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GeneXpert Ultra- a New Diagnostic Era for Childhood Tuberculosis

Tahmida Haque[1*], Delwar Hossain[2], Tania Islam[3]

[1]Associate Professor, Department of Pediatrics, Combined Military Hospital, Dhaka, Bangladesh
[2]Professor, Department of Pediatrics, Institute of Child and Mother Health, Dhaka, Bangladesh
[3]Assistant Professor, Department of Pediatrics, Institute of Child and Mother Health, Dhaka, Bangladesh

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***Corresponding author:** Tahmida Haque
Associate Professor, Department of Pediatrics, Combined Military Hospital, Dhaka, Bangladesh

Abstract **Original Research Article**

Introduction: Tuberculosis (TB) is a serious public health problem and a significant diagnostic and therapeutic challenge worldwide. Molecular diagnostic techniques are crucial in the World Health Organization's new tubercular control strategy. ***Objective:*** The study aims to investigate the efficacy of GeneXpert Ultra in the diagnosis of childhood tuberculosis. ***Methods:*** A prospective cross-sectional study was carried out in the pediatric pulmonology ward and general pediatric ward of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka. Child ages ranging from two months to fifteen years who were admitted to the pediatric pulmonology ward and general pediatric ward of ICMH during the period of December 2020 to November 2021 with a history of fever, cough, chest pain, evening rise of temperature, night sweats, weight loss were included in this study. ***Results:*** Among the study population (N=99), almost all the patients (91, 91.2%) had cough, thirty-six patients (36, 36.3%) had cough for 2 weeks, and about half of the patients (49, 49.5%) had a productive cough, ninety patients (90, 90.9%) had fever, around one-fifth of the patients, had a fever more than two weeks. About half of the patients (47, 47.5%) had a high-grade intermittent fever. Evening rise of temperature was seen in around one-fourth of the patients (24, 24.2%). MT test was positive for two patients (2, 2.0%) and doubtful for two patients (2, 2.0%)., Seventeen patients (17, 17.1%) specimens were collected from induced sputum, around three-fifths of the patients' (70, 70.7%) specimens were collected from gastric aspirate, eighty-two patients (82, 82.8%) specimens were collected from the stool and one patient's specimen (1, 1.0%)was collected from pus produced in BCG vaccine site. GeneXpert ultra was detected in thirteen patients (13, 13.1%) and was trace detected in six patients (6, 6.0%). Of the 13 patients, 7 patients (7, 53.8%) were diagnosed with pulmonary TB, two patients (2, 15.4%) were diagnosed with t pleural effusion, two patients (2, 15.4%) were diagnosed with abdominal TB, one patient (1, 7.7%) was diagnosed with tuberculous lymphadenitis and one patient (1, 7.7%) was diagnosed with BCG abscess by GeneXpert ultra. ***Conclusion:*** The findings of this study confirm that GeneXpert ultra may become a new diagnostic era for the diagnosis of pulmonary TB and extra-pulmonary TB due to its efficacy.

Keywords: Tuberculosis (TB), GeneXpert Ultra, Pulmonary Tuberculosis (TB).

INTRODUCTION

2020 WHO Global Tuberculosis Report estimates of 10 million people fell ill from TB, and among them 56% were men, 32% were women and 12% were children under 15 years of age [1]. This accounts for an estimated 1.2 million children around the world who fell ill with TB in 2019 [1]. WHO End TB the strategy has envisioned a goal with a timeline to reduce TB burden, death from TB, and catastrophic cost due to TB. Whereas recent determinations have decreased the incidence of TB, the infection still has a momentous global burden. In spite of advances in rapid molecular testing for pulmonary tuberculosis (TB), the microbiologic validation of TB in children remains a challenge and has donated to high morbidity and mortality [2]. In recent years, nucleic acid intensification tests (NAATs) have been applied for TBM diagnosis. The most distinguished tests are GeneXpert MTB (Xpert) and the re-engineered GeneXpert MTB Ultra (Xpert Ultra), however, other commercial and 'in-house' NAATs have been used as well[3]. Microbiologic confirmation of pulmonary tuberculosis (PTB) in children remains significant, as clinical and radiologic diagnosis lack specificity [4]. Gene Xpert MTB (Xpert, Cepheid, Sunnyvale, California) is a cartridge-based nucleic acid amplification assay, that licenses fast recognition of Mycobacterium tuberculosis complex and identification of rifampin resistance [5]. Two important changes were made along with other methodological

