ORIGINAL ARTICLE

Diagnostic Role of Cartridge-Based Nucleic Acid Amplification Test (CB-NAAT) in Both Pulmonary and Extra-Pulmonary Pediatric Tuberculosis: A Hospital-Based Study

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Abstract

Background: Early diagnosis of pediatric tuberculosis (TB) still remains a challenge due to the paucibacillary nature of the disease. **Objectives:** To assess the role of CB-NAAT in diagnosing pulmonary and extra-pulmonary tuberculosis as compared to AFB smear and Mycobacterium tuberculosis culture in clinically diagnosed pediatric tuberculosis cases. **Study design:** Hospital-based prospective study. **Methods:** A total of142 patients with strong clinical suspicion of pulmonary or extra-pulmonary tuberculosiswere enrolled over a period of two yearsand then results of CB-NAAT were compared to conventional methods (culture/ AFB smear) on follow-up. **Results:** In subjects who had clinically diagnosed tuberculosis, culture was positive in 18.9% of cases, AFB smear was positive in only 12.1% whereas CB-NAAT was positive in 55.4% of subjects. The sensitivity, specificity, PPV, and NPV of CB-NAAT in patients with culture proven tuberculosis were100%, 83.3%, 47.5%, and 100% respectively with a diagnostic accuracy of 93.3%. and 60%, 100%, 100%, and 65.8% respectively with a diagnostic accuracy of 80.2% in clinically diagnosed pulmonary cases. **Conclusion:**CB-NAAT has true diagnostic potential with good sensitivity in confirmed cases and significantly better sensitivity than conventional methods in clinically diagnosed cases.

Keywords

CB-NAAT, Tuberculosis, Pediatric, Pulmonary, Extra-pulmonary.

Introduction

Tuberculosis (TB) is one of the world's most common infectious causes of morbidity and mortality. Globally, about one million cases of pediatric TB occur every year [1], of which 70-80% have pulmonary tuberculosis, 15-20% have extra-pulmonary tuberculosis, with mortality rate as high as around 25% [2]. The cornerstone of TB control remains in early diagnosis and treatment. Diagnosis of pediatric pulmonary and extra-pulmonary TB is challenging and is primarily on basis of history, clinical examination and radiological findings without definite laboratory evidence. Rapid diagnosis of TB significantly facilitates early treatment initiation thereby reducing transmission rates [3]. Newer diagnostic technologies are being introduced but no single test currently is rapid yet easy and affordable. While smear microscopy has poor sensitivity for pediatric tuberculosis and issues related to quality control, conventional culture, the best available reference standard for TB diagnosis, requires 6-8 weeks[4]. Nucleic acid amplification tests like in-house

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Polymerase chain reaction (PCR) developed for rapid diagnosis and identification of drug-resistance, require sophisticated equipment and well-trained staff which are rare in resource-poor settings. Also conventional Nucleic acid amplification tests (NAAT) for TB have chances of cross-contamination during sample processing[5]. XPERT MTB /RIF or CB-NAAT(Cartridge based nucleic acid amplification test), based on nested real-time PCR and molecular beacon technology, is a self-contained cartridge-based fully automated DNA testing platform, that can accurately detect both Mycobacterium tuberculosis and resistance to rifampicin[2]. Studies show that NAATs have high specificity and positive predictive value with highly variable sensitivity**,** especially in children and extrapulmonary tuberculosis[6]**.** Patients with high risk of tuberculosis like presumptive HIV-associated TB and pediatric presumptive TB cases including extra-pulmonary cases with negative AFB smear examination are most likely to be benefitted from CB-NAAT[7]. This study aimed to assess the diagnostic role of CB-NAAT in both pulmonary and extra-pulmonary pediatric tuberculosis.

Materials and Methods

This hospital based prospective studywas conductedover a period of two years in a tertiary care centre after ethical clearance from Hospital Ethics Committee. 142 pediatric patients admittedwith clinical suspicion of pulmonary/ extra-pulmonary tuberculosis were enrolled in the study after taking written informed consent from the caretaker/ guardian of patients. Patients already on antitubercular medications for more than two weeks were excluded. Samples from these patients were collected and sent to designated laboratory for diagnostic testing. A minimum of three ml sample was taken, half ml was used for AFB microscopy (Ziehl-Neelsen staining), half ml was processed and then subjected to cultivation on solid medium (egg based Lowenstein- Jensen medium) in Microbiology laboratory; rest two ml sample was collected in Falcon tube (15 ml) with aseptic precautions and was subjected to CB-NAAT. CB-NAAT was performed using GXMTB-RIF-50 version of cartridges as per manufacturer's instructions (Cepheid Inc). Unprocessed samples were used directly for performing tests and no frozen samples were used for evaluation.

