

Original Research Article

Diagnostic accuracy of sputum gene xpert MTB /RIF and stool gene xpert-ultra in children with pulmonary tuberculosis in a resource constraint country

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Received: 05 September 2024

Accepted: 07 October 2024

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ABSTRACT

Background: Diagnosis of tuberculosis in children remains a challenge especially in resource limited settings. This study aimed to determine the diagnostic accuracy of sputum Gene Xpert MTB/RIF and stool Xpert-Ultra for the diagnosis of PTB.

Methods: This retrospective study was conducted in paediatric pulmonology ward of Bangabandhu Sheikh Mujib Medical University from March 2022 to December 2023. Consecutive 85 children aged 0-14 years with presumptive pulmonary tuberculosis (PTB) who provided both sputum and stool sample for GeneXpert and Xpert-Ultra respectively were included in the study. Diagnosis of PTB was confirmed by clinical, radiological, bacteriological or molecular evidence and taken as reference.

Result: A total 119 children were enrolled; 85 of whom complete microbiological examination. PTB was diagnosed in 25 children. Of these 18(18/25) children was found positive in stool Xpert -Ultra and 6 children were tested positive by sputum /GA Gene Xpert MTB/RIF. All 6 positive sputum/GA samples were also positive on stool testing. The rate of concordance between stool and sputum was moderate with a kappa value of 0.441 ($p < 0.001$). Overall sputum GeneXpert and stool Gene Xpert-Ultra showed Sensitivity of 24% and 72%, specificity of 100% and 100%, positive predicted value of 100% and 100%, negative predicted value of 75.95% and 89.55% and accuracy of 77.65% and 91.76% respectively.

Conclusion: Stool gene xpert-ultra and sputum Gene Xpert both tests are highly specific for diagnosis of PTB. But sensitivity of stool Xpert-Ultra is more than the sputum Gene Xpert.

Keywords: Children, Sputum gene Xpert MTB/RIF, Stool Gene Xpert ultra, Tuberculosis

INTRODUCTION

Tuberculosis (TB) remains among the top 10 causes of death worldwide and the leading cause from a single infectious agent. According to World Health Organization (WHO) report 2019, an estimated 10 million people developed TB disease of whom 12% were children <15 years. The estimated incidence of TB per 100,000 is 221 in Bangladesh, with a mortality rate of 24

per 100,000 population.¹ Approximately 80% of all TB cases in Bangladesh are pulmonary TB.² The diagnosis of pulmonary tuberculosis in young children remains a challenge because of nonspecific clinical presentation, difficulty in obtaining sputum specimen, paucibacillary nature of sputum and lack of sensitive diagnostics tests.^{3,4} Sputum sample is the most frequently used specimen in diagnosis of pulmonary tuberculosis but most of the children are unable to expectorate adequate amount of

sputum, that's why sputum induction and gastric aspiration are used to confirm the diagnosis by microbiologically but requires specific skills and equipment which are usually not available in low level health care facilities and in remote settings.⁵ In addition, Mycobacterium tuberculosis culture requires long incubation period (3-4 weeks) which makes delay in the diagnosis as well as treatment initiation.⁶

That's why it is required to diagnose PTB in non-sputum-based specimen. Recent studies showed that the use of Xpert ultra in various sample such as stool, urine are beneficial on detection of childhood tuberculosis as these samples are easy to collect.⁷⁻⁹ Stool sample is an alternative sample for detection of tubercle bacilli's DNA from swallowed sputum in children with pulmonary tuberculosis.^{10,11} Since 2022 stool Xpert-Ultra has been recommended by WHO as an alternative sample to diagnose childhood pulmonary tuberculosis.^{12,13} Recent studies showed that stool Xpert MTB/RIF has higher sensitivity ranging from 32% to 83.3%^{11,14}. The study was aimed to determine the diagnostic performance of sputum gene Xpert MTB/RIF and stool Xpert-Ultra in children with PTB.

METHODS

Study design

A retrospective study was conducted among admitted children with presumptive pulmonary tuberculosis (aged 0-14 years) in paediatric pulmonology ward of Bangabandhu Sheikh Mujib Medical University.

Study duration

The study period was from March 2022 to December 2023.

Inclusion criteria

Children aged <15 years and having sign-symptoms of pulmonary tuberculosis whose both sputum/gastric lavage and stool samples were sent for Gene Xpert and Gene Xpert-Ultra respectively were included in this study.

