



ORIGINAL RESEARCH PAPER

Microbiology

RAPID DIAGNOSIS OF PEDIATRIC TUBERCULOSIS CASES USING CBNAAT

KEY WORDS:

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ABSTRACT

Tuberculosis (TB) in India accounts for about a quarter of the world's TB cases and children are at high risk of tuberculosis infection and TB disease. TB diagnosis in children continues to be a challenge due to difficulty in getting appropriate samples and paucibacillary disease. Therefore Cartridge based nucleic acid amplification test (CBNAAT) is now considered as WHO recommended simple rapid diagnostic test with a well-established role in diagnosis of Pulmonary TB (PTB) and Extrapulmonary TB (EPTB) cases as compared to culture and ZN staining. Therefore, this study was conducted to determine the role of ZN staining, culture and CBNAAT from PTB and EPTB samples for diagnosis of paediatric tuberculosis. **Methods:** A Prospective study in a tertiary care hospital on 284 samples from randomly selected patients suspected of TB with age group 0-12 yrs. All the pulmonary and extrapulmonary samples were compared by ZN staining, Culture and CBNAAT. **Result:** Total no. of 284 samples were processed. Out of them 21, 18 and 09 samples were positive by CBNAAT, culture and ZN microscopy respectively. In our study we found that 7.3% positivity by CBNAAT, 6.3% by culture and 3.1% by ZN staining. All the 21 positive cases were Rifampicin sensitive. **Conclusion:** The study showed MTB detection in 7.3 % of total pediatric cases by CBNAAT. Though ZN staining is the old, rapid and cost effective method but is less sensitive as compared to culture and CBNAAT. Although culture is gold standard but it is time consuming. As CBNAAT is rapid, accurate, highly sensitive and specific with the additional advantage of Rifampicin sensitivity in the same turn. Hence CBNAAT can be a best substitute, point of care test for TB diagnosis in pediatric age group.

INTRODUCTION

Tuberculosis remains the major public health threat in India. ⁽¹⁾ Out of 10 million cases 2.6 million are present in India. ⁽²⁾ If we see the paediatric age group, there are 1 million cases globally. ⁽³⁾ Out of these 8.9% (0.29 million) cases are present in India. ⁽³⁾ Although child TB in India is estimated to be approximately 10% of the total adult incidence, only 6% of the total cases reported to the program are children. ⁽⁴⁾ A large number of children with TB remain undiagnosed each year which makes it difficult to assess the actual magnitude of the childhood TB epidemic. ⁽⁴⁾ Samples in pediatric age are difficult to obtain especially sputum in cases of Pulmonary Tuberculosis. ⁽⁵⁾ Therefore, in case of Pulmonary Tuberculosis gastric lavage is considered ideal sample. ⁽⁵⁾ In Extrapulmonary TB samples are obtained from organs affected. It is difficult to diagnose EPTB as samples are to be taken from deep seated sites. ⁽⁶⁾ Also, diagnosis of EPTB is difficult as it is paucibacillary disease. ⁽⁶⁾

The challenges in diagnosing TB in children include the paucibacillary nature of the disease in many, difficulty to obtain a sample from young children, and low sensitivity of the commonly used smear microscopy technique. ⁽⁴⁾ Although the role of pediatric TB in the transmission of disease may be lower than that of adult patients, pediatric TB can be a reservoir which constitutes a significant number of future adult cases. ⁽⁴⁾ Newer advanced diagnostic tools coming into play are genotypic assays (LPA, CBNAAT, LAMP, TruNAAT etc.) that are rapid molecular tests, and culture methods (liquid culture media) with standard drug susceptibility testing assays. ⁽⁸⁾ The pressing demands of early diagnosis could only be fulfilled with rapid screening/diagnostic methods offered by molecular technologies based on capturing nucleic acid and detecting mycobacterium. ⁽⁸⁾

In 2013, the WHO endorsed the use of CBNAAT for TB diagnosis in pediatric presumptive pulmonary and extrapulmonary tuberculosis (EPTB) cases. CBNAAT, a tool with a quick turn-around time, which simultaneously detects TB and rifampicin resistance, offers a promising solution to achieve the global objective of improved TB care and control and early TB case detection. ⁽¹²⁾

MATERIAL AND METHODS

A total of 383 Pulmonary and Extrapulmonary specimens from patients of Paediatric Wards with clinical signs (radiological) and symptoms of local & disseminated tuberculosis and history of close contact were included in this observational study and processed in the Clinical Microbiology laboratory, Tertiary Care Hospital over a period of 2.5 yrs (July to December 2021). Each sample was analysed by CBNAAT, ZN smear, fluorescent microscopy and culture.

