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Original Research Paper

Utility of stool- CBNAAT testing for the diagnosis of pulmonary tuberculosis in children

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ABSTRACT:
Objectives: Paediatric tuberculosis (TB) is among one of the major health issues with diagnostic challenges globally. Collecting respiratory sample for analysis is traumatic for kids. Therefore, non-respiratory samples are being studied as a non-invasive diagnostic method. This study focussed on evaluating cartridge-based nucleic acid amplification test (CBNAAT) using stool sample in clinically suspected cases of paediatric pulmonary tuberculosis (PTB) and analyzing its accuracy with that of gastric aspirates (GA)-CBNAAT. **Materials and Methods**: This single-centric cross-sectional study included 50 children aged 0.25-16 years, suspected as PTB. GA and stool sample were obtained from the enrolled patients and CBNAAT diagnosis was performed. **Statistical analysis**: Statistical analysis was done using Microsoft Excel and SPSS v23. Continuous variables are represented as mean±standard deviation and categorical data as percentages and frequency. **Results:** Out of 50 patients, 28 were microbiologically confirmed, 7 were clinically diagnosed as TB and 15 were diagnosed other than TB. All microbiologically confirmed cases were GA-CBNAAT positive, but only 18 patients were stool-CBNAAT positive. Both GA and stool CBNAAT had strong relation with high Cohen's kappa value of 0.613 and highly acceptable significant value 0.0001. Stool-CBNAAT had sensitivity, specificity, PPV, NPV and diagnostic accuracy of 64.3%, 100%, 100%, 68.8%, and 80%, respectively as compared to GA-CBNAAT test. **Conclusion**: Stool-CBNAAT was found to have comparable accuracy with that of GA-CBNAAT. It can be considered as a feasible, reliable, and non-invasive diagnostic tool for PTB.

Keywords: Cartridge-based nucleic acid amplification test, Pediatric tuberculosis, Pulmonary tuberculosis, Stool-CBNAAT, GA-CBNAAT, Diagnosis

INTRODUCTION:

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a global health challenge. In 2022, the WHO estimated 10 million TB cases globally, with India accounting for 26% and children under 15 comprising 11% of these cases.[1] India records about 3.33 lakh pediatric TB cases annually, representing 28% of the global childhood burden.[2] Pediatric TB, which includes both pulmonary (PTB) and extra-pulmonary (EPTB) forms, is severe, especially in immunocompromised children.[3] Diagnostic limitations result in significant under-detection and high mortality, with 56% of children and 65% of those under five remaining undiagnosed compared to 25% of adults.[4] Early diagnosis and treatment are critical, though challenges persist due to nonspecific symptoms, paucibacillary nature, and difficulty in obtaining adequate samples. CBNAAT (Cartridge-based Nucleic Acid Amplification Test) and the Xpert MTB/RIF assay have enhanced TB detection and rifampicin resistance identification, yet microbiological confirmation in children remains difficult.[5]

Gastric aspirate (GA) has been the standard for microbiological diagnosis in pediatric TB but is invasive. Non-invasive alternatives like stool testing offer promise since swallowed sputum retains TB DNA.[6] Studies have explored the efficacy of stool CBNAAT for pediatric TB diagnosis.[6–11] WHO recommended in August 2021 the Xpert MTB/RIF assay on GA or stool samples for TB diagnosis in children under 10.[1] This study aims to evaluate the utility of stool CBNAAT in presumptive pediatric TB cases.

METHODOLOGY:

Study design and sample size:

This prospective study was conducted at a tertiary care hospital in South East Delhi, India, from November 2022 to May 2024. Children aged 1 month to 18 years with suspected TB were enrolled with parental consent. TB diagnosis followed pediatric TB management guidelines (2022),[12] based on symptoms like persistent fever and weight loss despite proper nutrition. Children already on anti-tuberculosis treatment (ATT) were excluded. The sample size was calculated based on a sensitivity of 73% for stool CBNAAT in pediatric PTB,[10] and 50 participants were estimated using formulae: $\alpha 2*S*(1-S)/P*W2$; where, S= sensitivity, 0.73, P= Proportion of subjects of Target Condition (0.373 or 37.3%), Type I error (α) = 5% Z $1-\alpha/2$ = 1.96, W= Width of Confidence Interval (0.2 or 20%).

