Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV


Abstract

Background: Diagnosing tuberculosis (TB) in people living with HIV/AIDS (PLHIV) is challenging as sputum microscopy is negative in more than half of the patients due to lack of caseous necrosis. Sputum culture is a slow method which takes 4 - 8 weeks for growth of the mycobacteria. Delayed treatment for TB in PLHIV is associated with increased mortality. The role of a newly launched cartridge-based nucleic acid amplification test (CBNAAT) with a potential to diagnose TB and rifampicin resistance within 2 hours in PLHIV is promising.

Aims and objectives: The current study compared the efficacy of sputum microscopy and CBNAAT for diagnosing pulmonary TB in PLHIV. The detection rates of rifampicin resistance of CBNAAT and line probe assay (LPA) were also compared.

Method: One hundred PLHIV with age > 18 years having either cough for > 2 weeks or chest X-ray suggestive of pulmonary TB were included in this analytical cross-sectional study. Blood samples for CD4 count and sputum samples for microscopy and CBNAAT were sent. The sputum samples detected to be rifampicin resistant on CBNAAT also underwent LPA for multi-drug resistant TB (MDR-TB).

Results: Eleven patients (11%) were positive by sputum microscopy for acid-fast bacilli and 40 (40%) were positive by CBNAAT. This difference was statistically significant (p value < 0.001). Mean time taken for detection of TB was 2 days for microscopy and less than 2 hrs for CBNAAT. Out of the 40 patients positive by CBNAAT, rifampicin resistance was detected in 10 patients (25%) out of which 9 had multi-drug resistant tuberculosis (MDR-TB) as detected by LPA. The mean CD4 count of the patients was 230 cells/ml and there was no statistically significant difference in CD4 counts between CBNAAT positive and negative patients (p value = 0.264).

Conclusions: CBNAAT helps in increased case detection in lesser time to diagnose pulmonary TB in PLHIV as compared to conventional sputum microscopy. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB for the purpose of starting category IV anti-tubercular therapy (ATT) early.

Keywords: Tuberculosis, people living with HIV/AIDS, cartridge-based nucleic acid amplification test, multi-drug resistant tuberculosis.

Introduction

Tuberculosis remains the most common opportunistic infection among PLHIV, and HIV-TB co-infected individuals are at high-risk of death1. Standard sputum based methods to detect pulmonary tuberculosis include sputum microscopy and culture. However, in PLHIV, there is scanty sputum production, lack of caseous necrosis leading to decreased number of bacilli in sputum, and high incidence of non-tubercular mycobacterial infection. These factors decrease the sensitivity and specificity of sputum microscopy as a diagnostic tool.

To overcome these shortcomings, sputum culture and sensitivity for mycobacteria can be used. But it is a slow test usually taking 4 - 8 weeks, not widely standardised, and not economical for screening purposes. This delays initiation of anti-tubercular treatment especially for drug-resistant forms of TB, increases risk of transmission of (drug-resistant) TB in the community and increases the risk of spread to extrapulmonary sites within the patient2. Cartridge-based nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction (PCR) based method for detection of TB. It also detects rifampicin resistance as it targets the rpoB gene of mycobacteria. CBNAAT is a Mycobacterium tuberculosis-specific automated, cartridge-based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 100 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the rpoB gene of M. tuberculosis, which is the critical gene associated with rifampicin resistance.

No cross-reactions have been observed with many other bacterial species tested, including a comprehensive panel of mycobacteria, thereby excluding non-tubercular mycobacteria (NTM). Being a PCR based method, clinical validation trials done in four distinctly diverse settings have shown that 92.2 per cent of culture-positive patients were

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detected by a single CBNAAT test with a specificity of 99 per cent as compared to the sensitivity of a single direct sputum smear of 59.5%.

It's role in diagnosing TB in PLHIV has not been studied widely in India. This study was carried out to evaluate the role of CBNAAT in early diagnosis of TB in PLHIV and detection of M. tuberculosis in sputum by CBNAAT compared to conventional sputum microscopy in pulmonary TB.

