Diagnostic accuracy of Gene Xpert MTB/RIF and AFB smear compared to culture for the detection of Tuberculosis using bronchio-alveolar lavage (BAL) fluid

Imranullah Khan[1], Muhammad Shah Fahad[2], Muhammad Irfan[3], Kashif Aziz[3], Raza Ullah[4]

[1]Rehman Medical Institute, Peshawar - Pakistan

[2]Shaukat Khanam Memorial Cancer Hospital and Research Centre, Peshawar - Pakistan

[3]Department of Cardio pulmonary and Medicine, Aga Khan University, Karachi - Pakistan

[4]Department of pulmonology and critical care, Hayat Abad Medical Complex, Peshawar - Pakistan

Address for correspondence
Raza Ullah
Departments of pulmonology and critical care, Hayat Abad Medical Complex, Peshawar – Pakistan.
E mail: dr.raza127@gmail.com

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Author Contributions

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Abstract

Background: A disease caused by Mycobacterium, known as Tuberculosis (TB) is a global health problem, in a developing country like Pakistan. The widely available medical therapy has a high cure rate but the main issue with TB is the need for the rapid diagnostic test with high accuracy to commence an early treatment.

Objective: This study was done to assess the diagnostic accuracy of GeneXpert and acid-fast bacilli (AFB) smear in comparison to the culture to detect TB using a Broncho alveolar lavage (BAL) fluid.

Methodology: A prospective observational study was carried out in the Department of Pulmonology, Rehman Medical Institute (RMI) Peshawar, from January 2018 to December 2019. A standardized proforma was used for data collection. Demographic, laboratory findings, clinical and radiological features were recorded and subsequently entered Statistical Package for Social Sciences (SPSS). After completing data entry, the statistical analysis was performed using SPSS version 23.0. Frequency of AFB smears, GeneXpert assay and mycobacterial cultures were calculated by percentages. Sensitivity, specificity and predictive values were calculated with 95% class intervals (CIs). A $P < 0.05$ was considered statistically significant.

Results: A total of 88 patients fulfilling the inclusion criteria were included in the study. The mean age of the patients was 49.5±18.01. About 59 patients (67%) were males while the rest were female patients. The most common symptoms included fever (78.4%), weight loss (71.6%), cough (64.8%), anorexia (43.2%) and breathlessness (29.5%). Out of 88 patients who were tuberculosis suspect, 73 (82.9%) cases were confirmed as pulmonary tuberculosis on mycobacterial cultures which was taken as a gold standard. On GeneXpert assay 72 (81.8%) cases and on AFB (BAL) smear only 52 (59.1%) were positive for MTB.

Discussion: GeneXpert had a high sensitivity than AFB smear to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity), in those cases of pulmonary tuberculosis who have either smear negative or sputum scarce disease. This test has the advantages of being inexpensive, requires less manpower and gives results on the same day.

Key Words: Gene Xpert; MTB; Pulmonary TB; Broncho alveolar lavage

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Introduction

A disease caused by Mycobacterium, known as Tuberculosis (TB) is a global health problem, in a developing country like Pakistan. The widely available medical therapy has a high cure rate but the main issue with TB is the need for the rapid diagnostic test with high accuracy to commence an early treatment. This study was done to assess the diagnostic accuracy of GeneXpert and acid-fast bacilli (AFB) smear in comparison to the culture to

detect TB using a Broncho alveolar lavage (BAL) fluid.

According to the 2013 estimates of World Health Organization (WHO), around 9.0 million people suffered from TB and 1-1 /2 million deaths occurred due to it. In the analysis, new cases were found to be 56% occurring mostly in South-East Asia and the Western Pacific Regions.[1] 0.3–0.5 million cases were reported in Pakistan, making it one of the top 5 countries reporting the highest number of cases. In a survey conducted by WHO in Pakistan, the following parameters were assessed per hundred thousand population, incidence (230), prevalence (310) and mortality (39).[2]

Stopping transmission of the mycobacterium is the mainstay of achieving control on this disease. It can be achieved by detecting cases in early stages and effectively treating them.[3] As per the current treatment guidelines, the mycobacterium should be isolated from a sputum sample in order to confirm the diagnosis of tuberculosis. One of the commonly used and most readily accessible test is sputum acid-fast bacilli (AFB) staining, however, it is not very specific.[4] Though WHO recommends confirmation of the disease by the detection of AFB in respiratory specimens.[5]

