

Original Research Article

ROLE OF CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT-GENEXPERT) AMONG SUSPECTED PULMONARY TUBERCULOSIS OF PEDIATRIC AGE GROUP

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ABSTRACT

Background: Tuberculosis (TB) remains a major global health challenge, with pediatric TB accounting for approximately 31% of the global burden in India. Bacteriological confirmation is difficult in children because of the paucibacillary nature of the disease and challenges in obtaining quality specimens. Given the limited studies on pediatric pulmonary TB diagnosis using CBNAAT in South India, this study was conducted in Andhra Pradesh to evaluate the role of cartridge-based nucleic acid amplification test (CBNAAT-GeneXpert) in suspected pediatric pulmonary TB cases, excluding neonates.

Materials and Methods: A hospital-based prospective cross-sectional study was conducted involving 98 children aged 1 month to 18 years with suspected pulmonary tuberculosis after obtaining ethical clearance. Children with prolonged fever, cough (>2 weeks), weight/appetite loss, and TB contact history were included. Those with extrapulmonary TB, prior treatment, immune deficiencies, or chronic systemic illnesses were excluded. Clinical data were recorded using a structured proforma. Sputum, induced sputum, or gastric lavage samples were tested using CBNAAT GeneXpert. Additional investigations were done as needed. Data analysis was performed using IBM SPSS Statistics version 21.

Results: In this study, 39.8% of subjects showed abnormal chest X-ray findings. CBNAAT-GeneXpert and Mantoux tests yielded positivity rates of 9.2% and 14.3%, respectively, while smear microscopy showed 4.0% positivity. Among 16 confirmed cases, 56.3% tested positive with GeneXpert, with a higher positivity in males (66.6%) compared to females (33.4%). GeneXpert demonstrated greater sensitivity than smear microscopy across sample types: gastric lavage samples showed 6.9% positivity with GeneXpert versus 2.7% with smear microscopy; sputum samples showed 15.3% positivity with GeneXpert compared to 7.6% with smear microscopy. All GeneXpert-positive cases were rifampicin-sensitive.

Conclusion: GeneXpert demonstrates superior diagnostic performance compared to sputum microscopy, with higher test positivity rates across both gastric lavage and sputum samples. Notably, all cases identified as positive by GeneXpert were found to be sensitive to rifampicin.

Keywords: Tuberculosis, CBNAAT-GeneXpert, Sputum sample, Gastric lavage sample, Smear Microscopy.

INTRODUCTION

Tuberculosis (TB) continues to pose a significant global health challenge. According to the World Health Organisation (WHO) Global Tuberculosis Report 2021, an estimated 10.6 million individuals were infected with TB worldwide, including 1.2 million children. The global incidence rate exceeds 500 new cases per 100,000 population annually. In India, approximately 306,000 children (aged 0–18 years) contract TB each year, representing around 11% of all cases reported under the National Tuberculosis Elimination Program (NTEP).^[1]

India bears a disproportionate burden of pediatric TB, accounting for nearly 31% of the global pediatric TB caseload. Despite its prevalence, pediatric drug-resistant (DR) TB remains underreported and poorly understood, both globally and within India.^[2]

Diagnosing TB in children is particularly challenging due to its atypical clinical presentation and the paucibacillary nature of the disease.^[3] Pediatric TB often lacks the classic symptoms seen in adults, leading to underdiagnosis or overdiagnosis. Clinical and radiological assessments remain the primary diagnostic tools, as bacteriological confirmation is difficult to obtain due to challenges in collecting high-quality specimens. Culture-based isolation of *Mycobacterium tuberculosis* is considered the diagnostic gold standard. However, it requires 4–8 weeks for results and demands sophisticated laboratory infrastructure, which is often unavailable in resource-limited settings.^[3] Serological tests such as TB ELISA are discouraged due to poor sensitivity and specificity.^[4] PCR-based diagnostic methods, while promising, suffer from variability in performance and require multiple manual steps, limiting their utility in decentralised healthcare environments.^[5]

Recent meta-analyses have highlighted the utility of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), also known as GeneXpert, in TB diagnosis. CBNAAT demonstrates high specificity and variable sensitivity across different specimen types^[6]. This automated molecular technique can detect *Mycobacterium tuberculosis* and rifampicin resistance within two hours, making it a valuable tool for rapid diagnosis.

Endorsed by WHO since 2010 for adult pulmonary TB and since 2013 for pediatric cases, CBNAAT is particularly advantageous in low-resource settings. It is simple to perform, requires minimal technical expertise, and does not necessitate advanced biosafety infrastructure. Moreover, it is applicable for both pulmonary and extrapulmonary TB diagnosis in children.

