Performance of Cartridge Based Nucleic Acid Amplification Test for the Diagnosis of Smear Negative Suspected Tuberculosis - A Study from a Teaching Hospital in Thrissur, Kerala, India

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ABSTRACT

BACKGROUND

In India, everyday more than 6000 people develop tuberculosis (TB) and more than 600 people die of TB (2 death every 5 minutes).[1] World Health Organization (WHO) has recently endorsed cartridge based nucleic acid amplification test (CBNAAT) which has the potential to lead a revolution in the diagnosis of tuberculosis. This study intends to assess the performance of CBNAAT for the diagnosis of suspected smear negative pulmonary and extra pulmonary tuberculosis.

METHODS

The cross-sectional study was carried out in Department of Microbiology, Government Medical College, Thrissur, Kerala, India. CBNAAT was done in district tuberculosis center, Thrissur. The study was done from December 2016 to December 2017. Samples were sent for microscopy, culture and CBNAAT.

RESULTS

A total of 250 patients were evaluated. Majority of the specimens collected were sputum (61.2 %) followed by bronchial wash (17.6 %). Culture was positive in 48 specimens. CBNAAT was positive in 58 specimens. Both culture and CBNAAT were positive in 47 patients. CBNAAT was negative in 1 specimen but positive in culture test. CBNAAT detected an additional 10 samples. Taking culture as gold standard, culture positives were taken as true positives and culture negatives were taken as true negatives. Accordingly, true positive was 48, true negative was 202, false positive was 10 and false negative was 1. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were respectively 97.95 %, 95.2 %, 82.75 % and 99.5 %. CBNAAT missed out a sputum sample which was culture positive.

CONCLUSIONS

We found CBNAAT to be an important diagnostic modality especially in smear negative patients for early diagnosis and treatment of TB. Culture of mycobacteria is considered as a gold standard method, but it takes weeks to obtain a positive result and simultaneous detection of rifampicin resistance is not possible with this method.

KEY WORDS

Tuberculosis, Smear Negative TB, ZN Stain, AFB, Petroff's Method, CBNAAT, RNTCP, Culture

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BACKGROUND

"I have no business to live this life if I cannot eradicate this scourge from mankind" - Robert Koch (Lecture at Berlin University regarding discovery of TB, 1882).[2] TB is an ancient yet still a major disease that causes significant morbidity and mortality. It continues to be a major health problem and has a huge socioeconomic impact. An increasing number of cases converting to multi drug resistant tuberculosis could escalate these costs dramatically.[3]

Tuberculosis in the World

TB is the 9th leading cause of death worldwide. Most of the cases occurred in WHO Southeast region (45 %), the WHO African region (25 %) and the WHO Western Pacific region (17 %). The top five countries, with 56 % of estimated cases, were (in descending order) India, Indonesia, China, the Philippines and Pakistan. Globally, the TB mortality rate is falling at about 3 % per year. The decline since 2010 has exceeded 4 % per year in several high TB burden countries, including Ethiopia, Kenya, Lesotho, Namibia, the Russian Federation, the United Republic of Tanzania, Zambia and Zimbabwe. Regionally, the fastest declines in the TB mortality rate were noted in the WHO European Region and the WHO Western Pacific Region since 2010.[4]

Tuberculosis in India

India accounts for one fourth of the global TB burden. India has highest burden of both TB and multidrug resistant tuberculosis (MDR TB) based on estimates reported in Global TB Report 2016. An estimated 1.3 lakh incident multi-drug resistant TB patients emerge annually in India which includes 79000 MDR TB patients estimates among notified pulmonary cases. India bears second highest number of estimated human immunodeficiency virus (HIV) associated TB in the world.[5]

Tuberculosis is a major public health problem and socioeconomic issue in India. Early diagnosis and treatment are the most important armour against this deadly infectious disease. A rapid diagnostic test with high sensitivity and specificity is a long-awaited dream and is quite essential to win the battle against this 'captain of men of all deaths.' So, the present study was undertaken to assess the importance of CBNAAT in smear negative tuberculosis in the field of diagnosis by comparing with culture and to find out rifampicin resistance if present by CBNAAT.

METHODS

The cross-sectional study was done in the Department of Microbiology, Government Medical College, Thrissur, Kerala, India. CBNAAT was done in the district tuberculosis centre, Thrissur. Study was conducted over a period of one year from December 2016 to December 2017. Patients with suspected tuberculosis sent to RNTCP unit of Government Medical College, Thrissur for CBNAAT were taken as subjects.

All smear negative suspected cases of pulmonary and extra pulmonary tuberculosis were included, and all smear positive cases of tuberculosis were excluded from the study.