optimizations. First, each cartridge includes a larger chamber for DNA amplification (50 compared to 25), thus helpful twice the volume of sample for the PCR. Second, two additional multicopy molecular targets for Mycobacterium tuberculosis, IS1081, and IS6110 were introduced, alongside 4 rpoB gene probes, subsequent to a decrease in the limit of detection (LOD) in vitro from 113 bacilli per ml of sputum for Xpert to 16 for Ultra, with a trace category added for lowest bacillary load [6]. Ultra runs on the similar GeneXpert platform as Xpert MTB (using software version 4.7b or later) as well as on the GeneXpert Omni platform. Nevertheless, sensitivity for the diagnosis of pediatric PTB is limited; 62% for sputum samples in a current meta-analysis. The challenge is how to correctly assess this novel investigative assay in the nonappearance of a perfect gold standard and in a population where obtaining high-quality samples is inherently problematic. The culture-based reference standard is widely recognized to be defective, with results in true childhood TB cases being often misclassified; culture techniques can miss up to 40% of childhood pulmonary TB cases [7]. Such misclassification by the reference ordinary types is problematic to evaluate any novel assay, as true TB cases detected only by the novel assay may be incorrectly labeled as false positive, thus wrongly reducing the specificity of the novel test. Xpert and culture frequently notice cases that the other modality misses and Xpert's negative prognostic value is only 84–94%, meaning that while Xpert is cooperative if optimistic, it cannot successfully rule out TBM [8]. At present, access to Xpert is increasing though still not satisfactory. The aim of the study is to investigate the efficacy of GeneXpert Ultra in the diagnosis of childhood tuberculosis.

METHODS

A prospective cross-sectional study was carried out in the pediatric pulmonology ward and general pediatric ward of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka. Child ages ranging from two months to fifteen years who were admitted to the pediatric pulmonology ward and general pediatric ward of ICMH from December 2020 to November 2021 with a history of fever, cough, chest pain, evening rise of temperature, night sweats, weight loss were included in this study. Data were collected in a pretested questionnaire. All study populations underwent Mantoux Test (MT) for TB. Seventeen induced sputum, seventy gastric aspirates, and eighty-two stool specimens were collected for GeneXpert ultra. Fresh samples like stool, gastric lavage, sputum, etc. were collected from hospitalized patients. Samples were sent to the ICDDRB lab maintaining a temperature of 4º-8ºc in an icebox. For the direction of mycobacterium tuberculosis, cartridge-based nucleic acid amplification was done. Thirteen children were diagnosed as TB or extrapulmonary TB patients. Those patients underwent WHO-recommended TB regimen treatment. Those who were trace detected were advised to come after 1 month for follow-up. Verbal consent was taken before recruiting the study population. Ethical clearance was taken from the hospital authority. The information is kept confidential and only be used for the study purpose.

Inclusion Criteria

- Children in the age group 2 months-15 years.
- Children presenting with symptoms of TB (fever, cough, weight loss, chest pain, raised temperature etc.)

Exclusion Criteria

- Critically ill patients with chronic respiratory illness, bronchial asthma, and cystic fibrosis.

MT Test:

The tubercular skin test is called the Mantoux test. It is performed by injecting a small amount of fluid which is called tuberculin into the skin on the ventral part of the lower forearm. A person who underwent the tuberculin test should be returned within 48 to 72 hours to the healthcare worker to check the reaction of the forearm. The reaction should be measured in millimeters of induration. Indurated area is identified by specific criteria those are palpable, raised, hardened, and swelling. In a healthy person whose immune system is normal, induration greater than or equal to 10 mm is considered a positive MT test for TB [9].

GeneXpert MTB Assay:

GeneXpert MTB assays were conducted on 169 specimens collected from 99 samples. 1.0ml of GeneXpert MTB assay sample reagent was added to 500µL of each specimen using a sterile pipette [10]. A new GeneXpert MTB/RIF Ultra (hereafter referred to as Xpert Ultra) assay has been developed to overcome the limitations of the old Xpert MTB/RIF G4 assay with improved sensitivity in the detection of TB and RIF resistance. The Xpert Ultra assay is a rapid assay that uses improved assay chemistry and cartridge design. It incorporates two different multicopy amplification targets viz., IS6110 and IS1081, and the RIF resistance-determining region (RRDR) of the *rpo*B gene [11, 12].

