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Brief Research

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Diagnosis of Pulmonary and Extra Pulmonary Tuberculosis: How Best is CBNAAT when Compared to Conventional Methods of TB Detection?

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ABSTRACT

Background: Globally, India is a home for more than 25% of global Tuberculosis (TB) burden. The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. We compared the cartridge-based nucleic acid amplification test (CBNAAT) results for diagnosis of pulmonary and extrapulmonary tuberculosis with the conventional methods like sputum smear and solid culture examination.
Methods: A descriptive study was conducted at Government General and Chest Hosptial, Hyderabad, India during 2014 to 2016. The study population included all the pulmonary and extrapulmonary presumptive TB cases who were subjected for further investigations.
Results: Of the two hundred samples received, 110 (55%) were sputum samples and 90 (45%) were extrapulmonary samples. For pulmonary samples, the sensitivity and specificity for CBNAAT samples were 79.2% and 89.5% respectively; while that for sputum smear were 41.5% and 98.2% respectively. For extrapulmonary samples, the sensitivity and specificity for CBNAAT samples were 85.7% and 93.5% respectively; while that for sputum smear were 60.7% and 100% respectively.
Conclusion: CBNAAT is one of the rapid diagnostic tests available in the country and it should be routinely used under the public and private health sector effectively to detect a tuberculosis case.

KEY WORDS: Cartridge-based nucleic acid amplification test (CBNAAT); Tuberculosis; Sputum smear.

ABBREVIATIONS: TB: Tuberculosis; CBNAAT: Cartridge-Based Nucleic Acid Amplification Test; PCR: Polymerase Chain Reaction; DNA: Deoxyribonucleic acid; LED: Light Emitting Diode; FM: Fluorescent Microscopy; LJ: Lowenstein-Jensen; FNAC: Fine Needle Aspiration Cytology; ATT: Anti-Tuberculosis Treatment; RNTCP: Revised National TB control Programme; BAL: Bronchoalveolar Lavage; MDR-TB: Multi-drug resistant TB; MTB: Mycobacterium Tuberculosis; RIF: Resistance to Rifampicin.

INTRODUCTION

India has the highest number of Tuberculosis (TB) cases in the world, with over two million TB cases every year.[1] Annually, one fourth of the global incident TB cases occur in India. Early and accurate diagnosis is the first critical step in controlling TB. The control of TB is hampered by diagnostic methods with sub-optimal sensitivity, particularly for the detection of drug resistant forms and in patients with human immunodeficiency virus (HIV) infection. Early detection is essential to interrupt transmission and reduce the death rate, but the complexity and infrastructure needs sensitive methods which limit their accessibility and effect.

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According to WHO global TB report, the estimated incidence of TB (including TB with HIV) is 2.2 million and prevalence is 2.5 million with mortality (excluding TB with HIV) of 0.22 million.[1] There were 580,000 estimated new cases of MDR-TB (Multi-drug resistant TB) and Rifampicin resistant TB (RR-TB); among them 125,000 (20%) were enrolled. India, China and the Russian Federation accounted for 45% of all estimated MDR/RR-TB cases (henceforth to be called as MDR-TB). India, one of the countries with high burden of TB, has an estimated 79,000 MDR-TB cases among notified pulmonary TB cases. The estimated incidence of MDR-TB is 2% among new cases and 15% among re-treatment cases.

The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. Culture methods and drug susceptibility testing is complex, time consuming, and takes around 6-8 weeks. While patients await diagnosis, they are likely to receive inappropriate or in effective treatment and consequently disease may progress. This results in an increased chance of morbidity from tuberculosis. They continue to transmit drug-resistant TB to others; especially for family members and the resistance might have amplified.

To address this issue there was a need for a simple and rapid diagnostic tool at least for high-burden countries and a new diagnostic test cartridge based nucleic acid amplification test (CBNAAT) was developed which was rapid, fully automated and was based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimens and also detects rifampicin resistance.[2] This diagnostic test was designed to purify, concentrate, amplify and identify targeted *rpoB* nucleic acid sequences, and delivered the results in about 120 minutes.

In this study, we compared the CBNAAT results for diagnosis of pulmonary and extrapulmonary tuberculosis with the conventional methods like sputum smear and solid culture examination.

METHODS

A descriptive study was conducted in Department of Pulmonary Medicine, Government General and Chest Hosptial, Hyderabad, Telangana, India during January 2014 to January 2016. The study population included all the pulmonary and extrapulmonary presumptive TB cases who were subjected for further investigations. To diagnose tuberculosis the investigations included sputum smear microscopy by light emitting diode (LED) fluorescent microscopy (FM), solid culture and liquid culture examination, CBNAAT and Fine needle aspiration cytology (FNAC) depending upon the type of specimen.

