

Original article

COMPARISON OF GENEXPERT MTB/RIF ASSAY AND AFB SMEAR MICROSCOPY IN DIAGNOSIS OF PULMONARY TUBERCULOSIS AMONG HIV PATIENTS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Tuberculosis (TB) has been the most common acute opportunistic infection in HIV-positive patients and accounts for more than half of all AIDS cases in underdeveloped nations. Hence, rapid laboratory diagnosis of *M. tuberculosis* is needed for vulnerable patients.

Methodology: In this study, 72 sputum samples were collected from ART-naive and patients using ART for 6 months. The samples were tested with the GeneXpert MTB / RIF assay to diagnose TB and drug resistance and AFB smear microscopy as per NTP guidelines.

Results: Among 72 sputum samples studied, males were more diagnosed with TB than females. People aged 25-56 years were more infected with HIV-TB co-infection. Four patients (5.56%) were found to be TB positive with both techniques, 7 (9.72%) were GeneXpert MTB/RIF positive but smear-negative and 61 patients (84.72%) were negative with both methods. The positivity for MTB detected for GeneXpert MTB/RIF method in females was 3 (15%) and the males were 8 (15.38%), while for the ZN staining method the positivity in females was 2 (10%) and the males were 2 (3.85%). The GeneXpert MTB/RIF assay was also capable of detecting TB in smear-negative cases.

Conclusion: This study showed that the GeneXpert MTB/RIF assay is an effective tool for the early diagnosis of TB among HIV patients as compared to AFB smear staining method.

Keywords: *Mycobacterium tuberculosis*, Ziehl-Neelsen Acid Fast Bacilli staining (ZN-AFB), Human Immunodeficiency Virus (HIV), GeneXpert MTB/RIF.

INTRODUCTION

Tuberculosis (TB) is considered a highly significant infectious disease through the course of human history which can affect nearly any organ in the body but mostly affects the lung.¹ Tuberculosis is transmitted to people via minute droplets released through coughing or sneezing. The illness is typically chronic, and its main signs include

persistent cough with or without expectoration, intermittent fever, loss of appetite, weight loss, chest pain, and hemoptysis.² Hence, it results in high global morbidity and mortality.³

Furthermore, according to 2017 WHO Global Tuberculosis report, 490,000 MDR cases were reported, with less than 50% survival in patients who received WHO-recommended treatment regimens possessing a serious threat to its management. While drug resistance (DR) continues to be a serious crisis and threatens health safety, TB / HIV coinfection is another major tuberculosis problem in Nepal.⁴ The TB-HIV co-infection rate is 2.4% in Nepal.⁵ According to the Global TB Report 2021, the TB mortality rate was 214000 among HIV-

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positive people globally. As a result, those with HIV have a 19-fold increased risk of developing active TB making it a deadly combination; accelerating each other's progression.⁴

For many decades, conventional methods such as smear microscopy and culture techniques were used to diagnose TB.⁶ Culture is the gold standard and the most sensitive method for diagnosis of TB but has a limitation of a long turnaround time of 2-8 weeks as well as the complexity of the procedure demands highly skilled staff along with a biosafety level III lab.⁷ Further, plenty of time is consumed to produce drug resistance patterns. As a result, its applicability as a diagnostic test gets limited.⁸ Comparatively smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive but it has poor sensitivity. Bacilli concentrations as high as 5000-10,000 per ml of the specimen are normally required for the smear to be positive.⁷

Hence, WHO recommended the use of the rapid test GeneXpert MTB/RIF in 2010 that was implemented in Nepal in 2011/12. The GeneXpert MTB / RIF Assay is a unique hands-free integrated cartridge with incorporated chemicals and reagents for sample processing and hemi-nested real-time PCR analysis in a sequential manner for tuberculosis diagnosis and quick RIF resistance detection in clinical samples on GeneXpert platform.⁹ This diagnostic has advantages of high sensitivity (79%) specificity (99%)¹⁰, as well as determining rifampicin resistance as an indicator for MDR TB and providing the result in two hours. It is simple to conduct and is safe as it produces no cultivable aerosols. As a result, WHO has taken Gene Xpert MTB/RIF as one of the essential tests for all patients registering HIV care.¹¹