Study procedure:

Institutional ethical clearance (HIMSR/IEC/00191/2024) from the committee and consent/assent from guardians and children (>7 years) was obtained before the initiation of the work. A structured questionnaire captured history, demographics, and physical examination. Tuberculin Skin Tests (TST) and chest X-rays (CXR) were performed, along with additional tests like CBC, ESR, and LFT. GA and stool samples were collected and processed for CBNAAT.

Sample collection and processing:

Gastric aspirate (GA) was collected as per Singhal et al. [13] Patients fasted for 4–6 hours before a lubricated Ryle's tube was inserted into the stomach. Gastric contents (10–15 mL) were aspirated, centrifuged, and processed for CBNAAT. The pellet was re-suspended in 67 mM Phosphate/H2O buffer, and 0.5 mL was transferred to a screw-capped tube for CBNAAT. To this, 1.5 mL of CBNAAT reagent was added, vortexed for 10–20 minutes, and the test was conducted.

For stool samples, 2–3 g was collected in a sterile container and immediately processed to maintain the cold chain. A 0.5 g portion of stool was mixed with 10 mL Sheather's solution, vortexed for 2 minutes, and allowed to settle for 30 minutes at room temperature. The supernatant (0.5 mL) was transferred to a fresh centrifuge tube, and 1.8 mL of CBNAAT reagent was added. The mixture was vortexed and incubated at room temperature for 15 minutes. One mL of this was mixed with 2 mL sample diluent and transferred to the Xpert MTB/RIF cartridge. The GeneXpert device was used according to the manufacturer's instructions.

Diagnosis:

Cases were categorized as: (1) microbiologically confirmed TB (GA-CBNAAT positive), (2) clinically diagnosed TB (GA-CBNAAT negative but radiologically diagnosed, ATT started), and (3) non-TB cases. ATT initiation was based on clinical and radiological findings.

Statistical analysis:

Data were analyzed using SPSS v23 (IBM Corp.). Continuous variables were expressed as mean±standard deviation, and categorical data as percentages/frequency. Chi-square, independent t-tests, and ANOVA were applied, with statistical significance set at $p \leq 0.05$. Sensitivity, specificity, PPV (Positive Predictive Value), NPV (Negative Predictive Value), and diagnostic accuracy of stool-CBNAAT were calculated using GA-CBNAAT as the reference. Diagnostic accuracy was assessed with Cohen's Kappa (κ) to measure agreement between stool-CBNAAT and GA-CBNAAT.

RESULTS:

General characteristics of patients:

Fifty TB-suspected patients (1 month-18 years) were enrolled with consent/assent. Table 1 summarizes the baseline profile of the patients. The mean age was 10.16±4.97 years, with median 11.5 years (interquartile range 0.25-5.25). Maximum patients (56%, n=28) were >10 years, 20% (n=10) of patients were 2.1-5 years and 18% (n=9) were between 5.1 and 10 years. There was female dominance of 56% over male patients (44%). Anthropometric measurements showed 4% of children had weight for age <-3 SD, while 4% (n=2) had weight for height/BMI for age <-3SD.

Table 1: Baseline characteristics of patients included in the study

Characteristics		n (%)
Age (Years)	**Median (IQR)**	**11.5 years (0.25-5.25) years**
	0-1	2 (4)
	1.1-2	1 (2)
	2.1-5	10 (20)
	5.1-10	9 (18)
	>10	28 (56)
Sex	Male	22 (44)
	Female	28 (56)
Weight for age	< -3 SD	2 (4%)
	-3 to -2 SD	13 (26%)
	-1.9 to +2 SD	35 (70%)
	> +2 SD	0
Height for age	< -3 SD	0
	-3 to -2 SD	4 (8%)
	-1.9 to +2 SD	42 (84%)
	> +2 SD	4 (8%)
Weight for height/ BMI for age	< -3 SD	2 (4%)
	-3 to -2 SD	15 (30%)
	-1.9 to +2 SD	33 (66%)
	> +2 SD	0