Inclusion Criteria:

- 1) Age <12 yrs.
- 2) Fever and cough for >2 weeks.
- 3) History of weight loss or no weight gain in past 3 months.
- 4) Hillar lymphadenopathy on chest x-ray.
- 5) Tuberculin skin test positive.
- 6) Abdominal USG suggestive of tuberculosis.
- 7) CSF findings consistent with tubercular meningitis.
- 8) Cranial CT suggestive of tuberculoma.

Exclusion Criteria:

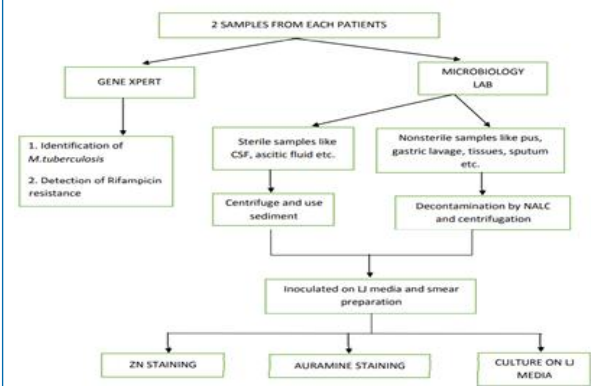
Cases of non-tubercular pleural effusion, nontubercular ascites, pyogenic meningitis, malignant & inflammatory lymphadenopathy, patients above 12 yrs of age were excluded

STUDY TECHNIQUE

Patients were selected as per the inclusion criteria. Detailed history taking, physical examination and relevant laboratory investigations were done. History was taken about socio-economic profile, vaccination status and history of contact and also obtained data based on clinical profile and investigations after explaining the purpose of the study and obtaining informed consent from the parent/guardian of the child in writing.

All the samples were collected in falcon tubes and were labelled. Samples collected were divided into two halves. Then samples were classified as sterile and non-sterile according to site from where samples were taken. Thereafter, one set of each sample were subjected to culture after decontamination if sample was non sterile and directly if sample was sterile. Further, after culture from same set of

samples smears were made and Zn staining and fluorescent staining was done and examined under light microscope and fluorescent microscope respectively. Other set of samples were subjected to CBNAAT.



Cbnaat
 CBNAAT (GeneXpert Assay) was performed according to the manufacturer's instructions (CEPHEID GeneXpert). Our machine contains 4 cartridges so 4 samples were processed foreach run. According to standard operating procedure the sampling reagent (containing NaOH and isopropanol) was added at2:1 ratio to the sample and kept for 15 minutes at room temperature with intermittent shaking. 3ml of this treated sample was transferred to the cartridge and the cartridge was inserted in the module of CBNAAT machine. An automatic process completed the remaining assay steps and the results were displayed on the monitor attached to Gene Xpert after 1hr and 50 minutes.

Afb Smear
 Smears were made after inoculation of samples for culture and ZN staining and Fluorescent staining was done.The slides of ZN staining were examined under 100X oil immersion objective lens. The slides were systematically scanned in a zig-zag manner covering the whole smear from left to right. The slides of Fluorescent staining were examined under 40X magnification LED fluorescent microscope.The Pulmonary TB smear of both staining was graded according to the latest RNTCP guidelines.

Culture
 Culture The concentrated sample obtained after decontamination was inoculated for culture in BACTEC mycobacterium growth indicator tube (MGIT) for liquid culture. Culture was considered as gold standard for analysis of results. When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturer's instructions. All tubes were checked for positivity till 42 days. Mycobacteria other than tuberculosis (MOTT) and Mycobacterium tuberculosis testing from positive culture tubes were done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturer's instructions.

Data Analysis
 All recorded data were analyzed using standard statistical methods (SPSS software). Sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT were calculated. Values of P < 0.05 were considered as statistically significant.