Patients who are smear positive amount to about 20 - 40% (presently 28%),6,7 the rest are either have a negative smear or sputum-scarce disease. The key to quick and precise diagnostic modality is obtaining a proper specimen. In order to obtain a quality specimen, Bronchoscopy with BAL is performed routinely for suspected cases.[8]

TB Culture for the drug sensitivity patterns is a slow and cumbersome process. It requires sequential procedures starting with isolation of mycobacteria from biological specimens and letting it grow.[1,7,9] A potential solution is the semi-automated liquid culture, although promising, it is yet to be sufficiently validated.[10]

The morbidity and mortality increase with failure to diagnose quickly and treating the affected accordingly. Secondary resistance develops with the ongoing transmission of the disease.[11] In 2007, 0.5 million cases of muti-drug resistant tuberculosis (MDR-TB) were reported. In each case, the organism infecting its host was resistant to at least one of the anti-tubeculous drugs. In the same study, at least 1 case of extensively drug resistant TB (XDR-TB) was also reported from 55 countries. Patients with decreased immunity or comorbidities are at a greater risk.[12]

It is essential to detecting cases, detect them early and identify its drug sensitivity at an early stage. This will not only help in improving the patients' health but will also assist in decreasing its transmission in the community at large.[13] GeneXpert had a high sensitivity than AFB smear to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity), in those cases of pulmonary tuberculosis who have either smear negative or sputum scarce disease. This test has the advantages of being inexpensive, requires less manpower and gives results on the same day. It is a rapid and effective tool compared to the bronchial wash and reduces the time constraint associated with culture-based diagnosis. It also determines Rifampin Resistance simultaneously. The WHO endorsed the Xpert MTB/RIF assay in early 2011. It is a PCR based automated real-time test that detects the disease and the sensitivity of the drug rifampicin. Results are generated in two hours and patients can be started on treatment early.[14,15] It is a highly sensitive and specific test, low cost, less

Table 1. Demographics Details of Suspected Cases (N=88)

Age	Mean 49.5	SD 18.01	Range 18-80
Gender	N (%)		
Male	59 (67%)		
Female	29 (33%)		
Presenting Complaints			
Fever	69 (78.4%)		
Cough	63 (71.6%)		
Weight loss	57 (64.8%)		
Anorexia	38 (43.2%)		
Breathlessness	26 (29.5%)		
Hemoptysis	11 (12.5%)		
Chest pain	10 (11.4%)		
Night sweats	06 (6.80%)		

complexity and can be made widely available. This test has played a vital role in the diagnosis and therapeutic management of suspected cases of TB.[16]

The aim of this study was to analyze the sensitivity and specificity of the GeneXpert assay and AFB smear and compare it with the traditional mycobacterial cultures for TB diagnosis at a tertiary care hospital of Peshawar.

Methodology

A prospective observational study was carried out in the Department of Pulmonology, Rehman Medical Institute (RMI) Peshawar, from January 2018 to December 2019. The study sample was 88 patients with suspected tuberculosis. A patient was suspected for tuberculosis based on the clinical and radiological evidence that corresponded to pulmonary TB. A case was considered smear negative if it did not show any acid-fast bacilli on microscopic examination using a zeihl nelson stain on three consecutive early morning specimens. Sputum scarce disease was defined as patients having less than 1ml of sputum. A confirmed case of pulmonary tuberculosis was one in whom Mycobacterium tuberculosis (MTB) grew on BAL mycobacterial cultures by LJ medium, that was taken as the gold standard. Any patient 18 years or older who had visited the pulmonology unit and was suspected for Pulmonary Tuberculosis (PTB) with a history of cough, fever and weight loss ≥2-3 weeks, had taken any of the anti-tubeculous medications for less than 7 days and a chest radiograph showing signs of TB were included in the study. Smear positive cases, disseminated or extra pulmonary tuberculosis, and those that were on anti-tubercular therapy (ATT) for 2 weeks or more in the past 3 months, not fit medically or not willing to undergo bronchoscopy, and patient that did not show on follow-up appointments were excluded from the study.