There have been many types of research around the world to evaluate the effectiveness of the Xpert assay for tubercle bacillus culture, acid-fast bacillus staining and fluorescence microscopy, among TB suspected patients and the results show that the Xpert assay is an innovative invention.¹² But to the best of my knowledge, the study regarding the gene Xpert assay for prompt diagnosis, especially in ART-naive HIV patients is not conducted till now in Nepal. This study aims to provide valuable data regarding the application of gene Xpert assay for prompt diagnosis, especially in vulnerable populations such as HIV-infected patients. This study is carried out to compare AFB staining and GeneXpert MTB/RIF to consider whether Xpert

service can be one of the baseline examinations for HIV-infected patients in TB-abundant countries like Nepal.

METHODOLOGY

This study was conducted at Sukraraj Tropical and Infectious Disease Hospital (STIDH), Teku, Kathmandu, Nepal from December 2019 to July 2020. This study compares the Genexpert MTB/RIF assay to AFB staining in the sputum samples of ART-naive HIV patients and patients using ART for 6 months. The samples were collected following stop TB guidelines.¹³ A total of 72 sputum specimens were collected from clinically TB suspected HIV patients with their consent. While the sample containing only saliva and scanty sample were excluded for processing. Also, the occurrence of sample error during GeneXpert MTB/Rif assay analysis were excluded. Using the Ziehl-Neelsen technique, direct smear microscopy was used to detect the presence of AFB in sample II. Then, WHO criteria were used to report the AFB reports. Similarly, the GeneXpert MTB RIF assay was performed as per standard protocol provided by the manufacturer (Cepheid Inc., Sunnyvale, USA). The GeneXpert DX System interpreted the results from measured fluorescent signals and embedded calculation algorithms, which were displayed in the GeneXpert machine's "View Results" window.

Ethical Approval was taken from Nepal Health Research Council Reference number 110/2020 MT along with informed consent from all the suspected pulmonary tuberculosis HIV patients involved in this study by counseling and making them understand the study regarding the confidentiality and implication of the result.

All data were entered, structured and analyzed using SPSS 26.0 version software. Chi-Square test was employed to determine the associations between ZN AFB smear microscopy and GeneXpert MTB/RIF assay at a 95% confidence interval (95% CI).

RESULTS

Among 72 TB suspected HIV patients, 72.2% were males and 27.8% were females. More than half (69.4%) were in the age group of 15-45 years (**Table 1**).

Table 1: Gender and age-wise distribution of patients (n=72)

Age group (in years)	No.	Male %	No.	Female %	No.	Total %
<15	2	3.9	-	-	2	2.8
15-45	37	71.2	13	65.0	50	69.4
>45	13	25.0	7	35.0	20	27.8
Total	52		20		72	100

Among 72 samples, 11 samples were found to be positive by GeneXpert assay whereas the remaining 61 samples were negative. Out of the 11 GeneXpert MTB/RIF assay positive samples, only 5.6% samples were AFB positive (Table 2).

Table 2: Distribution of samples based on AFB staining and GeneXpert MTB/RIF assay results (n=72)

AFB smear	No.	%	GeneXpert assay	No.	%
1+	1	1.4	VL	3	4.2
2+	2	2.8	L	3	4.2
3+	1	1.4	M	4	5.7
N	68	95.8	H	1	1.4
			N	61	84.72
Total sample	72	100		72	100

Note: VL: Very low, L: Low, M: Medium, H: High and N: Negative

GeneXpert MTB/RIF assay gave positive result in seven AFB negative samples. Out of which 3 showed very low load, 2 showed low load and the next two showed a medium load of tuberculosis bacilli respectively. In this data, a statistically significant relationship was found between AFB smear results and gene Xpert test results (Table 3).

