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Diagnostic Accuracy of Gene Xpert vs Acid Fast Smear in HIV Patients Suspected for Tuberculosis

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Abstract
The risk of developing tuberculosis (TB) is found to be 20 times greater in people living with Human immunodeficiency virus (HIV) than among those without HIV infection.
Objective: *To evaluate the accuracy of Gene Xpert for detection of MTB in HIV patient suspected for tuberculosis and comparing with conventional acid fast smear examination.*
Methodology: *The study was conducted in Medicine OPD and Pulmonology OPD of R L Jalappa Hospital, Tamaka, and Kolar. ART center and RNTCP center, SNR Government Hospital.*
Study Design: *Cross sectional study.*
Study Period: *1 year.*
Sample Size: *225 subjects*
Results: *Smear microscopy has Sensitivity 45.03%, Specificity 100%, NPV 11.76% and PPV 100% for pulmonary specimens, Smear microscopy has Sensitivity 16.67%, Specificity 100%, NPV 44.44% and PPV 100% for extra pulmonary specimens. Overall Smear microscopy has Sensitivity 43.35%, Specificity 100%, NPV 16.06% and PPV 100%.Gene Xpert has Sensitivity 87.43%, Specificity 92.86%, NPV 35.14% and PPV 99.40% for pulmonary specimens, Gene Xpert has Sensitivity 83.33%, Specificity 100%, NPV 80% and PPV 100% for extra pulmonary specimens. Overall Smear microscopy has Sensitivity 87.19%, Specificity 95.45%, NPV 44.68% and PPV 99.44%.*
Conclusion: *The Gene Xpert MTB is a rapid, sensitive and a reliable diagnostic test for TB than smear microscopy in both pulmonary as well as extra pulmonary TB.The results of this study indicate that the implementation of the Gene Xpert (MTB/RIF) assay could dramatically improve the rapid diagnosis of extra pulmonary tuberculosis as well in HIV infected patients.*
Keywords: *Smear Microscopy, Xpert MTB, HIV, Pulmonary Tuberculosis, Extra Pulmonary Tuberculosis.*

Introduction

Mycobacterium tuberculosis (MTB) among the infectious diseases is the number one killer worldwide[1]. In 2017, new cases of ruberculosis (TB) were estimated around 10.0 million (9.0–11.1 million) of which 0.88 million patients were *Human immunodeficiency virus* (HIV) positive. 87% of all incident cases of TB occurred in the 30 high TB burden countries, India accounting for 27% of these cases. HIV positive TB incidence in

India was estimated around 86,000. India accounts for about 10% of the global burden of HIV-associated TB ranking 2nd in the world. Mortality in this group is very high and every year 11,000 people succumb to the disease.[1,2]

Human immunodeficiency virus (HIV) infection is the most important risk factor for developing tuberculosis. The risk of developing tuberculosis (TB) is found to be 20 times greater in people living with HIV than among those without HIV infection.[1,3] Patients with HIV have a higher incidence of active tuberculosis as HIV increases both the risk of reactivating latent TB infection, as well as the rapid TB progression after infection. [4] They also potentiate each other by mechanisms that disrupt immunological functions. [3] The annual risk of developing TB in patients with HIV can exceed 10% compared to a lifetime risk of 10-20% in HIV negative patients. [5]

TB is the most common cause of death in HIV-positive individuals living in developing countries, despite being a preventable and treatable disease.[6] There were an estimated 1.3 million (1.2–1.4 million) deaths from TB among HIV-negative people, and an additional 300,000 (266,000–335,000) deaths among HIV-positive people, most of them in developing countries. [1] The most important reason for this high fatality is the difficulty in diagnosing tuberculosis in the HIV population. Among the 2,826,486 pulmonary TB cases that were notified in 2017, only 59% were bacteriologically confirmed. [1]Accurate identification and prompt treatment of tuberculosis infection is a priority as this will prevent deaths and decrease morbidity.

Previously smear examination, culture were the main methods used in detecting tuberculosis. Sputum smear microscopy still remains the basis for diagnosis of pulmonary TB in developing countries for its fast and relatively inexpensive results. [7] However, around 10,000 bacilli/ml of sputum needs to be present for detection. This results in poor and varying sensitivity ranging from 20% to 80%, especially in HIV infected people with higher rates of smear-negative disease.[7,8] Many new tools and methods have been studied to improve the sensitivity, yet still inadequate in HIV-TB coinfection.[9]

Culture of *Mycobacterium tuberculosis* still remains the gold standard for diagnosis and permits the diagnosis of drug resistance, including the emerging mutations, but it has a long turnover time (6-8 weeks for solid and 1-2 weeks for liquid media).[6,9] Such diagnostic delay is unacceptable given the possibility of further spread of the disease, high morbidity and mortality among HIV-TB patients.

