Role of Cartridge based nucleic acid amplification test (CBNAAT) in diagnosis of tuberculosis in a tertiary care teaching institute: A retrospective study

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ABSTRACT
Objective: To determine the utility of cartridge based nucleic acid amplification test (CBNAAT) in smear negative pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB) cases. To watch for Rifampicin resistance in MTB positive cases on CBNAAT analysis.

Materials and methods: Total 125 suspected pulmonary and extrapulmonary smear negative tuberculosis cases in a period of 8 months from June 2018 to January 2019 were included in this retrospective, observational record based study. All the samples were sent for CBNAAT and data was analysed. Data was expressed as mean±SD and percentages.

Results: Out of 125 CBNAAT samples, 92 were sputum/BAL samples and 33 were extra-pulmonary samples. Patients included were in the age range of 11 to 80 years with mean age of 40.56±17.02 years. Male preponderance was seen with 74 (59.2%) male and 51 (40.8%) female patients. Out of 125, CBNAAT could identify MTB in 32 sputum smear negative cases, of which, 23 (71.88%) were Rifampicin sensitive and 3 (9.38%) were Rifampicin resistant cases.

Conclusion: CBNAAT is an efficient test to detect presence of MTB as well as Rifampicin resistance in both PTB and EPTB in AFB smear negative cases. These enable early treatment initiation and accelerate implementation of MDR-TB control measures, ultimately reducing incidence TB cases.

Keywords: CBNAAT, pulmonary tuberculosis, extrapulmonary tuberculosis, MDR-TB

INTRODUCTION
Worldwide, Tuberculosis (TB) is one of the top ten causes of death. According to WHOM, in 2017, 10 million people got infected with TB out of which 1.6 million died of the disease. About 54 million lives were saved through diagnosis and treatment of TB between 2000 and 2017. (WHO) In India, TB is an important health problem and is a prime concern for public health due to scarce diagnostic assays. (Munje)

Early and accurate diagnosis is very important in controlling TB. Available culture methods and drug susceptibility tests are complex and time consuming leading to inappropriate or ineffective treatment and increased morbidity. To overcome this issue, a new diagnostic test, cartridge based nucleic acid amplification test (CBNAAT) was developed which is rapid, fully automated test delivering results in about 120 minutes. CBNAAT is based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimen and also detects rifampicin resistance. (Munda) It marked an important development in the field of rapid molecular TB diagnostics. Sputum samples can be
analyzed with very minimal processing, yielding positive diagnoses in 99–100% of patients with smear-positive pulmonary tuberculosis (PTB) and 57–83% of patients with smear-negative PTB in clinical evaluation studies. (Lawn) Globally, it has been estimated that 3.3% of new TB cases and 20% of previously treated cases have MDR-TB. (Basawraj) The use of CBNAAT was promptly sanctioned by the WHO in December 2010 in place of sputum smear microscopy, mainly in the settings where high rates of HIV-associated TB and multidrug-resistant TB were noted. (Who website and Lawn) CBNAAT does not have particular mandatory pre-requisites for the set-up and does not demand much technical training. Also, the reagent used for processing is bactericidal and tubercle bacilli are inactivated in vitro, eliminating the biosafety risks and enabling its use as a rapid point-of-care diagnostic test. (Munda)

CBNAAT has two main advantages compared with conventional smear microscopy (smear) for the diagnosis of tuberculosis (TB). Firstly, it is highly sensitive both for PTB as well as extra-pulmonary tuberculosis (EPTB) and secondly it detects mutations in rpoB gene that confer rifampicin resistance (RR-TB). (Youngs) We planned a record review research from a retrospective data of patients with both pulmonary and extrapulmonary smear negative TB cases in which diagnosis was confirmed with CBNAAT. This study was conducted with the aim to evaluate the utility of CBNAAT in detecting MTB in smear negative pulmonary and extrapulmonary TB cases and to watch for Rifampicin resistance amongst MTB positive cases according to CBNAAT reports.

MATERIALS AND METHODS:

This study was a retrospective observational record based analysis of total 125 patients. Suspected pulmonary and extrapulmonary smear negative tuberculosis cases in a period of 8 months from June 2018 to January 2019 were included in this study. All the samples were sent for CBNAAT and data was analysed. Specimen subjected to CBNAAT was either sputum, bronchoalveolar lavage (BAL) or extrapulmonary fluid sample (lymph node, pus, Pleural fluid, ascitic fluid, cerebrospinal fluid, endometrial biopsy).

