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Cartridge Based Nucleic Acid Amplification Test (Cbnaat) For Diagnosis of Tuberculosis in Children

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ABSTRACT

Introduction: Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis, has an estimated global annual incidence of 9.6 million with 2.2 million cases in India according to World Health Organization (WHO) Global tuberculosis Report (2015) Thus, 23% of global annual TB incidents occur in India making it the highest TB burden country. Bacteriological confirmation of TB in children is challenging due to difficulty in obtaining quality specimens, in the absence of which diagnosis largely depends on clinical judgement. Lack of high sensitivity tests adds to the diagnostic challenge. This study focuses on finding the sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT for diagnosis of TB in pediatric population in pulmonary and extra pulmonary specimens.

Methodology: This study was carried out at department of Pediatric, MLB Medical College, Jhansi from May 2018 to May 2019. Total 200 children of age less than 18 years showing symptoms and signs of suspected localized and/or disseminated tuberculosis or having history of close contact with diagnosed tuberculosis patients admitted in our hospital during the study period were included in this study. Samples(pulmonary and extrapulmonary) were collected from the subjects and put to test for CBNAAT, Zeihl-Neelsen (ZN) smear and culture.

Results: Majority of patients belonged to age group of 11-18 year (47.5%) with maximum number of patients were from class V socioeconomic status (53%). Out of 200 samples, 61 were pulmonary samples (sputum, induced sputum), other 139 were extrapulmonary samples (gastric lavage 87, cerebrospinal fluid 29, pleural fluid/ascetic 4, congenital TB 2). Out of 200 samples AFB-ZN smear method detected tuberculosis in 69 cases (34.5%). Out of 200 samples, CB-NAAT detected tuberculosis in 82 cases (41%). CB-NAAT detected tuberculosis in 65 (94%) of 69 ZN smear positive cases while in 17 (12.5%) of the ZN smear negative cases. Sensitivity, specificity, positive predictive value and negative predictive value of CB-NAAT in comparison to ZN smear staining were 94%, 87%, 80% and 97% respectively. CB-NAAT detected rifampicin resistance in 47 of the 200 presumptive pediatric tuberculosis patients, among which 16 patients had history of tuberculosis contact.

Conclusion: CB-NAAT was more sensitive and specific than ZN smear microscopy, not only for acid fast bacilli detection but also detection of rifampicin resistance. Study demonstrated the feasibility of extending X-Pert testing to non sputum specimens with a high proportion of interpretable results. Revised National Tuberculosis Control Programme (RNTCP) is also currently using X-Pert MTB/RIF to diagnose pediatric pulmonary tuberculosis, extra pulmonary tuberculosis and rifampicin resistance by WHO 2013 policy recommendations.

Keywords: CBNAAT; MTB; ZN smear; Culture

I. INTRODUCTION

Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis, has an estimated global annual incidence of 9.6 million with 2.2 million cases in India according to World Health Organization (WHO) Global TB Report (2015) Thus,23% of global annual TB incidents occur in India making it the highest TB burden country[(1)]. While, globally the exact burden of childhood TB is not well documented, it is estimated that childhood TB constitutes about 10–20% of all TB cases, in high burden countries[(2,3)] and TB remains one of the leading cause of childhood mortality and morbidity[(4)]. In 2013, 63,919 pediatric TB cases were notified accounting for 5% of notified TB cases[(5)] in India, under the Revised National Tuberculosis Control Programme (RNTCP).

Diagnosis of pulmonary TB in children is challenging, more so in resource-limited, tuberculosis-endemic countries and is largely based on clinical and radiological findings and medical history [(6,7)]. Bacteriological confirmation of pulmonary TB is challenging due to difficulty in obtaining good quality sputum specimens from children. In the absence of quality specimens, one has to rely on testing alternative specimens which is challenging due to difficulties in obtaining these specimens, inadequate clinical sample volumes and paucibacillary nature of biological samples[(8,9)].