Exclusion criteria

Patients already getting antitubercular therapy before enrolment and whose symptoms and sign were suggesting extrapulmonary tuberculosis was excluded from the study.

Children up to 14 years were screened for pulmonary tuberculosis and those with presumptive TB were enrolled in this study. Chest Xray was done for radiological evaluation and blood samples were drawn for routine hematological and biochemical assays. Participants were asked to give two specimen, one

respiratory specimen and another stool specimen in two separate containers. After collection two samples were sent to the laboratory with standard protocol for Gene Xpert MTB/ RIF of respiratory specimen and Xpert-Ultra in stool specimen. Respiratory samples were collected as per standard of care.

Gastric aspirate was collected by treating physician at early morning after fasting of at least 6 hours mostly in <3 years children. Induced sputum was collected instead of gastric aspirate who were able to produce sputum as per National guidelines for the management of tuberculosis in children, Bangladesh.

In this study, PTB diagnosis was a composite derived from clinical, radiological, and bacteriological or molecular evidence. All the patients were confirmed to have at least one positive, either bacteriological or molecular evidence: sputum AFB or Gene Xpert assay results (positive Gene Xpert MTB/ RIF of sputum or gastric aspirate or Xpert-Ultra in stool).

Data collection

Data were collected in a structured questionnaire by researcher herself. Sociodemographic characteristics were collected.

Statistical analysis

Quantitative data were expressed as mean±standard deviation (SD). Qualitative data were presented as frequency and percentage. The Chi-square test was used to assess the association between categorical variables for the two diagnostic procedures. Additionally, diagnostic validity tests, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were conducted to evaluate the performance of sputum Gene Xpert MTB/RIF and Gene Xpert-Ultra of stool in diagnosing PTB in children. A p value of <0.05 was considered statistically significant. All analyses were conducted using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Initially 119 children of <15 years with presumptive pulmonary tuberculosis were enrolled in the study. Finally, 85 participants whom both samples were studied were included in this study (Figure1).

Overall, 18 out of 85 study participants had microbiologically confirmed pulmonary tuberculosis (positive results on stool gene Xpert-Ultra /or sputum/GA Gene Xpert). Another, 7 participants who were microbiologically negative were diagnosed as Pulmonary TB by clinicians. Total 25 participants were diagnosed as confirmed PTB (either clinically, radiologically or bacteriologically). Among 85 children with suspected pulmonary tuberculosis, most (58.8%) of the children

were aged 5-14 years, with a mean age of 6.2 years (SD±3.91, range 0.20-14 years). Regarding sample types, all participants provided stool samples (100%), 88.2% provided sputum samples, and 11.8% provided gastric aspirates. Most of the patients reported with cough (89.4%), weight loss (63.5%), fever (30.6%) and history of contact with TB cases were present in 22 participants (25.9%) (Table-1).

Among 25 confirmed PTB, 18 participants (21.2%) were tested positive by stool Xpert-Ultra and 6 participants (7.1%), were positive by sputum/ GA examination as shown in figure 2. Out of 18 children with MTB detected in sputum or stool samples rifampicin resistance was not seen any of the sample.

Table 1: Baseline characteristics of study participants (n=85).

Variables	Frequency	(%)
Age group (in years)		
<5	35	41.2
5-14	50	58.8
Mean±SD	6.2±3.91 (0.20-14)	
Gender		
Male	52	61.2
Female	33	38.8
Sample type		
Sputum	75	88.2
Gastric aspirate	10	11.8
Stool	85	100.0
Presenting symptoms		
Cough	76	89.4
Fever	26	30.6
Contact history	22	25.9
Weight loss	31	63.5

Table 2: Comparison of Xpert-Ultra results of stool and sputum/gastric aspirates Gene Xpert from children with presumptive TB (n=85).

		MTB detected from stool		Ka ppa	P value
		Positive	Negative		
MTB detected from sputum/ GA	Pos-itive	6 (7.1%)	0 (0.0%)	0.441	<0.001
	Neg-ative	12 (14.1%)	67 (78.8%)		
Total		18 (21.2%)	67 (78.8%)		

P value obtained by Chi-square test, p<0.05 was considered as a level of significant.