Sample Size

According to Kerala 2015 status the prevalence of smear negative tuberculosis is 40 %.[6]

Formula: $n = 4 pq / d^2$, where p = 40 %, q = 100-p, d = 20 % of p

Sample Size – 150

Informed consent from all subjects, ethical clearance and institutional approval were taken. The patients who were sent to RNTCP unit of Government Medical College, Thrissur for CBNAAT were assessed with clinical history. Samples were sent for microscopy, culture and CBNAAT.

Microscopy

The samples of the patient were taken for smear microscopy which was done by Ziehl Neelsen (ZN) staining.

Culture

Concentration of specimen was done prior to inoculation into Lowenstein Jensen (LJ) medium by Petroff's method.

CBNAAT

The sample was poured into a single use disposable cartridge that was placed in the Xpert Dx module. The sample was diluted with three times the reagent, incubated at room temperature and loaded into the cartridge for automated analysis with results in 2 hours. It interpreted all the results from measured fluorescent signals, into following categories: invalid, positive or negative. If positive, strains were categorised as susceptible or resistant to rifampicin.[7]

Statistical Analysis

In order to check whether the effectiveness of two tests are equal or not, Mann Whitney and two sample binomial tests are conducted. The results are given below. Mann Whitney test for equality of two groups.

Null Hypothesis: Two tests are equally effective (Table). Since the P-value is greater than 0.05 (0.33 > 0.05) we reject the hypothesis that two tests are equally effective.

Two sample binomial tests: Since the observations in culture data and CBNAAT are dichotomous, the most appropriate test for testing the null hypothesis: Two tests are equally effective, is two sample binomial tests. The results are given below. (Table 2)

Therefore, the effectiveness of two tests is different. CBNAAT has more positive result significantly. This could be since CBNAAT, being a nucleic acid amplification test can even give positive results with dead bacilli. Culture, being the gold standard test for the diagnosis of TB detects only viable tubercle bacilli and also helps in the identification of non-tuberculous mycobacterium (NTM) where CBNAAT is negative.

RESULTS

Of the 250 patients 167 were males and 83 were females. Majority of the study population belonged to 41 - 60 years of age. Majority of the specimen collected were sputum followed by bronchial wash. The samples were 153 sputum, 44 bronchial wash, 15 pleural fluid, 10 cerebrospinal fluid (CSF), 10 pus aspirate, 8 lymph node aspirate, 6 ascitic fluid and 4 gastric aspirates. Ascitic fluid, gastric aspirate and pus aspirate were negative both in culture and CBNAAT. (Table 3)

Culture was positive in 48 specimens. CBNAAT was positive in 58 specimens. Both culture and CBNAAT was positive in 47 patients. CBNAAT was negative in 1 specimen which was culture positive. CBNAAT detected an additional 10 samples. Taking culture as gold standard, culture positives were taken as true positives and culture negatives were taken as true negatives. Accordingly, true positive was 48, true negative was 202, false positive was 10 and false negative was 1. CBNAAT missed out a sputum sample which was culture positive. (Table 4) and (Table 5). The specimen was sent to RNTCP state laboratory (IRL, Trivandrum) for speciation and the result came out to be *Mycobacterium abscessus*.

	Observed Values
Mann-Whitney U test	30125.000
Wilcoxon W test	61500.000
Z	- .974
P-Value	.330

Table 1. Test Statistics Grouping Variables-Culture vs. CBNAAT

	Category	N	Observed Prop.
Culture group 1	Negative	202	.80
Group 2	Positive	48	.20
Total		250	1.00
CB NAAT group 1	Positive	58	.24
Group 2	Negative	192	.76
Total		250	1.00

Table 2. Two Sample Binomial Test (Based on Approximation)

Tests	Positive	Negative
Culture	48	202
CBNAAT	58	192

Table 3. Results of CBNAAT and Culture

Sensitivity	97.95 %
Specificity	95.2 %
PPV	82.75 %
NPV	99.5 %

Table 4. Results of Our Study (Overall Sensitivity, Specificity, PPV and NPV)

Samples	Sensitivity	Specificity	PPV	NPV
Sputum	97.7 %	96.4 %	91.5 %	99.1 %
Bronchial wash	100 %	95.1 %	71.4 %	100 %
Lymph node aspirate	100 %	70 %	25 %	100 %

Table 5. Sensitivity, Specificity, PPV and NPV of Each Sample

DISCUSSION

In this study, we have evaluated the diagnostic yield of CBNAAT in smear negative tuberculosis and to detect rifampicin resistance if present. A total of 250 smear negative tuberculosis suspected patients were considered. Majority of the samples were sputum followed by bronchial wash. Mycobacterial cultures for detection of *Mycobacterium tuberculosis* was done in LJ medium. In our study CBNAAT showed a sensitivity, specificity, negative predictive value (NPV), positive predictive value of 97.95 %, 95.2 %, 99.5 % and 82.75 % respectively.