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Diagnostic efforts are also undermined by the lack of simple diagnostic tests with high sensitivity that can be applied at the point of clinical care[8]. Isolation of mycobacteria by culture, while considered as gold standard for diagnosing TB, takes 4–8 weeks and often requires expensive and sophisticated laboratory facilities which cannot be afforded in most resource-limited settings[9]. Both antigen and antibody TB ELISA tests are poorly sensitive and specific and are not recommended for diagnosis of tuberculosis [10]. Interferon gamma release assays (IGRAs) are being used in place of the skin test in low prevalence countries to detect latent TB infection. However, these expensive tests do not differentiate the TB infection from disease. Also its exact utility in high burden situation is still not clear[11].

The specificities and sensitivities of the polymerase chain reaction (PCR) based diagnostic tests are quite variable[8,11]. Further, these tests involve multiple manual steps and long turnaround time, making them unsuitable for decentralized deployment. A series of meta-analyses have shown cartridge based nucleic acid amplification test (CBNAAT)/ Xpert MTB/ RIF to have a high specificity with variable sensitivity in different type of specimens for TB diagnosis[8,12-14]. In 2013, the WHO endorsed the use of CBNAAT for TB diagnosis in pediatric presumptive pulmonary and extra-pulmonary tuberculosis (EPTB) cases[15,16]. 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[17].

Since there is paucity of data regarding the usefulness of CBNAAT test in pediatric tuberculosis, the present study was undertaken to assess the efficacy of CBNAAT test in diagnosis of suspected case of pediatric tuberculosis and also in children who came in contact with adult tuberculosis patients.

II. MATERIAL AND METHODS

The present study was carried out on cases of pediatric tuberculosis, suspected cases of tuberculosis and those children who came in contact with adult tuberculosis patients were taken for present study. Approximately 200 children of age less than 18 years will form the study group, after taking institutional ethical approval from the ethical committee of college. Patients having symptoms and signs of suspected localized and/or disseminated tuberculosis or having history of close contact with diagnosed tuberculosis patients were included in the study. The exclusion criteria being parents not giving consent.

Diagnostic criteria - suspect-children with persistent fever and/or cough for more than 2 weeks, loss of weight/no weight gain (History of unexplained weight loss or no weight gain in past 3 months; loss of weight will be defined as loss of more than 5% body weight as compared to highest weight recorded in last 3 months) and/or history of contact with infectious TB cases (In a symptomatic child, contact with a person with any form of active TB within last 2 years maybe significant)(1). Clinical history regarding current complaints of fever, cough, sputum production, haemoptysis, and weight loss will be taken. All patients will be evaluated for headache, seizures, chest pain, breathlessness and neck swelling or any other evidence of tubercular meningitis and abdominal tuberculosis.

Presumptive extrapulmonary TB - presence of organ specific symptoms and signs like swelling of lymph node, pain and swelling in joints, ascitis, neck stiffness, disorientation, seizure, etc and/or constitutional symptoms like significant weight loss, persistent fever for more than 2 weeks, night sweats.

Laboratory investigations- Complete hemograms, erythrocyte sedimentation rate (ESR), Mantoux test, Zeihl- Neelsen (ZN) smear, fine needle aspiration cytology (FNAC), histopathology (HPE) of samples.

Radiological Investigations. Chest radiograph, ultrasonography (USG), computed tomography (CT) scan, if indicated. CBNAAT of samples [sputum/induced sputum, gastric aspirate/ lavage, cerebrospinal fluid (CSF), pleural fluid, ascitic fluid, bronchoalveolar lavage (BAL), FNAC material].

Study technique Patients will be selected as per the inclusion criteria and recruited in the study. Detailed history taking, physical examination and relevant laboratory investigations were done. A pre designed semi structured proforma was used to obtain data based on socio-economic profile, 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 well labelled falcon tubes. In pulmonary cases two sputum/induced sputum (Sputum induction was done in a well-ventilated room with an ultrasonic nebuliser and nebulisation done with 10-20mL of 3% hypertonic saline until patient coughed up at least 2 mL of sputum or a maximum of 15 minutes.) samples were collected: one early

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morning and other supervised spot specimen. Smears of both the sputum samples will be made, stained by ZN procedure, examined under light microscope.
Both positive and negative samples are used for CBNAAT test for diagnosis of pediatric tuberculosis.