A comparison of stool Gene Xpert-Ultra and sputum /gastric aspirate Gene Xpert was done to see the concordance of stool Gene Xpert-Ultra and sputum Gene Xpert result (Table-2). Finding shows that

Mycobacterium tuberculosis (MTB) was detected in 7.1% (6/85) of sputum/GA samples and 21.2% (18/85) of stool samples. Notably, all 6 positive sputum/GA samples were also positive on stool testing, while an additional 12 stool samples were positive despite negative sputum/GA results. The Kappa statistic of 0.441 indicates moderate agreement between the two sample types, and the p-value of <0.001 suggests that the findings are statistically significant.

Table 3: Diagnostic performance index of MTB detected from sputum/GA taking confirmed TB as reference (n=85).

Statistic	Value	95% CI
Sensitivity	24%	9.36%-45.13%
Specificity	100%	94.04%-100%
Positive predictive value	100%	54.07%-100%
Negative predictive value	75.95%	71.70%-79.74%
Accuracy	77.65%	67.31%-85.97%

Table 4: Diagnostic performance index of MTB detected from stool taking confirmed TB as reference (n=85).

Statistic	Value	95% CI
Sensitivity	72%	50.61%-87.93%
Specificity	100%	94.04%-100%
Positive predictive value	100%	81.47%-100%
Negative predictive value	89.55%	82.05%-94.14%
Accuracy	91.76%	83.77%-96.62%

The diagnostic performance of Mycobacterium tuberculosis (MTB) detection from sputum/gastric aspirate (GA) samples was evaluated using confirmed TB as the reference as shown in table 3. The sensitivity of sputum/GA testing was 24%, specificity was 100%, positive predictive value was 100%, negative predictive value was 75.95%, and overall accuracy was 77.65%. These results indicate that while sputum/GA testing is highly specific and accurate, with excellent positive predictive value, its low sensitivity suggests that a significant number of clinically confirmed TB cases may not be detected by sputum/GA testing alone.

Table 4 showed the diagnostic performance of Mycobacterium tuberculosis (MTB) detection from stool samples was evaluated using clinically confirmed TB as the reference. These results indicate that stool testing is highly specific and accurate, with excellent positive predictive value, though its sensitivity indicates some clinically confirmed TB cases may not be detected by stool testing alone.

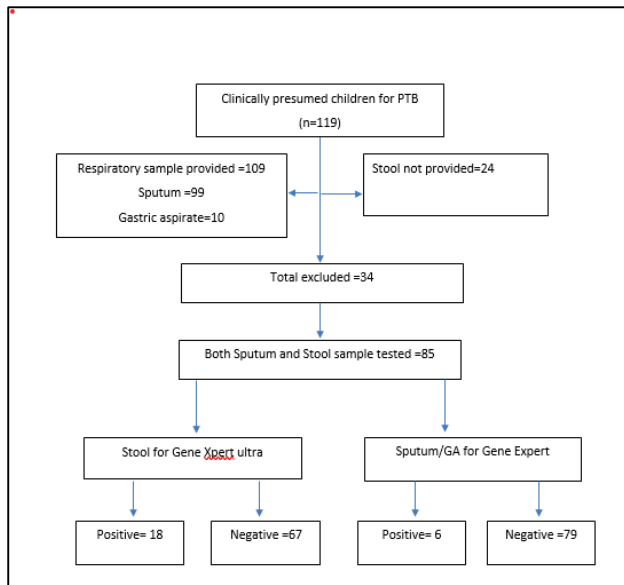


Figure 1: Flow chart describing the study workflow.

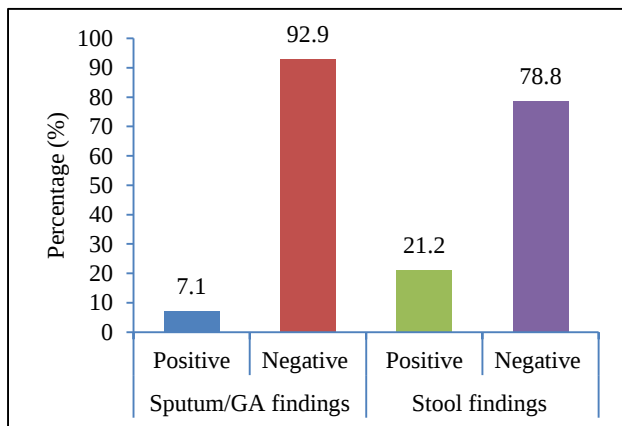


Figure 2: Distribution of the study subjects by sputum, stool findings.