A standardized proforma was used for data collection. Demographic, laboratory findings, clinical and radiological features were recorded and subsequently entered Statistical Package for Social Sciences (SPSS). In order to obtain the sample, each patient had a flexible bronchoscopy through the trans-nasal route. The procedure involved the inspection of the bronchial tree and bronchial alveolar lavage was taken in three tubes and later on transferred to one: falcon tube.

It was sent for ZN stain and mycobacterial cultures by Lowenstein Jenson (LJ) medium to the Hospital Laboratory and to WHO sponsored Hayatabad Medical Complex (HMC) Laboratory, Peshawar, for GeneXpert studies. BAL specimens were processed by HMC laboratory using standardized protocols and quality assurance procedures for the Xpert MTB/RIF assay. The results of all tests were read by a trained technologist and reported for detection of MTB and

Table 2. Details of the Suspected Cases (N=88)

Variables	Percentage
Smoking history	
Current	13 (14.8%)
Ex-smoker	15 (17.0%)
Co-morbidities & Contributory Factors	
Diabetes mellitus	23 (26.1%)
Chronic liver disease	07 (8.0%)
Alcohol	06 (6.8%)
Malignancy	06 (6.8%)
Chronic renal failure	03 (3.4%)
HIV	01 (1.1%)
Radiological Findings	
Infiltrates	74 (84.1%)
Cavitation	11 (12.5%)
Milliary pattern	06 (6.8%)
Fibrosis	04 (4.5%)
Overall Cases	
New cases	76 (86.4%)
Relapse	04 (4.5%)
Default	03 (3.4%)
Failure	01 (1.1%)

presence or absence of rifampicin resistance. BAL specimens for smear microscopy and mycobacterial cultures were evaluated at Rehman Medical Institute Laboratory and results were available after 1-2 days and 6 weeks respectively.

After completing data entry, the statistical analysis was performed using SPSS version 23.0. Frequencies of AFB smear, GeneXpert assay and mycobacterial cultures were calculated by percentages. Sensitivity, specificity, and predictive values were calculated with 95% class intervals (CIs). A $P < 0.05$ was considered statistically significant.

Results

A total of 88 patients were seen to fulfill the inclusion criteria. The mean age of patients being 49.5±18.01 years. About 59 patients (67%) were males while the rest were female patients. The common presenting complaints included fever (78.4%), weight loss (71.6%), cough (64.8%), anorexia (43.2%), breathlessness (29.5%), hemoptysis (12.5%) chest pain (11.4%) and night sweats (6.8%). The demographic features of are presented in Table 1.

Radiological features compatible with the diagnosis of tuberculosis included infiltrates (84.1%), cavitation (12.5%), milliary shadows (6.8%) and fibrosis (4.5%). Around 76 (86.4%) were newly diagnosed cases of tuberculosis, 04 (4.5%) were relapse, 03 (3.4%) were default and only 01 (1.1%) case was of failure. The history of smoking and comorbidities along with other risk factor of these patients are presented in Table 2.

Out of 88 patients who were tuberculosis suspect, 73 (82.9%) cases were confirmed as pulmonary tuberculosis on mycobacterial cultures which was taken as a gold standard. On GeneXpert assay 72 (81.8%) cases and on AFB (BAL) smear only 52 (59.1%) were positive for MTB. Table 3.

The AFB smear test had 71.2% sensitivity, 100.0% specificity with 100.0% PPV and 41.7% NPV compared to culture report (p~0.00). The GeneXpert test had better sensitivity 98.6% and good specificity 86.7% with 97.3% PPV and 92.9% NPV compared to culture test (p~0.00). The frequency, sensitivity, specificity, positive predictive value, and negative predictive values for BAL ZN stain and GeneXpert are presented in Table 4.

Four patients (4.5%) had rifampicin resistance detected on BAL GeneXpert, while the rest had susceptible strains. Five patients (5.37%) had rifampicin resistance detected on mycobacterial cultures; there was only one patient with rifampicin resistance detected on mycobacterial cultures that was not detected on GeneXpert, hence BAL GeneXpert was able to detect rifampicin resistance in 83.33% of cases in this study with a specificity of 100%.

Table 3. Comparison between AFB smears microscopy, GeneXpert MTB/RIF assay, and MTB culture of suspected patients.