RESULTS:

Retrospective analysis of 125 samples was done. Out of 125 CBNAAT samples, 92 were sputum/BAL samples and 33 were extra-pulmonary samples. Patients included were in the age range of 11 to 80 years with mean age of 40.56±17.02 years. Most of the patients were in the age group 20 to 50 years. Maximum cases were in the age group 21-30 years i.e. 42/125 (33.6%). Male preponderance was seen with 74 (59.2%) male and 51 (40.8%) female patients. Out of 125, CBNAAT could identify MTB in 32 sputum smear negative cases. Agewise distribution of CBNAAT results was as depicted in (figure 1). The genderwise distribution of CBNAAT results was as shown in (figure 2). Results of CBNAAT in PTB and EPTB patients was as depicted in (table 1). MTB was detected in 32 (25.6%) samples, out of which 23 (71.88%) were Rifampicin sensitive and 3 (9.38%) were Rifampicin resistant cases. There was no rifampicin resistance detected in extra pulmonary samples. TB and HIV co-infection was seen in 2.17% (2/92) patients with PTB. CBNAAT results among different PTB and EPTB samples were as depicted in (table 2 & table 3).

DISCUSSION:

Early and precise diagnosis is the prime step in control of TB which is hampered mainly by diagnostic methods having sub-optimal sensitivity, particularly while detecting drug resistant forms and in patients of human immunodeficiency virus (HIV). Early detection is very important to interrupt transmission and reduce the death rate, but infrastructure needs sensitive methods which limit their accessibility and effect. (Kandi) It is important to diagnose active smear-negative PTB, which represents most of the TB cases. 2015 European Centre for Disease Prevention (ECDC), in Italy, reported that in 2014, out of all TB cases, 68.1% were smear-negative TB cases leading to delayed diagnosis as well as optimal treatment. (European centre 2015)

CBNAAT is a fully automated, real-time, heminested PCR system which detects MTB and rifampicin resistance as well. According to the World Health organization, CBNAAT is the most rapid sensitive test for TB diagnosis in respiratory samples. This test needs less than 2 hours to be performed. The main steps in CBNAAT include bacterial lysis, DNA extraction, amplification, and organism detection.
CBNAAT has very high sensitivity in diagnosis of smear-negative pulmonary TB and has a crucial role to play especially in low- to middle-income countries. CBNAAT is the only fully automated real-time DNA-based test that can detect both MTB and resistance of rifampicin. (Opota O)

Globally, EPTB constitutes 20% burden of TB. EPTB is a pauci-bacillary disease as the number of bacteria are less to detect and are deep seated in the organs. Conventional methods including histology and smear microscopy are never diagnostic and diagnostic methods such as culture methods are time consuming. (Munje) People living with HIV (PLHIV) have more than a 20-fold increased risk of TB compared to HIV-uninfected people. However, detecting TB among HIV is still challenging. (Rathinam) Hence, newer and faster diagnostic methods like nucleic acid amplification techniques like Gene Xpert (CBNAAT) are needed. (Munje)

In our study, mean age of the patients included was 40.56±17.02 years with maximum patients 42/125 (33.6%) were in the age group of 20 to 30 years. In consistency with our study, in a studies by Sedky et al. and Youngs et al., the mean age of patients was 45.5±17.7 years and 44 years respectively. Also, Rai and colleagues, in their study, found that most of i.e. 53/106 (50%) patients were in the age range of 15 and 30 years.

In this study, male predominance was seen with 74 (59.2%) male and 51 (40.8%) female patients. This was in accordance with study by Youngs et al in which 58% of patients were males. Also, in study by Sedky et al. there was a predominance of male patients (80%) than females. However, in a study by Mohamed and colleagues, results were in disagreement with our study where females were 70.87% and males were 29.13%. They explained the higher incidence of TB in females in their study by the routines in Upper Egypt, where females, especially farmers, have a main role of work outside or inside the home which has a higher chance of exposure to infection.

In this study, CBNAAT was able to detect MTB in 32/125 (25.6%) PTB and EPTB patients who were smear negative. Similarly, in a study by Rai et al., MTB was detected in 37 (34.9%) patients out of 106 smear negative patients. Also, in a study by Sedky et al. CBNAAT detected MTB in 11/30 (36.7%) patients and negative in 19/30 (63.3%) patients. In our study, MTB was detected in 25/92 (27.17%) patients of PTB and in 7/33 (21.21%) patients of EPTB. Kumar et al., in their study reported that MTB was detected by CBNAAT in 48/110 (43.63%) patients of PTB and 28/90 (31.11%) patients of EPTB. Munda and colleagues, in their study included 200 specimens; 173 were pulmonary and 27 were extrapulmonary. The sensitivity of CBNAAT was 79% for pulmonary samples compared to 42% for sputum smear samples. The sensitivity of CBNAAT for extrapulmonary samples was 86% as compared to sputum smear samples which was 61%.

