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Comparison of the Diagnostic Accuracy of the Xpert MTB/RIF Assay with Acid-Fast Staining and Culture Methods for Prompt Detection of *Mycobacterium tuberculosis* Isolates

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ABSTRACT

Background: The aim of this study was to compare the diagnostic accuracy of the GeneXpert with acid-fast stain (AFB) and culture methods in patients with suspected pulmonary tuberculosis (SPT).
Methods: A total of 484 sputum and BALF (bronchoalveolar lavage fluid). Samples were analyzed from 428 individuals, of which 78.3% were men and the average age was 49.9 years.
Results: The GeneXpert test had a sensitivity of 95.7% and a specificity of 43.9% for MTB culture, and a sensitivity of 91.9% and a specificity of 52.8% for a definitive diagnosis. The sample had a sensitivity of 93.7%, specificity of 69.6%, and accuracy of 82.2% for MTB culture, and sensitivity of 86.9%, specificity of 82.2%, and accuracy of 85.3% for the final diagnosis. Overall, the AFB exam had the highest diagnostic accuracy of the three tests at 85.3%.
Conclusion: The study indicates that while the study's AFB test is usually used as the first diagnostic test for pulmonary TB due to its high accuracy, the GeneXpert test can be used in samples that are AFB negative, but strongly suspected for TB, because it has a higher sensitivity. Choosing the appropriate test based on the clinical context is critical for accurate diagnosis and treatment of TB.

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Introduction

Tuberculosis is a preventable and mostly treatable disease. In 2022, TB remains the second leading cause of death from an infectious agent in the world, after COVID-19 and twice as deadly as AIDS/HIV. In 2020, the estimated incidence of TB in Iran was around 10,000 cases (13,000-7,400) and the incidence rate per 100,000 population was 11 (15-3/8). The incidence of MDR-RR TB was 200 cases (370-34). The estimated proportion of MDR-RR TB cases in new cases in 2022 was 1.7% and in previously treated individuals, it was 6.1% (9.7-4.4) (1).

The reported cases of tuberculosis in Iran by the Ministry of Health include a total of 7261 new cases and relapses, with 3% of cases tested through rapid diagnostic methods, 79% of cases being pulmonary TB, 88% of cases confirmed by bacteriological methods, 3% of cases in children aged 0-14 years, 43% in women (age >14), 54% in men (age >14), and a total of 7413 (2).

In many areas with a high prevalence of TB, sputum the smear AFB is the primary diagnostic method for evaluating individuals showing signs and symptoms of TB. However, sputum the smear AFB is a relatively insensitive test with a detection limit of 5000 to 10000 bacilli per milliliter of sputum. Furthermore, sputum the smear AFB cannot differentiate drug-sensitive strains from drug-resistant strains (3).

Although culture-based methods are used as the primary diagnostic method in many countries with a high prevalence of TB, they are not used due to cost, required infrastructure, and the long time needed to obtain results (1 to 3 weeks for a positive result and up to 6 weeks for a negative result). Nevertheless, common microscopy and culture tests are still necessary for monitoring treatment responses in patients. Culture methods remain important in diagnosing TB in children and extrapulmonary TB cases with low bacillary load and in the differential diagnosis of non-tuberculosis mycobacterial infections (3).

In recent years, several nucleic acid amplification-based TB testing devices have become available and are currently the preferred first-line diagnostic methods. One of these devices, the Xpert MTB/RIF assay, allows for rapid and highly sensitive TB detection (very close to culture-based methods in liquid media) using fully automated and rapid nucleic acid amplification technology. This method simultaneously identifies *Mycobacterium tuberculosis* (TB) and rifampicin resistance in less than two hours and requires minimal bio-safety precautions and training. In diagnosing pulmonary TB, this test has an overall sensitivity of 85%, with a sensitivity of 98% in positive the smear AFB samples and 70% in negative the smear AFB samples, and a specificity of 98%. In comparison to phenotypic drug resistance determination methods for simultaneous rifampicin resistance detection, the Xpert MTB/RIF assay has a sensitivity of 96% and specificity of 98% (4).

