



## A STUDY ON THE ROLE OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) AND MANTOUX TEST FOR DIAGNOSIS OF PEDIATRIC TUBERCULOSIS

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### ABSTRACT

**Introduction:** India accounts for one-fifth of the global tuberculosis (TB) incidence, with TB remaining one of the leading causes of childhood mortality and morbidity. 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 role of CBNAAT for diagnosis of TB in pediatric population in pulmonary and extra pulmonary specimens.

**Methodology:** The study was done in the new hospital medical college unit of department of Pediatrics, Govt. Medical College, Kota Rajasthan, from July 2017 to July 2019. A total of 130 patients presenting with symptoms & signs of suspected pulmonary or extrapulmonary tuberculosis or having history of contact with diagnosed tuberculosis patients admitted in our unit during the study period were included in this study. Samples (pulmonary and extrapulmonary) were collected from the subjects and put to test for CBNAAT. Mantoux test was done in all suspected cases & interpretation done at 48 hours of injection. Presence of BCG scar was noted as a proof of BCG Vaccination. Other haematological & radiological investigations were done as per requirement.

**Results:** A total of 130 cases of suspected Tuberculosis were subjected for CBNAAT test in the study. Majority patients belonged to the age group of 0 to 5 years (60%). Remaining 23% were in age group of 5 to 10 years & 16.9% in the ages between 10 to 18 years. Males were more affected than females (45%). Most common form of Pediatric Tuberculosis cases in the study was the Progressive Pulmonary tuberculosis, 2 cases were of TB Lymphadenitis, 4 cases of TB Meningitis, 2 with Tuberculoma with seizures, 2 cases of Abdominal TB & one case presented with Acute Pain Abdomen with TB salpingitis in an adolescent girl of 16 years. Miliary tuberculosis was seen in 2 cases. While 8 cases reported with Tubercular Pleural effusion. One with TB of Dorsolumbar spine & Gibbus. Most of the patients presented with fever & cough > 2 weeks (78%), 85% presented with weight loss/ no weight gain & Anorexia. Out of these 12(63.15%) patients had a positive Mantoux Test while 7(36.84%) patients with a positive CBNAAT had a negative MT. BCG scar was not present in 19 cases. 17 patients (15.39%) with CBNAAT detected had a BCG scar while 94 cases with a BCG scar did not have MTB detected by CBNAAT. Only 2 patients with positive CBNAAT did not have a BCG scar (10.5%).

**Conclusion:** CBNAAT assay is a rapid test which identifies both the presence of Mycobacterium tuberculosis (MTB). It also correlates well with Mantoux Positivity in Pediatric Population.

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### 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]. India is 17th among 22 high burden countries in terms of overall TB incidence rate. 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.

In most settings the diagnosis in children is traditionally based on the basis of contact tracing and very few attempts have been made for active case detection. This is mainly due to lack of pathognomic clinical presentation in paediatric Tuberculosis and lack of sensitive diagnostic tools. Tuberculosis in children has been relatively neglected mainly because clinical diagnosis has low specificity, radiological interpretation is subject to inter-observer variability and the tuberculin skin test is a marker of exposure, not disease. Infants and young children do not expectorate but instead swallow their sputum. Aspiration or lavage of gastric content is best procedure in them for obtaining specimen from which to culture

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*Mycobacterium tuberculosis*. Cartridge based nucleic acid amplification test is a nucleic acid amplification test which simultaneously detects DNA of *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampicin (RIF) in less than 2 hours. This system integrates and automates sample processing, nucleic acid amplification and detection of the target sequences.

The recent introduction of Cartridge based nucleic acid amplification test has significantly transformed the diagnostics of tuberculosis in adults but its application for Paediatric Tuberculosis is under evaluation. Therefore, authors conducted a study on role of gastric aspirate examination by ZN stain and Cartridge based nucleic acid amplification test in the diagnosis of childhood Tuberculosis. Simultaneously, all suspected patients were subjected to Mantoux test & results studied at 48 hrs of test.

**METHODS**

The study was a prospective hospital-based study from July 2017 to July 2019 consisting of 130 selected patients suspected of tuberculosis who had their gastric aspirate tested for CBNAAT and ZN stain for acid fast bacilli (AFB) along with Mantoux test and other routine investigations. Gastric aspirate was taken after six hours of fasting . other samples like ascitic, pleural and Cerebrospinal fluid were also sent for CBNAAT if required . Mantoux Testing done in all suspect cases with 0.1 ml intradermal injection of Purified Protein Derivative. The result was interpreted at 48 hrs after injection. A positive response was considered if induration was measured >10 mm.

**Inclusion criteria for suspected TB cases were**

- Fever & cough > 2 weeks
- Weight loss ( loss of > 5 % weight) or no weight gain in past 3 months.
- Chest X ray findings :- Hilar lymphadenopathy, homogenous opacity, pleural effusion
- Positive MT
- Tubercular Ascites or Sub acute obstruction, Abdominal Usg findings of TB
- TB Meningitis/ CNS Tuberculoma.
- Contact history of positive case.

All eligible cases were subjected to detailed history, general physical & systemic examination. Hematological investigations for CBC, ESR & HIV done. All patients were subjected to intradermal MT test & results interpreted at 48 hours.

