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A STUDY OF EVALUATION OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) IN PAEDIATRIC PATIENTS SUSPECTED OF TUBERCULOSIS IN A TERTIARY CARE HOSPITAL IN CENTRAL INDIA

Paediatric Medicine

Dr. Akshay Kamle Junior Resident, MGM Medical College Indore
Dr. Nilesh Jain* Associate Professor, MGM Medical College Indore *Corresponding Author
Dr. Pramila Ramawat Assistant Professor, MGM Medical College Indore
Dr. Hemant Jain Professor & Head of the Pediatric department MGM Medical College Indore

ABSTRACT

Background: Tuberculosis (TB) is a leading cause of morbidity and mortality worldwide. Pediatric patients are underdiagnosed and underreported, have a significantly high mortality risk. CBNAAT is a good diagnostic method to detect mycobacterium tubercule bacilli (MTB). **Material and methods:** A Prospective observational study was conducted in a tertiary care hospital. 171 pediatric patients with clinical features suggestive of presumptive pulmonary or extrapulmonary tuberculosis based on RNTCP guidelines were included in the study. Relevant demographic, clinical and laboratory details were recorded. Patients were labelled as bacteriologically confirmed tuberculosis (if found positive on microscopy, culture and CBNAAT) while labelled as clinically diagnosed, in absence of bacteriological confirmation but imaging findings, other diagnostic tests and /or clinical findings were suggestive of pulmonary or extrapulmonary tuberculosis. **Results:** CBNAAT detected TB bacilli in 57.9% patients hospitalized with clinical suspicion of TB. CBNAAT was also positive in all Z N smear positive patients (n-8) [p <0.05] while 55.8% of smear negative cases were detected by CBNAAT. The detection rate of Rifampicin resistant TB was 2.9% in the study population (p<0.05). **Conclusions:** CBNAAT not only identifies TB bacilli and Rifampicin resistance but also useful in early treatment initiation. It is a good diagnostic tool for diagnosis of TB in children who mostly remain underreported and improperly treated.

KEYWORDS

Tuberculosis, extrapulmonary tuberculosis, CBNAAT, antitubercular medication

INTRODUCTION

Mycobacterium tuberculosis infection is one of the most lethal diseases among communicable diseases.[1] It has been affecting mankind since ages and has a huge influence on healthcare around the world.

WHO reported about 10 million new tuberculosis cases worldwide in 2018; of which children accounted for 11%. India's share was around 27%, making it globally first ranking country. Among 2.74 million cases of tuberculosis, 2.24 lakh cases were from pediatric age group.(1) Childhood tuberculosis poses a diagnostic challenge because it is considered as noninfectious, paucibacillary in nature and the erroneous belief that BCG provides effective protection against pediatric TB.[2]

Bacteriological confirmation is seldom accomplished because of the difficulties in collecting good quality sputum sample. Culture considered to be the gold standard for TB diagnosis but it is of limited use as it takes 4-8 weeks to produce the outcome and also requires infrastructural support and technical assistance.[3] Paediatric case detections were poor due to the lack of diagnostic instruments and the lack of any available gold standard.[4] Contact tracing, history, tuberculin skin sensitivity testing, radiology and lack of response to antibiotics aids in diagnosis of paediatric tuberculosis.

In 2013, WHO endorsed the use of Cartridge-based nucleic acid amplification test (CBNAAT) for TB diagnosis in pediatric presumptive pulmonary and extra-pulmonary (EPTB) cases that simultaneously detects TB and rifampicin resistance provides a promising solution for early case identification and management of TB.[5]

The data regarding the tuberculosis prevalence in the pediatric age group is under-reported because of lack of proper diagnostic facilities. Hence, we undertook this study to evaluate the usefulness of CBNAAT in the diagnosis of tuberculosis in pediatric age group patients with the purpose to detect tuberculosis along with drug resistance early which may play a role in reducing the mortality among the cases and preventing the spread of tuberculosis in the community.

METHODOLOGY

The present study was conducted during the study period from June 2018 to June 2019 after obtaining the ethical clearance from Institutional Ethics Committee. All hospitalized pediatric patients of age group 1 month to 15 years with clinical suspicion of tuberculosis were enrolled in the study, patients whose parents did not give consent were excluded.

