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Sensitivity and Specificity of Xpert MTB/RIF for Diagnosis of Pulmonary Tuberculosis, Detection of RIF Resistance and its Concordance with Gene Sequencing for RIF Resistance

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ABSTRACT

Background: *Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent. The rapid diagnosis of tuberculosis and detection of rifampicin (RIF) resistance are essential for early disease management. The diagnostic accuracy of GeneXpert need is an important issue to be considered.* ***Objective:*** *To determine the diagnostic accuracy of GeneXpert in the diagnosis of rifampicin resistance in pulmonary tuberculosis keeping the culture-based test as gold standard for drug sensitivity.* ***Material and methods:*** *A cross-sectional validation study was conducted in pulmonology department of PIMS, Islamabad. The study sample size was 352 calculated by using WHO formula. The study duration was 6 months (Oct 2015-March 2016). Non-Probability consecutive sampling was done for selection of participants. Consent forms were taken from all the participants.* ***Results:*** *The study found out that out of all participants 352 (100%), there were 169 (48%) females and 183 (52%) males. The mean age of participants was 29.3 ± 11.5 SD. The sensitivity of expectorated sputum was 98.5 (94.7-98.2), specificity was 50 (49.2-42.9), positive predictive value was 98 (98.5-99.9) and negative predictive value was 50 (49.3- 41.0). A statistically significant association was found between culture, age, gender, recurrence, and treatment failure ($p<0.05$).* ***Conclusion:*** *Our study highlights that Xpert MTB/RIF has high sensitivity and moderate specificity for diagnosis of pulmonary tuberculosis, high rates of detection of RIF resistance and greater concordance with gene sequencing for RIF resistance when compared with culture.*

Keywords: Xpert MTB/RIF, Pulmonary tuberculosis, Sensitivity

INTRODUCTION

Tuberculosis (TB) is one of the most important infectious causes of morbidity and mortality around the world. According to the WHO report in 2010, 8.8 million people developed TB disease (active TB) for the first time [1]. Most of the people affected are living in underdeveloped or developing countries. Pakistan ranks sixth in the global burden of TB. The prevalence of TB in Pakistan is 310 cases per 100000 population or 0.4% and the mortality rate is 39 cases per 100000 population.

TB is a curable disease and when detected in time can be managed effectively. However, if not caught it can be a fire in the jungle as it is lethally infectious and communicable. It is caused by the bacterium *Mycobacterium tuberculosis* and is spread from person to person through the air. TB most commonly affects the lungs (pulmonary TB) but may affect any organ or tissue of the body (pulmonary TB). Signs and symptoms of pulmonary TB include cough for at least two weeks, fever, chills, night sweats, weight loss, hemoptysis (coughing up blood), and fatigue [2].

Xpert is an automated Polymerase Chain Reaction (PCR) test (molecular test) utilizing the GeneXpert® platform [3]. Xpert is a single test that can both detect M. tuberculosis complex and rifampicin resistance within two hours after starting the test, with minimal hands-on technical time. The Xpert MTB/RIF test was approved by World Health

Organization (WHO) in 2010. The GeneXpert test has shown sensitivity above 90% for culture-positive tuberculosis, with high specificity in sputum samples. Sensitivity in individuals with HIV co-infection is over 80% [4-6]. A recent Cochrane smear review concluded for rifampicin resistance detection, Xpert achieved a sensitivity of 94% and specificity of 98%. In high MDR TB settings, the presence of rifampicin resistance alone may serve as a proxy for MDR TB [7].

TB treatment regimens must contain multiple drugs to which the organisms are sensitive to be effective. The treatment of MDR-TB is complex, usually requiring two years or more of therapy and drugs that are less potent and more toxic than the drugs used to treat drug-susceptible TB. WHO guidelines on TB treatment are based on the evidence assessed according to the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach for developing health care recommendations [1-3]. On the other hand, several studies have reported successful use of the Xpert MTB/RIF test on pulmonary samples [2-8]. Thus, this test can be safely and accurately utilized in all types of tuberculosis.

Rationale

We plan to conduct a validation study to assess the diagnostic accuracy of Xpert MTB/RIF in the diagnosis of tuberculosis keeping culture-based tests as the gold standard. Pakistan is a high TB epidemic country and due to its highly communicable and infectious nature the burden of the disease is on the rise. It is very important for healthcare workers and program managers to find out ways to timely diagnose and manage tuberculosis so that further spread may be avoided. Due to the urgency of diagnosis in suspected cases because of a rapid decrease of survival chances with the increase of severity, a rapid, accurate diagnostic test like Gene/Xpert that also is able to identify rifampicin resistance could have a great impact on the survival of these patients, moreover, by treating these patients, its control could be finally ascertained.