RESULT
Table 1: CBNAAT Positivity and Rifam picin Resistance

Total cases= 383	CBNAAT Positive		CBNAAT Negative
	78(20.36%)		305(79.63%)
	Rifampicin Sensitive	Rifampicin Resistant	305 (79.63%)
	72(92.30%)	6(7.69%)	

Table 1 shows CBNAAT Positive Cases and Rifampicin Resistance Pattern. Out of total 383 Suspected Paediatric TB cases 20.36% (78 out of 383) were CBNAAT positive and 79.63% (305 out of 383) were CBNAAT negative. Out of 78 TB positive cases 7.69% (6 out of 78) were Rifampicin Resistance and 92.30% (72 out of 78) were Rifampicin Sensitive.

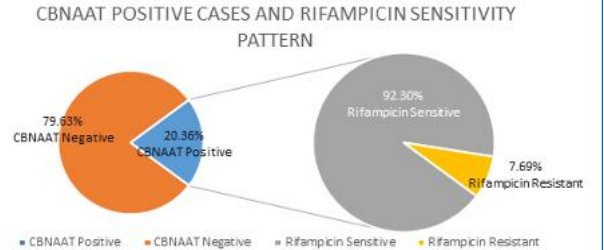


Table 2: Demographic Profile of Cases.

Parameters	No. of Suspected Cases N= 383	No. of Positives (%) n = 78 (20.36%)
Gender		
Male	264	62 (16.18%)
Females	119	16 (4.17%)
Age		
0-4 yrs	202	52 (13.57%)
4-8 yrs	92	16 (4.17%)
8-12 yrs	89	10 (2.61%)
H/O Contact		
Yes	98	70 (18.27%)
No	285	08 (2.08%)
Mantoux test		
Positive	35	33 (8.61%%)
Negative	348	45 (11.74%)
BCG vaccination		
Taken	310	22 (5.74 %)
Not Taken	73	56 (14.62%)
Malnutrition		
Present	84	68 (17.75%)
Absent	299	10 (2.61%)

In our study 383 samples were included of which 264 were from males and 119 were of females. Of these 16.18% (62/383) males and 4.17% (16/383) females were positive respectively, with male:female ratio of 2:1. Maximum positivity was seen in 0-4 yrs of age 13.57% (52/383), youngest patient being positive was of 6months of age. Only 18.27 % of patients had history of contact with TB patient and 8.61% of patients showed positive Mantoux test. Higher number of positivity was seen in cases who did not took BCG vaccination 14.62 (56/383) and also higher number of positivity 17.75% (68/383) was seen in malnourished children.

Table 3: Specimen-wise distribution of CBNAAT Positive Patients

Pulmonary (n = 249)	Types of Samples	Total Patients n = 383	CBNAAT Positive N= 78
	Gastric Lavage	137 (55.02 %)	46 (33.57 %)
	Sputum	112 (44.97 %)	18 (16.07 %)
	Total	249 (65.01%)	64 (25.70%)
Extrapulmonary (n=134)	LN aspirate	45 (33.58 %)	06 (13.33 %)
	CSF	43 (32.08 %)	05 (11.62 %)
	Ascitic fluid	22 (16.41 %)	02 (9.09 %)
	Pus	24 (17.91 %)	01 (4.16 %)
	Total	134 (34.98%)	14 (10.44%)

Of total 383 samples, 65.01% (249/383) samples were from pulmonary tb cases of these 25.70% (64/249) were positive by CBNAAT. Pulmonary samples consisting of 55.02% (137/249) Gastric lavage samples and 112/249 (44.97%) sputum samples. Out of Gastric lavage samples 46 (33.57%) were detected positive and of sputum samples 18(16.07%) were detected positive by CBNAAT.

Out of 134 samples from extrapulmonary tb cases maximum samples were of lymph node aspirates i.e., 45 of which 6 (13.33%) were positive followed by, 43 CSF samples of which 5 (11.62%) were positive, 22 were ascitic fluid of which 2 (9.09%) were positive and lastly 24 pus samples of which 1 (4.16%) was detected positive by CBNAAT

Table 4: Comparison of Zn staining and Culture

Zn Staining	Culture	
	Positive	Negative
Positive (23)	19 (25.33%)	04 (1.29%)
Negative (360)	56 (74.66%)	304 (98.70%)
Total = 383	75	308

This table shows comparison of Zn staining with Culture. Out of 383 samples from children suspected with TB, Zn staining was positive in 23 (6.00%) samples and negative in 360 (94.25%) samples. Whereas, Culture was positive in 75 (19.58%) samples and was negative in 308 (80.41%) samples. Out of Zn staining positive samples 25.33% (19/75) were also positive by Culture while, 1.29% (04/308) samples were positive by Zn staining but negative by Culture. Out of Zn staining negative samples 74.66% (56/75) were positive by Culture while, 98.70% (304/308) samples were negative by both the methods.