**Materials and methods**

The study group consisted of 100 subjects who were diagnosed as HIV infected by ELISA rapid and simple (ERS) test according to NACO guidelines between January 2013 and December 2013 and who presented to the chest and TB centre of Lok Nayak Hospital with productive cough for 2 weeks and/or chest X-ray findings suggestive of pulmonary tuberculosis. It was an analytical cross-sectional study. All such patients underwent the following:

1. **History and examination**
   All patients included in the study underwent a detailed history and clinical examination. History of presenting complaints, past illnesses, mode of transmission of HIV and high-risk behaviour was taken. Clinical history regarding current complaints of fever, cough, sputum production, haemoptysis, weight loss was taken. History regarding previous treatment for tuberculosis was also taken. All patients were evaluated for headache, seizures, chest pain, breathlessness and neck swelling or any other evidence of extrapulmonary tuberculosis.

2. **Immune status assessment**
   CD4 lymphocyte counts of all the patients were determined by flow cytometry.

3. **Sputum analysis**
   Sputum microscopy for acid-fast bacilli (AFB) – two samples of at least 1 ml sputum were sent for microscopy 24 hours apart to the chest and TB centre at Lok Nayak Hospital in sterile containers. Sputum smears after Ziehl-Neelsen staining were examined under oil immersion microscopy. A minimum of 1 slide positive even for single AFB/100 fields were taken as positive for Mycobacterium tuberculosis and a minimum of two sputum samples negative for AFB evaluated for 100 fields were declared as negative.

   Sputum for CBNAAT: One sputum sample of 1 ml was collected in a sterile container and was analysed by CBNAAT on Xpert® MTB/RIF manufactured by Cepheid, endorsed by WHO (2010). The sample was diluted with three times the reagent, incubated at room temperature and loaded into the cartridge for automated analysis with results in 100 minutes. Detection of mycobacteria and rifampicin resistance was carried-out in the same setting.

   Rifampicin resistant samples were further analysed by LPA. The three steps for LPA test included DNA extraction, multiplex polymerase chain reaction (PCR) amplification and reverse hybridisation.

**Statistical analysis**

Data analysis was done using SPSS (statistical package for social sciences) software SPSS (version 22.0.0.0). Diagnostic efficacy of sputum microscopy and microscopy were compared with McNemar test.

**Results**

The mean age of the study population was 35 ± 9 years. Most patients (69%) were in the age group of 20 to 40 years as seen in Table I. Majority (76%) of the patients in the study were men, there were 21% women and three transgenders. The mean CD4 count of the subjects was 230 cells/ml. Thirty two patients had CD4 count less than 100 cells/ml. Distribution of CD4 count is summarised in Table II.

**Table I: Age distribution of patients.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number (n)</th>
<th>Percentage (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 - 20</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>21 - 40</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>41 - 60</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>61 and above</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**Table II: CD4 counts and their relation with CBNAAT result.**

<table>
<thead>
<tr>
<th>CD4 count (cells/ml)</th>
<th>CBNAAT Negative Number</th>
<th>CBNAAT Positive Number</th>
<th>Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upto 100</td>
<td>18</td>
<td>14</td>
<td>32</td>
</tr>
<tr>
<td>101 - 200</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>201 - 350</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>351 - 500</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>&gt;500</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Eleven patients out of 100(11%) were positive by sputum microscopy for acid-fast bacilli and 40 (40%) were positive by CBNAAT. Thus, tuberculosis detection rate increased by more than three times using CBNAAT. There is a highly
significant statistical difference in the diagnostic ability of CBNAAT when compared to sputum microscopy (the two-tailed p value is less than 0.001). Results were obtained within 2 hours by CBNAAT, whereas the mean time of detection of sputum microscopy was 2 days. CBNAAT positivity was seen across all ranges of CD4 counts and there was no statistically significant difference between CD4 count of CBNAAT positive and CBNAAT negative patients (p value = 0.264).

CBNAAT also diagnosed 10 (25%) cases of rifampicin resistance among the 40 Mycobacterium tuberculosis positive cases. On LPA, all these were confirmed to be resistant to rifampicin. CBNAAT had 100% specificity for detection of rifampicin resistance. History of previous ATT intake was found in 7 out of 10 (70%) rifampicin resistant patients. LPA further revealed that 9 of 10 patients detected rifampicin resistant by CBNAAT also had isoniazid resistance.

**Discussion**

This study was conducted to evaluate the role of CBNAAT in diagnosing pulmonary tuberculosis in PLHIV and detection of rifampicin resistance among these patients. Tuberculosis is a major challenge for anti-retroviral therapy (ART) services in resource-limited countries like India where patients typically enroll with advanced immunodeficiency. These risks are heightened when patients have MDR-TB. To address these challenges, there is a critical need in such settings for rapid, effective screening for TB and detection of drug resistance for early initiation of appropriate treatment.

