Detection of Rifampicin Resistance in HIV Seropositive Individuals with Suspected Pulmonary Tuberculosis by Using CBNAAT

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Tuberculosis (TB) remains the most common opportunistic infection among people living with HIV (PLHIV) and diagnosing it becomes a challenging task as sputum microscopy is negative in more than half of the patients. Delayed treatment for TB in PLHIV is associated with increased mortality. Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a recently introduced Polymerase chain reaction based method for detection of Mycobacterium tuberculosis, which also detects Rifampicin resistance as it targets the rpoB gene of mycobacteria. To determine the prevalence of Rifampicin resistance in HIV seropositive patients with suspected pulmonary tuberculosis in a tertiary care hospital by using CBNAAT. HIV seropositive patients with clinically suspected tuberculosis were included in the study. Two sputum samples from each patient were collected and subjected to sputum microscopy by LED-fluorescent Microscope. Detection of mycobacteria and Rifampicin resistance was carried out by CBNAAT on Gene Xpert MTB/RIF. Out of the total 576 sputum, 74 (12.84%) patients were positive by sputum microscopy for acid fast bacilli and 137 (23.78%) were positive by CBNAAT. Rifampicin resistance was detected in 12 (8.75%) cases. CBNAAT helped in increased case detection in lesser time as compared to 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, CBNAAT, Rifampicin resistance, HIV sero-positive.

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, particularly in the HIV-infected population.

Tuberculosis remains the most common opportunistic infection among people living with HIV. People living with HIV are 20 to 37 times more likely to develop tuberculosis than those without HIV and Tuberculosis accounted for 1 in 4 deaths among HIV-positive individuals. If diagnosis and appropriate therapy are delayed, mortality is as high as 70% to 87%.

Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014: 5.4 million men, 3.2 million women and 1.0 million children. Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive.

Standard sputum based methods to detect pulmonary tuberculosis include sputum microscopy and culture. Sputum smears are stained with Ziehl-Neelsen (ZN). Each smear examination
requires on average 5–10 min, creating considerable workloads for laboratories with limited resources. An alternative technique to ZN smear microscopy, fluorescence microscopy (FM), is reported to be more sensitive than ZN smear microscopy and FM smears can be examined in a fraction (about 25%) of the time needed for ZN smears. However, in people living with HIV, 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.

This delays initiation of anti-tubercular treatment especially for drug-resistant forms of Tuberculosis, increases risk of transmission of (drug-resistant) tuberculosis in the community and increases the risk of spread to extrapulmonary sites within the patient. Culture is a more sensitive way to detect paucibacillary forms of Tuberculosis, which are common in HIV patients and children. However, in several resource-limited settings, mycobacterial culture is inaccessible to the majority of the population. Even when available, the conventional solid culture on Lowenstein–Jensen media (L–J) is time-consuming and growth of mycobacteria is typically detected after 4-8 weeks. Not economical for screening purpose. This is associated with delayed detection and initiation of TB treatment, and a higher mortality rate compared with patients who are smear-positive.

Since 2007, the pipeline for new Tuberculosis diagnostics has expanded. In parallel, WHO has endorsed several new tests for increased and more rapid tuberculosis diagnosis.

The molecular test XpertMTB/Rif (Cepheid GeneXpert System, Sunnyvale, CA) is based on quantitative real-time PCR for detection of Tuberculosis. This is the first molecular method for Tuberculosis detection to be fully automated and to integrate all the steps required for PCR-based DNA testing which is a Cartridge-based nucleic acid amplification test (CBNAAT). It also detects Rifampicin resistance as it targets the rpoB gene of mycobacteria. Results are reported within three hours and with high accuracy. Both processed and unprocessed sputum can be used in this test.

The emergence of multidrug-resistant Tuberculosis (MDRTB) and extensively drug-resistant TB (XDR-TB) in the past decade has highlighted the urgent need for both accurate diagnosis and Drug susceptibility testing. Approximately 3.6% of all new TB cases are caused by MDR strains, of which 10% are XDR-TB.

Present study was carried out to determine the prevalence of Rifampicin resistance in HIV seropositive patients with suspected pulmonary tuberculosis in a tertiary care hospital by using CBNAAT.

**MATERIALS AND METHOD**

A cross-sectional study. HIV seropositive patients with clinically suspected tuberculosis during the period between January 2013 and December 2013 were included in the study.

The study group consisted of HIV seropositive individuals with productive cough for 2 weeks or more, and/or chest X-ray findings suggestive of pulmonary tuberculosis.

HIV seropositivity was detected as per NACO guidelines strategy III using COOMBS AIDS, SD BIOLINE and HIV SIGNAL kits.

All patients included in the study underwent a detailed history and clinical examination. History of presenting complaints, regarding current complaints of fever, cough, sputum production, haemoptysis, weight loss, past illnesses, previous treatment for tuberculosis was recorded.