Data Analysis

The study coordinators performed random checks to verify data collection processes. Completed data forms were reviewed, edited, and processed for computer data entry. The data analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 25.0.

RESULTS

Among the study population (n=99), patients who were admitted to the hospital were aged from 2 months to 15 years. Most of the patients (33, 33.4%) aged were below 1, and about one-fourth of the patients' (24, 24.2%) age was between 5 to 18. The majority of the patients (61, 61.6%) were female. Most of the patients (83, 83%) had no education, one patient (1, 1.0%)

studied in class 4, and only three (3, 3.0%) patients studied in class 7. Around one-fifth of the patients' fathers (20, 20.2%) occupation was microbusiness, ten patients' fathers (10, 10.1%) were farmers, and around one-fifth of the patients' fathers (19, 19) were day laborers. None of the patients was previously diagnosed as TB patients, one patient had TB affected by family members, but three patients had exposure to other TB patients, except family members (**Table 1**). The mean, maximum, and minimum height of the patients were 84.78±SD, 156 cm, and 48 cm, the mean weight of the patients was 11.06kg±SD, and the maximum weight of the patients was 44 kg. The mean mid-upper arm circumference (MUAC) was 13.5cm±SD, maximum MUAC was 19 cm and minimum MUAC was 8cm. Ten patients (10, 10.1%) had severe malnutrition, twenty-nine patients had moderate malnutrition and the majority of the patients (60, 60.6%) had mild malnutrition. Only three patients (3, 3.0%) had nutritional edema (**Table 2**). Around three-fifths of the patients (60, 60.6%) completed EPI vaccination, and most of the patients (60, 60.6%) had prominent BCG vaccine scars. Of them, one child developed a local abscess at the BCG vaccine injection site (**Table 3**). Almost all the patients (91, 91.2%) had to cough, thirty-six patients (36, 36.3%) had coughed for 2 weeks, about half of the patients (49, 49.5%) had a productive cough, ninety patients (90, 90.9%) had fever, around one-fifth of the patients, had a fever more than two weeks. About half of the patients (47, 47.5%) had a high-grade intermittent fever. Evening rise of temperature was seen in around one-fourth of the patients (24, 24.2%). Most of the patients (59, 59.6) experienced a loss of taste, around two-fifth of the study population (37, 37.4%) experienced breathlessness, the majority of the patients (60, 60.6%) experienced a loss of activities, one patient (1, 1.0%) had swelling on BCG vaccination site, most of the patients had a previous history of the same episode and among them, three patients experienced it in 2019 and sixty-seven patients experienced it in 2020 (**Table 4].** Among the study population (N=99), four patients had gland swelling (4, 4.0%), two patients (2, 2.0%) had pleural effusion for 18 days, two patients (2, 2.0%) had ascites, two patients (2, 2.0%) had vomiting, one patient (1, 1.0%) had abdominal pain for 12 days and one patient (1, 1.0%) had swelling and redness on BCG vaccine site (**Table 5).** MT test interpretation was positive for two patients (2, 2.0%) and doubtful for two patients (2, 2.0%), and negative to ninety-five patients (95, 95.1%) (**Table 6**)**.** A total of one hundred seventy specimens were collected from ninety-nine study populations (N=99) for GeneXpert ultra. Of them, seventeen patients (17, 17.1%) specimens were collected from induced sputum, around three-fifths of the patients' (70, 70.7%) specimens were collected from gastric aspirate, eighty-two patients (82, 82.8%) specimens were collected from the stool and one patient's (1, 1.0%) specimen was collected from pus produced in BCG vaccine site (**Table 7**)**.** Among the study population (N=99), gene expert ultra was detected in thirteen patients (13, 13.1%) and was trace detected in six patients (6, 6.0%) (**Table 8**)**.** Among thirteen study populations (n=13), the majority of the patients (7, 58.3%) were diagnosed with pulmonary TB, two patients (2, 16.6%) were diagnosed with pleural effusion, two patients (2, 16.6%) were diagnosed with abdominal TB, one patient (1, 8.33%) was diagnosed with tuberculous lymphadenitis and one patient(1, 1.0%) was diagnosed with BCG abscess by GeneXpert ultra **(Table 9**)**.**

Table 1: Distribution of study population based on characteristics, (n=99)

