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Original Article

Evaluation of cartridge-based nucleic acid amplification test for diagnosis of tuberculosis in children in various body fluids

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ABSTRACT

Introduction: The paucibacillary nature presents a major challenge in the diagnosis of tuberculosis (TB) in children. The utilization of cartridge-based nucleic acid amplification test (CBNAAT) for the diagnosis of TB presents itself with added advantages such as detection to resistance to rifampicin and short turnaround time. **Objectives:** The aim of the study is to evaluate the diagnostic yield of CBNAAT in various body fluids and to compare with BACTEC-MGIT 960 and acid-fast bacilli (AFB) microscopy in children with suspected TB and to see the prevalence of rifampicin resistance in the study population using CBNAAT. **Materials and Methods:** This cross-sectional study included participants <14 years with suspected TB. Gastric aspirate samples obtained from pulmonary TB cases and body fluid specimens obtained from extrapulmonary TB cases were processed for the detection of *Mycobacterium tuberculosis* (MTB) using CBNAAT, BACTEC-MGIT 960, and AFB microscopy. The results obtained using CBNAAT were compared to other laboratory tests using an appropriate statistical method. **Results:** Fifty patients diagnosed with TB (34 pulmonary, 10 pleural effusion, and 6 abdominal) were included in the study, and clinical fluid specimens obtained from study participants were processed for the detection of MTB. Out of 34 gastric aspirate samples, 28 (82%) were positive by CBNAAT which was statistically higher than BACTEC-MGIT 960 ($P < 0.05$). Among extrapulmonary TB cases, only 2 pleural fluid specimens were positive by CBNAAT, whereas BACTEC-MGIT 960 and AFB microscopy could not detect MTB. Out of 34, 4 (11.76%) patients with newly diagnosed pulmonary TB were found to be rifampicin resistant using CBNAAT. **Conclusions:** CBNAAT showed promising results as a diagnostic tool in detecting MTB and rifampicin resistance in pulmonary TB using gastric aspirate. It, however, did not show good results in children with extrapulmonary TB in the clinical fluid specimen. The present study also showed the presence of high rifampicin resistance in treatment naïve pulmonary TB patients.

Key words: *Acid-fast bacilli microscopy, BACTEC 960, Cartridge-based nucleic acid amplification test, Children, Diagnosis, Tuberculosis*

Tuberculosis (TB) is one of the leading causes of death from infectious disease worldwide. Globally, 10.4 million of new cases of TB disease were reported in the year 2015 by the WHO, with an estimated 1 million children <14 years of age [1]. Rapid emergence of drug resistance in recent years is another major issue in the management of TB. Globally, an estimated 3.6% of new TB cases and 20% of previously treated cases have multidrug-resistant TB [1]. Diagnosis of TB in children is challenging because of paucibacillary nature. The drawback of the conventional culture method is that it is positive in <50% of cases and is time-consuming [2].

BACTEC-MGIT 960 culture system allows rapid detection of mycobacteria and has reported a sensitivity of 51.5% and 48.6% in pulmonary and extrapulmonary TB participants, respectively [3,4]. Cartridge-based nucleic acid amplification test (CBNAAT) needs only 150 bacilli per ml of clinical specimen to be positive [5]. Meta-analysis study conducted showed that pooled sensitivity of CBNAAT was observed to be 78% in gastric aspirate samples, 34% in pleural fluid, and <1% for non-pleural fluid samples [6]. CBNAAT can simultaneously detect resistance to rifampicin and has the advantage of short turnaround time [7].

Hence, this study was undertaken to assess the diagnostic yield of CBNAAT in comparison to BACTEC-MGIT 960 and Zeihl–Neelson microscopy in gastric aspirate specimens of pulmonary TB cases and in specimens obtained from serous cavities in extrapulmonary TB cases. Detection of resistance to rifampicin was also assessed in these participants using CBNAAT.

MATERIALS AND METHODS

A cross-sectional study was conducted in children up to 18 years of age from the period of March 2015 to June 2017. The inclusion criteria consisted of participants with suspected TB attending the

Department of Pediatrics of tertiary care hospital during the study period. The cases were diagnosed in accordance to the National Guidelines on the Diagnosis of Pediatric [8] TB. Participants with pulmonary TB and extrapulmonary TB with serous cavity involvement were included in the study. Patients with a history of receiving antitubercular therapy and with central nervous system TB were excluded from the study. Ethical clearance was obtained from the institutional ethical committee. Informed consent was obtained from the caretaker/guardian of the participants enrolled in the study.