AFB Smear

A minimum of 1 slide positive even for single AFB/100 fields were taken as positive for Mycobacterium tuberculosis and a minimum of two samples negative for AFB evaluated for 100 fields were declared as negative(13).

CBNAAT

CBNAAT samples were sent to District tuberculosis Lab. CBNAAT will be performed according to the manufacturer's instructions. 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 will be transferred to the cartridge and the cartridge will be inserted in the module of CBNAAT machine.
After 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.

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.

III. RESULTS

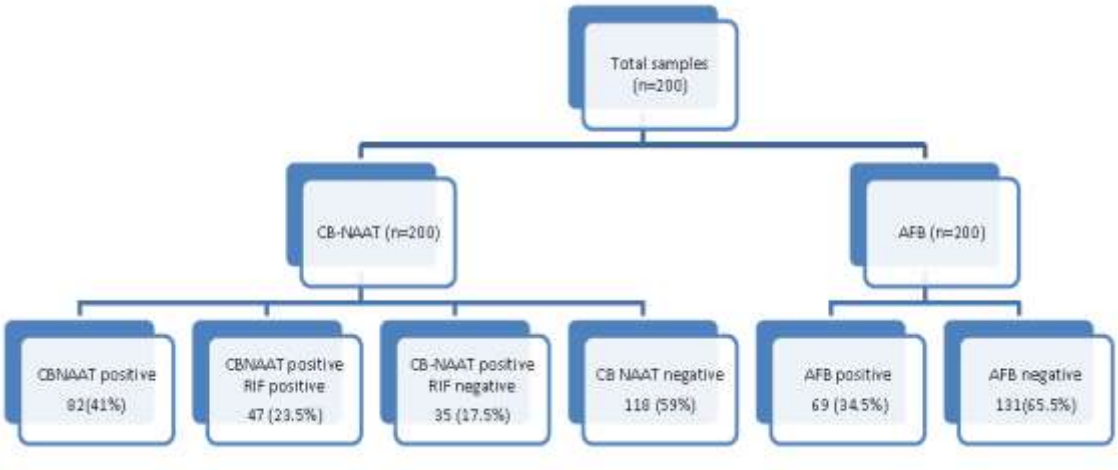

Table : Demographic profile of cases

Sex	No. of cases	CB NAAT positive cases	
		No.	%
Male	99	39	39.3
Female	101	43	42.5
Total	200	82	41
Age group			
0-1 yr	21	4	19.04
13 month – 5 yr	38	5	13.15
5-10 yr	46	5	10.87
11 yr -18 yr	95	68	71.5

Table : Sample wise ZN-smear-AFB, RIF and CB-NAAT positive cases

	CB NAAT positive	AFB positive	RIF
Sputum (61)	34 (55.0%)	33 (54.0%)	33 (54.0%)
GL (87)	33 (37%)	28 (32.0%)	10 (11.5%)

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CSF (29)	6 (17%)	3 (10.3%)	2 (6.9%)
Pleural fluid (8)	4 (50%)	3 (37.5%)	1 (12.5%)
Ascetic fluid (4)	2 (50%)	2 (50%)	1 (25.0%)
Lymphnode (9)	3 (33%)	1 (11.1%)	0 (0%)
Congenital TB (2)	0(0%)	0 (0%)	0 (0%)
Total	82	69	

Table: CBNAAT vs AFB

Cases	No. of cases	AFB Positive	AFB Negative	Total
CBNAAT positive	82	65	17	82
CB NAAT negative	118	4	114	118
Total	200	69	131	200
		P<0.05		