Characteristics	(n, %)
Age	
<1	33, 33.4%
1-3	28, 28.2%
>3-5	14, 14.2%
5-18	24, 24.2%
Sex	
Male	38, 38.4%
Female	61, 61.6%
Education	
No Education	83, 83.9%
Class 1	5, 5.0%
Class 2	4, 4.0%
Class 3	1, 1.0%
Class 4	1, 1.0%
Class 6	2, 2.0%
Class 7	3, 3.0%
Father's Occupation	
Farmer	10, 10.1%
Day labor	19, 19.2%
Small Trader	20, 20.2%
Job holder	31, 31.3%
Driver	10, 10.1%
Abroad	9, 9.0%
Number of family members	
≤4	40, 40.4%
>4	59, 59.6%
Contact with TB patients	
Yes	4, 4.1%
No	95, 96.9%

Table 2: Distribution of study subjects by anthropometry, (n=99)

Height	
Mean	84.78±SD
Maximum	156 cm
Minimum	48 cm
Weight	
Mean	11.06±SD
Maximum	44 kg
Minimum	3 kg
MUAC	
Mean	13.5±SD
Maximum	19 cm
Minimum	8 cm
Severe malnutrition	10, 10.1%
Moderate malnutrition	29, 29.3%
Mild malnutrition	60, 60.6%
Nutritional edema	3, 3.0%

Table 3: Distribution of study population by vaccination status and prominent BCG mark, (n=99)

Completed EPI vaccination	60, 60.6%
Prominent BCG vaccine scars	60, 60.6%

Table 4: Distribution of study population based on symptoms, (n=99)

Symptoms	**(n, %)**
Cough	91, 91.2%
Cough >2 weeks	36, 36.3%
Productive cough	49, 49.5%
Fever	90, 90.9%
Fever >2 weeks	22, 22.3%
Type of fever	
High grade intermittent **High grade continuous** **Low grade intermittent**	47, 47.5% 2, 2.0% 4, 4.0%
Evening rise of temperature	24, 24.2%
Night Sweat	17, 17.1%
Chest pain	9, 9.0%
Loss of taste	59, 59.6%
Breathlessness	37, 37.4%
Weight loss	20, 20.2%
Loss of activities	60, 60.6%
Swelling on BCG vaccination site	1, 1.0%
Previous history of the same episode	70, 70.7%

Table 5: Distribution of study population based on signs of TB, (n=99)

Diagnosis	**(n, %)**
Gland swelling	4, 4.0%
Pleural Effusion for 18 days	2, 2.0%
Abdominal pain for 12 days	1, 1.0%
Ascites	2, 2.0%
Vomiting	2, 2.0%
Swelling and redness on BCG vaccine site	1, 1.0%

Table 6: Distribution of study population based on MT test interpretation, (n=99)

MT test interpretation	**(n, %)**
Positive	2, 2.0%
Doubtful	2, 2.0%
Negative	95, 95.1%

Table 7: Distribution of study population based on specimen collection (n=99)

Specimen collection	**(n, %)**
Induce Sputum	17, 17.1%
Gastric Aspirate	70, 70.7%
Stool	82, 82.8%
Pus from BCG vaccine site	1, 1.0%

Table 8: Distribution of study population based on gene expert ultra-detection, (n=99)

Detection	**(n, %)**
Gene Expert Ultra Detection	13, 13.1%
Trace Detected	6, 6.0%

Table 9: Distribution of study population based on diagnosis, (n=13)

Diagnosis	**(n, %)**
Pulmonary TB	7, 53.8%
Pleural Effusion	2, 15.3%
Tuberculous Lymphadenitis	1, 7.7%
Abdominal TB	2, 15.3%
BCG Abscess	1, 7.7%

DISCUSSION

The present study was conducted with a total of 99 patients who were between the age of 2 months to 15 years and were presenting symptoms of TB. Among the study participants, over half the participants were 3 years of age or younger. This was in line with the general understanding that TB has a higher prevalence among children under the age of 5 compared to the older population [13,14]. This is primarily due to the still-developing immune system of children that are not able to combat TB, and get affected by a much smaller number of them compared to the older population with fully developed immune systems. In our study, a high female prevalence was observed, which was quite different from the global understanding that TB is generally more common among the male population [15, 16].

