limitations of poor specificity and high inter-observer variability [17].

In this study, Mantoux test was suggestive of TB in 69.2% patients. A significant association was found between positive Mantoux test and CBNAAT positivity (p=0.02). Mantoux test serves as an indirect evidence for diagnosing paediatric TB, its positivity in 69.2% cases highlighting the relevance of this cheap, easily available and simple test in diagnosis of TB. Positivity was very high when compared to a study done in the Institute of child health, Chennai which reported Mantoux positivity of 34.7% in various forms of paediatric TB [19]. Vijayasekaran D et al., found that the positivity of Mantoux was highest in lymph node TB (53%) and lowest in CNS TB (21.2%) [20].

ESR was raised in 76.6% of patients but there was no association between raised ESR and CBNAAT. An elevated ESR was observed by Al-Marri MR and Kirkpatrick MB in children with TB, but they found that one-third of children with TB had a normal ESR at the time of diagnosis, and consequently there would be little value in using ESR as a diagnostic test for childhood TB [21].

CECT was advised in 34 cases and was suggestive of TBM in 29 (85.3%) patients, which is comparable to Ozates M et al., who reported abnormal CT head findings in 88% cases of TBM [10]. The advent of CT has provided insight into disease progression, and gives prognostic and diagnostic information. As CECT findings are considerably high in cases of TBM in children, few authors recommend CECT should be part of the initial work-up in every child suspected of having central nervous system TB [22].

CSF cytological and biochemical analysis was suggestive of TBM in 29 (42.0%) cases of the 69 clinically diagnosed TBM patients. Out of these 29 patient, 10 (34.5%) were confirmed by CBNAAT. Similar to a previous study done by Mittal M et al., found 34% of the TBM patient had positive CBNAAT in CSF [23]. This study shows significant association between CSF analysis suggestive of TBM and CBNAAT positivity.

In the present study, Rifampicin resistance was not detected in any case by CBNAAT. Das PK et al., reported point and periodic prevalence of Rifampicin resistant among the children vary from 0.2% to 0.5% [13]. The possible reason may be less incidence of drug resistance in children or the small sample size.

Limitation(s)

The limitation of the study was small sample size. If the study is extended to a wider sample size or is conducted as a multi-centric study, it may give better results. This study was done in rural area of central UP. There can be difference in prevalence of TB in urban population. So, results of this study cannot be projected in urban population.

CONCLUSION(S)

In the present study, CBNAAT positivity showed significant association with positive Mantoux test and CSF analysis suggestive of TBM. Drug resistance for the Rifampicin was also evaluated. Use of CBNAAT like newer diagnostic modalities, will help in early diagnosis and management of paediatric TB.

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