All baseline demographic parameters were noted. History regarding presenting complaints, history of contact, duration of illness, relevant physical examination and laboratory finding were recorded in preformed Performa. According to the RNTCP new updated guidelines cases were labeled as pulmonary or extra-pulmonary tuberculosis.[6]

Gastric aspirate was collected in labeled tubes in morning following proper collection method. Expectorated sputum or induced sputum specimen was collected after nebulization with 3% hypertonic saline. In cases with extrapulmonary findings, cerebrospinal fluid was collected using lumbar puncture; fine needle aspiration of the lymph node; pleural fluid and ascitic fluid by tapping according to organ and system involved. In suspected cases, MRI brain / spine and USG of the affected part was done if needed.

CBNAAT samples were sent for processing which was carried out according to the manufacturer's instructions (CEPHID, Sunnyvale, CA, USA). CBNAAT results are provided as Positive / Negative. Based on the results of CBNAAT, the child was considered as bacteriologically confirmed TB or not and Rifampicin resistance was also confirmed.

Definition of the terms:

Bacteriologically confirmed case: A patient who has a microbiological diagnosis of TB, based on positive microscopy, culture or a validated PCR-based test or gene expert (CBNAAT).

Clinically diagnosed case: A patient with negative microbiological tests for TB (microscopy, culture and validated PCR-based tests), but with strong clinical suspicion and other evidence of TB, (imaging findings, histology, diagnostic tests or response to antitubercular treatment.)

The statistical software IBM SPSS Version 22.0.0.0 was used for analysis. Association between CBNAAT findings and other non-parametric variables was done using Pearson Chi-square test. A p value of <0.05 was taken as statistically significant.

RESULTS

171 cases with suspicion of tuberculosis were included in the study. 57.9% cases were found to be positive on CBNAAT (N-99) and rest 42.1% were negative (N-72).

Cases age ranged from 1 month to 15 years. Higher incidence of CBNAAT positivity seen in infants (<1 year age) and in 10-15 years old. Majority of patients were in the 10-15 years age group (52.5%), CBNAAT positivity was also high in this age group which is statistically significant. (p = 0.001) Females (56.1%) were more than males (43.9%) in the study, CBNAAT positivity was also high in females (60.6%) but it is not statistically significant (p = 0.168). [Table 1]

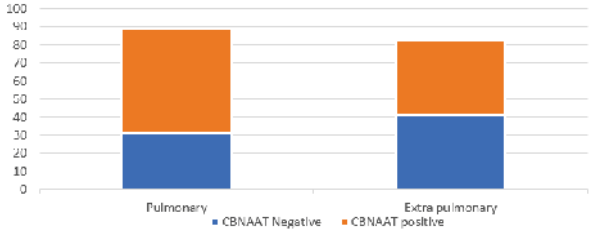
BCG vaccination was received in 85.9% of CBNAAT positive cases which had no significant association with CBNAAT findings (p=0.835). (Table 1) History of contact was seen in 22.8% of the study population and it was positive in 25.3% CBNAAT positive cases. There was no significant association seen between CBNAAT positivity and history of contact (p>0.05). HIV test was positive in 3.5% of study population and 2% CBNAAT positive cases. It is not statistically significant (p = 0.215). (Table 1)

Table 1: Association between CBNAAT and Demographic and clinical parameters

Demographic and clinical parameters		CBNAAT		Total (N – 171)	Chi-square value	P value
		Negative (N- 72)	Positive (N – 99)			
Age Group	< 1 Year	2 (2.8%)	13 (13.1%)	15 (8.8%)	21.886	0.001*
	1-5 Year	22 (30.6%)	13 (13.1%)	35 (20.5%)		
	5-10 Year	28 (38.9%)	21 (21.2%)	49 (28.7%)		
	10-15 Year	20 (27.8%)	52 (52.5%)	72 (42.1%)		
Sex	Female	36 (50.0%)	60 (60.6%)	96 (56.1%)	1.904	0.168
	Male	36 (50.0%)	39 (39.4%)	75 (43.9%)		
History of contact	Negative	58 (80.6%)	74 (74.7%)	132(77.2 %)	0.799	0.371
	Positive	14 (19.4%)	25(25.3%)	39 (22.8%)		
HIV Test	Negative	68 (94.4%)	97 (98.0%)	165 (96.5%)	1.539	0.215
	Positive	4 (5.6%)	2 (2.0%)	6 (3.5%)		
BCG vaccination	Negative	11 (15.3%)	14 (14.1%)	25(14.6 %)	0.043	0.835
	Positive	61 (84.7%)	85(85.9%)	146 (85.4%)		

Pearson chi-square test applied. P value < 0.05 was taken as statistically significant, p value *0.001 for age group was showing statistical significant value.