Table 3: Comparative table showing M.tuberculosis load in AFB smear-positive and negative samples comparative to GeneXpert MTB/RIF assay (n=72)

AFB smear result	Gene-Xpert test results					Total (n)	p-value
	VL	L	M	H	N		
1+	-	-	1	-	-	1	0.001
2+	-	1	1	-	-	2	
3+	-	-	-	1	-	1	
Negative	3	2	2	-	61	68	
Total	3	3	4	1	61	72	

Note: VL: Very low, L: Low, M: Medium, H: High and N: Negative

In GeneXpert MTB/RIF assay, 7 samples were found positive in ART-naive whereas in patients using ART for 6 months 4 samples were positive. Similarly, 2 samples were found positive in both patients. In this data, a statistically insignificant relationship was found between ART-naive and patients using ART for 6 months as shown in (Table 4).

Table 4: Distribution of tuberculosis in ART-naive and patients using ART for 6 months as shown by GeneXpert MTB/RIF assay (n=72)

Types of HIV patients	Total no. of Samples (n %)	AFB smear Positive (n %)	GeneXpert MTB/RIF assay positive (n %)	p-value
ART naïve	27 (37.5%)	2 (2.7%)	7 (9.7%)	0.905
Patients using ART for 6 months	45 (62.5%)	2 (2.7%)	4 (5.5%)	
Total	72 (100%)	4 (5.5%)	11 (15.3%)	

Out of the 11 samples shown positive by GeneXpert MTB/RIF assay, 10 samples were found to be sensitive to rifampicin whereas 1 sample was found to be indeterminate. No samples were found to be resistant to rifampicin (Table 5).

Table 5: Gene Xpert MTB/RIF assay rifampicin sensitivity results (n=72)

Rifampicin sensitivity	Gender		Total (n)
	Male (n%)	Female (n%)	
Sensitive	7 (70%)	3 (30%)	10
Resistance	-	-	-
Indeterminate	1 (100%)	-	1
Total	8	3	11

DISCUSSION

TB poses a concern to HIV-positive individuals both before and after starting antiretroviral medication, is difficult to diagnose, is rapidly lethal when drug-resistant, and is spreading in clinics and hospitals.¹⁴ Thus, early diagnosis of TB is necessary to disrupt the disease transmission chain. In this study, among seventy-two samples from HIV patients suspected of pulmonary tuberculosis, smear

positivity was found to be 4 (5.6%) and GeneXpert positivity for MTB on the same samples remained 11 (15.3%) respectively which is similar to a study, that showed 67.5% smear positivity and 77.4% of GeneXpert.¹⁵ However, a study conducted in Benin city¹⁶ among HIV patients showed 41.6% positive cases by ZN microscopy and 62.1% positive cases by GeneXpert MTB/RIF. This is because sputum smear microscopy has a particularly low sensitivity for detecting TB among people living with HIV (PLHIV).¹⁷ Diagnosis of TB among HIV patients are particularly challenging also because people hesitate to visit health facilities that delay not only their ART initiation but also screening for TB. Hence, people in later stages of HIV infection and with a weakened immune system often release fewer organisms into their sputum, at concentrations below the threshold for visual detection under a microscope.¹⁷ However, the variation in this study and previous studies may be explained by differences in the physiological and thus medical conditions of the samples.