A detailed history and thorough clinical examination were done in all the study participants. Chest X-ray and tuberculin skin test were also performed in all the participants. Five milliliter of serous fluid sample (pleural/ascitic/pericardial fluid) from extrapulmonary TB cases and 5 ml of gastric aspirate samples were obtained from pulmonary TB cases under aseptic conditions. These samples were transported to laboratory maintaining the cold chain on the same day for the following investigations: cytological and biochemical tests, acid-fast bacilli (AFB) microscopy, culture on BACTEC-MGIT 960, and CBNAAT.

Data were collected and analyzed using SPSS software version 20. The sensitivity of CBNAAT, BACTEC-MGIT 960, and AFB microscopy was calculated. McNemar's Chi-square test was applied to compare the sensitivity CBNAAT with BACTEC-MGIT 960 and AFB microscopy. A $p \leq 0.05$ was taken as statistically significant. Taking BACTEC-MGIT 960 as gold standard, specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), negative likelihood ratio, and positive likelihood ratio of CBNAAT were calculated.

RESULTS

A total of 50 patients with diagnosed TB cases were registered during the study period: 34 were pulmonary TB, 10 were pleural effusions, and 6 were abdominal TB. The clinical specimen was obtained from these patients (34 gastric aspirate, 10 pleural fluid, and 6 ascitic fluid).

Fever was the most common clinical presentation in the study participants (80% case). According to the WHO classification, 80% of cases were malnourished; 36% were moderately; and 44% were severely malnourished. Clinical and demographic profile of the study participants is depicted in Table 1. Chest X-ray was abnormal in 32 (94%) of 34 pulmonary TB cases, showing hilar lymphadenopathy in 26 cases (76%), pulmonary infiltrates in 12 cases (24%), consolidation in 5 cases (10%), and military shadow in 4 cases (8%). Out of 6 cases of abdominal TB, only 2 (33.3%) showed hilar lymphadenopathy in chest X-ray.

The yield of CBNAAT, BACTEC-MGIT 960, and AFB smear microscopy for gastric aspirate, pleural, and ascitic fluid samples is shown in Table 2. On comparing CBNAAT with AFB smear microscopy and BACTEC-MGIT 960 in gastric aspirate samples, there was statistically higher yield (P = 0.000003 and 0.00814, respectively). Taking BACTEC-MGIT 960 as the gold standard, sensitivity of CBNAAT was 100% (95% confidence interval [CI]: 83.89–100%) and specificity was 46.15% (95% CI: 19.22–74.87%), positive likelihood ratio was 1.86 (95% CI: 1.12–3.07%), negative likelihood ratio was 0, PPV was 75% (95% CI: 55.13–89.31%), and NPV was 100% (95% CI: 54.07–100%).

Table 1: Clinical and demographic profile of tuberculosis suspect cases (n=50)

Variables	n (%)
Demographic	
Mean age	4.97±2.82
Male gender	29 (58)
History of contact	29 (58)
BCG vaccination	23 (46)
Mantoux positive	28 (56)
Clinical	
Persistent fever	40 (80)
Cough >2 weeks	36 (72)
Loss of weight/no weight gain	27 (54)
Anorexia	19 (38)
Respiratory distress	18 (36)
Abdomen distension	6 (12)
Pain abdomen	3 (6)
Diarrhea/constipation	4 (8)

BCG: Bacillus Calmette–Guérin

CBNAAT detected *Mycobacterium tuberculosis* in 2 (20%) specimens of pleural fluid but could not detect in any of the ascitic fluid specimens. BACTEC-MGIT 960 and microscopy could not detect MTB in pleural and ascitic fluid specimens. There was no significant difference seen in comparing CBNAAT with other diagnostic tests for body fluid samples. Out of 34 pulmonary TB cases, 4 (11.76%) were found to have rifampicin resistance and no resistance was detected among extrapulmonary cases.

DISCUSSION

CBNAAT has been endorsed by the WHO to be used for an initial diagnostic test in children suspected of having pulmonary as well as extrapulmonary TB [7,8]. In the present study, sensitivity of CBNAAT was assessed in gastric aspirate, pleural fluid, and ascitic fluid specimens obtained from pulmonary, pleural, and extrapulmonary TB participants, respectively. In the present study, the mean age of the study participants was 4.97 ± 2.82 years. A similar observation has been made in a study by Anane and Grangaud, showing that the risk of acquiring TB was higher in younger children [9]. Male predominance was seen in the present study as also reported by Jani *et al*. [10].