Sensitivity and specificity of GeneXpert in sputum assay in our study was 97.7 % and 96.4 % that is in line with the study of Sharma et al.[8] For bronchial wash the sensitivity and specificity of our study were 97.5 % and 95.2 % respectively which were comparable with other studies.[9,10,11,12] For smear negative samples, sensitivity and specificity of GeneXpert were 97.5 % and 95.2 % in our study that correlates well with other studies from 57 % - 75 % and 97 % - 100 % respectively.[13,14,15]

In a study conducted by Monika et al. in 2016, the overall sensitivity, specificity, PPV and NPV of CBNAAT were 86.8 %, 93.1 %, 78.5 % and 96 % respectively and for bronchoalveolar lavage (BAL) sample, 81.4 %, 93.4 %, 73.3 % and 95.7 % respectively and for acid fast bacilli (AFB) negative samples, sensitivity and specificity were 79.1 % and 93.1 % respectively.[16]

In a study conducted by Vadwai et al. the sensitivity of the CBNAAT was 64 % for smear-negative cases and 96 % for smear-positive cases, with a specificity of 99.6 %. The Xpert test correctly identified 98 % of phenotypic rifampin (RIF) resistant cases and 94 % of phenotypic RIF-susceptible cases.[17] A study conducted by Zeka et al. in pulmonary specimens, sensitivities were 100 % and 68.6 % for smear-positive and smear-negative specimens, respectively. It had a lower sensitivity with extra pulmonary specimens, 100 % for smear-positive specimens and 47.7 % for smear-negative specimens.[18]

In patients with incongruous results of smear microscopy and CBNAAT but high clinical evidence of pulmonary tuberculosis like HIV positive or critically ill, clinicians may exercise their clinical decision to start anti tubercular treatment after sending sample for culture.[19]

As compared to culture, sensitivity and specificity values were 80.0 % and 98.6 % for CBNAAT and 25.0 % and 95.8 % for smear microscopy. The sensitivity of CBNAAT was significantly higher than that of smear microscopy. CBNAAT found out additional cases, compared to smear microscopy.[9] The findings in subgroup analysis of Chang et al. the accuracy of CBNAAT was higher in smear-positive specimens and the sensitivity of diagnosing pulmonary TB in adults was higher than that in children.[20]

A study done in 2011 in Hyderabad showed incremental case detection of 10.8 % when CBNAAT was used to diagnose tuberculosis over and above fluorescent microscopy.[21] In smear negative-culture positive cases, the test had a sensitivity of 77.7 %. The sensitivity and specificity for detecting rifampicin resistance was 94.5 % and 97.7 % respectively with respect to culture as reference standard. Hence, while culture still forms the gold standard for diagnosis of TB, CBNAAT proposes to be a strong first line diagnostic tool for TB cases.

CBNAAT had 100 % specificity for detection of rifampicin resistance in a study conducted on retro positive individuals.[22] It shows that a patient with a negative GeneXpert can still have TB with *Mycobacterium tuberculosis* or mycobacterium other than tuberculosis (MOTT). Our study further strengthens the need of CBNAAT in smear negative tuberculosis. GeneXpert does not eliminate the need of conventional microscopy,

culture and anti-tubercular drug sensitivity that plays an important role in monitoring the progression of treatment and to detect resistance to drugs other than rifampicin.[23]

CONCLUSIONS

CBNAAT can be a useful diagnostic method in patients with suspected tuberculosis either AFB smear negative or positive due to its rapidity and simultaneous detection of rifampicin resistance especially beneficial in patient with extra pulmonary, MDR and HIV associated tuberculosis. CBNAAT negative and culture positive cases could be infections with MOTT. CBNAAT positive and culture negative cases could be due to dead bacilli which could be detected in CBNAAT due to amplification of genetic material whereas culture requires viable bacteria to be present for growth on culture media. In specimens like CSF, the number of mycobacteria is too low to be detected in culture which requires 10 - 100 viable bacilli for growth to appear in which condition CBNAAT has an edge over culture.

Limitations of the Study

Clinical characteristics of the subjects were not taken into consideration in this study. Treatment outcome was not followed up in this study. Comorbidities and vulnerability of the patients were not assessed.

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