BMI: Body Mass Index; SD: Standard Deviation

Table 2: Clinical profile of the enrolled patients

Variables		n (%)
Symptoms	Fever	43 (86)
	Cough	35 (70)
	Weight loss	25 (50)
	Abdominal pain	9 (18)
	Seizure	2 (4)
	Lymphadenopathy	2 (4)
Contact history	Present	12 (24)
	Absent	38 (76)
Mantoux test	Positive	33 (66)
	Negative	17 (34)
Chest findings	Clear	13 (26)
	Crepitations	19 (38)
	Decreased air entry	8 (16)
	Rhonchi	2 (4)

	Bronchial breathing	1 (2)
	Crepitations + Decreased air entry	5(10)
	Crepitations + Rhonchi	1 (2)
	Rhonchi + Bronchial breathing	1 (2)
CXR findings	Normal	8 (16)
	Lobar consolidation	18 (36)
	Pleural effusion	10 (20)
	Fibro cavitary lesion	7 (14)
	Lymph nodes (Mediastinal/Hilar)	6 (12)
	Non-specific infiltrates	1 (2)

CXR: Chest X-Ray

Table 3: Distribution of patients according to their demographic details

Variables	**Confirmed (n=28)**	**Clinically diagnosed (n=7)**	**Diagnosed other than TB (n=15)**	**p-value**
Age (Years)				
0-1	0	0	2 (13.33%)	**0.001**
1.1-2	0	0	1 (6.67%)	
2.1-5	2 (7.14%)	3 (42.86%)	5 (33.33%)	
5.1-10	2 (7.14%)	2 (28.57%)	5 (33.33%)	
>10	24 (85.71%)	2 (28.57%)	2 (13.33%)	
Gender				0.31
Male	10 (35.71%)	3 (42.86%)	9 (60%)	
Female	18 (64.29%)	4 (57.14%)	6 (40%)	
BMI for age/weight for height				
< -3 SD	2 (7.14%)	0	0	0.529
-3 to -2 SD	8 (28.57%)	1 (14.29%)	6 (40%)	
-1.9 to +2 SD	18 (64.29%)	6 (85.71%)	9 (60%)	
> +2 SD	0	0	0	

p-value ≤0.05 was considered significant.

Table 4: Clinical profile of patients in different diagnosed groups

Variables	**Confirmed (n=28)**	**Clinically diagnosed (n=7)**	**Diagnosed other than TB (n=15)**	**p-value**
Primary symptoms				
Fever	27 (96.43%)	5 (71.43%)	11 (73.33%)	0.06
Cough	20 (71.43%)	4 (57.14%)	11 (73.33%)	0.72
Weight loss	20 (71.43%)	3 (42.86%)	2 (13.33%)	**0.001**
Other symptoms				
Abdominal pain	7 (25%)	2 (28.57%)	0	0.09
Seizure	1 (3.57%)	0	1 (6.67%)	0.75
Lymphadenopathy	2 (7.14%)	0	0	0.44
H/o contact	10 (35.71%)	1 (14.29%)	1 (6.67%)	0.08
Mantoux Positivity	28 (100%)	5 (71.43%)	0	**0.0001**
CXR findings				
Normal	4 (14.29%)	1 (14.29%)	3 (20%)	0.88

Lobar consolidation	11 (39.29%)	0	7 (46.67%)	0.06
Pleural effusion	5 (17.86%)	3 (42.86%)	2 (13.33%)	0.26
Fibro cavitary lesion	4 (14.29%)	1 (14.29%)	2 (13.33%)	1.00
Lymph nodes (Mediastinal/Hilar)	3 (10.71%)	2 (28.57%)	1 (6.67%)	0.33
Non-specific infiltrates	1 (3.57%)	0	0	0.68