The Xpert MTB/RIF assay test was approved by WHO in 2011. This test has the ability to identify both live and non-live bacteria. The sensitivity of a single the Xpert MTB/RIF assay in cases of positive the smear AFB and positive culture was 98.2%, while the sensitivity in cases of negative the smear AFB and positive culture was 72.5%, and its sensitivity increases with repeated testing. The Xpert MTB/RIF assay is a cost-effective method for diagnosing the smear AFB -negative TB compared to conventional methods in resource-limited countries (5-7).

This study compared the diagnostic accuracy of the Xpert MTB/RIF assay with acid-fast staining and culture methods for the rapid identification of Mycobacterium tuberculosis and clinical isolates at the Razi Education and Medical Center in Rasht.

Materials and Methods

Study population

The study population consisted of patients suspected of pulmonary tuberculosis referred to the tuberculosis laboratory of Razi Educational and Medical Center in Rasht between 2021 and 2022. As it were those patients who are 'sputum-scarce' with suspected pneumonic TB were included within the consider. All other patients with sputum production, either spontaneous or induced, and those with history of taking anti-TB treatment (ATT), were excluded from the study. So also, patients who were dying and seem not withstand the bronchoscopy method for BALF, were prohibited. The consider was endorsed by the Morals Committee. A completely educated composed assent was gotten from all the subjects who are portion of this think about.

The patient presenting with symptoms suggestive of pulmonary tuberculosis is experiencing persistent cough, chest pain, and a recent onset of fatigue. They have also noticed weight loss and a decreased appetite, along with night sweats. These symptoms, coupled with a history of being in close contact with someone diagnosed with tuberculosis, raise suspicion for the disease. The healthcare provider will likely order diagnostic tests such as a chest X-ray, sputum analysis, and possibly a TB skin test or interferon-gamma release assay to confirm the diagnosis. Prompt identification and treatment of pulmonary tuberculosis are essential to prevent the spread of the disease and mitigate potential complications. The patient will be closely monitored and provided with appropriate treatment, including a regimen of antibiotics tailored to combat the specific strain of Mycobacterium tuberculosis (8).

Diagnosis of pulmonary tuberculosis

The diagnosis of active pulmonary tuberculosis was based on a positive culture of the M. tuberculosis complex, which is the gold standard. Patients who were either Xpert MTB/RIF positive, BALF, or had PTB and Xpert MTB/RIF-negative clinical radiological evidence w ere considered “probable PTB”. Patients with a negative BALF Xpert MTB/RIF and AFB cell sample and imaging features suggestive of pulmonary tuberculosis on chest X-ray or chest HRCT were excluded from ATT and evaluated for other causes and treated accordingly, culture, AFB results. They were classified as non-TB if their AFB culture was negative. HRCT of the chest was performed when the chest X-ray was equivocal or in the differential diagnosis and for "probable" PTB (8).

Procedure for BAL fluid

In this study was used a flexible bronchoscope to perform the BAL procedure for a breath sample. Also, was applied the BAL procedure to all patients to avoid the influence of the procedure on the diagnostic performance of the sample. Of the samples thus obtained, half was sent for a smear AFB sample and culture for Mycobacterium tuberculosis complex (MTBC) and the other half for the Xpert MTB/RIF assay. All samples were processed at tuberculosis laboratory of Razi Educational and Medical Center in Rasht.

Microbiology and molecular biology

Half of the BAL sent for sampling and culture was decontaminated using a standard protocol using sterile N-acetyl-L-cysteine/4% NaOH and centrifugation at 3000x g for 20 min. After Ziehl-Neelsen staining, one smear was examined for the smear AFB. For culture, the above sample was inoculated into the Lowenstein-jensen medium. The Xpert MTB/RIF assay, an automated cartridge-based molecular technique, from was performed on the other half of the BAL sample, according to the manufacturer's instructions. BAL fluid without prior decontamination was loaded into the Xpert-cartridge and test reported as ‘detected’ or ‘not detected’ (Cepheid GeneXpert System, Sunnyvale, US) (8).

