Utility of GeneXpert for Detection of *Mycobacterium tuberculosis* and Rifampicin Resistance in Pediatric Tuberculosis

Ashwani K Sood[1], Mohit Bajaj[2], Ravinder Kumar[3], Anil Kanga[4]

Abstract

Background: Childhood tuberculosis (TB) is an important cause of morbidity and mortality in the developing countries. In 2014, the World Health Organization (WHO) recommended the use of GeneXpert as the initial diagnostic test for detecting all cases of pediatric TB. Only limited data are available on the utility of GeneXpert in the diagnosis of pediatric TB. The present study was done to evaluate the efficacy of GeneXpert as diagnostic tool for pediatric TB detection.

Objectives: To understand the utility of GeneXpert for detecting *Mycobacterium tuberculosis* and rifampicin resistance in pediatric TB.

Materials and methods: This was a prospective cross-sectional study done in a tertiary care teaching hospital over a period of 1 year. After obtaining consent, sputum/induced sputum (IS)/gastric aspirate (GA)/fine needle aspiration cytology (FNAC)/cerebrospinal fluid (CSF)/pleural tap samples were obtained and subjected for cartridge-based nucleic acid amplification test (CBNAAT) and *Mycobacterium* growth indicator tube (MGIT) culture, respectively.

Results: Based on the inclusion criteria, 128 children with presumptive TB were enrolled; of which 76 children were diagnosed with TB [30 with pulmonary TB (PTB) and 46 with extrapulmonary tuberculosis (EPTB)]. In EPTB, lymph node TB was most commonly seen in 37.0% subjects. In pulmonary TB, GeneXpert sensitivity and specificity were 85.71% (95% confidence interval, CI 62.64–96.23%) and 77.7% (95% CI 40.19–96.05%), respectively. Observed positive predictive value (PPV) and negative predictive value (NPV) of GeneXpert were 90% (95% CI 66.87–98.24%) and 70% (95% CI 35.36–91.90%), respectively. Sensitivity and specificity in EPTB was observed to be 50% (95% CI 28.80–71.19%) and 83.33% (95% CI 61.81–94.52%) with PPV of 73.33% (44.82–91.08%) and NPV of 64.51% (45.38–80.17%), with an overall sensitivity and specificity of GeneXpert of 67.39 and 92.68%, respectively, and PPV and NPV of 83.78 and 83.51, respectively. All the recruited subjects were found to be rifampicin sensitive.

Conclusion: GeneXpert is a very useful test with a high sensitivity and specificity for diagnosing pediatric TB and for detection of rifampicin resistance in a short period of time.

Keywords: Cartridge-based nucleic acid amplification test, Childhood, GeneXpert, MGIT culture, Tuberculosis.

Pediatric Infectious Disease (2019): 10.5005/jp-journals-10081-1229

Introduction

Tuberculosis is an infectious disease that has plagued humans since the Neolithic times, with the earliest reported case dating back to 5,000 B.C.[1] In 2016, an estimated 10.4 million new cases of TB was reported, which included 5.9 million men and 3.5 million women and 1.0 million children with an estimated 14 lakh deaths.[2] India, Indonesia, and China were the largest contributors of cases (23%, 10%, and 10% of the global total, respectively). The high prevalence and incidence of TB in developing countries such as India are probably due to a high prevalence of TB in adults acting as contacts, poor socioeconomic condition, malnutrition, overcrowding, delayed diagnosis, and HIV coinfection.

It is estimated that with an annual risk of infection of 2–3%, close to 40% of the population may be infected by the age of 15[3]. In adults, it is easy to make bacteriological confirmation of TB, while it is difficult in pediatric TB as it is paucibacillary in nature. The diagnosis of childhood PTB is based on history, clinical examination, Mantoux test and radiological evidence, and those diagnosis without microbiological confirmation lead to both over- and underdiagnosis. The newer approved molecular tests by WHO help in making a faster and accurate diagnosis, and CBNAAT is one such promising test. In December 2010, the WHO recommended the use of the GeneXpert *Mycobacterium tuberculosis* (MTB)/ rifampicillin resistance (RIF) assay;

[1,2]Department of Pediatric Medicine, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India

[3]Department of Health, World Health Organization, Revised National Tuberculosis Control Programme, India

[4]Department of Microbiology, Dr Yashwant Singh Parmar Government Medical College Nahan, Nahan, Himachal Pradesh, India

Corresponding Author: Ashwani K Sood, Department of Pediatric Medicine, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India, Phone:+91-7018400045, e-mail: doc.aksood@gmail.com

and in October 2013, the WHO revised recommendations for using GeneXpert as the initial diagnostic test in all cases of pediatric TB.[4] As there is limited data regarding the utility of CBNAAT in the diagnosis of pediatric TB, the current study was done to evaluate the diagnostic accuracy of CBNAAT in detecting pediatric TB and rifampicin resistance in the recruited subjects.