p-value ≤0.05 was considered significant. CXR: Chest X-ray; TB: Tuberculosis

Table 5: Symptom wise distribution

Symptoms	Confirmed (n=28)	Clinically diagnosed (n=7)	Diagnosed other than TB (n=15)	p-value
Only fever	2 (7.14%)	2 (28.57%)	3 (20%)	0.25
Only cough	0	1 (14.29%)	2 (13.33%)	0.13
Only weight loss	0	0	0	0
Cough+ fever	3 (10.71%)	0	7 (46.67%)	**0.007**
fever + weight loss	2 (7.14%)	0	1 (6.67%)	0.77
Cough + weight loss	0	1 (14.29%)	1 (6.67%)	0.88
Cough + others	0	0	1 (6.67%)	0.18
Fever + others	2 (7.14%)	0	0	0.44
Cough + fever + weight loss	12 (42.86%)	1 (14.29%)	0	**0.007**
Fever +cough + others	1 (3. 57%)	1 (14.29%)	0	0.28
Fever + weight loss + others	2 (7.14%)	1 (14.29%)	0	0.39
Cough + weight loss +others	1 (3.57%)	0	0	0.67
Cough + fever + weight loss + others	3 (10.71%)	0	0	0.28

p-value ≤0.05 was considered significant.

Table 6: Contact history vs Mantoux test

Mantoux test	H/o contact		p-value
	Positive (n=12)	Negative (n=38)	
Positive	11 (91.67%)	22 (57.90%)	**0.031**
Negative	1 (8.33%)	16 (42.10%)	

p-value ≤0.05 was considered significant.

Table 7: GA- and Stool- CBNAAT diagnosis in three groups

Tests	Confirmed (n=28)	Clinically diagnosed (n=7)	Diagnosed other than TB (n=15)	p-value
GA-CBNAAT				
Positive	28 (100%)	0	0	**0.0001**
Negative	0	7 (100%)	15 (100%)	
Stool-CBNAAT				
Positive	18 (64.29%)	0	0	**0.0001**
Negative	10 (35.71%)	7 (100%)	15 (100%)	

p-value ≤0.05 was considered significant. CBNAAT: Cartridge-based nucleic acid amplification test; GA: Gastric Aspirate; TB: Tuberculosis

Table 8: Comparison between stool- and GA- CBNAAT

Stool-CBNAAT	GA-CBNAAT		Weight kappa	
	Positive	Negative	κ-value	p-value
Positive (n=18)	18 (100%)	0	0.613	**0.0001**
Negative (n=32)	10 (31.25%)	22 (68.75%)		

p-value ≤0.05 was considered significant. CBNAAT: Cartridge-based nucleic acid amplification test; GA: Gastric Aspirate; TB: Tuberculosis

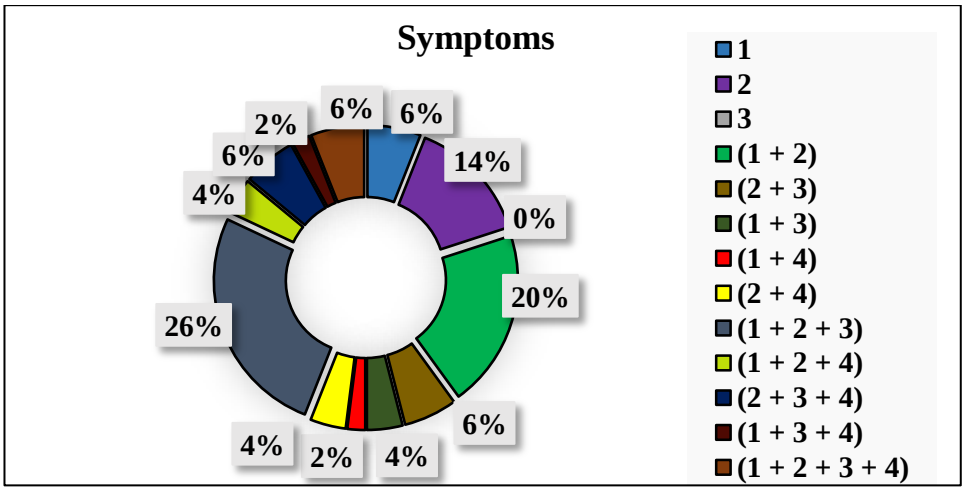

Figure 1: Symptoms in suspected patients; where 1: Cough; 2: Fever; 3: Weight loss; 4: Others (abdominal pain, seizure, lymphadenopathy)

