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Role of GeneXpert® MTB/RIF assay for early diagnosis of pulmonary tuberculosis in people living with HIV

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Abstract:

Introduction: Tuberculosis (TB) is one of the commonest opportunistic infections and the leading cause of death in HIV patients in developing countries. Diagnosing tuberculosis (TB) in people living with HIV/AIDS is challenging as sputum microscopy is negative in more patients due to lack of caseous necrosis. This study was done to find out the co-prevalence of TB among HIV infection patients, to determine the prevalence of MDR tuberculosis in HIV patients using GeneXpert MTB/RIF assay and comparing GeneXpert efficacy with fluorescent microscopy.

Materials and methods: Four hundred and thirty three patients samples with age > 20 years having symptoms suggestive of pulmonary TB in HIV patients were included in this cross sectional study. Sputum for fluorescent microscopy and GeneXpert were processed. Rifampicin resistance was detected by GeneXpert.

Results: Out of 433 samples**,** 36 sputum samples were positive by GeneXpert and 28 samples were positive by fluorescent microscopy. 'p' value was (<0.001) statistically significant, 5 cases of Rifampicin resistance were detected by Genexpert.

Conclusion: GeneXpert helps in increased early case detection in lesser time to diagnose pulmonary TB in people living with HIV as compared to fluorescent microscopy and also to detect Rifampicin resistance with high specificity; it can be used for screening for MDR-TB for starting anti-tubercular treatment early.

Key words: Tuberculosis, people living with HIV (PLHIV), GeneXpert, fluorescent microscopy, multi-drug resistant tuberculosis

Introduction:

In the global tuberculosis report (2015), W.H.O. reported that, 10.4 million people developed TB, including 1.2 million cases among people who were HIV positive. Global burden of multi-drug resistant TB (MDR-TB) was estimated to be 4,80,000 cases leading to estimated 210,000 deaths[1]. 25% of global annual TB incidents occur in India making it highest tuberculosis burden country[2]. Prompt and accurate TB diagnosis are prerequisites for early and effective treatment thereby reducing the tuberculosis burden[2].

In PLHIV, there is scanty sputum production, lack of caseous necrosis leading to decreased number of bacilli in sputum and also high incidence of non-tubercular mycobacterial infection .These factors decrease the sensitivity and specificity of sputum microscopy as a diagnostic tool[3]. Sputum culture and sensitivity takes 4-8 weeks. 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 extra-pulmonary sites within the patient[4].

GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA ,USA) 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. GeneXpert is a *Mycobacterium tuberculosis*-specific automated, GeneXpert/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 [2,3].

This study was carried out to evaluate the role of CBNAAT/GeneXpert in early diagnosis of TB in PLHIV and detection of *M. tuberculosis* in sputum by CBNAAT compared to fluorescent microscopy in pulmonary TB.

Objectives:

1. To evaluate the role of Gene Xpert MTB/RIF assay in early diagnosis of tuberculosis in people living with HIV.
2. Comparing the efficacy of sputum fluorescent microscopy and GeneXpert MTB/RIF assay.
3. Detection of Rifampicin resistance.

Materials and Methods:

Cross sectional comparative study was conducted in Sri Chamarajendra Hospital, Hassan Institute of Medical Sciences, Hassan. Suspected pulmonary tuberculosis in HIV patients were referred to the microbiology laboratory for sputum microscopy and GeneXpert assay from Pulmonary Medicine Department from July 2016 to December 2016.

Inclusion criteria:

Suspected cases of pulmonary tuberculosis in people living with HIV**.**

Exclusion criteria:

All cases of pulmonary and extra-pulmonary tuberculosis without HIV.

Sputum microscopy/ Fluorescent staining: 1 ml sputum was collected for microscopy in a sterile container.

Procedure:

1. The slide was placed on rack with smear facing up.
2. Smear was flooded with freshly filtered auromine O and allowed to stand for 20 minutes, then rinsed in running water.
3. Then it was decolourised by covering completely with acid alcohol solution for 3 minutes, rinsed well with water.
4. Counterstain with 0.5% Potassium permanganate, then examine under fluorescent microscope.

GeneXpert:

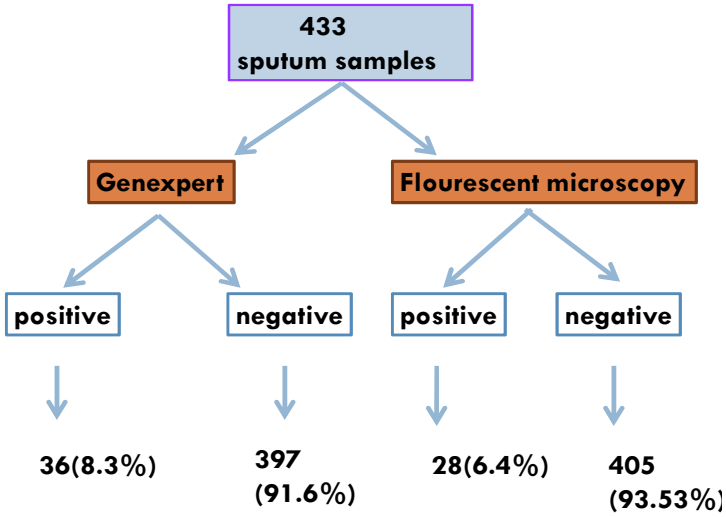
1. One ml sputum sample was collected in a sterile container and analysed by Gene Xpert® MTB/RIF manufactured by Cepheid.
2. The sample was diluted three times with the reagent, incubated at room temperature and loaded into the cartridge for automated analysis.
3. Detects DNA sequence specific for *Mycobacterium tuberculosis* and Rifampicin resistance by polymerase chain reaction and both were carried out in the same setting and results are obtained in 100 minutes.