Sputum microscopy for AFB is a simple, economical, and easy-to-do test for diagnosing pulmonary tuberculosis. However, as it needs at least 10,000 bacilli per ml to give a positive result and being a highly subjective (operator dependant) test, it’s sensitivity has been shown to range from 20% to 60% under different conditions. This sensitivity is further decreased in PLHIV due to lower rates of caseous necrosis and sputum production. In the present study only 11 patients were found to be sputum positive for AFB by direct microscopy and 29 cases were missed and reported as sputum smear negative. The consequences of this can be several, including delayed or misdiagnosed cases, contributing to delayed treatment with increased morbidity and mortality rates and continued spread of TB to contacts. From Africa, Cattamanchi et al have also reported that the sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 per cent, and in Tanzania, Matee et al found it to be only 55%. In the past, these (false) smear-negative cases would initially be treated with broad-spectrum antibiotics, followed up after 7 - 14 days and re-assessed for TB if symptoms persisted. There would be additional delays to TB treatment if these patients did not come back for a re-assessment or actually felt better with the initial treatment. Delay in initiating ATT in PLHIV is also associated with higher mortality.

CBNAAT, on the other hand, detected 40 patients with pulmonary tuberculosis. Further, rifampicin resistance was detected in 10 patients of which 9 turned out to be MDR-TB by LPA. Both these results were available within 2 hours. Past studies on drug resistance have shown that rifampicin resistance is seldom detected alone and 90% of rifampicin resistant patients turn out to be MDR-TB. In the present study, 9/10 rifampicin resistant samples also demonstrated resistance to isoniazid. Hence CBNAAT can be a useful test for screening for MDR-TB. This is of particular reference to TB endemic areas like India where there is high prevalence of MDR-TB of around 3% in new cases and 12 - 18% in old treated cases, and sputum microscopy is the only screening test used to diagnose tuberculosis.

HIV-TB co-infection has been shown to substantially decrease the sensitivity of sputum microscopy (to 47%), but it does not significantly affect CBNAAT performance. Studies from high HIV endemicity areas in Peru have also shown that HIV status does not affect the performance of CBNAAT. Sensitivity and specificity of CBNAAT were reported to be > 95%. In a study from Cape Town, South Africa, the combination of smear microscopy and CBNAAT had a significantly better sensitivity than smear microscopy alone in patients infected with HIV with a CD4 count less than 200 cells/ml (69.6% vs 39.1%; p = 0.05). Thus CBNAAT was found to be additive over microscopy alone.

There are only a few studies on CBNAAT from India. 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. However, HIV status of the patients was not evaluated in the study. A multicentre assessment at five trial sites in Peru, Azerbaijan, Cape Town, Durban and India by Boehme et al demonstrated sensitivity of nearly 100% by CBNAAT. Under RNTCP, impact study on CBNAAT found that additional 2,493 patients were diagnosed of pulmonary TB by CBNAAT in 2012 among more than 30,000 TB suspects as compared to sputum microscopy.

The WHO policy guidance on the use of CBNAAT was issued in December 2010. The recommendations were that it should be used as the initial diagnostic test in individuals at risk of having MDR-TB or HIV-associated TB (strong recommendation), and that it could be used as a follow-on test to microscopy in settings where MDR and/or HIV is of lesser concern, especially in smear-negative specimens (this was a conditional recommendation).
recognising major resource implications). This recommendation applied to the use of CBNAAT in sputum specimens only, as data on its performance (sensitivity and specificity) for testing of extrapulmonary specimens at that time were limited15.

RNTCP adopted CBNAAT in India in April 2012. In the government set up, CBNAAT was launched in 2012 as a pilot project in Maharashtra by the State tuberculosis department. By the end of 2012, under EXPANDx-TB project, 12 CBNAAT labs were established all over India across different states14. CBNAAT is currently being made available at more centres with the aim to establish it at every hospital associated with a medical college throughout the country and also in private institutions.

Conclusion
CBNAAT detects pulmonary TB in PLHIV with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so that early therapy can be started, thus decreasing the incidence of MDR-TB. WHO recommends CBNAAT for diagnosis of pulmonary tuberculosis and detection of rifampicin resistance, especially in PLHIV and re-treatment cases who are at risk of MDR-TB11.

References