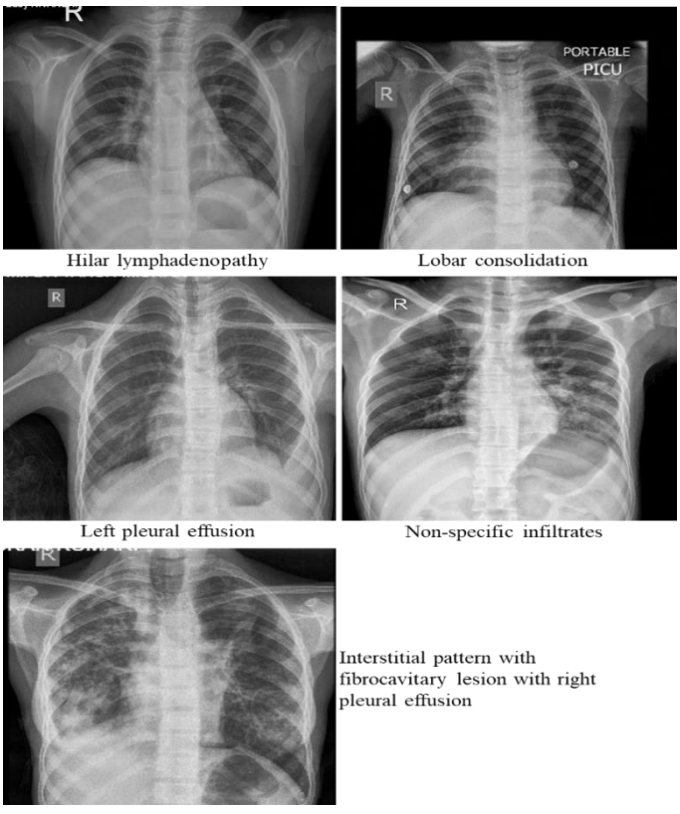

Figure 2: CXR images showing different pathological conditions

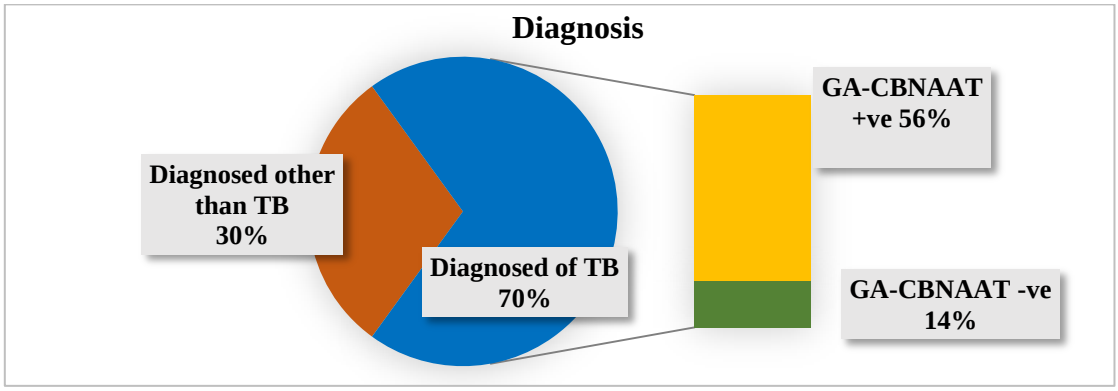

Figure 3: Diagnosis of patients

***Clinical profile**:*

Table 2 summarizes the clinical profile of the patients. Cough (86%), fever (70%), and weight loss (50%) were the most common symptoms. Abdominal pain (18%) was linked to abdominal TB, and 4% had seizures due to Global Development Delay and Hypocalcaemia, 4% also had lymphadenopathy. Figure 1 shows the presence of major symptoms individually and in combination with other symptoms which clearly shows that combination of Cough + fever + weight loss (26%) was the most prominent symptom, followed by Cough+ fever (20%). Amongst 50 patients, only 24% had contact history with TB patients and most of the patients (66%) were Mantoux (TST) positive.