MATERIALS AND METHODS

This was a prospective cross-sectional single-center study carried out in a teaching tertiary care institute in North India from August 1, 2016, for 1 year. Children in the age-group of 3 months to 18 years presenting with symptoms suggestive of TB based on Revised National Tuberculosis Control Program (RNTCP) 2017 guidelines[5] were recruited after approval of the study protocol from Institutional Ethics Committee (IEC).

Children with presumptive TB (PTB/EPTB) were enrolled in study which included:

- Fever and/or cough for 2 weeks,
- With or without weight loss or no weight gain (5%) or more.
- Neurological symptoms like irritability, refusal to eat, headache, and vomiting.

Written informed consent was obtained from the parents <7 years of age and assent was obtained from children aged >7 years.

After recording the demographic profile of enrolled subjects, in suspected PTB/EPTB patients, two samples of fasting gastric lavage or sputum/induced sputum of patient were collected separately at the same time, according to the RNCTP guidelines;[5] and one was sent for GeneXpert and the other sample for MGIT culture, respectively. All enrolled subjects underwent Mantoux test and chest radiograph (CXR). Tuberculin skin test (TST) was performed using 2 tuberculin unit (TU) purified protein derivative (PPD) injected intradermally in the upper third of the left forearm, resulting in a wheal of about 6 mm, and it was recorded 48–72 hours later and the positivity test was defined according to the RNTCP guidelines.[5] The CXR changes such as hilar/lymphadenitis with or without parenchymal lesion, miliary shadows, and fibrocavitary pneumonia were considered highly suggestive of PTB.

In suspected TB meningitis patients, two different separate CSF samples were collected at the same time, and one sample was subjected to CBNAAT and other was subjected to MGIT culture.

In pleural effusion, two different pleural fluid samples were collected at the same time, and one sample was analyzed by CBNAAT and the other was subjected to MGIT culture in the Department of Microbiology under the supervision of a coauthor (professor and head, Department of Microbiology, IGMC Shimla). The person involved in the processing of samples was not aware of the result of GeneXpert.

In patients with suspected TB lymphadenitis, two samples were collected at the same time, and one sample of FNAC or biopsy specimen was sent for CBNAAT and second sample for MGIT culture, in addition to all the relevant investigation.

Computed tomography (CT) and magnetic resonance imaging (MRI) brain were done in cases of suspected central nervous system (CNS) meningitis and ultrasonogram USG of abdomen in suspected abdominal TB.

One sample was subjected to GeneXpert, per the WHO protocol, wherein to a minimum sample of 1 mL, twice the quantity of buffer was added and was shaken vigorously and left for 15 minutes. If mixture was homogenized, then proceeded further, if not, again shake the mixture and leave it for another 10 minutes. At least 2 mL of the homogenized sample was pipetted and added to the cartridge and the result was read within 2 hours.

The second sample was inoculated into MGIT 960 tube per the manufacturer's instructions.[6] Growth was reported as positive if *Mycobacterium tuberculosis* growth was found. Culture was labeled as "negative" after 56 days of inoculation if no growth of *Mycobacterium tuberculosis* was seen.

DIAGNOSIS AND MANAGEMENT

Based on clinical findings, CXR, TST, and reports of CBNAAT, children were classified as having microbiologically confirmed TB (positive for *Mycobacterium tuberculi*), clinically diagnosed TB (signs and symptoms suggestive of TB but microbiologically negative for *Mycobacterium tuberculi*) with highly suggestive CXR, contrast-enhanced computed tomography (CECT), or MRI or reactive Mantoux test, and not having TB; and the treatment was started according to the standard fixed dose combination (FDC) protocol without waiting for culture results.