**Exclusion criteria**

- Unwilling subjects
- Those who had already received ATT drugs

**RESULTS**

A total of 130 cases of suspected Tuberculosis were subjected for CBNAAT test in the study. Majority patients belonged to the age group of 0 to 5 years ( 60%). Remaining 23% were in age group of 5 to 10 years & 16.9% in the ages between 10 to 18 years. Males were more affected than females (45%). Most common form of Pediatric Tuberculosis cases in the study was the Progressive Pulmonary tuberculosis, 2 cases were of TB Lymphadenitis, 4 cases of TB Meningitis, 2 with

Tuberculoma with seizures, 2 cases of Abdominal TB & one case presented with Acute Pain Abdomen with TB salpingitis in an adolescent girl of 16 years. Miliary tuberculosis was seen in 2 cases. While 8 cases reported with Tubercular Pleural effusion. One with TB of Dorsolumbar spine & Gibbus. Most of the patients presented with fever & cough > 2 weeks (78%), 85% presented with weight loss/ no weight gain & Anorexia.

Table 1 shows the correlation of positive MT with CBNAAT detection. 34 patients had a positive Mantoux Test. Of these, CBNAAT was detected in 12 patients while 22 cases failed to detect MTB in gastric aspirate sent for CBNAAT ( p value <0.002 significant).

Table II shows the correlation between CBNAAT & a positive history of Contact with a case of TB. 31 patients had a history of close contact with a case of TB. Out if these, MTB was detected in 11 (57.8%) cases, while 8 cases who had a positive CBNAAT were devoid of any close contact with a case of TB.

BCG scar was absent in 19 cases. 17 cases with detected CBNAAT had the presence of BCG scar while 2 patients with a positive CBNAAT did not have a BCG scar.

**Table I** Association between CBNAAT & Mantoux Test

	CBNAAT	Mantoux Test		Total
		Positive	Negative	
CBNAAT	MTB Detected	12(63.15%)	7(36.84%)	19 (14.6%)
	MTB Not detected	22( 19.81%)	89(80.18%)	111(85.38%)
Total		34(26.15%)	96(86.48%)	130

P < 0.002

**Table II** correlation between CBNAAT & History of Contact of TB

	CBNAAT	History of Contact		Total
		Yes	No	
CBNAAT	Detected	11 (57.8%)	8 (42.1%)	19
	Not Detected	20(18.01%)	91(81.98%)	111
Total		31 (23.84 %)	99 (76.15 %)	130

**Table III** Presence of BCG scar in suspected cases of Tuberculosis

	CBNAAT Detected	CBNAAT Not detected	Total
BCG Scar present	17(15.39%)	94(84.68%)	111
BCG scar not present	2(10.5%)	17(89.47%)	19
Total	19 (14.6%)	111(85.38%)	130

**DISCUSSION**

The recent introduction of the CBNAAT assay has significantly revolutionized the diagnostics of tuberculosis in adults, but its application for the diagnosis of pediatric TB is under evaluation. To date, there are only a few studies on the application of CBNAAT for the diagnosis of pediatric tuberculosis in India, more so in eastern India. The Gene Xpert MTB/Rif test is a cartridge-based fully automated NAAT (nucleic acid amplification test) for TB case detection and rifampicin resistance testing, suitable for use in disease-endemic countries. It purifies, concentrates, amplifies (by rapid, real-time PCR) and identifies targeted nucleic acid sequences in the TB genome, and provides results from

unprocessed sputum samples in less than 2 hours, with minimal hands-on technical time.

In the present study, 130 patients were included & subjected for CBNAAT & MT. A total of 19 patients had MTB detected by CBNAAT (14.6%). Out of these 12 (63.15%) patients had a positive Mantoux Test while 7 (36.84%) patients with a positive CBNAAT had a negative MT.

Similar results were found in studies done by Qing-Qin Yin *et al* who demonstrated positivity of Xpert associated significantly with history of contact with tuberculosis present in patients (p value 0.010).<sup>12</sup> We have observed a statistically significant association (p value <0.002) between positive rates of Cartridge based nucleic acid amplification test and reactivity of tuberculin sensitivity test.

Also another study by Sekadde *et al* obtained significant association between positive tuberculin sensitivity test and positive Xpert (p value 0.002) which is in accordance to present study.<sup>13</sup>

The positive rate of CBNAAT Test in patients with history of tuberculosis contact was significantly higher (p value 0.02) than in those with no history of contact. Sekadde *et al* obtained significant association between positive history of contact and positive Xpert (p value 0.03).<sup>11</sup>

BCG scar was not present in 19 cases. 17 patients (15.39%) with CBNAAT detected had a BCG scar while 94 cases with a BCG scar did not have MTB detected by CBNAAT. Only 2 patients with positive CBNAAT did not have a BCG scar (10.5%). This is in accordance with the study of Prasad *et al*.

## CONCLUSION

Majority patients belonged to the age group of 0 to 5 years (60%). Remaining 23% were in age group of 5 to 10 years & 16.9% in the ages between 10 to 18 years. Males were more affected than females (45%).

Out of these 12 (63.15%) patients had a positive Mantoux Test while 7 (36.84%) patients with a positive CBNAAT had a negative MT.

BCG scar was not present in 19 cases. 17 patients (15.39%) with CBNAAT detected had a BCG scar while 94 cases with a BCG scar did not have MTB detected by CBNAAT. Only 2 patients with positive CBNAAT did not have a BCG scar (10.5%).

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