Variables of study

Demographic and clinical data were included age, gender, history of previous TB treatment, history of incarceration, history of drug abuse, specimen type (sputum or BAL), results of culture, smear AFB specimen and Xpert MTB/RIF assay, final disease diagnosis, and resistance to rifampicin.

Sample size

Given that the main goal of the project was to d etermine the diagnostic value of the Xpert MTB/RIF assay compared to culturing Mycobacterium tuberculosis in the diagnosis of pulmonary tuberculosis, the Buderer formula (3) was used to determine the sample size. Based on the study by Bajrami et al. (9) and by determining a type I error of 0.05, a sensitivity of 82.3% and specificity of 97.6% for the Xpert MTB/RIF assay, and a prevalence of 20.5% (4), and w of 10.0, the sample size required for sensitivity and specificity were calculated to be 273 and 12 individuals, respectively. Considering the importance of both sensitivity and specificity, a maximum sample size of 273 individuals was chosen as the final sample size.

Statistical analysis

In this study, the values of quantitative variables are shown as "(standard deviation) mean" and the values of qualitative variables are shown as "(percentage) frequency". Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and 95% confidence intervals were calculated to evaluate the diagnostic power of the Xpert MTB/RIF assay compared to the methods of culturing *Mycobacterium tuberculosis* and acid-fast staining. These indicators were calculated considering the culturing method and the final diagnosis as the standard reference method. The data were analyzed using SPSS version 16 and MedCalc version 19.5.3 software, and a significance level of 0.05 was considered.

Results

In this study, 484 sputum and BAL samples from 428 patients suspected of pulmonary tuberculosis were examined. These samples were collected from June 2021 to March 2022 in the Razi TB Laboratory of the Razi Educational and Medical Center in Rasht and each sample was investigated by three diagnostic methods for Mycobacterium tuberculosis; culture, the smear AFB, and Xpert MTB/RIF assay. Out of 428 patients, 335 (78.3%) were male and 93 (21.7%) were female. Out of 484 samples, age information was available for 360 (74.4%). The average age of the patients studied was 49.9 (with a standard deviation of 6.16) years, ranging from 16 to 89 years. The highest number of patients were in the age group of 60 years and older with 113 patients (31.4%), followed by ages 49-40 years with 75 patients (20.8%), ages 59-50 years with 70 patients (19.4%), ages 39-30 years with 52 patients (14.4%), ages 29-20 years with 44 patients (12.2%) and ages 19-10 years with 6 patients (1.7).

Out of 428 patients studied, 55 patients (12.8%) had a history of imprisonment and 38 individuals (8.8%) had a history of drug addiction. Among the 428 patients, 63 individuals (14.7%) had a history of receiving anti-TB treatment. Patient information is summarized in Table 1.

Out of 484 samples, 417 samples (86.2%) were sputum samples and 67 samples (13.8%) were BAL samples. Out of 484 samples, 254 samples (52.5%) had positive culture results and 230 samples (47.5%) had negative culture results. Out of 484 samples, 308 samples (63.6%) had positive the smear AFB results and 176 samples (36.4%) had negatived the smear AFB results. Out of 484 samples, 372 samples (76.9%) had positive results with the Xpert MTB/RIF assay and 112 samples (23.1%) had negative.

Out of 372 positives with the Xpert MTB/RIF assay, 328 samples (88.2%) were sensitive to rifampicin, 6 samples (1.6%) were resistant to rifampicin, and 38 samples (10.2%) were reported as rifampicin resistance unknown. The 6 samples resistant to rifampicin belonged to 4 patients. Out of 484 samples, 321 samples (66.3%) had a final diagnosis of tuberculosis, and 163 samples (33.7%) did not have a final diagnosis of tuberculosis. In the evaluation of the diagnostic accuracy of the smear AFB and the Xpert MTB/RIF assay, considering culture as the standard reference test, for the the smear AFB method; sensitivity of 93.7% (95% CI 90.96-96.4), specificity of 69.6% (95% CI 63.2-75.4), positive predictive value of 77.3% (95% CI 73.6-80.6), negative predictive value of 90.9% (95% CI 86.1-94.2), diagnostic accuracy of 82.2% (95% CI 78.5-85.5) were calculated; and for the Xpert MTB/RIF assay; sensitivity of 95.7% (95% CI 92.4-97.8), specificity of 43.9% (95% CI 37.4-50.6), positive predictive value of 65.3% (95% CI 62.6-67.9), negative predictive value of 90.2% (95% CI 83.5-94.3) and diagnostic accuracy of 71.1% (95% CI 66.8-75.1) were calculated. The results are summarized in Table 2.