In this study, sputum samples were common 76/92 as compared to bronchial wash samples 16/92 in cases of PTB for CBNAAT analysis. MTB was detected in 18/76 (23.86%) of sputum samples and 7/16 (43.75%) of bronchial wash samples. Maximum EPTB cases were of pleural effusion, hence, pleural fluid was the most common sample processed (13/33) for CBNAAT with 23.08% of MTB detection rate amongst EPTB cases. This was in accordance with study by Gupta et al in which out of 300 samples of EPTB patients 203 were pleural fluid samples.

Out of total 32 CBNAAT positive cases for MTB, 23 (71.88%) were Rifampicin sensitive and only 3 (9.38%) were Rifampicin resistant cases of PTB which can be correlated with findings of the study by Suresh K et al., in which, out of the 45 CBNAAT positive cases, 42 (93.3%) were rifampicin sensitive and 3 (6.7%) were rifampicin resistant. But they noted rifampicin resistance in EPTB cases. However, in another study done by Dewan et al., found rifampicin resistance of 10%. This high resistance to rifampicin may be due to higher prevalence of MDR TB in north India and also referred cases from multiple states. We found TB and HIV co-infection in 2.17% (2/92) patients of PTB. Similarly, in a study by Munda et al., TB and HIV co-infection was seen in 3.3% patients.

Limitations: This was a retrospective study. As the data was collected in a shorter period of six months, it will be difficult to generalise study findings.

CONCLUSION:

CBNAAT is one of the rapid diagnostic tests that are accessible and it should be efficiently used in routine
practice under the public and private health sectors to detect a tuberculosis cases. The CBNAAT assay is a gold standard to diagnose TB due to its high sensitivity and specificity for diagnosing smear-negative PTB as well as EPTB cases and to detect resistance to rifampicin associated with mutation of rpoB gene. This can enable early and appropriate treatment initiation, as well as accelerating the implementation of MDR-TB control measures, and ultimately reducing TB case incidence.

REFERENCES:

TABLES AND FIGURES:

**Fig 1: Agewise distribution of CBNAAT results**

![Age distribution chart](image1)

**Fig 2: Genderwise distribution of CBNAAT results**

![Gender distribution chart](image2)

<table>
<thead>
<tr>
<th>CBNAAT Results</th>
<th>Pulmonary TB(%)</th>
<th>Extrapulmonary TB(%)</th>
<th>Total(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTB detected</td>
<td>25(27.17%)</td>
<td>7(21.21%)</td>
<td>26(20.8%)</td>
</tr>
<tr>
<td>MTB not detected</td>
<td>67(72.82%)</td>
<td>26(78.8%)</td>
<td>99(79%)</td>
</tr>
</tbody>
</table>

Table 1: Results of CBNAAT in study patients
### Table 2: CBNAAT results among different PTB samples

<table>
<thead>
<tr>
<th>Sample processed</th>
<th>No. of cases</th>
<th>MTB detected(%)</th>
<th>MTB not detected(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial Wash</td>
<td>16</td>
<td>7(43.75%)</td>
<td>9(56.25%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>76</td>
<td>18(23.86%)</td>
<td>58(76.31%)</td>
</tr>
</tbody>
</table>

### Table 3: CBNAAT results among different EPTB samples

<table>
<thead>
<tr>
<th>Sample processed</th>
<th>No. of cases</th>
<th>MTB detected(%)</th>
<th>MTB not detected(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>9</td>
<td>2(22.22%)</td>
<td>7(77.78%)</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>4</td>
<td>0</td>
<td>4(100%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>13</td>
<td>3(23.08%)</td>
<td>10(76.92)</td>
</tr>
<tr>
<td>CSF</td>
<td>2</td>
<td>2(100%)</td>
<td>0</td>
</tr>
<tr>
<td>LN</td>
<td>3</td>
<td>0</td>
<td>3(100%)</td>
</tr>
<tr>
<td>Endometrial</td>
<td>2</td>
<td>0</td>
<td>2(100%)</td>
</tr>
</tbody>
</table>