Despite its potential, limited research has been conducted on the use of CBNAAT for diagnosing pediatric pulmonary TB in South India. To address this gap, the present study was undertaken in Andhra Pradesh to evaluate the diagnostic utility of

CBNAAT (GeneXpert) among children suspected of having pulmonary tuberculosis, excluding neonates.

MATERIALS AND METHODS

A hospital-based prospective cross-sectional study was conducted over one year among children aged 1 month to 18 years admitted to Sri Venkateswara Ramnarayan Ruia Government General Hospital, a tertiary care teaching hospital in Tirupati after obtaining ethical clearance from the Institutional Ethics Committee of Sri Venkateswara Medical College.

Based on a 2019 study conducted in Odisha^[7], which reported a CBNAAT positivity rate of approximately 40% among pediatric patients suspected of having pulmonary tuberculosis, the sample size for this study was calculated using the following formula:

$N = Z\alpha^2 \cdot PQ / L^2$, Where

N is the required sample size.

Z α is the 2-tailed Z value for the given alpha error (0.05) at 95% confidence intervals = 1.96

P is the assumed proportion of complete antenatal care = 40

Q is given by (100 - P) = (100 - 40) = 60

L is the allowable error in terms of absolute precision 10%

Substituting the values, the calculated sample size was 92.16, rounded to 93. Accounting for a 5% non-response rate, the final adjusted sample size was 98. Before data collection, all participants' parents or guardians were briefed about the study objectives, and written informed consent was obtained. Children aged 1 month to 18 years presenting with clinical features suggestive of pulmonary tuberculosis were enrolled^[8]. Exclusion criteria included extrapulmonary tuberculosis, ongoing anti-tubercular therapy, primary immunodeficiency disorders, and pre-existing chronic systemic illnesses.

A detailed clinical history and physical examination were documented using a structured proforma. Respiratory samples—either sputum, induced sputum, or gastric lavage (in cases where sputum could not be obtained)—were collected and subjected to CBNAAT (GeneXpert) testing.

Additional investigations were performed as needed, including Complete blood hemogram, Mantoux test, Chest X-ray and CT scan of the chest (when indicated)

Children with clinical, radiological, and laboratory findings suggestive of pulmonary tuberculosis were managed according to the National Tuberculosis Elimination Program (NTEP) guidelines.

All collected data were entered into Microsoft Excel and analysed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarised using frequencies and percentages. Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median with interquartile ranges (IQR), depending on

the distribution assessed using the Kolmogorov-Smirnov test.

Statistical comparisons were made using: Chi-square test (with or without Yates' correction) or Fisher's exact test for proportions and Unpaired t-test for comparing means. A p-value < 0.05 was considered statistically significant.

RESULTS

In the present study, the majority of participants belonged to the 6–10 years age group (40.9%), followed by children aged ≤5 years (33.6%) and those aged 11–18 years (25.5%). Females constituted a

slightly higher proportion of the study population (52.2%).

Regarding clinical presentation, the most commonly reported symptom was a persistent cough lasting more than two weeks, observed in 72.4% of subjects. This was followed by fever (61.2%), weight loss (61.2%), and loss of appetite (58.2%). Hemoptysis was reported in a small fraction of cases (4.1%).

Only 16.3% of children had a documented history of contact with a tuberculosis patient. However, a large majority (89.7%) had received the Bacillus Calmette Guérin (BCG) vaccine.

Among the 14 malnourished children aged ≤5 years, 57.14% were classified as having severe malnutrition, while 42.85% had moderate malnutrition.

Table 1: Distribution of study subjects

Age Group (Years)	Frequency (n)/N = 98	Percentage
≤5	33	33.6
6-10	40	40.9
11-18	25	25.5
Gender		
Male	47	47.8
Female	51	52.2
Symptom distribution		
Cough for 2 weeks	71	72.4
Fever	60	61.2
Loss of weight	60	61.2
Loss of appetite	57	58.2
Hemoptysis	4	4.1
TB contact history		
Present	16	16.3
Absent	82	83.7
BCG Immunization		
Immunized	87	89.7
Not immunized	11	10.3
WHO Classification of Nutritional Status in <5 years		
Moderate acute Malnutrition	6	42.85
Severe acute Malnutrition	8	57.14

In the present study, abnormal respiratory findings were noted in 74.4% of the subjects. Crepitations were observed in nearly half of the participants (49.9%), with bilateral crepitations being more common (39.8%) than unilateral (10.2%). Reduced breath sounds were documented in 14.2% of cases, while bronchial breath sounds were present in 10.2%.