DISCUSSION

According to the Bangladesh National guidelines for the management of tuberculosis in children, WHO estimates 10% child TB among all detected TB cases, Bangladesh could only report 4.2%, which indicates under diagnosis and reporting.¹⁵ The national guidelines of Bangladesh recommend various diagnostic procedures for detection of childhood TB from appropriate clinical samples include sputum, gastric aspirates, nasopharyngeal aspirate, stool and other relevant material.

In this current study, we have done sputum/GA Gene Xpert MTB/RIF and stool Gene Xpert-Ultra for confirming the PTB. In this study male child outnumbered female child among presumptive pulmonary tuberculosis. According to the WHO, Global TB report 2019 also reported a higher male preponderance among TB case.¹ Cough, fever and weight loss were the most common symptoms in our patients

which was similar to that reported by Anmol Goyal et al, positive contact history was found in 25.95% participants similar to the study by Yohannes et al which showed 20.9%.^{16,17} This study shows that stool specimens had a higher proportion of MTB detection (18%) than sputum specimens (7.1%) (Figure 1).

This could be due to stool samples may contain MTB DNA from other sites such as abdominal lymph nodes, intestines and pleura and also due to younger children cannot produce adequate amount of sputum. A similar finding was reported by Yohannes et al where stool sample yielded a higher proportion of MTB detected than sputum specimens.¹⁷ Another study in Ethiopia and Vietnam also showed the similar result reported by de Haas et al.^{18,19}

In our study the Xpert-Ultra result of stool sample had moderate concordance with sputum Gene Xpert results (Table 2). A study carried in Ethiopia by Yohannes babo et al. showed a very good concordance between these two samples where as in a study of China reported the moderate and good quality of the agreement between stool and sputum Xpert-Ultra assays.^{20,21}

In this study sensitivity of sputum/GA Gene Xpert was 24% for MTB detection taking confirmed TB as a reference (Table 3). A study conducted in Shanghai, China by H.Zhang et al. had sensitivity of sputum GeneXpert was 68%.²² Possibility of this lower sensitivity is due to some participants who had positive sputum were missed as corresponding stool sample were not tested. Another cause may be the paucibacillary nature of the disease and at least 136 bacilli/ml of the specimen is required for positive Gene Xpert MTB/RIF.²³

We have also found specificity of sputum Gene Xpert was 100% (95%CI 94.04%-100%) similar to a study conducted in Nyamira by G M Orina et al. also showed higher sensitivity (99.4% with 95% CI 98.3%).²⁴ In this present study diagnostic accuracy of stool Gene Xpert-Ultra were tested taking confirmed TB as a reference (Table 4), sensitivity and specificity were 72% and 100% respectively, similar to a study conducted in Bangladesh where the sensitivity and specificity of Xpert-Ultra on stool were 58.6% and 88.1% respectively taking “bacteriologically confirmed on induced sputum” as a reference.²⁵ According to Esther n et al. specificity of stool Gene Xpert was 93.4% which was almost similar to our study.²⁶

This study had several limitations. Firstly, sample size was small as both sputum/GA and stool sample could not be collected in all patients with presumptive PTB. Secondly, though MTB culture is the gold standard test for the diagnosis of tuberculosis as it can detect as low as 10 bacilli/ml of the specimen but we could not take culture as reference because of not doing culture in most of the cases.²¹

CONCLUSION

Sputum Gene Xpert is highly specific whereas stool Xpert-ultra is highly specific and sensitive for diagnosis of pulmonary tuberculosis in children. Moreover, MTB detection rate is more in stool Gene Xpert-Ultra than sputum Gene Xpert. As a non-invasive procedure stool can be an alternative specimen for diagnosis of pulmonary tuberculosis. This study recommends the stool Gene Xpert-Ultra test in preference to sputum Gene Xpert to avoid missed diagnosis of pulmonary tuberculosis in children.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Bhadra S, Rahman A, Alam R, Mahbuba S. Diagnostic accuracy of sputum gene xpert MTB /RIF and stool gene xpert-ultra in children with pulmonary tuberculosis in a resource constraint country. *Int J Contemp Pediatr* 2024;11:1506-11.