Fever was the most common clinical presentation followed by loss of weight, decreased appetite which was similar to the observations made by Anane and Grangaud [9]. Tuberculin test was found to be positive in about 50% participants in our study. Mehta *et al*. also found positive tuberculin testing in 40% of children with TB [11]. Lower tuberculin positivity could be due to coexistent undernutrition in the study participants. In our study, a

Table 2: Comparison of cartridge-based nucleic acid amplification test, BACTEC-MGIT 960, and acid-fast bacilli microscopy in various body fluids

Clinical specimen, n (%)	CBNAAT positive	BACTEC-MGIT 960 positive	AFB microscopy positive
GA (n=34)	28 (82)	21 (67.7)	6 (18)
PF (n=10)	2 (20)	0	0
AF (n=6)	0	0	0
Total (n=50)	30 (60)	21 (42)	6 (12)

GA: Gastric aspirate, PF: Pleural fluid, AF: Ascitic fluid, CBNAAT: Cartridge-based nucleic acid amplification test, AFB: Acid-fast bacilli

large number of patients was malnourished; a similar observation was made in a study by Jaganath and Mupere which showed high TB prevalence in severely malnourished children [12].

In the present study, the yield of microscopy, BACTEC-MGIT 960, and CBNAAT was compared in gastric aspirate samples. In the present study, smear microscopy was positive in the small number of gastric aspirate samples. In a study conducted by Bates *et al*., smear microscopy was positive in 25% of gastric aspirate samples [13]. BACTEC-MGIT 960 was positive in more than half participants who were higher than the study conducted by Ruiz Jiménez *et al*. who showed culture to be positive in 47.1% in gastric aspirates [14]. In our study, BACTEC-MGIT 960 performed slightly better than the previous studies probably due to a better sample collection technique.

Sensitivity of CBNAAT was 82% which was higher than the sensitivity reported by Bates *et al*. which was 68.8% (95% CI: 53.6–80.9%) [13]. In a meta-analysis conducted by Maynard-Smith *et al*., pooled sensitivity estimate of CBNAAT in gastric aspirate was 78% (95% CI: 69–86%) [6]. In the present study, CBNAAT when compared with BACTEC-MGIT 960 taken as reference test showed 100% sensitivity. In another study conducted by Pang *et al*., CBNAAT compared with BACTEC-MGIT 960 showed sensitivity of 64.7% and specificity of 70.1% [15]. Hence, CBNAAT can be used as an initial diagnostic test for diagnosing pulmonary TB using gastric aspirate samples.

Extrapulmonary TB cases were evaluated in the study using various diagnostic tools. BACTEC-MGIT 960 and microscopy were negative in pleural and ascitic fluid samples. In a study conducted by Chakravorty *et al*., smear microscopy in extrapulmonary TB showed positivity of 3.9% [16]. Zmak *et al*. found only 1 out of 42 pleural fluid and 3 out of 10 ascitic fluid to be culture positive [17]. Low sensitivity in the present study was probably due to paucibacillary nature of pleural and ascitic fluid coupled with a small sample size of the study group. In a study conducted by Friedrich *et al*., sensitivity of CBNAAT assay in pleural fluid was 25% [18]. In a meta-analysis conducted by Maynard-Smith *et al*., pooled sensitivity estimate of CBNAAT in pleural fluid was 34% (95% CI: 24–44%) and for non-pleural serous fluid was <1% [6].

In the present study, rifampicin resistance was detected at an alarmingly high rate in new cases of pulmonary TB cases using CBNAAT as also observed in another study by Sharma *et al*. [19]. A meta-analysis by Chang *et al*. has shown effectiveness and rapidity in diagnosis of rifampicin resistance [20]. The major limitation that the present study faced was the small number of patients in the study population. Hence, to overcome the outcome from this limitation, further studies should be designed with larger number of participants in the study population.

CONCLUSIONS

CBNAAT showed promising results for the diagnosis of pulmonary TB using gastric aspirate in children. It had higher sensitivity compared to currently available diagnostic modalities including smear microscopy and BACTEC-MGIT 960. CBNAAT, however, does not show good results in children with extrapulmonary TB in pleural and ascitic fluid. The study also shows high rifampicin resistance in new cases of pulmonary TB. To conclude, CBNAAT may be a promising diagnostic test as compared to conventional methods for an initial diagnostic test in children with suspected pulmonary and extrapulmonary TB and to detect rifampicin resistance.

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