MATERIALS AND METHODS

This is a cross-sectional validation study held in the Department of Pulmonology, PIMS Hospital, Islamabad. The study was completed in 6 months (October 2015-March 2016) after the approval of synopsis by using non-probability consecutive sampling technique.

Sample Size

Using sensitivity and specificity based calculator with the following assumptions:

Alpha error=5%

Confidence level=95%

Sensitivity of GeneXpert in diagnosing TB=94% (d) 6% [7]

Specificity of GeneXpert in diagnosing TB=98% (d) 2% [1]

Approximate prevalence of MDR TB in Pakistan=12% [1]

The sample size was found to be 352.

Patient Selection

Inclusion criteria:

- Adult patients above 18 years to 70 years of age
- Patient of both genders
- All those patients who have known drug-resistant TB contact
- Treatment failure cases or Relapse cases or Recurrence cases

Exclusion criteria:

- Patients having chronic pulmonary co-infections and lung cancer

- Those refusing to consent for participation in the study
- Patients already confirmed as a TB case and on treatment
- Other types of TB

Data Collection Procedure

After the approval of permission from the hospital ethical committee, patients coming in the hospital OPD and A and E department with suspicion of pulmonary TB were screened. The details and benefits of the study were explained to the patient and written informed consent was taken.

After a detailed physical exam and history including personal bio-data and medical history was recorded. These include demographic and clinical characteristics (age, sex, height and weight, signs and symptoms of TB). The patient sample of sputum sent for GeneXpert diagnosis and also for culture-based detection.

The results of the test were noted on patient proforma specifically designed for this study. The study outcome was determined in terms of diagnostic accuracy of GeneXpert MTB/RIF in the diagnosis of MDR TB keeping culture-based test as gold standard. All the data collection was done by the researcher himself to limit the selection bias and maintain data quality. The data was collected by using Performa.

Data analysis procedure: Data was entered and analyzed using SPSS version 12. Descriptive statistics were used to calculate mean and standard deviation from continuous variables like age. A two by two table was generated for GeneXpert and culture-based test to estimate the values of true positive (TP), false positive, false negative (FN) and Tue negative (Table 1). The diagnostic accuracy was calculated using standard formulae as mentioned below:

Sensitivity: TP/TP+FN × 100

SPECIFICITY: TN/FP+TN × 100

PPV: TP/TP+FP × 100

NPV: TN/FN+TN × 100

DA: TP+TN/TP+FP+FN+TN

Table 1 GeneXpert and culture-based test

Gene/Expert	Culture	
	Positive	Negative
Positive	TP	FP
Negative	FN	TN

Effect modifiers like age and gender were controlled to see the effect on outcome variable. Post-stratification chi-square was applied. p-value ≤ 0.05 was considered significant.

RESULTS

The study included 352 patients from the department of pulmonology, PIMS hospital Islamabad. The study found out that out of all participants 352 (100%), there were 169 (48%) females and 183 (52%) males. The mean age of participants was found to be 29.3 ± 11.5. Out of all the participants 352 (100%), there were 89 (25%) in 18-28 years age group, 168 (48%) in 29-38 years age group, 80 (23%) 39-48 years age group, 15 (4%) in 49-58 years age group (Table 2). The entire sample 352 (100%) consist of 96 (27%) drug-resistant TB contact cases, 177 (50%) recurrence cases and 79 (23%) treatment failure cases (Figure 1). The study found out that sensitivity of expectorated sputum was 98.5 (94.7-98.2). The specificity expectorated sputum was 50 (49.2-42.9). The positive predictive values Expectorated sputum was 98 (98.5-99.9). The negative predictive value expectorated sputum was 50 (49.3- 41.0) (Table 3). The study finds out that out of all GeneXpert positive cases, there were 337(TP) culture-positive and 5 (FP) culture-negative while out of all 10 GeneXpert negative, there were 5 (FN) culture-positive and 5 (TN) culture-negative (Table 4). The study finds out that out of all GeneXpert positive cases, there were 337 (TP) culture-positive and 5

(FP) culture-negative while out of all 10 GeneXpert negative, there were 5 (FN) culture-positive and 5 (TN) culture negative.