Among pulmonary TB patients 65.2% were detected by CBNAAT while in extrapulmonary cases 50% were detected by CBNAAT. (Graph - 1)



Graph 1 – Distribution of TB cases according to type of TB and CBNAAT positivity

In all collected samples of patients for CBNAAT (n= 263), 49% were of gastric lavage and aspirate, 23.4% of sputum, 15.5% were CSF. Case detection rate of TB cases by CBNAAT was 37.6% in this study. 69.2% of CBNAAT positive cases were detected by sputum and gastric lavage/aspirate while 19.2% were by CSF. 75% of lymph node specimens, 66.6% of pus sample, 48.9% of CSF samples, 56.5% of sputum, 28.5% of gastric and 25% of pleural fluid were CBNAAT positive.

Table 2 – CBNAAT positivity in various samples of study population

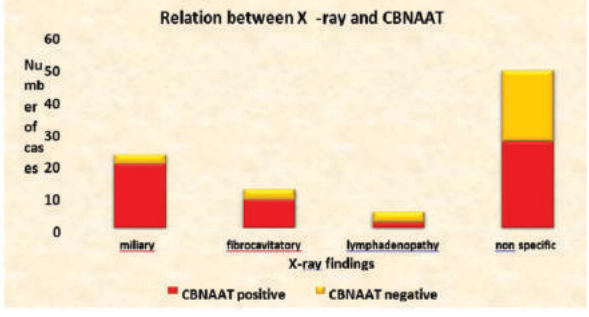
Sample type	CBNAAT POSITIVE (N= 104)	CBNAAT Negative (N= 156)	Total (n = 263)
Gastric aspirate	37 (28.5%)	93 (71.5%)	130 (49%)
Sputum	35 (56.5%)	27(43.5%)	62 (23.4%)
CSF	20 (48.9%)	21 (51.1%)	41 ((15.5%)
Pleural fluid	3 (25%)	9 (75%)	12 ((4.5%)
Ascitic fluid	0 (0.0%)	3 (100%)	3 (1.1%)
Lymph node specimen	9 (75%)	3 (25%)	12 (4.5%)
Pus	2 (66.7%)	1 (33.3%)	3 (1.1%)

CBNAAT was found to be more positive in cases who were also positive on ZN stain. Of the 163 negative cases on ZN stain, 91 cases were positive on CBNAAT. ZN stain was positive in 4.6% (n-8) cases and negative in 95.3% (n-163) cases. There was a good association between ZN stain and CBNAAT findings (p = 0.001). Tuberculin skin test (TST) was found to be positive in 14.6% of the patients. 12.1% patients who were CBNAAT positive were also found to be TST positive, while 18.1% CBNAAT cases were TST positive and 81.95 were TST negative. The association between CBNAAT and TST was not statistically significant (p>0.05). (Table 3)

Table 3 - Association of CBNAAT with other investigations for TB

Type of test		CBNAAT Positive (– 99)	CBNAAT Negative (– 72)	Total (n – 71)	Chi Square test	P Value
ZN Staining	Positive	8 (8.1%)	0 (0.0%)	8 (4.6%)	5.166	0.023
	Negative	91 (65.8%%)	72 (44.2%)	163 (95.3%)		
TST	Positive	12 (12.1%)	13 (18.1%)	25 (14.6%)	1.176	0.278
	Negative	87 (87.9%)	59 (81.9%)	146 (85.4%)		

Patients with suspected pulmonary tuberculosis in chest x-ray, positive signs were noted in 44.9% of patients. Among 40 patients with highly suggestive chest x-ray, 77.5% were CBNAAT-positive. Commonest x-ray finding was miliary shadow and 87% of them were positive on CBNAAT, CBNAAT was positive in 75% of fibrocavitatory and 40% of lymphadenopathy. 49 cases had non-specific x-ray finding, 55.1% of them were CBNAAT positive. (Graph 2) A significant association was seen between x-ray findings and CBNAAT (p<0.05).



Graph 2: Bar graph showing relation between x-ray and CBNAAT in cases of pulmonary TB

Among 171 cases, 99 were diagnosed by CBNAAT, 94 cases were not drug resistant while 5 cases were showing Rifampicin resistance. Drug resistance was found in 2.9% cases. A good statistical association was seen between drug resistance and CBNAAT findings (p = 0.001).