According to microbiological, radiological and clinical findings at enrollment and follow-up visits, participants were classified into three groups: Confirmed tuberculosis, clinically diagnosed tuberculosis and no tuberculosis (Table1). Regular follow-up of patients was doneover a period of six months.These patients were observed for clinical symptoms and response to ATT. Those patients with clinical symptoms suggestive of pulmonary or extra-pulmonary TB and having improvement in clinical symptoms with ATT were classified as clinically diagnosed TB cases even if culture for TB was negative, whereas those with culture-positive were categorized as confirmed TB cases. The diagnostic utility of CB-NAAT was evaluated both in confirmed TB cases and clinically diagnosed cases in comparison to conventional methods. Statistical analysis of the data was done using IBM SPSS version 23 and sensitivity, specificity, positive predictive value (PPV) andnegative predictive value (NPV) of each diagnostic test were calculated. Wilson's binomial method was used to calculate 95% confidence intervals. By taking the culture method as a reference, samples that were positive and negative in culture were considered true positives and true negatives. Hence results were tabulated.

Results

A total of 142 pediatric patients were included in the study with a median age of 8.1 years and a mean weight of 19.7 ± 4.7 kg of which 52.2% were males and 47.8% were females. Sixty patients were classified as "no TB" cases based on predefined criteria (*Table 1*). Three patients died during the study period and three patients were lost to follow-up. For two patients CB-NAAT gave the result as "error" or "invalid" and hence were excluded from the study. A total of 74 patients were left to be included for final analysis in (*Fig 2*). Among these14 (18.9%) were culture-positive "conûrmed TB" cases and 60 (81.1%) were "clinically diagnosed TB" cases (*Table 1*). Out ofthese74cases, 52 had pulmonary tuberculosisand 22 had extra-pulmonary tuberculosis. The diagnostic algorithm of suspected pulmonary and extra-pulmonary tuberculosis is depicted in Diagram 3. In culture positive cases of tuberculosis, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CB-NAAT were 100%, 83.3%, 47.5% and 100% respectively with a diagnostic accuracy of 93.28% while sensitivity, specificity, PPV and NPV ofAFB smear was 21.4%, 88.5%, 33.3% and 91.2% respectively. Overall CB-NAAT detected 100% of smear positive cases.The sensitivity, speciûcity, PPV and NPV of CB-NAAT in clinically diagnosed pulmonary caseswas 60%, 100%, 100% and 65.8% respectively with diagnostic accuracy of 80.2%.In extra-pulmonary samples, there was variable sensitivity (*Table 2*).In subjects with clinically diagnosed tuberculosis, Mycobacterium tuberculosis culture was positive in 18.9 % cases, AFB smear in 12.1% whereas CB-NAAT was positive in 55.4% cases

Table 1: Categorization of Study Participants

Category	AFB smear	Culture	Signs/ Symptoms	Mantoux Test	Radiology	Histo-pathology	Response to ATT/ persistent symptoms on follow up
Confirmed TB	+/-	+	+	+/-	+/-	+/-	+
Clinically diagnosed TB	+/-	-	+	+/-	+	+/-	+
No TB	-	-	+	-	-	-	-

ATT – antitubercular therapy, AFB – acid fast bacilli, TB- tuberculosis

Table 2: CB-NAAT Positivity Rate in Various Samples Taken From Clinically Diagnosed Pulmonary or Extra-Pulmonary TB cases.

Specimen	Clinically diagnosed cases	CB-NAAT + ve cases	CB-NAAT positivity rate (%)
Sputum	28	17	60.7
Gastric lavage	24	15	62.5
Cerebrospinal fluid	9	3	33.33
Pus	3	3	100
Ascitic fluid	3	1	33.33
Pleural fluid	7	2	28.57

Table 3: Diagnostic Accuracy of CB-NAAT in Comparison to MTB Culture.

Study	Sensitivity	Specificity	PPV	NPV
Palud PL *et al.*[16]	80	98.6	88.4	97.2
Lee HY *et al.*[17]	81.6	100	100	92.1
Barnard DA *et al.*[18]	92.3	87.7	80	75.5
Khalil KF *et al.*[15]	91.86	71.42	97.53	41.66
Sharma SK *et al.*[12]	90	100	100	98.1
Agrawal *et al.*[14]	81.4	93.4	73.3	95.7
Singh S *et al.*[19]	82.7	98.3	93.5	90.2
Present study	100	83.3	47.5	100

NPV – negative predictive value, PPV- positive predictive value

indicating that CB-NAAT had significant diagnostic accuracy.