Table : CB-NAAT vs history of TB contact

History	Total cases	History of TB contact Positive	History of TB contact Negative	P value
CB-NAAT positive	82	27	55	<0.005
CB-NAAT negative	118	15	103	
Total	200	42	158	
		Chi square 11.917		

Table : History CB-NAAT vs Mantoux

History	Total cases	Mantoux Positive	Mantoux Negative	P value
CB-NAAT positive	82	67	15	<0.05
CB-NAAT negative	118	23	95	
Total	200	90	110	

Table : History of TB contact vs RIF positive

RIF	Total cases	H/o tuberculosis contact Positive	H/o tuberculosis contact Negative	P value
Rifampicin resistance Positive	47	16	31	0.01
Rifampicin resistance Negative	153	26	127	
Total	200	42	158	

Table : Rifampicin resistance in history of ATT intake

	H/o ATT intake (+)	H/o ATT intake (+) + RIF resistant (+)	RIF resistant (+) H/o ATT (-)
Sputum (61)	21	21 (100%)	12
GL (87)	-	-	11
CSF (29)	-	-	1
Ascitic fluid (4)	1	1 (100%)	-
Pleural fluid (8)	1	1 (100%)	-
FNAC (9)	-	-	-
Congenital TB (2)	-	-	-
Total	23	23	24

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IV. DISCUSSION

The recent introduction of the CB-NAAT assay has significantly revolutionized the diagnostics of tuberculosis in adults but its application for the diagnosis of pediatric tuberculosis is under evaluation. To date there are only a few studies on the application of CB-NAAT for the diagnosis of pediatric tuberculosis in India. The Gene X-pert MTB/rifampicin test is a cartridge based fully automated nucleic acid amplification test for tuberculosis case detection and rifampicin resistance testing, suitable for use in disease endemic countries[18,19].

Under this study X-Pert testing for first time was extended to various type of non sputum and non respiratory specimens to assess the performance of this assay under uncontrolled field conditions.

In the present study 200 patients were included, youngest patient being 3 days and oldest patient 17 years 10 month old. Male : female ratio 0.98:1. Majority of patients belonged to age group of 11-18 years (47.5%). Out of 200 samples 61 were respiratory samples (sputum) other 139 were non respiratory samples. Gastric lavage -87, CSF-29, Pleural fluid -8, ascitic fluid-4, FNAC-9, congenital TB-2. Out of 200 patients, 21% of patients had history of TB contact and 11.5% patient had history of ATT intake while 47 patient had rifampicin resistance (23.5%).

Among the pulmonary samples, CB-NAAT detected MTB in 34 of the 61 sputum samples (55.7%) and 33 out of 87 gastric lavage samples (37%). 6 out of 29 CSF samples (17%), 4 out of 8 pleural fluid samples (50%), 3 out of 9 FNAC samples, 2 out of 4 ascitic fluid samples, 0 out of 2 congenital TB samples had positivity of CB-NAAT.

CB-NAAT was positive in 82 (41%) and negative in 118 (59%) of the 200 cases. However, ZN smear detected MTB in 69 (34.5%) cases and failed to detect MTB in 131 (65.5%) cases. We tried to arrive at correlation between CB-NAAT and AFB-staining by ZN smear.

CB-NAAT detected MTB in 65 of the 69 (94%) ZN smear positive cases and 17 (12.5%) of the ZN smear negative cases. Similarly a correlation between CB-NAAT test as well as MTB positivity by ZN smear and MTB negative cases was also derived. This shows that CB-NAAT detected MTB in 65 cases in ZN-positive cases and 17 of the ZN-smear negative cases. Our observation that CB-NAAT was positive even in ZN-smear negative cases, signifies that CB-NAAT is more sensitive in detection of tuberculosis than AFB-staining by ZN-smear method. On statistical analysis done by chi square test, the sensitivity, specificity positive predictive value and negative predictive value of CB-NAAT in comparison to AFB staining were 94%, 87%, 80% and 97% respectively. CB-NAAT also detected rifampicin resistance in 47 of the 82 MTB positive cases. Among the 200 cases, 23 patient had history of ATT intake and X-Pert detected rifampicin resistance in all of those 23 patient, along with 24 cases without history of ATT intake.