The diagnosis of tuberculosis still remains a challenge especially in HIV positive patients. Therefore, there is an urgent need for implementing new diagnostic methods for tuberculosis in resource-limited setting with high HIV prevalence.[10]

To close the gaps in detection and treatment the Revised National Tuberculosis Control Programme (RNTCP) has adopted certain technological advances recommended by WHO in its national network of designated microscopy centers and intermediate reference laboratories to direct appropriate use of limited resources for tuberculosis (TB) control.[6,11] These include Cartridge-based nucleic acid amplification test (CB-NAAT), also called Gene Xpert and Line probe assay (LPA).[7]

Gene Xpert (Xpert MTB/RIF) is a molecular cartridge-based nucleic acid amplification test which simultaneously detects both TB and resistance to rifampicin. The limit of detection is 5 genome copies of purified DNA per reaction or 131 colony forming units per mL of sputum [12] Rifampicin resistance is reported on the basis of late or absent probe signals by the Xpert MTB/RIF software. [13] It is automated and provides results within 2 hrs.[9]

However, despite all data available about detection of TB by microscopy and Gene Xpert, there is sparse data on the accuracy of diagnosis of Tuberculosis by Gene Xpert in HIV patients. So we take this potential scope to find out the accuracy of Gene Xpert over sputum microscopy

in early detection of tuberculosis in HIV positive individuals.

Objective

To evaluate the accuracy of Gene Xpert for detection of MTB in HIV patient suspected for tuberculosis and comparing with conventional acid fast smear examination.

Materials and Methods

Study Setting: The study was conducted in Medicine OPD and Pulmonology OPD of R L Jalappa Hospital, Tamaka, and Kolar. ART center and RNTCP center, SNR Government Hospital**.**

Study Design: Cross sectional study**.**

Study Period: 1 year

Sample Size: 225 subjects

Method of Data Collection

This study was conducted in Kolar which has a high incidence of tuberculosis.

Inclusion Criteria

Diagnosed cases of HIV who are registered with ART center suspected for pulmonary and extra pulmonary tuberculosis are taken for the study. HIV patients with cough of any duration, weight loss, fever, other respiratory and constitutional symptoms, radiological findings consistent with pulmonary tuberculosis and any findings consistent with extra pulmonary TB are included.[6]

Exclusion criteria-

Patients who had been treated with anti-TB drugs previously are excluded.

Processing of Samples

Two sputum samples (1 spot and 1 morning) were collected and at the same time another sputum sample was collected in falcon's tube. Samples such as pleural fluid, ascitic fluid, cerebrospinal fluid, lymph node biopsy were collected as per symptoms (for extra pulmonary TB).

All samples were subjected to acid fast smear, GeneXpert and culture inoculation at RNTCP certified lab where it is done free of cost.

1. AFB smear microscopy by ZN staining following the WHO recommended protocol.

2. Gene Xpert: Samples were processed directly from falcon tube. Sample reagent is added in a 2:1 ratio to unprocessed sample in 15 ml falcon tube and the tube is manually agitated twice during a 15minute incubation period at room temperature. 2 ml of the inactivated material is transferred to the test cartridge by a sterile disposable pipette (provided with kits). The interpretation of data from MTB/RIF tests is software based and not user dependent.

3. Culture: Samples were inoculated in Mycobacteria Growth Indicator Tube (MGIT) which is a liquid culture. MTB was identified by colony morphology and growth rate. Culture was taken as the reference standard.

Statistical Analysis

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data represented as mean and standard deviation. The sensitivity and specificity of each test was be calculated. P value (Probability that the result is true) of <0.05 is considered as statistically significant after assuming all the rules of statistical tests. Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) is used to analyze data

Results

In our study we had included 225 HIV patients with suspected for pulmonary and extra pulmonary tuberculosis.

Table 1: Distribution of subject according to age group

Age Group	Frequency	Percent
1-20yrs	10	4.4
21-40yrs	123	54.7
41-60yrs	78	34.7
61-80yrs	14	6.2
Total	225	100.0

Majority 54.7% of the subjects were between 21-40yrs age group followed by 41-60yrs age group

about 34.7%, 6.2% were above 60yrs and only 4.45 were between 1-20yrs .Mean age of subjects was 40.01 ±12.65 yrs

Table 2: Distribution of subject according to Sex

SEX	Frequency	Percent
Female	109	48.4
Male	116	51.6
Total	225	100.0

Majority 51.6% of the subjects were male and 48.4% were female.

Table 3: Distribution of subject according to Specimens

Specimens	Frequency	Percent
Pulmonary	205	91.1
Extra pulmonary	20	8.9
Total	225	100.0

Out of 225 subjects 205 samples (91.1%) were obtained from pulmonary and only 20samples (8.9%) were obtained from extra pulmonary.

Among pulmonary samples 191 samples were culture positive and 14 pulmonary samples were culture negative. Among extra pulmonary samples 12 samples were culture positive and 8 extra pulmonary samples were culture negative.

Overall out of 225 samples 203 were culture positive and 22 were culture negative.

Using culture as a reference standard, the sensitivity, specificity, NPV and PPV for smear microscopy and Gene Xpert are calculated separately for pulmonary and extra pulmonary samples.

