Pak. J. Chest Med. 2022;28(04)

Original Article

journal homepage: https:/www.pjcm.net/

Pakistan Journal of Chest Medicine

Official journal of Pakistan Chest Society

Comparative Sensitivity of Smear Microscopy for Acid Fast Bacilli versus Gene Xpert MTB/Rif Assay on Endobronchial washings of the Sputum Smear Negative/Sputum Scarce patients with suspected Pulmonary Tuberculosis keeping Culture as Gold standard

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Article History:

Received:	Oct 15, 2022
Revised:	Nov 25, 2022
Accepted:	Dec 01, 2022
Available Online:	Dec 02, 2022

Author Contributions:

MY ST MAK conceived idea, MY ST SYMS MA drafted the study, MY FM MA collected data, MY ST MAK SYMS did statistical analysis and interpretation data, MY ST did critical reviews manuscript. All approved final version to be published.

Declaration of conflicting interests
All authors declare that they have no conflicts of interest.

How to cite this article:

Yasin M, Mustaan F, Abbass M, Tareen S, Khan MA, Shah SYM. Comparative Sensitivity of Smear Microscopy for Acid Fast Bacilli versus Gene Xpert MTB/Rif Assay on Endobronchial washings of the Sputum Smear Negative/Sputum Scarce patients with suspected Pulmonary Tuberculosis keeping Culture as Gold standard. Pak J Chest Med 2022;28(4):465-470

ABSTRACT

Background: Tuberculosis (TB) is the world's tenth most common cause of death, and it continues to be a serious global health problem, generating considerable morbidity and mortality. According to the World Health Organization (WHO) Global Tuberculosis Report 2020, there were an estimated 10 million new tuberculosis cases and 1.4 million tuberculosis deaths globally in 2019. TB incidence and mortality rates have decreased since the WHO end TB Strategy was adopted in 2014; nonetheless, the rate of decline has been far slower than predicted, highlighting the need for better, more focused implementation of this strategy.

Objectives: To determine the comparative sensitivity of smear microscopy (SM) for acid fast bacilli (AFB) versus Gene Xpert MTB/RIF assay on endobronchial washings of the Sputum Smear Negative/Sputum scarce patients with suspected Pulmonary tuberculosis (PTB) keeping culture as gold standard.

Methodology: This Cross-sectional validational study was carried out in the Department of Pulmonology, Ayub Teaching Hospital Abbottabad, Pakistan from 28th March to 28th September 2022. All patients of any gender having age 20 to 75 years with TB who were sputum smear negative or had scarce sputum were included. Patients who were already diagnosed as PTB and those who were already on anti tuberculous treatment were excluded.

Results: Mean age of the patients was 47.02±14.65 years ranging from 20 to 75 years with 44(50.6%) males and 43(49.4%) females. In comparative sensitivity of Gene Xpert with respect to culture as gold standard the sensitivity of Gene Xpert was 93.24% while its specificity was 84.62% with positive predicted value (PPV) of 97.18%, negative predicted value (NPV) of 68.75% and accuracy of 91.95%. In comparative sensitivity of AFB SM with respect to culture as gold standard, the sensitivity of SM was 74.32% and specificity was 53.85%, PPV was 90.16%, NPV was 26.92% and the accuracy was 71.26%.

Conclusion: Gene Xpert is more sensitive than SM to detect mycobacterium TB and rifampicin resistance. Gene Xpert can be utilized for earlier detection of rifampicin resistant TB cases on the endobronchial washings while waiting for detailed culture results in suspected PTB cases having either sputum negative or sputum scarce disease.

Key words: Comparative sensitivity; Smear microscopy; Gene Xpert; Endobronchial washings; Sputum smear.

Introduction

Tuberculosis (TB) is the world's tenth most common cause of death, and it continues to be a serious global health problem, generating considerable morbidity and mortality.[1] TB incidence and mortality rates have decreased since the WHO end TB Strategy was adopted in 2014; nonetheless, the rate of decline has been far slower than predicted, highlighting the need for better, more focused implementation of this strategy.[2] The early detection and treatment of tuberculosis is a critical component of this strategy. The microbiologic diagnosis of obtained specimens determines the appropriate treatment prescription.[3]

Till now, pulmonary tuberculosis (PTB) has been diagnosed in 84% of documented TB cases, but only 57 % of PTB cases worldwide and about 50 % in China have been bacteriologically verified.[1] Smear microscopy (SM) and culture are two common laboratory procedures for identifying Mycobacterium tuberculosis (MTB) infection.[4] Sputum SM is a frequent approach for diagnosing PTB because of its speed, low cost, and high specificity (98–99%), but its sensitivity is only about 50%, implying that many PTB patients are sputum-smear negative or have little sputum.[5]