In the examination of the diagnostic accuracy of culture tests, the smear AFB and Xpert MTB/RIF assay considering the final diagnosis of the disease as the standard reference, for the culture method; sensitivity 77.6% (95% CI: 72.6-82%), specificity 96.9% (95% CI: 93-99%), positive predictive value 98% (95% CI: 95-99.4%), negative predictive value 68.7% (95% CI: 64.1-72.9%), and diagnostic accuracy 84.1% (95% CI: 80.5-87.2%), for the the smear AFB method; sensitivity 86.9% (95% CI: 82.7-90.4%), specificity 82.2% (95% CI: 75.5-87.7%), positive predictive value 90.6% (95% CI: 87-93.1%), negative predictive value 76.1% (95% CI: 70-81.5%), and diagnostic accuracy 85.3% (95% CI: 81.9-88.4%), and for the Xpert MTB/RIF assay; sensitivity 91.9% (95% CI: 88.4-94.6%), specificity 52.8% (95% CI: 44.8-60.6%), positive predictive value 79.3% (95% CI: 76-81.9%), negative predictive value 76.8% (95% CI: 69-83.1%), and diagnostic accuracy 78.7% (95% CI: 74.8-82.3%) were calculated. The results are summarized in Table 3.

Discussion

Currently, there are three different methods for TB diagnosis in Iran: culture, AFB stain and GeneXpert test. Since the Xpert MTB/RIF analysis is more expensive than traditional methods and not all health centers have the equipment or access to cassettes, it is not practical to use this method as the first diagnostic step for all samples of pulmonary TB in our country. It seems crucial to compare the diagnostic accuracy of the GeneXpert test with the AFB sample (10). Our analysis showed that the GeneXpert test shows a higher sensitivity than the AFB smear method compared to both culture as the gold standard and definitive diagnosis.

However, the Smear AFB method shows greater specificity and diagnostic accuracy than the GeneXpert test. In addition, evaluating the diagnostic accuracy of culture, AFB sampling and Xpert MTB/RIF analysis in relation to definitive disease diagnosis, the Xpert MTB/RIF analysis showed the highest sensitivity of 91.9%, while the culture method had the lowest. sensitivity 77.6%. The culture method had the highest specificity of 96.9%, while the Xpert MTB/RIF assay had the lowest specificity of 52.8%. Smear AFB method had the highest diagnostic accuracy of 85.3%. In comparison, the AFB smear method has a higher diagnostic accuracy of 85.3% compared to the Xpert MTB/RIF assay of 78.7%. The Central Laboratory of the Raz Education and Medical Center, the only culture reference laboratory in the province, receives samples at long intervals, resulting in inadequate culture conditions. This may have contributed to the low sensitivity of the culture method in our study, increasing the risk of false negative results. According to the results, the smear AFB technique is suitable for the initial diagnosis of pulmonary tuberculosis in a

Table 1. Demographic and clinical characteristics of patients with suspected PTB .

Characteristics	N (%)
Age (years), Mean (SD)	49.9 (16.6)
Gender, Number (%)	
Male	335(78.3)
Female	93 (21.7)
History of receiving anti-malarial treatment, Number (%)	63 (14.7)
History of imprisonment, Number (%)	55 (12.8)
History of addiction, Number (%)	38 (8.9)

Table 2. The diagnostic accuracy of the smear AFB and GeneXpert test compared to the culture method as the gold standard.