Discussion

Diagnosis of tuberculosis in children is difficult and is largely based on clinical and radiological findings and medical history.Conventional diagnostic methods including AFB smear and culture for MTB have poor sensitivity. High-burden countries like India need rapid diagnostic tests to recognize smear-negative cases that are actually a challenge for treating physicians to ensure effective TB control. Many studies from India and also from all over the world have evaluated the performance of CB-NAAT in diagnosing both pulmonary and extra-pulmonary TB. A number of studies have demonstrated the utility of CBNAAT in the diagnosis of pulmonary tuberculosis[8-12].Most of these studies have tried to find sensitivity and specificity in comparison to AFB smear in culture proven TB cases. However, it is well known that many cases of

clinical tuberculosis are culture negative. Many of these patients are diagnosed and treated on clinical grounds. The purpose of our study was to evaluate thediagnostic utility of CB-NAAT in clinically diagnosed cases in comparison to AFB smear and MTB culture. In the present study, sensitivity, speciûcity, PPV and NPV of CB-NAAT in clinically diagnosed pulmonary cases were 60%, 100%, 100% and 65.8% respectively with a diagnostic accuracy of 80.2%. In extra-pulmonary samples, there was variable sensitivity; highest (100%) in pus, moderate in CSF and ascitic fluid (33%) and lowest in pleural fluid (25%). All culture positive cases were also positive for CB-NAAT.This shows that CB-NAAT is a way more sensitive test than conventional methods in diagnosing pediatric TB. Both increased sensitivity in TB diagnosis and rapid availability of results can help in rapid diagnosis, early initiation of treatment, formulating preventive strategies as well as avoiding unnecessary medications. An added advantage of CB-NAAT is in identifying Rifampicin resistance which helps in the diagnosis and effective management of Multi-Drug Resistant Tuberculosis(MDR TB). No such case of MDR TB was detected in our study.Similar to our results, Raizada N et al[13]and Agrawal M et al[14]reported more than two-fold higher TB case detection on CBNAAT as compared to smear microscopy irrespective of the type of specimen. In comparison with culture as the gold standard, the sensitivity of CB-NAAT in the present study was 100% with a diagnostic accuracy of 93.28%but the specificity was relatively low (83.3%). Similar to this observation, Khalil *et al* [15] reported low specificity of CB-NAATof 71.2 % as compared to other studies. In both studies, only solid culture (LJ medium) was used whereas in other studies, liquid media ((BACTEC MGIT) were used for M. tuberculosis culture. *Table 3* shows the comparison of various studies evaluating the diagnostic utility of CB-NAAT.

There was a significant difference in the positivity rate of conventional methods than CB-NAAT in patients with strong clinical evidence of tuberculosis who responded to ATT. Mycobacterium tuberculosisculture was positive only in 18.9 % of the study population, AFB smear in only 12.1% of cases whereas CB-NAATin 55.4% of cases. Similar observations have been reported by other studies. R. Dewan R *et al* [20] found that among clinically suspected TB cases, CB-NAAT was positive in 40% of cases whereas sputum microscopy for AFB was positive in only 11% of cases. Ahmed *et al* [21] found that the CB-NAAT positivity rate was higher (17.8%) in clinically suspected cases than the culture-positive rate (16%) or positive AFB smear rate (11%).

The low sensitivity of culture in comparison with that of the CB-NAAT in clinically diagnosed cases can be explained as follows: (i) the paucibacillary nature of extrapulmonary specimens with a tendency of M. tuberculosis to form clumps leads to an uneven distribution of the bacilli; (ii) there is loss of viable bacilli during N-acetyl-l-cysteine-sodium hydroxide (NALC-NaOH) processing (due to decanting supernatant steps), unlike CB-NAATprocessing, wherein the entire volume of the processed specimen is used[6,22].

It is apparent from the present studythat CB-NAAT is the most sensitive and specific technique for quick identification of Mycobacterium tuberculosis, especially in cases diagnosed as negative using conventional diagnostic methods but are clinically diagnosed cases of pulmonary or extrapulmonary tuberculosis and have responded to ATT.Finally, not only M. tuberculosisdetection but also rapidlydetermining the patient's MDR status is of prime importance in bringing to an end the spread of MDR-TB and decreasing mortality. Faster methodsthat allow MDR regimens to be started early are urgently needed. However, CB-NAAT does not eliminate the need for conventional microscopy, culture, and anti-tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than Rifampicin.

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Conflicts of Interest

There are no conflicts of interest.

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