DISCUSSION

Majority of clinically suspected TB patient among 171 included cases belongs to 10 – 15 year age group (42.1%). Usually malnourished and children below 5 years of age are found to be more frequently have clinical features as reported in literature. In comparison to adults, disease progression is highest in adolescents and young adults.[7] This

may be due to out-of-household exposure to large number of contacts. Shresthta et al in their study reported that 63.4% of their cases belonged to 10-15 years age group.[8] Present study findings are also comparable as 52.5% cases in the age group 10-15 years were also positive on CBNAAT which could be due to the ease of collecting the samples in this age group, which has led to higher case detection.

In present study there is a female preponderance (55.05%) and 60.6% females are detected positive while only 39.4% males are detected positive on CBNAAT. Our results are supported by Kamath et al study who also reported female preponderance.[9]

BCG immunization was received in 85.4% cases in current study, all CBNAAT positive cases received BCG immunization. A study done by Gupta et al, reported that 86% cases with pulmonary and 73% cases with extrapulmonary tuberculosis were BCG vaccinated.[10] These findings reinstate that BCG vaccination is not completely efficacious in protecting from tuberculosis.

Positive history of contact was found in 22.8% cases of present study. CBNAAT positive cases, 25.3% gave positive history of contact. Goyal et al reported history of contact in 35.5% cases.[11] Majority of these children acquire this from an infected adult. A meta-analysis reported that 7.8% of the household contacts developed tuberculosis.[12] So, history of contact becomes a key factor in suspected cases of TB.

In this study, slightly higher proportion of cases (52.05%) had pulmonary tuberculosis. Brent et al reported 87% prevalence of pulmonary tuberculosis.[13] Another study done in Philippines reported it in 40.3% cases.[14]

CBNAAT is positive in 57.89% cases in this study. It is positive in 65.16% of pulmonary tuberculosis and 50% of extrapulmonary tuberculosis cases. CBNAAT positivity is shown to be highest in lymph node specimens (75%), followed by sputum (56.5%), CSF reported positive cases of CBNAAT (48.9%), gastric (28.5%), pleural fluid (25%). Kumar et al reported positive CBNAAT in 21.4% of sputum samples, 33% of gastric lavage/aspiration samples, 12.7% of CSF samples, 10% of pleural fluid samples, 0 in ascitic fluid samples and 66.7% of lymph node samples.[15] Das et al also registered detection levels of 2.3% for CSF, 8.3% for gastric lavage and 4.3% for general MTB detection.[16]

In this study, ZN stain and CBNAAT are related significantly ($p<0.05$). All cases with positive ZN staining were also CBNAAT positive while 55.8% cases with negative ZN staining were found CBNAAT positive. In just 4.68 per cent of cases, ZN stain was positive. Sowjanya 's study recorded a 70.24 percent CBNAAT case identification rate for pulmonary tuberculosis and 52.68 percent for AFB with sputum, suggesting a higher diagnostic value for CBNAAT.[17]

On chest x-ray in patients with suspected pulmonary tuberculosis, positive signs were noted in 44.9% cases. 40 patients with highly suggestive x-ray 77.5% were CBNAAT-positive, which corroborates with study done by Pandey et al in 2015.[18] CBNAAT was positive in 87% cases with miliary picture, in 75% with fibrocavitatory, in 40% with lymphadenopathy. Of 49 cases with non-specific x-ray finding 55.1% were CBNAAT positive, reason attributed was due to poor quality of films and intra-reader differences in x-ray interpretations. Significant association was seen between X-ray findings and CBNAAT ($p<0.05$).

Nearly half of the patients in the present sample have extrapulmonary tuberculosis (EPTB), indicating that EPTB remains an underdiagnosed condition in the absence of pulmonary findings. According to Mukherjee et al, the prevalence of EPTB has increased over the last 20 years.[19]

In present study a strong association was reported between CBNAAT and drug resistance ($p<0.05$). Rifampicin resistance is seen in only 2.9% of cases in this study. Dewan et al reported 25% resistance to Rifampicin, which is higher than this study. Although other studies have indicated that it is between 0.6% and 2.3%, which is comparable to current study.[20]

The limitation of this study is that MTB culture methods could not be used for better comparison.

CONCLUSION

Childhood tuberculosis is difficult to diagnose because of difficulty in getting the sputum from them. Extra-pulmonary samples such as CSF, lymph node apart from conventional samples have been of importance in diagnosing MTB. CBNAAT has ability to diagnose as well as evaluate the rifampicin resistance in the same test. It delivers results within 2 hours along with information about rifampicin resistance. Combined with other modalities, CBNAAT has added to case detection rate, becoming a useful armamentarium as a diagnostic tool.

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