Table 4: Comparison of sensitivity, specificity, NPV and PPV for smear microscopy and gene Xpert with respect to pulmonary and extra pulmonary

		Pulmonary	Extra Pulmonary	Overall
Sensitivity	Smear	45.03%	16.67%	43.35%
	Gene Xpert	87.43%	83.33%	87.19%
Specificity	Smear	100%	100%	100%
	Gene Xpert	92.86%	100%	95.45%
NPV	Smear	11.76%	44.44%	16.06%
	Gene Xpert	35.14%	80%	44.68%
PPV	Smear	100%	100%	100%
	Gene Xpert	99.40%	100%	99.44%

Smear microscopy has Sensitivity 45.03%, Sspecificity 100%, NPV 11.76% and PPV 100% for pulmonary specimens, Smear microscopy has Sensitivity 16.67%, Sspecificity 100%, NPV 44.44% and PPV 100% for extra pulmonary specimens. Overall Smear microscopy has Sensitivity 43.35%, Sspecificity 100%, NPV 16.06% and PPV 100%.

Gene Xpert has Sensitivity 87.43%, Sspecificity 92.86%, NPV 35.14% and PPV 99.40% for pulmonary specimens, Gene Xpert has Sensitivity 83.33%, Sspecificity 100%, NPV 80% and PPV 100% for extra pulmonary specimens. Overall Smear microscopy has Sensitivity 87.19%, Sspecificity 95.45%, NPV 44.68% and PPV 99.44%.

Discussion

Mycobacterium tuberculosis is the leading cause of death in HIV patients from infectious diseases.[1] The combination of HIV and *M. tuberculosis* infection increases the mortality rate resulting from infectious disease. In order to curb the menace due to *M. tuberculosis* there is need for speedy, accurate laboratory diagnosis which will eventually lead to adequate treatment. This study was aimed at carrying out comparative analysis of acid fast smear and Gene Xpert techniques for the diagnosis of tuberculosis in patients living with HIV.

Sputum smear microscopy is a simple and an inexpensive tool for diagnosing pulmonary TB but it has low and variable sensitivity. The sensitivity of smear microscopy in the diagnosis of pulmonary tuberculosis is 45.03% in our study. A study done by Jean Claude Semuto Ngabonziza et

al which had 648 participants showed 51% sensitivity of smear microscopy which is comparable to our study result.[14]

The sensitivity of acid fast smear microscopy in the diagnosis of extra pulmonary tuberculosis is 16.67% in our study. A Study done by Soumitesh Chakravorty et al has also shown alower sensitivity of 18.75% (3/16) when extra pulmonary TB samples were tested by smear microscopy.[15]

The sensitivity of Xpert MTB in diagnosing pulmonary TB in our study is 87.43% with a positive predictive value (PPV) of 99.4%.The results Xpert MTB sensitivity of our study are comparable to the WHO statistics which mentions the sensitivity of Xpert MTB for detecting culture-positive samples was 77.7%.[16] Xpert MTB is reasonably specific (92.86%) in excluding the pulmonary tuberculosis but with a poor negative predictive value. Hence the samples with a strong suspicion of TB which are tested negative by the Xpert MTB must be subjected to culture of MTB.

The sensitivity of Xpert MTB in our study is higher than the smear microscopy while in a study carried by Anwar sheed khan et al has shown a sensitivity 40-50% higher than microscopy technique.[17] Our study depicts similar results regarding sensitivity and specificity.

Sensitivity of Xpert MTB in diagnosing extra pulmonary TB in our study is 83.33% which is in parity with the WHO published report from a pooled sample which has shown a sensitivity of Xpert MTB in diagnosis of extra pulmonary TB as 66.1%.[16]

Mycobacterium tuberculosis examined in people living with HIV reveals that patients who were negative toacid fast smear (Ziehl-Neelsen staining) were found positive to Gene Xpert test. This could be due to the ability of Gene Xpert to detect very low numbers of bacilli compared to acid fast smear which may require higher number of bacilli. Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing acid fast smear microscopy.

Extra pulmonary tuberculosis is more common in HIV-infected patients than in the general population regardless of the CD4 lymphocyte count and has been associated with high morbidity and mortality.[18]Diagnosis of extra pulmonary tuberculosis in HIV and non-HIV-infected patients is challenging because of the lack of rapid diagnostic tools, especially in limited-resource settings where the traditional smear microscopy is frequently the only method available[19–21]. Although Xpert MTB/RIF was initially validated only for pulmonary specimens, the result of this study shows that the Xpert MTB/RIF assay can increase more than five times the rapid diagnosis of extra pulmonary tuberculosis compared to acid fast microscopy.

Conclusion

The Xpert MTB is a rapid, sensitive and a reliable diagnostic test for TB than smear microscopy in both pulmonary as well as extra pulmonary TB. The results of this study indicate that the implementation of the Xpert MTB/RIF assay could dramatically improve the rapid diagnosis of extra pulmonary tuberculosis as well in HIV infected patients.

The integration of molecular techniques such as Gene Xpert in the final identification of *M. tuberculosis* complex/*M. tuberculosis*is recommended as a routine practice as compared to smear examination as this can provide timely intervention in the diagnosis of tuberculosis and treatment in HIV positive patients.

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Declarations

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