All patients were evaluated for headache, seizures, chest pain, breathlessness and neck swelling or any other evidence of extrapulmonary tuberculosis.

Sputum microscopy for acid-fast bacilli (AFB) – As per RNTCP protocol, two samples of at least 1 ml sputum in sterile containers were sent to the Culture & DST centre TB Unit, Karnataka Institute of Medical Sciences, Hubli. Specimens were assessed macroscopically, and smears were prepared in duplicate for staining by Auramine O, counterstained with potassium permanganate, and examined by Fluorescent Microscopy (FM). LED-FM smears (in accordance with advice from the International Union Against Tuberculosis and Lung Disease Working Group on Smear Microscopy) examined at 200x, with confirmation of the results.
of positive smears at 400x magnification. Smears were classified as positive when ≥1 AFB was detected per 100 fields, and patients were considered smear-positive if they had ≥1 positive smear.

Sputum for CBNAAT (GeneXpert)-Sputum sample was analysed by CBNAAT on Gene Xpert® MTB/RIF manufactured by Cepheid. Detection of mycobacteria and Rifampicin resistance was carried-out in the same setting.

A sample reagent was added to the specimen in a 3:1 ratio. The mixture was incubated at room temperature for 15 minutes and was manually agitated. A total of 1 ml of sample was introduced into cartridge, which was then loaded into the GeneXpert instrument, where the subsequent steps of sample lysis, nucleic acid extraction, and amplification occurred automatically with results in 100 minutes.

A total of 576 Sputum samples collected were subjected to smear microscopy (Fluorescent microscopy) and Cartridge Based Nucleic Acid Amplification Assay (CBNAAT).

**RESULTS**

The age group of study population ranged from 10 to 70 years. The average age of the study population was 35 ± 5 years. Most patients (37.96%) were in the age group of 30 to 39 years. Majority (64.96%) of the patients were men. Female to Male ratio is 1:1.85

Seventy four patients out of 576(12.84%) were positive by sputum microscopy for acid-fast bacilli and 137 (23.78%) were positive by CBNAAT. Tuberculosis detection rate increased by more than two times using CBNAAT. There is a highly significant statistical difference in the diagnostic ability of CBNAAT when compared to sputum microscopy. Results were obtained within 2 hours by CBNAAT, whereas the mean time of detection of sputum microscopy was 2 days. CBNAAT also diagnosed 12 (8.75%) cases of Rifampicin resistance among the 137 Mycobacterium tuberculosis positive cases.

<table>
<thead>
<tr>
<th>Smear status</th>
<th>Smear microscopy</th>
<th>CBNAAT positive</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear positive</td>
<td>74</td>
<td>74 (100%)</td>
<td>S(at P value &lt; 0.05)</td>
</tr>
<tr>
<td>Smear negative</td>
<td>502</td>
<td>63 (12.54%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>576</td>
<td>137 (23.78%)</td>
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**DISCUSSION**

The HIV epidemic has led to large increase in the frequency of smear negative pulmonary tuberculosis which has poor treatment outcomes and excessive early mortality compared with smear positive disease.

HIV-positive individuals have a higher rate of smear-negative disease because they are less likely to have cavitary lesions due to the impairment of granuloma formation. Sensitivity of sputum microscopy in HIV ranges from 43% to 51%.

Fluorescence microscopy (FM), is reported to be 10% more sensitive than conventional ZN smear microscopy [2]. Since fluorescent acid fast bacilli (AFB) can be seen at lower magnification than ZN-stained AFB, FM
smears can be examined at a faster rate compared to time needed for ZN smears.

WHO definition of a case of smear negative pulmonary tuberculosis is sputum specimens negative for AFB, abnormalities on radiography consistent with active tuberculosis, no response to broad spectrum antibiotics.7

The World Health Organization (WHO) estimates that 8.7 million people develop tuberculosis (TB) each year worldwide. Of these, 13% are co-infected with HIV, while of the 1.4 million deaths that occur, 30% are HIV-related.5

Approximately 24% to 61% of HIV and TB co-infected patients are smear negative. 30-60% of people with HIV infection may die with tuberculosis often undiagnosed.8

In our study, 576 HIV positive patients were screened for TB as per WHO recommendations. Of these only 74 (12.85%) were smear positive and 502 (87.15%) smear negative. These patients have a higher mortality rate, probably due to profound immune suppression as well as delayed diagnosis. Furthermore, HIV-positive individuals often do not manifest typical symptoms of TB (prolonged cough, fever, night sweats, weight loss). HIV-positive patients also are more likely to have extrapulmonary TB than HIV negative patients. Although 40% of patients with extrapulmonary TB may have concurrent pulmonary TB, the most widely available method of diagnosis, sputum smear microscopy, is of little diagnostic value for the remaining 60%.2 Delay in initiating ATT in PLHIV is also associated with higher mortality.