In the present study, out of 200 samples CB-NAAT detected tuberculosis in 82 (41%) cases, in which CB-NAAT and AFB-ZN-smear both was positive in 32.5% cases, where CB-NAAT detected tuberculosis in AFB-ZN-smear method negative cases in 8.5%.

In study of Neeraj Raizad et al 2015 reported that the CB-NAAT was detected tuberculosis in 63.6% whereas were CB-NAAT and AFB-ZN-smear both were positive in 31.1%, CB-NAAT was detected tuberculosis in AFB-ZN-smear negative cases in 4.3%. So they found CB-NAAT method was better for diagnosis of tuberculosis in both AFB –ZM smear method positive and negative cases[20].

In the present study of 200 samples, CB-NAAT detected tuberculosis in pulmonary samples (sputum, induced sputum) in 55% of cases, whereas CB-NAAT was detected tuberculosis in extra pulmonary samples (GL, CSF, pleural fluid, Ascitic fluid, FNAC, congenital TB) in 34.5% if cases. Out of 200 samples, AFB-ZN-smear method detected tuberculosis in pulmonary samples (sputum, induced sputum) in 54% of cases, whereas AFB-ZN-smear method detected tuberculosis in extra pulmonary samples in 25% of cases.

Manish Kumar Munda et al 2018 reported that CB-NAAT detected tuberculosis in 101 (58.3%), out of 173 pulmonary samples; and 11 (40.74%) out of 27 extra pulmonary samples, whereas AFB-ZN-staining method detected tuberculosis in 38 (21.96%) out of 173 pulmonary samples and 6 (22.22%) out of 27 extra pulmonary samples.

In the present study, out of 200 samples, 61 samples were pulmonary (sputum/induced sputum) in which, AFB-ZN method detected tuberculosis in 33 samples (54%), whereas CB-NAAT detected tuberculosis in 34 cases (55%).

In the study of Sowjanya et al (2014) reported that the CB-NAAT detected tuberculosis in 144, out of 205 pulmonary samples (sputum, induced sputum) 70.24% whereas sputum for AFB detected tuberculosis only in 108 cases (52.68%). So, the present study shows there was not much

difference in the diagnosis of tuberculosis in sputum, induced sputum samples by CB-NAAT in comparison with AFB-ZN smear method.

In the present study, CB-NAAT detected tuberculosis in 34 (55%) of the 61 sputum /induced sputum samples, and 33 (37%) of the 87 gastric lavage samples, and 6 (17%) of the 29 cerebrospinal fluid samples, 4 (50%) of the 8 pleural fluid samples, 2 (50%) of the 4 ascitic fluid samples, 3 (33%) of the 9 FNAC samples, 0 (0%) of the 2 congenital tuberculosis patients. Sensitivity, specificity and positive predictive value, negative predictive value of CB-NAAT in reference to AFB-ZN-smear are 94%, 87%, 80% and 97% respectively.

Kumar Anshu et al 2018 reported that the CB-NAAT detected tuberculosis in 21 (21.4%) of the 98 sputum samples, 74 (33%) of 224 gastric lavage samples, 7 (12.7%) of the 55 CSF samples, 1 (10%) of the 10 pleural fluid samples, 2 (66.7%) of the 3 FNAC samples and none of the ascitic fluid samples.

Sensitivity, specificity and positive predictive value, negative predictive value of CB-NAAT in reference to AFB-ZN-smear was 100%, 90.68%, 71.2%, 100%. So inference of above comparison shows, CB-NAAT was more sensitive and specific than AFB-ZN-smear method for diagnosis of pulmonary and extra pulmonary tuberculosis[21].