Variables	Smear AFB		GeneXpert	
	Value (%)	**CI (95%)**	(%) Value	**CI (95%)**
Sensitivity	93.7	90-96.4	95.7	92.4-97.8
Specificity	69.6	63.2-75/.4	43.9	37.4-50.6
Positive predictive value	77.3	73.6-80.6	65.3	62.6-67.9
Negative predictive value	90.9	86.1-94.2	90.2	83.5-94.3
Positive accuracy ratio	3.1	2.5-3.7	1.7	1.5-1.9
Negative accuracy ratio	0.09	0.06-0.15	0.1	0.05-0.2
Test accuracy	82.2	78.5-85.5	71.1	66.8-75.1

Table 3. The diagnostic accuracy of culture test, the smear AFB, and GeneXpert compared to the final diagnosis of the disease as the gold standard.

Variables	Culture		Smear AFB		GeneXpert	
	Value (%)	**CI (95%)**	**Value (%)**	**CI (95%)**	**Value (%)**	**CI (95%)**
Sensitivity	77.6	72.6-82	86.9	82.7-90.4	91.9	88.4-94.6
Specificity	96.9	93-99	82.2	75.5-87.7	52.8	44.8-60.6
Positive predictive value	98	95.4-99.2	90.6	87.3-93.1	79.3	76.4-81.9
Negative predictive value	68.7	64.1-72.9	76.1	70.5-81	76.8	69-83.1
Positive accuracy ratio	25.3	10.6-60.1	4.9	3.5-6.8	1.9	1.6-2.3
Negative accuracy ratio	66.3	61.9-70.5	0.16	0.12-0.12	0.15	0.1-0.23
Test accuracy	84.1	80.5-87.2	85.3	81.9-88.4	78.7	74.8-82.3

symptomatic individual, while the Xpert MTB/RIF assay should be used only when tuberculosis is strongly suspected and the results of sputum staining are negative. In the present study, 88.2% of the isolates were also susceptible to rifampicin and 1.6% of the isolates were resistant to rifampicin. Subsequently, 10.2% of isolates were reported to have unknown rifampicin resistance.

Patel et al. investigated the diagnostic power, sensitivity and specificity of the GeneXpert test for spinal tuberculosis and rifampicin resistance at the Bombay Hospital and Medical Research Centre. The think about appeared that the Xpert MTB/RIF measure has 86.3% affectability and 85.3%

specificity compared to culture for the determination of spinal tuberculosis and 75.86% affectability and 96.12% specificity. In the GeneXpert test, four samples were false positive and 11 samples were false negative for RIF resistance (11).

Rimal et al investigated the diagnostic performance of the GeneXpert test in detecting MTB in AFB-negative presumptive tuberculosis patients. They found that 35 (21.60%) of these 162 samples from AFB-negative presumptive TB patients were positive for MTB, while the GeneXpert test detected MTB in only 31 (19.14%) samples. In addition, rifampicin-resistant MTB was confirmed in 4 (2.47%) (12). We know that about 0.5 million new rifampicin-resistant TB samples have been collected, of which 78% were multidrug-resistant TB (MDR-TB), but only 206,030 MDR-TB cases have been reported (13). We also know that the GeneXpert test detects rifampicin resistance. However, the Xpert MTB/RIF assay showed variable sensitivity and specificity for the detection of rifampicin resistance. Variation can be attributed to false-positive rifampicin-resistant strains due to genomic mutation, exclusion of mixed infections, and presence of both rifampicin-resistant and susceptible MTB isolates in the same samples (14).

Friedrich, Sven O. et al. worked to assess the sensitivity and specificity of the GeneXpert test as an early sputum biomarker of TB treatment response (15). They found that the GeneXpert test had a high sensitivity of 97.0% but a low specificity (48.6%) compared to the combined binary results of an AFB sample and culture as a reference standard. Friedrich, Sven O. et al. oppose this idea with the comes about of this consider. detailed that destitute specificity blocks the utilize of the Xpert MTB/RIF measure as a biomarker for checking TB treatment. They believed that the GeneXpert test should not replace standard AFB and culture (15).