As the majority of the study population were children, they had not started their education yet. It was observed that the majority of the patients' fathers were job holders (31.3%), micro businessmen (20.2%), day laborers (19.2%), etc. 59.6% of the participants were from families with over 4 members, and the remaining had 4 or fewer members. These findings were unique to developing countries like Bangladesh, Myanmar, and India, where many families are still joint families. The mean height of the patients was84.78cm, and the mean weight was 11.06 kg. Mean mid-Upper Arm Circumference (MUAC) was 13cm±SD, but due to the large variety of age among the participants, these values were not indicative of anything particular.

EPI vaccination and BCG vaccine scars were present in 60.6% of the participants. Of them, one child developed a local abscess at the BCG vaccine injection site. An abscess may develop on the injection site and can lead to mild or severe complications in children.

It was observed that over half the children (60.6%) had mild malnutrition, 29.3% had moderate malnutrition, and 10.1% had severe malnutrition. Malnutrition is an important factor in pediatric health, with or without TB. It has been observed that moderate and severe levels of malnutrition along with TB can greatly increase the mortality rates among TB patients, both pediatric and adult [17-19]. It was observed that cough and fever were the most common symptoms among the present study participants, and 22.2% of the participants suffered from fever for over 2 weeks. 47.5% of the participants had a high-grade intermittent fever, but only 2% had a high-grade continuous fever, and 4% had a low-grade continuous fever. Intermittent or recurrent fever is a common symptom among TB patients [20, 21]. Loss of taste, breathlessness, lethargy, and weight loss are some of the other common symptoms of TB, which were also supported by other studies [9, 18].

According to medical diagnosis, 4% of patients had gland swelling, ascites, and vomiting present for 2% of cases each, 2% had pleural effusion for 18 days, and 1 patient had pain in their abdominal region for 12 days, 1% of patients had swelling and redness on BCG vaccine site.

According to MT testing results, TB was positive for 2% of cases, doubtful for another 2% of cases, and negative for 95.1% of cases. MT testing often produced false-negative results when the immune system of the patients isn't functional yet [22]. As the majority of the present study patients were young children with a still-developing immune system, a high false-negative result of MT testing was to be expected.

For GeneXpert ultra testing, the specimen was collected from induced sputum in 17.1% of cases, from stool in 82.8%, gastric aspirate in 70.7% of cases, and pus produced from the BCG vaccine site in 1.0% of cases. By GeneXpert Ultra, 13.1% of patients were detected with TB, while trace detection of TB was observed in 6% of the cases. This was undoubtedly higher compared to MT testing alone. Previous studies with only smear-negative specimens showed high sensitivity of Ultra for TB detection [23, 24].

According to the GeneXpert ultra findings, it was observed that 53.8% of the TB-positive cases were pulmonary TB, and others were diagnosed with pleural effusion, abdominal TB, tuberculous lymphadenitis, and BCG abscess by GeneXpert ultra. GeneXpert Ultra is a well-suggested diagnostic tool for patients suspected of having extrapulmonary TB [25, 26].

Worldwide TB resistance to anti-bacillary treatments was estimated by WHO in 2017 at 18% in treated cases and 3.5% in new cases [27]. Rapid molecular diagnosis by GeneXpert ultra allows both the diagnosis of tuberculosis and its resistance to anti-bacillary agents [28]. The WHO recommend GeneXpert MTB in 2010 for the diagnosis of pulmonary TB and subsequent in the diagnosis of extra-pulmonary TB [27]. WHO recommendations for the integration of GeneXpert MTB in the TB diagnosis process are linked to its short time to results and demonstrated performance in childhood TB diagnosis [29].

Limitations of the study

The present study had several limitations. The sample size was really small, and the study was conducted at a hospital level, not at a community level. The study had a duration of 1 year, and extensive investigations such as specimen culture, bronchoscopy, etc were not done.

CONCLUSION

The findings of this study confirm GeneXpert MTB as a test of choice for the diagnosis of childhood pulmonary Tb and extra-pulmonary Tb due to its

efficacy. Its advantage is also highlighted in cases of tuberculosis with the negative MT test thus it substantially increases the sensitivity of tuberculosis and ensures early medical management of patients according to the WHO treatment regimen. The results embolden the integration of GeneXpert MTB into the tuberculosis control program in Bangladesh.

RECOMMENDATIONS

There is earnest demand for extensive research on GeneXpert ultra in detecting pulmonary and extrapulmonary childhood TB. To get robust data multicenter studies should be carried out to interpret the demonstrable scenario by policymakers. The result obtained by GeneXpert ultra might be checked by another molecular test like the DNA test [30].

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