Table 2 Demographic characteristics of participants

Characteristics	Frequency (N=352)	Percentage (100%)
Gender		
Male	183	52%
Female	169	48%
Age		
18-28 years	88	25%
29-38 years	164	48%
39-48 years	75	23%
49-58 years	25	4%

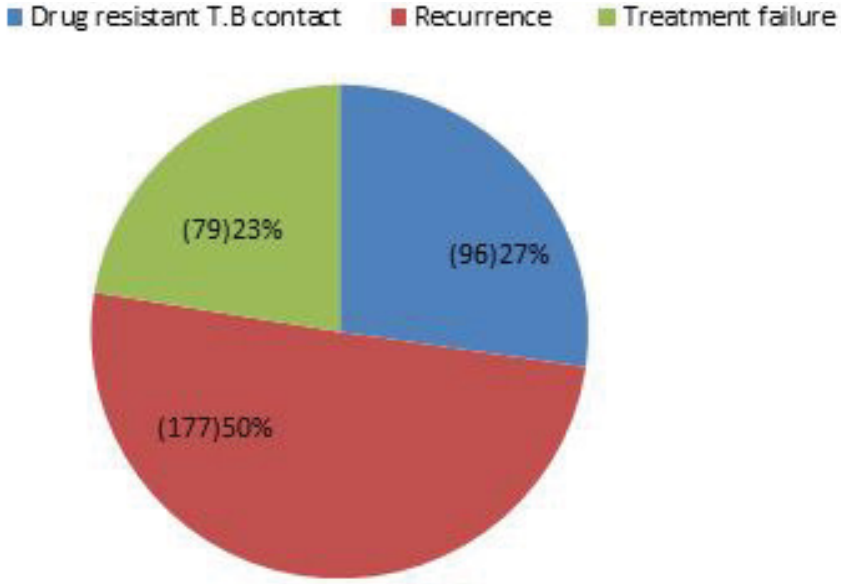

Figure 1 Clinical Characteristics of participants

Table 3 Diagnostic Accuracy of Xpert MTB/RIF in pulmonary tuberculosis

Sample Type	Expectorated Sputum
Sensitivity (%)	98.5 (94.7-98.2)
Specificity (%)	50 (49.2-42.9)
PPV (%)	98 (98.5-99.9)
NPV (%)	50 (49.3-41.0)
PPV: Positive Predictive Value; NPV: Negative Predictive Value	

Table 4 Evaluation of diagnostic accuracy of Xpert MTB/RIF

GeneXpert	Culture		Total
	Positive	Negative	
Positive	337 (TP)	5 (FP)	342 (97%)
Negative	5 (FN)	5 (TN)	10 (3%)
Total	342 (97%)	10 (3%)	352 (100%)

The study found out that among all those who have had a recurrence, 161 (97%) were found to be true positive while 5 (3%) were true negative, 5 (3%) false-positive and 5 (3%) were false-negative. Among those who do not have recurrence, 161 (97%) were true positive while 5 (3%) were true negative, 5 (3%) false-positive and 5 (3%) were false-negative. The automated reading software was able to distinguish between culture-positive TB and non TB patients with an area under the curve of 0.84 (95%CI 0.80-0.88). Including all *Mycobacterium tuberculosis* culture-negative patients, as the negative reference standard, CAD4TB performed slightly, but not significantly, worse: Az=0.81 (95%CI 0.77-0.85), p=0.28 as shown in Figure 2.

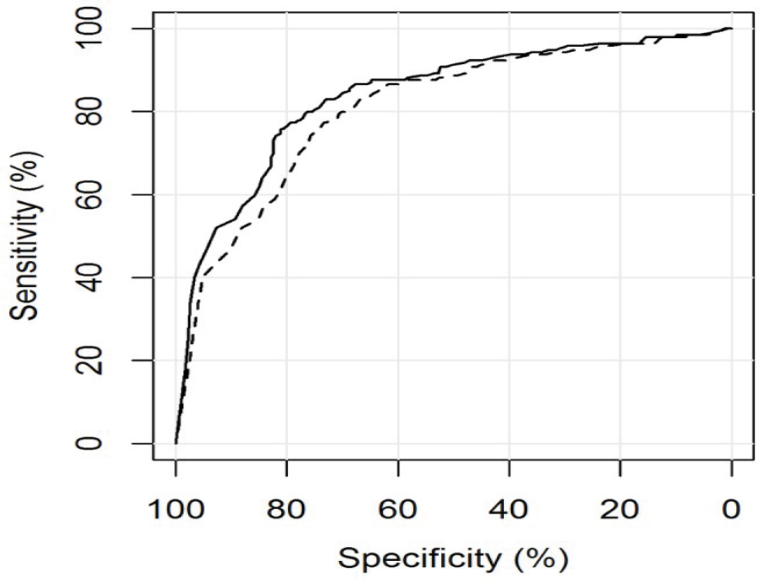

Figure 2 ROC analysis curve

The study found out that among all the males, there were 167 (97%) true positive cases, 5 (3%) were true negative cases, 5 (3%) were false-positive and 5 (3%) were false-negative cases. While among all the females, there were 155 (97%) true positive cases, 5 (3%) were true negative cases, 5 (3%) were false-positive and 5 (3%) were false-negative cases. The study found out that out of all those who showed treatment failure, there were 63 (94%) true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false negative. Among those who did not show treatment failure, there were 25s9 (94%) true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false negative.