Kabir, Shaila et al. worked on diagnostic challenges and the Xpert MTB/RIF assay for the detection of *Mycobacterium tuberculosis* in suspected pulmonary tuberculosis samples. They found that 21 of 185 presumptive AFB-negative samples were Xpert MTB/RIF positive, indicating drug resistance, and these results were confirmed by MTB culture showing resistance to isoniazid. Kabir, Shaila et al. shows that compared to AFB sputum, the GeneXpert test showed higher sensitivity and specificity with almost perfect accuracy. According to this study, the detection of pulmonary TB samples from presumptive samples with the GeneXpert test compared to primary detection using conventional tests had a significant impact, overcoming diagnostic challenges and uncertainties (16).

Biset, Sirak et al. reported trends in *Mycobacterium tuberculosis* and rifampicin resistance in northwestern Ethiopia using the Xpert MTB/RIF assay. They showed that out of 17,615 results, 10.5% were MTB-positive and 7.42% were RIF-resistant. However, they investigated the association between age, history of anti-TB treatment and year of diagnosis of MTB and rifampicin-resistant (RR)-MTB. Based on reports by Sirak Biset et al., the prevalence of TB was higher in productive age groups, while the prevalence of RR-TB was higher in the elderly. Regarding the year of diagnosis, the prevalence of tuberculosis and RR-TB showed a downward trend as the year progressed. MTB was detected in 12.8% of new and 22.2% of relapsed TB patients, while RR-MTB was detected in 8.5% of new and 18.5% of relapsed TB samples (17).

Jafari et al., evaluated the accuracy and ratio of the Alamar blue microplate assay for the rapid identification of clinical isolates of Mycobacterium tuberculosis and multidrug-resistant M. tuberculosis. They found that the microplate alar blue test had a sensitivity of 100, a specificity of 74.36%, a positive predictive value of 79.59% and a negative predictive value of 100%. The Rifampicin microplate Alamar blue test had a sensitivity of 100%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 100%. The microplate

Alamar blue test with isoniazid had a sensitivity of 84.38%, a specificity of 66.67%, a positive predictive value of 96.43% and a negative predictive value of 28.57%. Jafari et al. found high accuracy between the Alamar blue microplate assay containing rifampicin and the ratio assay. The rapid and inexpensive microplate Alamar blue test is a cheap and suitable test for the detection of rifampin-resistant tuberculosis in low-income countries (3).

Participant samples were processed as AFB followed by Xpert MTB/RIF analysis and cultured in Lowenstein-Jensen (LJ) medium in Nepal by Rimal et al. In the present study, Raksha Rimal et al found that the sensitivity, specificity, positive predictive value and negative predictive value of the GeneXpert test for AFB-negative sputum samples were 74.3%, 96.6%. 86.7% and 92%. The GeneXpert test has a remarkable 90.91% diagnostic agreement with culture (12).

Rasheed et al., found that the sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of the GeneXpert test were 84.48%, 100%, 100%, 65.38% and 88%, respectively (18).

The present study had some limitations. One of the limitations of our study was the relatively small sample size, which may have resulted in selection bias, reduced statistical power, unstable estimates, and wider confidence intervals. In addition, there were few rifampicin-resistant samples in the study, which could have limited the accurate assessment of the diagnostic accuracy of the GeneXpert test in detecting drug-resistant TB samples. In addition, rifampicin resistance detected by the Xpert MTB/RIF assay could not be fully confirmed by drug susceptibility testing, as the study was unable to confirm rifampicin-resistant and rifampicin-sensitive samples detected by the Xpert MTB/RIF assay.

Conclusion

In this study, the diagnostic accuracy of sputum AFB was higher than that of culture and Xpert MTB/RIF, so sputum AFB can still be the first line for the diagnosis of pulmonary tuberculosis. Be that as it may, considering that the affectability of the GeneXpert test is higher than culture and test AFB strategies, it ought to be utilized for the exact conclusion of AFB-negative tests when there's a tall clinical doubt of tuberculosis.

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Ethics approval and consent to participate

Ethical principles outlined in the Declaration of Helsinki were considered. The research protocol and procedures involving human participants were reviewed and approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1401.356).

Conflict of interest

The authors declare that they have no competing interests.

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