Diagnosis of TB

Criteria

For PTB:

- Positive *mycobacterium* bacilli staining of gastric aspirate/induced sputum by CBNAAT/MGIT.
- Any of the following:

  - History of contact with a tuberculosis patient.
  - Cough lasting more than 2 weeks.
  - Loss of weight (5%).
  - A reactive tuberculin skin test.
  - Radiological findings compatible with TB.

For extra-PTB:

- Fine needle aspiration cytology/biopsy specimen obtained from lymph nodes showing evidence of mycobacterium bacilli by CBNAAT/MGIT.
- Bacteriological evidence of TB in serous fluids including pleural, pericardial, ascetic, or CSF by CBNAAT/MGIT.

History suggestive of TB including history of contact with sputum-positive person, past contact history of TB or of anti-tubercular treatment, with clinical signs suggestive of TB on examination with strong corroborative investigative evidence in the form of one or more of the following.

- A reactive tuberculin test with PPD of 2 TU (10–15 mm after 48–72 hours).
- Radiographic findings compatible with TB
- Lymphocytosis in cytology of serous fluids including pleural, pericardial, ascitic, synovial fluid, and CSF.
- Computed tomography/MRI suggestive of tuberculoma or tuberculous meningitis.
- The USG of abdomen in case of suspected abdominal TB.

After clinical diagnosis, the disease was classified according to the system involved:

- Pulmonary TB
- Pleural effusion
- Tubercular lymphadenitis
- Central nervous system TB—tubercular meningitis/tuberculoma.
- Abdominal TB
- Disseminated TB with or without pulmonary involvement
- Musculoskeletal TB
- Renal TB
- Tubercular pericarditis.

All the enrolled children were followed up during the study period and for the next 6 months after completion of the study. All the samples were evaluated for rifampicin resistance by CBNAAT.

STATISTICAL ANALYSIS

Data collected in the study tool was transferred into MS Excel sheet for further processing and analysis. The SPSS version 22 (American) and Epi info version 7 software have been used for further analysis. Diagnostic accuracy has been determined by calculating sensitivity, specificity, and predictive values for CBNAAT using MGIT as the gold standard.

OBSERVATIONS (TABLES 1 TO 7)

Table 1: Gender-wise distribution of tuberculosis (TB) cases (*N* = 76) in the current study

| | | Type of TB | | |
| | | | Extra-PTB, | |
S. no	Gender	PTB, n (%)	n (%)	Total (%)
1	Male (*n* = 36)	13 (43.3)	23 (50)	36 (47.36)
2	Female (*n* = 40)	17 (56.7)	23 (50)	40 (52.64)
3	Total (*n* = 76)	30 (100)	46 (100)	76 (100.00)

PTB, pulmonary TB

Table 2: Age-wise distribution of tuberculosis (TB) cases (*N* = 76) in the current study

| | | Type of TB | | |
| | | | Extra-PTB, | |
S. no	Agegroup	PTB, n (%)	n (%)	Total
1	3 months to 1 year (*n* = 4)	3 (10)	1 (2.2)	4 (5.26)
2	1–5 years (*n* = 7)	2 (6.7)	5 (10.9)	7 (9.21)
3	6–10 years (*n* = 7)	2 (6.7)	5 (10.9)	7 (9.21)
4	11–18 years (*n* = 58)	23 (86.6)	35 (76.1)	58 (76.32)
5	Total (*n* = 76)	30 (100)	46 (100)	76

Median age and interquartile range (IQR) could not be calculated as these were subdivided into four groups
PTB, pulmonary TB

DISCUSSION

In this study, a total of 128 children in the age-group of 3 months to 18 years were enrolled; EPTB constituted the major group [46 (35.4)] followed by PTB [30 (23.4)], where most of the studies conducted earlier had documented preponderance of PTB followed by EPTB.[7] Higher proportion of EPTB can be attributed to tertiary healthcare institute where most of the cases of EPTB are confirmed and lack of diagnostic facilities in peripheral health institutes for EPTB. Further analysis in EPTB group revealed TB lymphadenopathy in 37.0% cases, which is higher than that reported by Garg et al.[7] (16.7%) and Franco et al.[8] (21.5%). Pleural effusion accounted for 26.1% of cases of EPTB which is similar to a study done by Franco et al.[8] In our study, only 9 (10.9%) cases of the confirmed CNS TB were observed, which is less than (16.67%) the incidence reported by Pontual et al.[9]