Variables	AFB Smear Microscopy N (%)	GeneXpert MTB/RIF Assay N (%)	MTB Culture N (%)
Positive	52 (59.1)	72 (81.8)	73 (82.9)
Negative	36 (40.9)	16 (18.2)	15 (17.1)
Total	88 (100)	88 (100)	88 (100)

Table 4. Comparison of AFB Smear and Gene Xpert to culture. (n=88)

Variables	Percentages						
	N (%)	Sensitivity	Specificity	PPV	NPV	Accuracy	P-value
MTB Test							
AFB Smear Positive Negative	 52 (59.1) 36 (40.9)	71.2%	100%	100%	41.7%	83.0%	0.000
GeneXpert MTB detected MTB not detected	 72 (81.8) 16 (18.2)	98.6%	86.7%	97.3%	92.9%		

Discussion

To the best of our knowledge, this study is the first of its kind that finds the diagnostic accuracy of GeneXpert and AFB smear in comparison to the mycobacterium culture in RMI, Peshawar. The study highlights that common presenting complaints, radiological features and associated comorbidities in patients with TB along with the biostatistical analysis comparing TB culture, GeneXpert and AFB smear.

Overall, 73 (82.9%) cases were confirmed as pulmonary tuberculosis on mycobacterial cultures, 72 (81.8%) cases on GeneXpert assay and only 52 (59.1%) on AFB (BAL) smear. While a study at central Punjab Pakistan showed that 48 (28.5%) cases were MTB positive on GeneXpert assay and 58 (34.5%) cases were positive for pulmonary tuberculosis on MTB culture test.[17]

In the same study only 1 case was detected as rifampicin resistance by GeneXpert assay. While in our study it was also a single case detected as rifampicin resistance by GeneXpert MTB/RIF assay.

Shah R et al[18] conducted a study which showed that sensitivity and specificity of GENE XPERT for MTB detection is 79.31% and 90.91 % & for rifampicin resistance is 64.29% & 100% respectively. While in our study the sensitivity and specificity of GeneXpert for MTB detection is 98.6% and 86.7% and for rifampicin resistance is 64.29% & 100% respectively.

Another study revealed that GeneXpert had 95.9% sensitivity and 94.4% specificity in diagnosing pulmonary TB and overall diagnostic accuracy of 95.5%.19 While in our study the GeneXpert had 98.6% sensitivity and 86.7% specificity in comparison to the culture and an overall diagnostic accuracy of 83.0%. In our study specificity and diagnostic accuracy is quite lower than the above study while sensitivity of the test is a little high.

A study on diagnostic accuracy of Xpert assay, taking TB culture as gold standard, found that for smear microscopy, the sensitivity (69.6%), specificity (95.5%), PPV (86.7%) and NPV (88.3%) whereas for GeneXpert they read 86.3%, 84.1%, 69.5%. 93.6% respectively.20 While in our study these readings for AFB smear microscopy were 71.2%, 100%, 100%, 41.7% respectively whereas for GeneXpert they were 98.6%, 86.7%, 97.3%. 92.9% respectively.

In the same study commonest symptoms of the patients were cough experienced by 86% (dry or productive), followed by fever 42.5% and weight loss 39.8%. While in our study the most common symptoms were fever, weight loss and cough; accounted for 78.4%, 71.6% and 64.8% respectively.

Bashir YU et al[21] conducted a study the results of which showed the sensitivity of the Xpert MTB/RIF assay was 97% while the specificity was 90%. The reference standard being the culture, the overall PPV and NPV for the Xpert MTB/RIF assay were and 63.4% and 99.4% respectively. The samples were aso tested by smear microscopy, which yielded a sensitivity of 36.7% and a specificity of 100%. PPV and NPV values for smear microscopy were 100% and 89.8% respectively. In our study the sensitivity and specificity of the Xpert MTB/RIF were 98.6%, 86.7% respectively and PPV and NPV were 97.3%, 92.9% respectively. The sensitivity and specificity for smear microscopy were 71.2%, 100% respectively and PPV and NPV were 100%, 41.7% respectively.

In the same study the only one patient was detected as rifampicin resistance by Xpert MTB/RIF assay while all other were rifampicin sensitive. While in our study it was also a single case detected as rifampicin resistance by GeneXpert MTB/RIF assay, others were rifampicin sensitive.

Conclusion

GeneXpert had a high sensitivity than AFB smear to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity), in those cases of pulmonary tuberculosis who have either smear negative or sputum scarce disease. This test has the advantages of being inexpensive, requires less manpower and gives results on the same day.

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