Chest X-ray evaluations revealed that the most frequent radiological pattern was nonspecific changes (36.8%), followed by bilateral infiltrates (24.4%), pleural effusion (12.2%), and consolidation (10.2%). Notably, many subjects exhibited overlapping radiological features, reflecting the complex presentation of pulmonary tuberculosis in the pediatric population.

Table 2: Distribution of Subjects based on clinic-radiological findings and Investigations

Respiratory Findings	Frequency (n)/N = 98	Percentage
Breath sounds		
Normal	25	25.6
Reduced	14	14.2
Unilateral	13	13.2
Bilateral	01	01
Bronchial Breath Sounds		
Right	6	6.1
Left	4	4
Total		
Crepitations		
Bilateral	39	39.8
Unilateral	10	10.2
Imaging findings (Chest X Ray and CT Chest)		
Non Specific changes	36	36.8
Consolidation	10	10.2

Cavity	2	2.1
Collapse	2	2.1
Bilateral Infiltrates	24	24.4
Pleural effusion	12	12.2
Hilar Lymphadenopathy	9	9.1
Bronchiectasis	4	4.1
Miliary pattern	1	1
Investigations		
Mantoux test	14	14.3
Chest X-Ray Findings	39	39.8
Smear Microscopy	4	4
CBNAAT- GeneXpert	9	9.2

In this study, abnormal chest X-ray findings were observed in 39.8% of the subjects. CBNAAT (GeneXpert) and Mantoux tests yielded positive results in 9.2% and 14.3% of cases, respectively, while smear microscopy showed positivity in only 4.0% of subjects.

Among the 16 cases tested with GeneXpert, 56.3% were found to be positive. Of these GeneXpert-positive cases, 66.6% were males and 33.4% were females, indicating a higher detection rate among male participants.

Table 3: Comparison of results of sputum microscopy and GeneXpert assay in sputum and gastric lavage samples

Sample	Sputum microscopy		GeneXpert	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Gastric lavage (n=72)	2(2.7)	70(97.3)	5(6.9)	67(93.1)
Sputum (n=26)	2(7.6)	24(92.4)	4(15.3)	22(84.7)

Among the gastric lavage samples, the test positivity percentage is higher in GeneXpert assay (6.9%) when compared with smear microscopy (2.7%). Similarly, in sputum samples the percentage of positivity is

higher in GeneXpert (15.3%) as compared with smear microscopy (7.6). [Table3]
Predictive efficacy of various investigations when compared with CBNAAT GeneXpert is illustrated in Table 4.

Table 4: Comparison of results of various investigations with GeneXpert Assay in study participants

Investigation	GeneXpert		Sensitivity of test	Specificity	False Positive	False Negative	Accuracy	P value
	Positive n(%)	Negative n(%)						
Smear Microscopy in gastric lavage sample								
Positive n (%)	2(40)	0(0.0)	40	100	0	60	95.8	<0.001
Negative n(%)	3(60)	67(100)						
Smear Microscopy in sputum sample								
Positive n (%)	2(50)	0(0)	50	100	0	50	92.3	<0.001
Negative n(%)	2(50)	22(100)						
Chest X ray								
Positive n (%)	9(100)	25(28.1)	100	71.9	28.1	0.0	74.4	<0.001
Negative n(%)	0(0)	64(71.9)						
Mantoux Test								
Positive n (%)	9(100)	5(5.6)	100	94.4	5.6	0	94.8	<0.001
Negative n(%)	0(0)	84(94.4)						

DISCUSSION

The present study primarily aimed to evaluate the diagnostic utility of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT–GeneXpert) in pediatric patients suspected of pulmonary tuberculosis (PTB), excluding neonates. A total of 98 children aged between 1 month and 18 years were enrolled, with the majority belonging to the 6–10 years age group. A higher prevalence of PTB was observed among male children, consistent with findings by Kumar A et al. [9] However, studies by Anand Kumar et al, [10] Mazta SR et al, [11] and Mohammed H et al, [12] reported a female

predominance, suggesting possible regional or demographic variations.

Cough was the most common symptom (72.4%), followed by fever and weight loss (61.2% each). This contrasts with Anand Kumar et al, [10] who reported fever as the predominant symptom. Such variations may be influenced by age, immune status, bacterial load, and co-infections. In our study, 16.3% of children had a history of TB contact, slightly lower than the 18.9% reported in a study from southern Haryana. [13] This discrepancy may be due to sample size limitations or regional differences in TB exposure.