GeneXpert was found to be a sensitive and specific point-of-care test for diagnosing pediatric TB against MGIT culture as the gold standard as GeneXpert can detect 100 colony-forming units (cfu)/mL.[10] The observed sensitivity of CBNAAT was 67.39% (95% CI 51.86–80.30%) for detecting pediatric TB which is comparable to the WHO[11]-reported sensitivity of 66 to 79.4%. All of these studies[12,13] used NGIT cultures as the gold standard and observed

Table 3: Pattern of extra-PTB cases

S. no	Type of TB	No.	(%)
1	Lymphadenopathy	17	22.36
2	Pleural effusion	12	15.78
3	CNS TB	9	11.84
4	Abdominal TB	5	6.57
5	Disseminated TB	2	2.63
6	Renal TB	1	1.31
	Total	46	100

Table 4: Duration of symptoms

S. no.	Duration	No. of cases	(%)
1	Less than 1 week	4	5.5
2	1 week–1 month	47	62.5
3	>1 month–3 months	20	25.8
4	>3 months	5	6.3

Median and IQR were not evaluated for these

Table 5: Symptom/sign-wise profile of tuberculosis cases in the current study (*n* = 76)

S. no	Symptoms	No.	%	Signs	No.	(%)
1	Fever	54	71.05	Pallor	32	42.10
2	Cough	47	61.84	Lymphadenopathy	17	22.36
3	Weight loss	30	39.47	Pleural effusion	15	19.73
4	Anorexia	23	30.26	Meningeal signs	10	13.15
5	Headache	10	13.15	Signs of raised ICT	7	9.21
6	Abdominal pain	09	11.84	Hepatosplenomegaly	5	6.57
7	Vomiting	09	11.84	Doughy abdomen	5	6.57
8	Seizure	05	6.41	Respiratory distress	2	2.63
9	Dyspnea	04	5.26	Ascites	2	2.63
10	Neurological deficit	03	3.84	Neurological deficit	2	2.63

Table 6: CBNAAT and MGIT positivity in PTB/EPTB

S. no	Type of TB		CBNAAT positive (%)	MGIT positive (%)	p value
1	Pulmonary TB (*n* = 30)		20 (66.67)	21 (70)	0.7834
2	Extra-PTB (*n* = 46)	Pleural effusion (*n* = 12)	0	2 (16.7)	0.1387
		Abdominal TB (*n* = 5)	1 (20)	2 (40)	
		CNS TB (*n* = 9)	5 (55.6)	4 (44.4)	
		Lymph node TB (*n* = 17)	8 (47.1)	12 (70.6)	
		Disseminated TB (*n* = 3)	1 (33.3)	2 (66.4)	
3	Total (*n* = 76)		35 (46.05)	43 (56.57)	0.1964

Table 7: Sensitivity and specificity of CBNAAT in PTB/EPTB in children using MGIT culture as gold standard

	PTB	EPTB	Pediatric TB
	% (95% CI)	% (95% CI)	% (95% CI)
Sensitivity	85.71 (62.64–96.23)	50.00 (28.80–71.19)	67.39 (51.86–80.30)
Specificity	77.77 (40.19–96.05)	83.33 (61.81–94.52)	92.68 (84.17–96.99)
PPV	90.00 (66.87–98.24)	73.33 (44.82–91.08)	83.78 (67.32–93.23)
NPV	70.00 (35.36–91.90)	64.51 (45.38–80.17)	83.51 (73.95–90.18)

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

lower specificity of 92.68% (95% CI 84.17–96.99%) for GeneXpert to the WHO-reported specificity of above 96.5%.

In our study, the sensitivity of the symptom-based approach in detecting TB was observed to be 60.9% (78/128) which is comparable to the study by Marais et al. which reported a sensitivity of 62.6% in detecting childhood TB.[14]

Culture positivity was significantly higher in the confirmed TB group, possibly due to the higher bacillary load in smear-positive specimens which is due to the superiority of MGIT culture to detect as few as 10 cfu/mL of specimen,[10] suggesting that around one-third culture-confirmed cases will be missed if only GeneXpert is used for the diagnosis of TB.

All the children were found to be rifampicin sensitive by CBNAAT in this study.