The explanation of higher detection of CB-NAAT test in detection of tuberculosis in our study, practically in all samples was higher than the study of Anshu et al, as the sample size in their study was much less than our study.

In the present study, AFB-ZN-smear method detected tuberculosis in 69 (34.5%) of the 200 total samples, whereas CB-NAAT detected tuberculosis in 82 (41%) of the 200 samples. CB-NAAT also detected rifampicin resistance in 47 (57.3%) of the 82 CB-NAAT positive tuberculosis patients. Out of 47 rifampicin resistance patients, 23 patients were having history of ATT intake (50%).

The study of R Dewan et al 2013 reported that the AFB-ZN-smear method detected tuberculosis in 11 (11%) of the 100 sputum samples, CB-NAAT detected tuberculosis in 49(40%) of the 100 sputum samples in which 10 patients were positive for rifampicin resistance (25%), 7 of the 10 rifampicin resistance patients having history of ATT intake in the past (70%). So we found that our study is in correlation with that of R. Dewan et al 2012 and that CB-NAAT was a better diagnostic test not for only for AFB, but also for rifampicin resistance detection[22,23,24].

The percentage of positivity in pulmonary and extra pulmonary samples. In this regards, in the present study, AFB-ZN-smear method detected tuberculosis in 36 (18%) of the 139 extra pulmonary samples. CB-NAAT detected tuberculosis in 48 (24%) of the 139 extra pulmonary samples, however CB-NAAT detected tuberculosis in 17 of the 103 AFB-ZN-smear method negative cases (16.5%).

Study of Shivprasad et al 2018 reported that the AFB –ZN-smear method detected tuberculosis in 17 (10.24%) out of 166 extra pulmonary samples, where CB-NAAT detected tuberculosis in 25 (15.06%), of the 166 extra pulmonary samples, in which CB-NAAT detected tuberculosis in 8 (4.8%) of the 149 AFB –ZN-smear method negative cases. So the results of our study which in correlation with Shivprasad et al (2018) and shows that CB-NAAT was a better test for diagnosis of tuberculosis in extra pulmonary cases.

Mantoux was positive in 90 (45%) of the 200 cases and negative in 110 (55%) of the 200 cases.

CB-NAAT detected MTB in 67 of the 90 mantoux positive cases (74.4%) and 15 of the 110 mantoux negative cases 13.6%.

As taken in literature mantoux positive cases denote only in later infection while in CB-NAAT becomes positive only in acute infection[25]. In our study, it was observed that out of 90 mantoux positive cases, 67 cases were positive by CB-NAT for detection of tuberculosis, which denotes that sensitivity of CB-NAAT as regards the detection of acute infection.

X-Pert performance in tuberculosis detection was excellent among various specimens tested as majority of specimen has yielded valid results. However highest positivity was observed in sputum/induced sputum specimen followed by pleural fluid, ascitic fluid, gastric lavage, FNAC, CSF. These findings are similar to findings from other study conducted in India. In presumptive TB cases, substantial numbers of rifampicin resistant TB patients were diagnosed in this study. Though limited data on levels of rifampicin resistant in pediatric population is available, our study findings are broadly similar to the findings from earlier studies conducted in India.

The data from the current study shows that rifampicin resistance in pediatric TB cases, correlated better with a positive history of ATT intake and history of TB contact. This finding suggest that history of ATT intake and history of TB contact can be considered as a more appropriate

risk factor for rifampicin resistance in pediatric population.

V. CONCLUSION

From our study, we conclude that CB-NAAT was more sensitive and specific than ZN smear microscopy, not only for acid fast bacilli detection but also detection of rifampicin resistance. This study demonstrated the feasibility of extending X-Pert testing to non sputum specimens with a high proportion of interpretable results. Revised National Tuberculosis Control Programme (RNTCP) is also currently using X-Pert MTB/RIF to diagnose pediatric pulmonary tuberculosis, extra pulmonary tuberculosis and rifampicin resistance by WHO 2013 policy recommendations.

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