A high proportion (89.7%) of children with PTB had received BCG vaccination, underscoring its limited

efficacy in preventing pulmonary TB. This highlights the need for complementary strategies such as early detection, contact tracing, and appropriate treatment. Malnutrition was prevalent among children under five, with more than half showing signs of severe or moderate malnutrition. Study conducted by Kaur K et al,^[14] reported 28.6% severe malnutrition, indicating variability across populations.

Nonspecific changes were the most common chest X-ray findings, followed by bilateral infiltrates, consolidation, and pleural effusion. Buonsenso et al,^[15] noted lymphadenopathy and consolidation as predominant features. In our study, 39.8% had abnormal chest X-rays, while Mantoux, CBNAAT, and smear microscopy showed positivity rates of 14.3%, 9.2%, and 4.0%, respectively.

Comparative studies show varied diagnostic yields. In Haryana, only 19% of suspected cases tested positive via ZN staining and CBNAAT. Alvarez-Uria G et al,^[16] reported 69% smear positivity and 75% CBNAAT positivity. Dewan R et al,^[17] found 11% positivity by ZN staining and 40% by CBNAAT. These differences may stem from sample collection techniques and bacillary load.

CBNAAT demonstrated superior sensitivity and specificity across multiple studies.^[18] Sowjanya et al,^[19] reported a 70.24% detection rate via CBNAAT versus 52.68% by smear microscopy. Raizada et al,^[20] found CBNAAT to be twice as effective as smear microscopy in detecting TB among children. Maynard-Smith et al,^[21] meta-analysis showed a median sensitivity of 83% and specificity of 98% for CBNAAT.

In our study, GeneXpert positivity was higher among males (66.6%) than females (33.4%), contrasting with Annamalai et al,^[22] findings of female predominance. The sensitivity and specificity of smear microscopy compared to GeneXpert were 55.5% and 100%, respectively. Similar results were reported in Odisha, with 31.1% sensitivity and 100% specificity for ZN staining. Kulkarni et al,^[23] found 33% sensitivity for gastric lavage and 57% for sputum samples. Bates M et al,^[24] reported 30% sensitivity and 97% specificity.

Kaur K et al,^[14] highlighted CBNAAT's ability to detect TB in cases missed by ZN staining, with statistically significant differences in diagnostic performance. Receiver Operating Characteristic (ROC) analysis in our study revealed that chest X-ray had a higher area under the curve than smear microscopy, indicating greater diagnostic accuracy. Mishra et al,^[25] reported CBNAAT sensitivity of 75%, specificity of 94.4%, and overall accuracy of 90%. Singh et al,^[26] found CBNAAT sensitivity and specificity to be 84.6% and 86.4%, respectively, when compared to culture.

In our study, sputum microscopy positivity was 7.6% for sputum and 2.7% for gastric lavage samples, while GeneXpert positivity was 15.3% and 6.9%, respectively. Annamalai et al,^[22] reported 55% CBNAAT positivity in sputum and induced sputum samples. Mishra et al,^[25] found CBNAAT detection

rates of 21.4% in sputum or induced sputum and 33% in gastric lavage samples.

All CBNAAT-positive cases in our study were rifampicin-sensitive. In contrast, Kaur K et al,^[14] identified Rifampicin resistance in 11.94% of CBNAAT-positive cases.

Due to the paucibacillary nature of pediatric TB, traditional smear microscopy has limited sensitivity. Clinical diagnosis remains common, but current NTEP and IAP guidelines emphasize bacteriological confirmation. CBNAAT offers rapid, sensitive, and specific detection of *Mycobacterium tuberculosis* and rifampicin resistance, making it a valuable tool in pediatric TB diagnosis. Despite advancements, many TB cases globally are still clinically diagnosed, underscoring the need for broader implementation of molecular diagnostics.

CONCLUSION

In this study, GeneXpert (CBNAAT) demonstrated a higher test positivity rate compared to conventional sputum microscopy across both gastric lavage and sputum samples. Notably, all GeneXpert-positive cases were found to be sensitive to rifampicin, underscoring its utility not only in rapid detection of *Mycobacterium tuberculosis* but also in identifying drug resistance. These findings reinforce the value of incorporating CBNAAT into routine diagnostic protocols for pediatric pulmonary tuberculosis, especially in settings where early and accurate diagnosis is critical.

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