Table 5: Comparison of Culture with Auramine Phenol O Fluorescent staining

Fluorescent Staining	Culture	
	Positive	Negative
Positive (27)	23 (30.66%)	04 (1.29%)
Negative (356)	52 (69.33%)	304 (98.70%)
Total = 383	75	308

This table shows comparison of Fluorescent staining with Culture. Out of 383 samples from children suspected with TB, Fluorescent staining was positive in 7.04% (27/383) samples and negative in 92.95% (356/383) samples. Whereas, Culture was positive in 19.58% (75/383) samples and was negative in 80.41% (308/383) samples. Out of Fluorescent staining positive samples 30.66% (23/75) were also positive by Culture while, 1.29% (04/308) samples were positive by Fluorescent staining but negative by Culture. Out of Fluorescent staining negative samples 69.33% (52/75) were positive by Culture while, 98.70% (304/308) samples were negative by both the methods.

Table 6: Comparison of CBNAAT with Lj culture

CBNAAT	Culture	
	Positive	Negative
Positive (78)	75 (96.1%)	03 (3.84%)
Negative (305)	0	305 (79.63%)
Total = 383	75 (19.58%)	308 (80.41%)

This table shows comparison between gold standard culture method and CBNAAT. Out of total 383 samples inoculated on Lj media 19.58% (75/383) samples showed growth on culture while 80.41% (308/383) samples did not growth on culture. While, detection rate of CBNAAT was close to Culture method i.e., CBNAAT was positive in 20.38% (78/383) samples and negative in 79.63% (305/383) samples. All 96.1% (75/78) samples that had grown on Lj media were also found to be positive by CBNAAT. While out of 308 samples that did not show growth on Lj media, 3.84% (3/78) were positive by CBNAAT.

Table 7: Sensitivity, specificity, Positive predictive value and Negative predictive value of CBNAAT, ZN staining, Fluorescent staining in reference to culture.

Test	Sensitivity	Specificity	Positive Predictive Value NPV	Negative Predictive Value NPV
Zn Staining	25.33%	98.70%	82.60%	84.44%
Fluorescent Staining	30.66%	98.70%	85.18%	85.39%
CBNAAT	100%	99.0%	96.15%	100%

Sensitivity, specificity, positive predictive value and negative predictive value of ZN staining with reference to culture are 25.33%, 98.70%, 82.60% and 84.44% respectively.

Sensitivity, specificity, positive predictive value and negative predictive value of fluorescent staining with reference to culture are 30.66%, 98.70%, 85.18% and 85.39% respectively.

Sensitivity, specificity, positive predictive value and negative predictive value of CBNNAT in reference to culture are 100%, 99.00%, 96.15% and 100% respectively.

DISCUSSION

Tuberculosis (TB) is the leading cause of death worldwide. It is one of the major causes of mortality and morbidity in childhood. It is challenging to diagnose Tuberculosis in paediatric age group due to difficulty in collection of samples, paucibacillary nature and resemblance of clinical symptoms of diseases with many other respiratory ailments.⁽⁹⁾ CBNAAT, a tool with a quick turn-around time, which simultaneously detects TB and rifampicin resistance, offers a promising solution to achieve the global objective of improved TB care and control and early TB case detection. Hence, in 2013 WHO endorsed Cartridge Based Nucleic Acid Amplification Test (CBNAAT) for diagnosis of TB.⁽¹⁰⁾

The present study was conducted in a tertiary care hospital in Central India. Study was carried out over the period of two years from October 2019 to September 2021. Total of 383 samples from children suspected with Pulmonary and Extrapulmonary tuberculosis were included in this study.

In our study out of total 383 Suspected Pediatric TB cases 20.36% (78/383) were CBNAAT positive and 79.63% (305/383) were CBNAAT negative. Out of 78 TB positive cases 7.69% (6/78) were Rifampicin Resistance and 92.30% (72/78) were Rifampicin Sensitive. World health organization (WHO) endorsed Xpert® MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis (MTB) and the mutations that confer Rifampicin resistance (RR).