Results:

Total sputum samples collected in PLHIV=433 from 321 male patients and 112 female patients. The mean age population was 35 ± 15 years; most patients (50%) were in the age group of 20 to 60 years as seen in **Table I**. Out of 433 sputum samples, majority of the patients in the study were(67%) men, and the rest were (33%) women; 36 were positive by GeneXpert and 5 showed Rifampicin resistance. Twenty eight specimens were positive by fluorescent microscopy (**Figure I**).

Table I: Age distribution

Age	Number	Percentage
1-20	0	0
21-40	18	50
41-60	18	50
>60	0	0



CHI SQUARE TEST

- 'p' value =0.0001 , <0.05 significance 'p' value

Discussion:

Tuberculosis in HIV is a leading cause of death worldwide. Globally, 12% TB cases in 2014 were HIV positive. End TB strategy is to reduce 90% deaths by 2030, reduce new cases by 80% and ensure that no family is burdened with catastrophic costs due to TB. GeneXpert/CB-NAAT is a new operational system recommended by W.H.O. after evaluating various studies. W.H.O. reported that the sensitivity in detecting TB was 70%-100% in culture positive patients and around 60% in those with smear negative disease and specificity ranged from 91%-100% and average Rifampicin sensitivity and specificity was around 98% and 99% [5].

In our study, males were affected more(67%) and common age group was 20-60 years; these results are similar to a study conducted by Desai K et al [6]; in this study, males were affected more (64.86%) and common age involved was 15-45years. Males are probably commonly affected due to their behavioural risks.

Bhatt CP et al [7] also found rates higher in males than females and fluorescent microscopy sensitivity at 73.9%.

A study Cattamanchi A et al [8], showed fluorescent microscopy sensitivity at 71% in HIV patients with suspected pulmonary tuberculosis and 77% sensitivity in HIV uninfected cases. Specificity was significantly lower (76%) in HIV and 98% in HIV uninfected cases; our study showed 77.7% sensitivity by fluorescent microscopy.

In a study conducted by Nicol MP et al [9] in 2009, MTB/RIF shows 100% sensitivity and specificity in HIV infected children with suspected pulmonary TB, both in one induced sputum and two induced sputum specimen and fluorescent microscopy showed 100% specificity in both with sensitivity of 38.6% and 37.9% respectively, In our study, GeneXpert MTB/RIF showing similar sensitivity and specificity and fluorescent microscopy sensitivity was more.

GeneXpert MTB/RIF assay is a molecular method, the sensitivity depends on the burden of organisms and thereby the target DNA present in the specimen. The published GeneXpert MTB/RIF detection

threshold is approximately 100-130 colony forming units (c.f.u.)/ml of sample [10, 11].

In the present study, GeneXpert shows high sensitivity than fluorescent microscopy and shows similar specificity. Increased use of molecular tests for TB helps early diagnosis and initiation of treatment and other measures to reduce TB transmission, which pays large dividends for global TB control [12,13].

GeneXpert identifies many smear-negative TB patients whom clinicians would otherwise fail to diagnose. CBNAAT sensitivity evaluated in our study was modest; it was also found that there was decreased time-to treatment initiation in smear-negative TB patients by almost four weeks. Smear-negative TB may account for upto half of all TB cases in endemic regions [1].

Patel and colleagues observed a similar threshold of 80–100 c.f.u./ml of CSF in this study [14]. The detection threshold is 10 c.f.u./ml for mycobacterial liquid culture and is 5000 c.f.u./ml for Ziehl-Neelsen staining for acid fast bacilli (AFB) via standard microscopy in sputum and for fluorescent microscopy 100-1000c.f.u./ml [12, 13, 15].

In real world clinical terms, this means that there is 98%–99% detection by GeneXpert MTB/RIF of AFB smear-positive pulmonary TB, and approximately 75% detection of smear-negative, culture-positive pulmonary TB[2,10,16]. So GeneXpert can be used as first line diagnostic method.

In India, the government has provided GeneXpert eqiupment under the Revised National Tuberculosis Control Program (RNTCP) to recognised medical centers and services can be availed free of cost thus making it a cost effective investigation for the detection of *Mycobacterium tuberculosis* and rifampicin resistance.

Conclusion:

We carried out a cross sectional comparative study to evaluate the accuracy and potential effectiveness of nucleic acid amplification testing for TB diagnosis in a hospitalized population with a high incidence of HIV and results showed excellent sensitivity, specificity, and a potential clinical impact. So, first line diagnostic modality technique can be used to diagnose TB accurately in peripheral laboratories in developing countries.

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