A predominance of co-infected people was observed in the economically active age range of 15-45 years. However, 4 (36.4%) out of 11 samples in the age group 25-45 were diagnosed MTB positive by both the ZN staining method and gene Xpert MTB/RIF assay. Likewise, a study was done in a South Asian general hospital in Nepal¹⁸ that showed ages ranging from 33-68 years for MTB-positive cases. Correspondingly, the mean age of HIV-associated tuberculosis samples was 38.0 years.⁸ This could be attributed to the weakening of the immune system due to HIV which quickly progress latent TB infection to TB disease. Even in the absence of HIV, this is the age group in the general population where reactivation of latent TB takes place.¹⁶ In this study, of the total samples, 27 samples were from ART-naive patients whereas the remaining 45 samples were from patients using ART for up to 6 months. Two samples from ART-naive patients and another two samples from patients using ART for up to 6 months were found to be AFB positive. Similarly, in GeneXpert MTB/RIF assay, seven samples from ART-naive patients and four samples from patients using ART for up to 6 months were found to be positive. Statistically, an insignificant finding was observed between ART-naive and patients using ART for up to 6 months. The data indicate that there was no difference in diagnosis of TB despite of initiation of ART for up to 6 months.

In this study, the highest sensitivity for rifampicin was seen in male samples 7(70%) as compared to female samples 3(30%) but no samples were found to be resistant to rifampicin. The gene Xpert MTB/RIF assay is considered a good rapid testing technology for the detection of MDR-TB, and it detects mutations in the *rpoB* gene which occur in 95-99% of the RIF resistant isolates.⁸ However, in subjects with no previous tuberculosis treatment, resistance was usually less frequent¹⁹; in a study carried out by Magar et al., 2019 (7), 66.67% and 28.85% were RIF-sensitive male and female cases respectively whereas 1.28% case was found to be RIF resistance which is higher compare to this study. Likewise, a wide range of resistance for MTB positivity by GeneXpert has been reported in earlier studies by Khunjeli et al.,²⁰ 3.2%, Bajrami et al.,⁶ 11.7% and Atashi et al.,²¹ 3.1%. Further, in this study, 1 sample was found to be indeterminate. The basis for rifampicin indeterminate is when the first probe CT is >34.5 and the last probe CT is >38 cycles.²² Though conventional culture-based drug sensitivity testing is considered a gold standard investigation to detect MDR, the sensitivity and specificity of GeneXpert MTB/RIF assay are comparable with conventional methods.²³

Because every previous study and meta-analysis has consistently shown a 99 percent specificity, culture for mycobacteria was not performed due to the non-availability of the test. In areas of high TB prevalence such as Nepal, the majority of suspected TB cases are assessed by sputum smear microscopy and, where available, by tuberculin test, ESR, ADA, and CXR. Patients are frequently assigned to pragmatic practical treatment practices based on symptomatic analysis or abnormal CXR alone.^{7,18} In these perspectives, GeneXpert excludes “false cases” from “true” smear-negative TB cases with cost-effectiveness reducing the burden out of the pocket of patients for their TB diagnosis and treatment.^{7,18,24} In addition, Nepal Government has made GeneXpert test free of cost for the patients.

That being the case, this study backs up the notion that the gene Xpert MTB/RIF test is superior to conventional AFB smear microscopy for rapid identification of *Mycobacterium tuberculosis* in an HIV and tuberculosis-in endemic zone. Due to higher sensitivity, gene Xpert assay provides both TB and rifampicin resistance results which will greatly improve the early diagnosis of HIV-

associated TB compared to microscopy.²⁵ Hence, the gene Xpert assay is recommended in the diagnosis of TB among HIV-positive patients of every age.¹¹ Nevertheless, GeneXpert does not take the place of traditional microscopy, culture and anti-tubercular drug sensitivity which are necessary to track the effectiveness of treatment.

CONCLUSION

From this study, it can be concluded that GeneXpert MTB/RIF assay was effective in detecting Mycobacterium tuberculosis compared to AFB smear microscopy. Therefore, the implementation of gene Xpert as a baseline analysis can be very effective in detecting pulmonary tuberculosis in vulnerable groups such as HIV patients. Furthermore, it is a suitable technique to detect rifampicin resistance leading to early treatment of patients.

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CONFLICT OF INTEREST

None

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