Our results of CBNAAT positivity were on higher side when compared to Agrawal et.al; in their study out of total 7380 Paediatric Presumptive TB cases 9.81% (724/7380) were MTb positive on CBNAAT, 6.49% (47/724) were rifampicin resistant and 93.50% (677/724) were rifampicin sensitive.⁽¹⁶⁾

Our results showed lower rate of positivity of CBNAAT when compared to Anjan Kumar Das et.al; study. Out of 565 cases CBNAAT detected 29.20% (165/565) and Rifampicin resistance in their study among the 165 cases was detected in 6.1% (10/165) by CBNAAT which was nearly same to our study.⁽¹¹⁾

In our study 383 samples were included of which 264 were from males and 119 were of females. Of these 16.18% (62/383) males and 4.17% (16/383) females were positive respectively, with male:female ratio of 2:1. Maximum positivity was seen in 0-4 yrs of age 13.57% (52/383), youngest patient being positive was of 6 months of age. Only 18.27 % of patients had history of contact with TB patient and 8.61% of patients showed positive Mantoux test. Higher number of positivity was seen in cases who did not took BCG vaccination 14.62 % (56/383) and also higher number of positivity 17.75 % (68/383) was seen in malnourished children.

Our results were close to results of Das S, Paul DK shows that majority of patients were males (67.3%). Majority of patients belonged to the age group and 13% of patients showed positive Mantoux test.⁽⁶⁾

Looking at the findings of our study were on higher side than study conducted by Sandhya et.al; out of total 50 patients 46% (23/50) of the patients had contact history of tuberculosis

while, 54% (27/50) had no history of contact. In their study they infer that, children end up contracting the disease, even without any H/o contact with tuberculosis positive patients. But it is also true that if contact history is positive, the chances of getting tuberculosis are higher.⁽¹²⁾

Our results of CBNAAT positivity in malnourished children were on higher side when compared to study of Chisti MJ et.al, in his study out of 747 there were 137 SAM children of which 21% (29/137) Sever acute malnutrition (SAM) cases were found to have tuberculosis. Chisti MJ in his review said that, children with SAM without the mention of HIV status might also have reduced immune status almost similar to that in HIV, and they have a reduced ability to clear infecting organisms, have reduced mucosal immunity and a favourable microenvironment at the point of organism deposition leading to a higher risk of pulmonary co-infection.⁽¹³⁾

Results of our study were close to study of Ahmet Soysal et.al., (119) 979 children were sputum smear positive TB out of which 13 children were diagnosed with active tuberculosis at enrolment. Out of 979 cases 770 were vaccinated with BCG and 209 were not vaccinated with BCG. Of 13 children that were diagnosed with active tuberculosis 23% (3/13) had a BCG scar (vaccinated) while 76.92% (10/13) did not had BCG scar (unvaccinated). His study also concluded that BCG vaccination protects against tuberculosis disease and tuberculosis infection.⁽¹⁴⁾

In our study amongst total 383 samples 65.01% (249/383) were from pulmonary TB cases and 34.98% (134/383) were from extrapulmonary TB cases. Of this higher positivity by CBNAAT was seen in pulmonary cases i.e.; 25.70% (64/249) and 10.44 % (14/134) in extrapulmonary cases.

Our results were similar to Das.S. et al; and Paul DK et.al; study, they found 29.23% pulmonary cases detected for MTB by CBNAAT and 13.88% extrapulmonary cases detected for MTB by CBNAAT. Also our findings were more or less similar to Anjana Kumar Das et.al; 30.8% pulmonary cases were detected for MTB by CBNAAT and 17.6% extrapulmonary were detected for MTB by CBNAAT.⁽⁹⁾

In present study out of 249 pulmonary samples consisting of 55.02% (137/249) Gastric lavage samples and 44.97% (112/249) sputum samples. Maximum positivity by CBNAAT was seen in Gastric lavage samples 33.57% (46/137) followed by 16.07% (18/112) sputum samples.

Our findings were comparable with Anjan Kumar Das et.al;⁽¹¹⁾ in their study also amongst samples from pulmonary cases maximum positivity was seen gastric lavage samples i.e.; 35.7% (110/308) .⁽¹¹⁵⁾ Our results were also similar to Das S. and Paul DK et.al;⁽¹⁰⁾ in his study among the total pulmonary samples, maximum positivity by CBNAAT was detected in 33% (74/224) of 224 gastric lavage/aspirate samples and 21.4% (21/98) positivity was seen out of 98 sputum/induced sputum samples by CBNAAT.⁽¹¹⁾

Out of total 134 samples from extrapulmonary tb cases maximum positivity of CBNAAT was found to be 13.33% (6/45) in lymph node aspirates.