The advantage of GeneXpert is that it provides results, including for rifampicin resistance, within 2 hours while it takes 7–15 days for culture to give results. The cost of running the Xpert system is currently almost the same as that of an automated liquid culture system, with similar per-assay running costs.[15] Power supply and temperature maintenance and skilled technician are mandatory, as the instrument ceases to function at 35.8°C and above. Despite these limitations, Xpert offers the advantages of being a sensitive and specific point-of-care test for diagnosing both TB and rifampicin resistance. It only requires minimal training of personnel and storage space. Furthermore, as steps are automated, specimen handling is minimized, thus reducing biosafety concerns. The results of this study are encouraging, with Xpert emerging as a promising point-of-care test for diagnosing pediatric TB.

Conclusion

Tuberculosis is a major global health problem and more research is needed for better and fast methods for detection and confirmation of pediatric TB. GeneXpert and MGIT culture both are capable of detecting TB in pediatric population, but the advantage of CBNNAT over MGIT culture is its easy handling, minimum training, and availability of results within 2 hours including rifampicin resistance. Thus, GeneXpert is a promising point-of-care test for diagnosing

pediatric TB. In every suspected pediatric TB patient, efforts should be made to collect sample/s, which should be subjected by CBNAAT for early detection and also to quantify rifampicin resistance.

What the Study Adds

GeneXpert is currently the best available rapid test to confirm pediatric TB with high specificity and sensitivity to detect *Mycobacterium tuberculosis* and rifampicin resistance and the results are available within 2 hours. However, one-third suspected children who are not detected with GeneXpert can continue to suffer from the disease. Thus, clinical profile of child along with optimum investigative workup is necessary for the diagnosis of pediatric TB.

Acknowledgments

Ethical Responsibilities of Authors

This article is our original unpublished work, and it has not been submitted to any other journal for reviews.

References

1. Sager P, Schalimtzek M, Moller-Christensen V. A case of spondylitis tuberculosa in the danish neolithic age. Dan Med Bull 1972;19(5): 176–180.
2. Global Tuberculosis Report 2016, WHO Report 2014. http://www.who.int/tb/publications/global_report/2017/en/index.html. accessed 31 October, 2017).
3. Udani PM. Tuberculosis in general. In: Parthasarathy A. Text book of Paediatrics. 1st ed., New Delhi: Jay Pee Brothers Publishers; 1991. pp. 995–1236. ; National Guidelines on diagnosis and treatment of Pediatric Tuberculosis 2017, https://tbcindia.gov.in/showfile.php?lid=2904 page 8–15.
4. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/

RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update. Geneva, Switzerland: WHO; 2013.

5. Kumar A, Gupta D, Nagaraja SB, et al. Updated national guidelines for pediatric TB in India, 2012. Indian Pediatr 2013;50:301–306.

6. Global laboratory initiative advancing TB diagnosis mycobacteriology laboratory manual April 2014. http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf. Accessed August 2014. pp. 33–46.

7. Garg P. Childhood tuberculosis in a community hospital from a region of high environmental exposure in north India. J Clin Diagn Res 2008:2:634–638.

8. Franco R, Santana M. Clinical and radiological analysis of children and adolescents with tuberculosis in Bahia, Brazil. Braz J Infect Dis 2003;7:73–81.

9. de Pontual L, Balu L, Ovetchkine P, et al. Tuberculosis in adolescents: a French retrospective study of 52 cases. Pediatr Infect Dis J 2006;25:930–932.

10. Van Zyl-Smit RN, Binder A, Meldau R, et al. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. PLoS ONE 2011;6(12):e28815. DOI: 10.1371/journal.pone.0028815.

11. World Health Organization (WHO). Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extra pulmonary TB in adults and children. Geneva: World Health Organization; 2011. Available from: http://apps.who.int/iris/ bitstream/10665/112472/1/9789241506335_eng.pdf?ua=1.

12. Rachow A, Clowes P, Saathoff E, et al. Increased and expedited case detection by Xpert MTB/ RIF assay in childhood tuberculosis: a prospective cohort study. Clin Infect Dis 2012;54; 1388–1396.

13. Sekadde MP, Wobudeya E, Joloba ML, et al. Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. BMC Infect Dis 2013;13;133.

14. Marais BJ, Gie RP, Hesseling AC, et al. A refined symptom based approach to diagnose pulmonary tuberculosis in children. Pediatrics 2006;118:e1350–e1359.

15. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010;1:229–232.