A study conducted by Sonnberg P, et al. reported that, as compared to people without HIV, people living with HIV have a 20-fold higher risk of developing TB and the risk continues to increase as CD4 cell counts progressively decline.9

The WHO recommends TB screening at the time that HIV infection is diagnosed, before the initiation of antiretroviral therapy and at regular intervals during follow up.10

In the present study all 74 smear positive samples were detected by CBNAAT (100% sensitive). Of the 502 smear negative, 63 (12.5%) were detected as TB positive by CBNAAT. Further, Rifampicin resistance was detected in 12 (8.75%).

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. 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.1

Substantial efforts are made to strengthen lab capacity to diagnose smear negative and MDR tuberculosis including use of solid and liquid culture, conventional DST, Line probe assay. Unfortunately these require extensive lab infrastructure and can be done only in reference lab.8

A realtime PCR assay that simultaneously detects Rifampicin resistance was developed on the GeneXpert platform, which integrates sample processing and greatly simplifies testing. This assay showed excellent performance in a multicentric study undertaken in reference lab.6,11

WHO endorsed the use of GeneXpert-Rif for rapid diagnosis of TB as well as rifampicin resistance among HIV-infected individuals with clinical suspicion of TB in 2010.12

Decentralised MTB/RIF test implementation is feasible and could lead improvement in tuberculosis care and control.6

The MTB/RIF test assay was designed specifically for use close to point of treatment in endemic disease settings and is the first of a new generation diagnostic tests that have potential to bring highly sensitive nucleic acid amplification testing to peripheral sections of the health system.

The only specimen processing required is the addition of a sample reagent that is bactericidal and results in 10^7 reduction in viable mycobacteria in the first 15min.

Unlike smear microscopy, the manual pipetting steps and automated portion of the assay do not generate viable mycobacterial aerosols. Together these results suggest that MTB/RIF test can be done without special biosafety precautions.8

In a study conducted by Andrea Rachow et al Xpert MTB/RIF Assay achieved 88.4% (95%CI = 78.4% to 94.9%) sensitivity among patients with a positive culture and 99% (95%CI = 94.7% to 100.0%) specificity in patients who had no TB. HIV status did not affect test performance (58.9% of all participants). Within non tuberculosis mycobacteria the assay’s specificity was 97.8%
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(95%CI = 88.2% to 99.9%). In a study by C.N. Deivanayagam, MDR-TB was detected in 33.3% patients. HIV seropositivity among MDR-TB was 4.42%. Significantly, 24.5% patients had tubercle bacilli resistant to one or more reserve drugs too (ethionamide, kanamycin and/or ofloxacin).14

Steingart K R et al., concluded that Xpert MTB/RIF assay showed a sensitivity of 95% and specificity of 98%; in which the prevalence of MDR TB was 5%, with a Positive predictive value (PPV) of 71% and Negative predictive value (NPV) of 100%.15

The association between MDR-TB and HIV infection remains unclear, but data from Estonia, Latvia, and Moldova suggest that HIV-positive individuals there are at higher risk for acquiring MDR-TB.2

Study in Peru showed that 43% of HIV&TB-coinfected patients had MDR-TB, compared with 4% of HIV-negative TB patients.16The overall sensitivity of XpertMTB for culture positive TB was 73.3% & specificity 99.2% compared to 28% & specificity 100% using smear microscopy.17Sensitivity & specificity for detection of Rifampicin resistance was 94.45% & 98.3%.8 According to a study by C.Padmapriyadarsini, et al, the sensitivity and specificity of Xpert MTB/RIF assay compared to culture were as follows: Sensitivity for AFB+/culture+ 98.2%, Sensitivity for AFB-/culture 72.5%, Specificity 99.0% Rifampicin resistance detection Sensitivity – 99.1%, Specificity – 100% .18

Studies have reported test sensitivity of 72-75% in case of smear negative tuberculosis and 98-100% in case of smear positive tuberculosis.8

The robustness of these data suggests that the test can be used in various resource scarce settings for detection and rapid decentralized screening of MDR in peripheral settings, including among patients with HIV is a break through in tuberculosis care & control.8

Several issues might restrict the applicability of the MTB/RIF test at small centres. The device requires stable electricity supply. Cartridges are confirmed to be stable at 2-28°C. The Gene Xpert device needs calibration yearly.8

The development of rapid, simple and accurate tuberculosis diagnostic tool with applicability at point of care & remote location is essential. To achieve these goals, greater political commitment, scientific interest and investment are needed.7

Conclusion: In this population of individuals, at high risk of tuberculosis, intensive screening using Gene Xpert increased case detection by 10.93% compared with smear microscopy. Prevalence of Rifampicin resistance was 8.75% in pulmonary tuberculosis with HIV seropositive patient. CBNAAT is simple and rapid identification system which is essential for more effective public health intervention.

REFERENCES