Pathological chest findings were present in 74%, including crepitations (38%) and decreased air entry (16%). CXR abnormalities were found in 58%, with lobar consolidation (36%) and pleural effusion (20%) being the most frequent (Table 2). CXR images showing various abnormal findings detected in enrolled patients has been shown in the Figure 2.

Figure 3 shows the final diagnosis of the patients on the basis of which patients were divided in three categories namely (1) Confirmed cases of TB (GA-CBNAAT +ve, 28/50); (2) Clinically diagnosed (GA-CBNAAT -ve, but ATT started on the basis of radiological findings, 7/50); and (3) Diagnosed other than TB (15/50). In the present study, 35 patients were diagnosed of TB (microbiologically confirmed + clinically diagnosed cases).

Clinical characteristics of patients in different diagnosed groups:

TB prevalence was significantly age-dependent (p=0.001). No confirmed or clinically diagnosed TB cases were in the 0–2 years group. Most TB cases (24/28 confirmed, 2/7 clinically diagnosed) were >10 years old. Among non-TB cases (n=15), 8 were 0–5 years, 5 were 5.1–10 years, and 2 were >10 years (Table 3). TB (both confirmed + clinically diagnosed) cases showed female dominance, while non-TB cases had fewer females. BMI analysis showed 7.14% of confirmed cases were severely undernourished (<-3 SD), and 28.57% were undernourished (-3 to -2 SD). In clinically diagnosed cases, 1 patient was under -3 to -2 SD, while 6 were within -1.9 to +2 SD. Among non-TB cases, 40% were under -3 to -2 SD, and 60% were within -1.9 to +2 SD (Table 3).

Fever was common in confirmed (96.4%), clinically diagnosed (71.4%), and non-TB cases (73.3%). Cough and weight loss were reported in 71.4% of confirmed cases. Weight loss was significantly associated with TB (p=0.001) (Table 4). A combination of cough, fever, and weight loss was significant in TB cases (p=0.007) (Table 5). Additional symptoms included abdominal pain (25% in confirmed, 28.6% in clinically diagnosed cases) and lymphadenopathy (7% in confirmed cases) (Table 4). Contact history was reported in 10 (35.7%) of confirmed cases but was not significantly associated with TB diagnosis (p>0.05) (Table 4).

Mantoux positivity was significantly associated with TB (p=0.0001), with all confirmed cases (28/28) and 5/7 clinically diagnosed cases testing positive, while all non-TB cases were negative. Among 12 patients with TB contact history, 11 had positive Mantoux (p=0.03) (Table 6).

CXR analysis in different groups revealed the ratio between normal and abnormal finding in confirmed, clinically diagnosed and other than TB diagnosed cases was 1:7, 1:6 and 1:4, respectively. Lobar consolidation (39.3%) and pleural effusion (17.9%) were prominent in confirmed cases, while 42.9% of clinically diagnosed cases had pleural effusion. Lobar consolidation was highest in non-TB cases (46.7%) (Table 4).

***Diagnostic results of GA- and stool- CBNAAT**:*

GA-CBNAAT was positive in all confirmed cases (28/28) and negative in all clinically diagnosed (7/7) and non-TB (15/15) cases (p=0.0001) (Table 4). Stool-CBNAAT was positive in 18/28 confirmed cases

(64.3%), while all clinically diagnosed and non-TB cases were negative (35.7%, n=10) (Table 7).

Comparative analysis of stool- and GA-CBNAAT diagnosis:

All stool-CBNAAT+ cases were also GA-CBNAAT+, but 31.3% of stool-CBNAAT− cases were GA-CBNAAT+. The weighted kappa coefficient (0.613, p=0.0001) indicated substantial agreement, supporting Stool-CBNAAT as a reliable, non-invasive TB diagnostic tool (Table 8). Stool-CBNAAT had 64.3% sensitivity, 100% specificity, 100% PPV, 68.8% NPV, and 80% diagnostic accuracy, making it a valuable alternative in resource-limited settings.

