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

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**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 PTB, 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, Gene xpert, MGIT culture, Tuberculosis.

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**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; 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.

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**Material 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 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, 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, 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. 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 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. 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 tuberculosis*), clinically diagnosed TB (signs and symptoms suggestive of TB but microbiologically negative for *Mycobacterium tuberculosis*) with highly suggestive CXR, contrast-enhanced computed tomography (CECT), or MRI or reactive Mantoux test, and not having TB; and the treatment was started accordingly 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 antitubercular 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 tuberculosis 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**

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

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

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

**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/signwise profile of tuberculosis cases in the current study (n = 76)**

| S. no | Symptoms | No. | % |
|-------|----------|-----|---|
| 1     | Fever | 54 | 71.05 |
| 2     | Cough | 47 | 61.84 |
| 3     | Weight loss | 30 | 39.47 |
| 4     | Anorexia | 23 | 30.26 |
| 5     | Headache | 10 | 13.15 |
| 6     | Abdominal pain | 9 | 11.84 |
| 7     | Vomiting | 9 | 11.84 |
| 8     | Seizure | 5 | 6.41 |
| 9     | Dyspnea | 4 | 5.26 |
| 10    | Neurological deficit | 3 | 3.84 |

**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. 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. (16.7%) and Franco et al. (21.5%). Pleural effusion accounted for 26.1% of cases of EPTB which is similar to a study done by Franco et al. 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.

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. The observed sensitivity of CBNAAT was 67.39% (95% CI 51.86–80.30%) for detecting pediatric TB which is comparable to the WHO reported sensitivity of 66 to 79.4%. All of these studies used NGIT cultures as the gold standard and observed
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,15 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 approximately 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. 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.

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