Our results were on lower side when compared to study of Anjan Kumar Das et.al; (115) in extrapulmonary samples had greater positive samples from 5 lymph node aspirate 60% (3/5) followed by 15 pleural fluid 20% (3/15) were positive.⁽¹¹⁾

In our study comparison of culture was done with ZN staining. Out of 383 samples from children suspected with TB, Zn staining was positive in 23 (6.00%) samples. Whereas, Culture was positive in 75 (19.58%) samples. Out of Zn staining positive samples 25.33% (19/75) were also positive by Culture while, Out of Zn staining negative samples 74.66%

(56/75) were positive by Culture while, 98.70% (304/308) samples were negative by both the methods. Sensitivity, specificity, positive predictive value and negative predictive value of ZN staining with reference to culture are 25.33%, 98.70%, 82.60% and 84.44% respectively.

Our study results were similar to Kumar A, Das S et;al, in their study Zn staining was positive in 18.89% and 27.7% were culture positive, having sensitivity, specificity, positive predictive value and negative predictive value of ZN staining with reference to culture were 39.09%, 88.85%, 57.33%, 79.19% respectively.⁽⁹⁾

In our study comparison of culture was done with Fluorescent staining. Out of 383 samples from children suspected with TB, Fluorescent staining was positive in 7.04% (27/383) samples. Whereas, Culture was positive in 19.58% (75/383) samples. Out of Fluorescent staining positive samples 30.66% (23/75) were also positive . Out of Fluorescent staining negative samples 69.33% (52/75) were positive by Culture while, 98.70% (304/308) samples were negative by both the methods. Sensitivity, specificity, positive predictive value and negative predictive value of fluorescent staining with reference to culture are 30.66%, 98.70%, 85.18% and 85.39% respectively.

Our study results were similar to Kumar P et;al, in their study Fluorescent staining was positive in 38% and 54% were culture positive, having sensitivity, specificity, positive predictive value and negative predictive value of Fluorescent staining with reference to culture were 70%, 100%, 100%, 73% respectively.⁽¹⁸⁾

In our study comparison of culture was done with CBNAAT. Out of total 383 samples inoculated on LJ media 19.58% (75/383) samples showed growth on culture while 80.41% (308/383) samples did not growth on culture. While, detection rate of CBNAAT was close to Culture method i.e., CBNAAT was positive in 20.38% (78/383) samples and negative in 79.63% (305/383) samples. All 96.1% (75/78) samples that had grown on LJ media were also found to be positive by CBNAAT. While out of 308 samples that did not show growth on LJ media, 3.84% (3/78) were positive by CBNAAT. Sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT in reference to culture are 100%, 99.00%, 96.15% and 100% respectively.

Our study results were similar to Kumar A, Das S et;al, in their study CBNAAT was positive in 26.44% and 27.7% were culture positive, having sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT with reference to culture were 92.7%, 98.9%, 97.1%, 97.2% respectively.⁽⁹⁾

CONCLUSION

Tuberculosis in India accounts for about a quarter of the world TB cases and children are at high risk of TB infection and TB disease. TB diagnosis in children is challenging due to paucibacillary nature of samples obtained with great difficulty from suspected pediatric cases. The study was done to evaluate the role of CBNAAT in diagnosing PTB and EPTB and rifampicin resistance. Amongst the methods which were used for the diagnosis of pediatric tuberculosis, CBNAAT was most advantageous as it could detect more cases which are missed by other conventional methods. CBNAAT has revolutionized TB diagnosis and treatment by allowing identification on MTB with high specificity sensitivity with Rifampicin resistance. It has shown good results and is nearly matching the results of gold std. culture methods. It rapid test which identifies both the presence of MTB and rifampicin resistance associated with mutation of rop B gene in a single test with turnaround time of only 2 hours. CBNAAT is now considered as a WHO recommended rapid diagnostic and is very simple test, with a well-established role in the diagnosis

of PTB and EPTB cases in comparison to AFB smear. Due to its automation, rapidity and simplicity, it has become attractive tool for rapid detection of TB. It does not require skilled staff, sophisticated laboratories or higher biosafety facilities. Thus, can be used in resource limited settings as a point of care test. Overall, CBNAAT has good clinical application in rapid diagnosis in Pediatric Tuberculosis and Rifampicin Resistance which is a key to TB control strategy. Therefore, CBNAAT should be offered upfront whenever TB is suspected.

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