The study found out that among all those who were in age group 18-28 years, 73 (94%) were true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false negative while among those who were in age group 29-38 years, 149 (94%) were true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false-negative. Among those who were in age group 39-48 years, 60 (94%) were true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false-negative while among those who were in age group 48-59 years, 10 (94%) were true positive, 5 (6%) were true negative, 5 (6%) were false positive and 5 (6%) were false-negative.

DISCUSSION

The present study included 352 patients from the department of pulmonology, PIMS hospital Islamabad. The study found out that out of all participants 352 (100%), there were 169 (48%) females and 183 (52%) males. Out of all the participants 352(100%), there were 88 (25%) in 18-28 years age group, 164 (48%) in 29-38 years age group, 75 (23%) 39-48 years age group, 25 (4%) in 49-58 years age group.

In this study, the performance of the MTB/RIF assay with pulmonary specimens obtained during the clinical routine was investigated. Previous studies of the MTB/RIF assay have reported test sensitivities of 57% to 76.9% in cases

of negative, culture-positive pulmonary tuberculosis and 98 to 100% in cases of positive, culture-positive pulmonary tuberculosis, while the test specificity remained at 99% to 100%. In our study, the sensitivity of the test with culture-positive pulmonary specimens was 100%, and the specificity was 98.3% [9-12], compatible with results presented in previous medical papers.

The study found out that among all those who have had a recurrence, 161 (97%) were found to be true positive while 5 (3%) were true negative, 5 (3%) false-positive and 5 (3%) were false-negative. Among those who do not have recurrence, 161 (97%) were true positive while 5 (3%) were true negative, 5 (3%) false-positive and 5 (3%) were false-negative.

In a study performed recently, the sensitivities of the test for pulmonary specimens have been reported to be 100%. In the present study, the sensitivity of the test was also 100% for pulmonary specimens. In all tuberculosis cases, the sensitivity of the MTB/RIF test for pulmonary specimens was statistically higher for pulmonary specimens (p=0.001). It could be because of the high negative rate for non-respiratory specimens [13-18]. Even though the sensitivity of the MTB/RIF test for positive culture specimen were higher and was found no statistically significant difference in the results of culture tests and MTB/RIF test.

The study finds out that out of all GeneXpert positive cases, there were 337 (TP) culture-positive and 5 (FP) culture-negative while out of all 10 GeneXpert negative, there were 5 (FN) culture-positive and 5 (TN) culture negative. Evidence exists for culture-positive specimens, the average turnaround time was 19 ± 9 days (range, 3 to 42 days) in liquid medium. Also, all positive and MTB/RIF test-positive samples had shorter turnaround times (p=0.001) [19-25]. This could be the result of a low number of organisms in pulmonary specimens. A previous study found that the MTB/RIF assay had a calculated limit of detection of 131 CFU/ml of sputum and was able to detect as few as 10 CFU/ml of sputum in 35% of samples. In the study, the longer turnaround time for the MTB/RIF test-negative samples could be due to low numbers of bacilli which were under the limits of detection of the test [26].

Another study reported that the diagnosis of tuberculosis at death was established based on microbiological and histopathological results. Eight out of 30 cases (26.7%) were diagnosed with tuberculosis. Xpert had a sensitivity to detect TB in lung tissue of 87.5% (95%CI 47.3-99.7) and a specificity of 95.7% (95%CI: 78.1-99.9). In-house DNA amplification methods and Xpert showed 93.6% concordance for lung tissue and 100% concordance for brain and liver tissues. The final cause of death was attributable to tuberculosis in four cases. Xpert MTB/RIF may represent a valuable, easy-to-perform technique for post-mortem TB diagnosis [27-29].

The study found out that out of all those who showed treatment failure, there were 63 (94%) true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false-negative. Among those who did not show treatment failure, there were 259 (94%) true positive, 5 (6%) were true negative, 5 (6%) were false-positive and 5 (6%) were false-negative. While similar studies considered other variables [30,31].

CONCLUSION

Our study highlights that Xpert MTB/RIF has high sensitivity and moderate specificity for the diagnosis of pulmonary tuberculosis, high rates of detection of RIF resistance and greater concordance with gene sequencing for RIF resistance when compared with culture.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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