Non-sterile clinical specimens were processed by conventional N-acetyl-l-cysteine–NaOH method. After decontamination, the smears were prepared by the auramine-rhodamine acid-fast staining method. The decontaminated specimens were inoculated into Lowenstein-Jensen (LJ) solid medium and MB/BacT liquid culture medium for growth detection. The smear-positive specimens were evaluated within two weeks at the latest, while the smear-negative specimens were studied immediately after the growth of culture.

For CBNAAT examination the sample reagent were added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge. All the specimens which were culture positive and mycobacterium tuberculosis/resistance to rifampicin (MTB/RIF) assay negative and specimens that were culture negative and MTB/RIF assay positive were retested twice. The last result was included for the analysis.

All the data were entered in Microsoft excel and the statistical analysis was performed using Epi-data analysis software (version V2.2.2.178).

Ethics

The Institutional Ethics Committee (IEC) of Osmania Medical College, Hyderabad had approved the conduct of the study.

RESULTS

There were 200 specimens collected during the study period. Of which 110 (55%) samples were sputum samples while the remaining 90 (45%) were extrapulmonary samples. Among the samples provided 120 (60%) were males and 12 (6%) were found to be reactive for HIV. The comparison of CBNAAT, sputum smear against solid culture is shown in Table 1.

Pulmonary Samples

The sensitivity and specificity for CBNAAT samples were 79.2% and 89.5% respectively; while that for sputum smear were 41.5% and 98.2% respectively. The positive and negative predictive value for CBNAAT was 79.2% and 89.5% respectively. The positive and negative predictive value for sputum smear was 41.5% and 98.2% respectively.

Extrapulmonary Samples

The proportion of the samples received from different anatomical sites were: lymph node 38 (19%), pleural fluid and bronchoalveolar lavage (BAL) 20 (10%) and cerebrospinal fluid 5 (2.5%). The sensitivity and specificity for CBNAAT samples were 85.7% and 93.5% respectively; while that for sputum smear were 60.7% and 100% respectively. The positive and negative predictive value for CBNAAT was 85.7% and 93.5% respectively. The positive and negative predictive value for sputum smear was 100% and 84.9% respectively.

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Table 1: Comparative Table Showing the Results for Culture, CBNAAT and Smear Examination for Pulmonary and Extra-Pulmonary Samples (N=200).

Type of samples →		Pulmonary (n=110)		Extra-pulmonary (n=90)	
Type of tests		Culture		Culture	
	Results	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)
CBNAAT	Negative	51(89.5)	11(20.8)	58(93.5)	4(14.3)
	Positive	6(10.5)	42(79.2)	4(6.5)	24(85.7)
Sputum smear	Negative	56(98.2)	31(58.5)	62 (100)	11 (39.3)
	Positive	1(1.8)	22(41.5)	0 (0)	17 (60.7)

DISCUSSION

Our study findings suggest that CBNAAT has higher sensitivity for detection of pulmonary and extrapulmonary tuberculosis cases. The WHO 2012 has also recommended the CBNAAT for routine use under programmatic conditions.[3]

In the present study, only 200 specimens were included; among them 110 were pulmonary and 90 were extrapulmonary. Among the 110 pulmonary presumptive TB cases, 15 were unable to produce adequate amount of quality sputum and hence were subjected to bronchoscopy and BAL was performed for collection of the sputum. The sensitivity of CBNAAT for pulmonary samples was 79% when compared to sputum smear which was 42%. The sensitivity of CBNAAT for extrapulmonary samples was 86% when compared to sputum smear which was 61%. The sensitivity of CBNAAT in smear-positive, culture-positive and smear-negative, culture-positive pulmonary samples were 100% and 66.67% respectively. Sensitivity of smear negative pulmonary samples can be increased by including more than one sample for diagnosis.

In a study done by Panayotis et al,[4] the sensitivity and specificity of CBNAAT in 80 pulmonary samples were 90.6% and 94.3% respectively. In a study done by Armand et al[5] the sensitivity of CBNAAT in 60 pulmonary samples which included sputum, BAL, bronchial aspirate and gastric aspirate was 79%. Among individual extrapulmonary samples, the sensitivity of CBNAAT was highest among lymph nodes (94.74%) when compared to sputum smear (73.68%). Inclusion of CBNAAT in the initial diagnosis of tubercular lymphadenopathy in addition to the FNAC would decrease the over diagnosis of tuberculosis and injudicious use of anti-tuberculosis treatment (ATT).

Various studies conducted across India has suggested the usage of CBNAAT up-front for people living with HIV (PLHIV).[6] The operational feasibility studies conducted under the Revised National TB Control Programme (RNTCP) have demonstrated the feasibility of the machine to efficiently work under Indian settings.[7]

The study has following limitations (a) it was an experimental study and it did not adapt a rigorous study design while the samples included were also small to make a generalised statements (b) the culture is known to be a suboptimal standard for extrapulmonary TB and the same was used as standard to compare with CBNAAT and sputum smear.

To conclude, CBNAAT is one of the rapid diagnostic tests available in the country and it should be routinely used under the public and private health sectors efficiently to detect a tuberculosis case.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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