DISCUSSION:

The present cross-sectional study assessed the efficacy of stool-CBNAAT in diagnosing pediatric pulmonary TB. While CBNAAT provides rapid and accurate TB diagnosis in adults, its effectiveness in children remains challenging due to low bacterial loads and difficulty in obtaining sputum samples. GA or induced sputum collection is invasive and stressful, often requiring hospitalization.[14-16] Stool, a non-invasive alternative, holds promise for pediatric TB diagnosis.[17]

Among 50 patients, 28 had confirmed TB, 7 were clinically diagnosed, and 15 had alternate diagnoses (13 pneumonia, 2 bronchial asthma). Similar to Agarwal et al.,[10] who reported 28 TB cases among 75 children (11 microbiologically confirmed, 17 clinically diagnosed), no TB cases were found in the 0–2 years group, suggesting lower TB prevalence in infants.[10] The median age for TB cases was 11.5 years (IQR: 5-14) versus 12 years for non-TB cases. Among children aged 2.1-5 years, 14.28% had confirmed TB, and among those aged 5.1-10 years, 11.43% had confirmed TB, indicating a significant burden of TB in preschool and school-aged children. However, 33.33% of children with alternate diagnoses were in the same age groups. TB prevalence was higher in children >10 years (74.28%), compared to 13.33% in the same age group with alternate diagnoses, showing a higher prevalence of TB in older children. This aligns with the epidemiological pattern where older individuals are at increased risk due to factors like prolonged exposure and social interactions. The results were in sync with previous studies.[7,8,18]

Among the confirmed TB cases (microbiologically confirmed and clinically diagnosed), a higher proportion of girls (62.86%, n=22/35) were observed as compared to boys (37.14%, n=13/35), indicating a higher prevalence of confirmed TB in female paediatric patients within the study cohort. Conversely, among those diagnosed other than TB, a higher proportion of males (60%, n=9/15) compared to females (40%, n=6/15) was observed. Similar to present study, Chipinduro et al.,[7] conveyed girl dominance (63%) in microbiologically TB confirmed group. However, more girls (56%) were also reported in non-TB group.

In confirmed TB cases (microbiologically and clinically diagnosed), fever was the most common symptom (91.4%, 32/35), followed by cough (68.57%, 24/35) and weight loss (65.71%, 23/35). In contrast, only 13.33% (2/15) of non-TB cases exhibited weight loss, with a significant p-value of 0.00068. Similarly, Singh et al.,[19] reported fever in 92.1% of TB-positive and 76.3% of non-TB cases, cough in 80.3% of TB-positive and 98.4% of non-TB cases, and weight loss in 84.3% of TB-positive and 60.5% of non-TB cases. Overlapping symptoms, such as cough + fever + weight loss, were observed in 68.6% of TB-positive and 31.6% of non-TB cases, while cough + weight loss occurred in 70.6% and 39.5%, respectively.[19]

Contact history was positive in 31.42% (11/35) of confirmed TB cases compared to 6.67% (1/15) in non-TB cases, reinforcing the link between close contact and TB risk in children. Similarly, Agarwal et al. [7] reported a 35.71% contact history among confirmed TB patients. Mantoux positivity was observed in 94.29% (33/35) of confirmed TB cases, whereas none of the non-TB cases tested positive. Only 5.71% (2/35) of TB cases were Mantoux negative, highlighting a significant difference (p=0.0001) and the test's diagnostic reliability. Copelyn et al. [20] reported Mantoux positivity in 77% of confirmed, 68% of unconfirmed, and 29% of unlikely TB cases.

This study showed abnormal CXR findings in each 85.71% confirmed and clinically diagnosed cases and 80% diagnosed other TB cases. In corroboration with present result, Copelyn et al.[20], reported abnormal CXR finding in 88% confirmed cases, 71% unconfirmed cases of TB and 58% unlikely cases of TB.

GA-CBNAAT is a rapid, highly sensitive, and specific TB diagnostic test, particularly effective in pediatric cases with low bacterial load. Unlike culture methods, GA-CBNAAT is less reliant on sample quality and is unaffected by non-tuberculous mycobacteria, reducing false negatives and diagnosis delays. WHO recommends GA-CBNAAT as the initial diagnostic tool for pediatric TB. Previous studies confirm its sensitivity (91.67%-100%) and specificity (75%-100%).[9, 27, 53-55] The present study found that all 28 confirmed TB cases and 7 clinically diagnosed cases were GA-CBNAAT positive, while all other cases were negative. Consistent with these findings, Dubale et al. confirmed all 8 GA-CBNAAT positive cases as TB [9], while Agarwal et al. found 10 out of 11 positive cases microbiologically confirmed.[10] Singhal et al. reported 57% positivity with GA-CBNAAT.[13]

This study found that 64.29% of confirmed TB cases (18/28) tested positive with Stool-CBNAAT, while 35.71% were negative. Similar to GA-CBNAAT, Stool-CBNAAT was negative in all clinically confirmed and other non-TB cases. While Stool-

CBNAAT is effective for diagnosing pediatric TB, its sensitivity is lower than GA-CBNAAT, missing 35.71% of confirmed cases. Previous studies highlight its effectiveness, with Chipinduro et al. reporting 68% sensitivity among microbiologically confirmed TB cases.[7] Agarwal et al. found 9 positive cases, 8 of which were confirmed microbiologically,[10] while Singhal et al. detected TB in 56% of suspected cases using Stool-CBNAAT.[13]

This study compared the effectiveness of Stool-CBNAAT and GA-CBNAAT in diagnosing pediatric TB. It found that all 18 positive Stool-CBNAAT cases were also positive with GA-CBNAAT, while none of the GA-CBNAAT negative cases were positive with Stool-CBNAAT. Among 32 Stool-CBNAAT negative cases, 10 were GA-CBNAAT positive, indicating lower sensitivity for Stool-CBNAAT. Statistical analysis showed a significant difference ($p=0.0001$) with strong agreement ($\kappa=0.613$). Singhal et al. reported similar findings with κ 0.94 and $p=0.0001$.[13] Agarwal et al. found a κ-value of 0.83 between the two tests.[10]

In the present study, Stool-CBNAAT had 100% specificity, 64.3% sensitivity, 68.8% NPV, and 100% PPV. Ainan et al. reported 62.5% sensitivity and 100% specificity for Stool-CBNAAT compared to GA/IS-CBNAAT/culture,[8] while Dubale et al. found 100% sensitivity, specificity, NPV, and PPV for Stool-CBNAAT.[9] Agarwal and Mathur reported a sensitivity range of 32-100% and specificity of 87.5%-100% for Stool-CBNAAT compared to GA/IS-CBNAAT.[17

The variability in stool-CBNAAT test sensitivities may be attributed to differences in laboratory processing techniques, as there are no standardized guidelines for handling stool specimens for Xpert MTB/RIF testing.[21] Effective outcomes depend on methods like centrifugation, filtration, and sedimentation, and standardizing these processes could improve test efficacy and acceptance.[17] This study used a simple processing method with distilled water and vortex mixing, omitting centrifugation, making it suitable for resource-limited settings. Samples were processed immediately or kept in a cold chain to maintain integrity. These findings support the use of stool Xpert assay alongside traditional methods for diagnosing pediatric TB, though further research is needed to implement this approach for identifying pulmonary tuberculosis.

The single-center design and small sample size limit the generalizability of the findings. A multicentric study with a larger, more diverse cohort is needed to validate the results and refine symptom-based algorithms. Nonetheless, the high effectiveness and agreement between GA- and stool-CBNAAT in this study suggest that stool-CBNAAT is an innovative, non-invasive, and rapid diagnostic approach for pediatric TB.

CONCLUSION:

Stool CBNAAT is an efficient, non-invasive diagnostic tool for pediatric TB, with comparable diagnostic accuracy to GA-CBNAAT. Global adoption of stool CBNAAT could reduce the need for trained personnel for gastric aspirate collection, minimizing patient trauma and providing a rapid, accessible diagnostic method for TB, especially in resource-limited settings.

Ethical approval:

This study was approved by the Institutional Ethics committee, number (HIMSR/IEC/00191/2024).

Declaration of patient consent:

All appropriate consent from the patient and their guardian were taken prior to investigation.

Conflict of interest: There are no conflicts of interest.

Financial support and sponsorship: None

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