Furthermore, while MTB culture is still the gold standard for detecting acid-fast bacilli (AFB), it is hampered by a poor sensitivity and time-consuming method (2–6 weeks) and is not frequently accessible.[5] The WHO authorized the Xpert MTB/Rifampicin (MTB/RIF) assay in 2010; it is a molecular-based test that can identify TB and RIF resistance in two hours and requires relatively basic technical skill. Additional data has established the Xpert assay as a first-line sputum test for diagnosing PTB and RIF-resistant TB, replacing SM, and culture.[6,2] In smear-negative PTB patients, however, the combined sensitivity of Xpert MTB/RIF assay was only 67%.6 The bacteriologic diagnosis of PTB in patients with sputum smear-negative or sputum-scarce is difficult.[7,2] Bronchoscopy can be beneficial for these individuals because it allows the physician to get acceptable specimens from the lower respiratory tract while evaluating all of the airways.[8] The literature on the diagnostic accuracy of the Xpert test on bronchoalveolar lavage fluid (BALF) is sparse, and most studies have employed small sample sizes.[9,10]

In a study conducted in Pulmonology Department, Pak Emirates Military Hospital Rawalpindi in which sensitivity, specificity, positive predicted value (PPV) and negative predicted value (NPV) of smear microscopy were 69.6%, 95.5%, 86.7%, 88.3% respectively whereas for Gene Xpert these were 86.36%, 84.1%, 69.5%. 93.66% respectively; when compared to culture considered as gold standard.[11]

Although WHO restricted recommendations on MTB Gene Xpert are for sputum samples, there are no special recommendations for bronchoscopy samples yet. Finally, there have been a small number of studies that have evaluated the Xpert MTB/RIF assay excellency using bronchoscopy samples for the diagnosis of TB in high prevalence countries.[11] Currently, there is also no published study in our region. The results of this study will provide us with local magnitude of the problem and data about sensitivity of AFB and gene expert. These results will then be compared with other internationally published data and based upon these comparisons, further steps can be taken or further line of action may be decided.

The objective of our study was to determine the comparative sensitivity of smear microscopy (SM) for AFB versus Gene Xpert MTB/RIF assay on endobronchial washings of the Sputum Smear Negative/Sputum Scare Patients with suspected PTB keeping culture as gold standard in our region.

Methodology

The Cross-sectional validational study was conducted in Department of Pulmonology, Ayub Teaching Hospital Abbottabad, Pakistan from 28th March 2022 to 28th September 2022. This hospital is situated on the Silk Road and is a tertiary care hospital with more than 1000 beds. It provides both undergraduate and postgraduate teaching facility. Hospital drains patients from whole Hazara Division of Khyber Pakhtunkhwa and adjacent areas of Hassan Abdal tehsil of Punjab province, Azad Jammu & Kashmir and Northern areas of Pakistan.

A total of 87 cases were enrolled via consecutive non-probability sampling technique. Sample size was calculated using sample size calculator for sensitivity and specificity with the following assumptions: Sensitivity= 86.36%; Specificity= 84.1%; Prevalence = 50%; Precision = 11%

Patients of age 20 to 75 years of any gender having high suspicion for TB who were repeatedly sputum smear negative or sputum scarce were included. Patients who were already diagnosed as pulmonary tuberculosis and those who were already taking anti tuberculous treatment were excluded. Permission from hospital ethical committee was taken followed by written informed consent from every patient.

All the demographic characteristics like age, gender, marital status and level of education of every individual were recorded. Endobronchial washings were collected from all study cases having smear negative PTB and patients with scarce sputum. Endobronchial washings were collected using fiber-optic bronchoscope following instillation of 20-30 ml of isotonic saline in affected lobes

guided by radiological findings. The collected aspirates were divided into three specimens, and 2ml of each specimen was sent to microscopy for AFB, MTB gene Xpert/RIF assay and culture for Mycobacterium tuberculosis using Lowenstein Jensen (LJ) medium. Keeping culture as a gold standard sensitivity of the SM to detect AFB was compared to MTB gene Xpert/RIF.

Data was analyzed by using statistical software SPSS version 25. For age and duration of illness, mean and standard deviation were used and frequencies and percentages were used for categorical variables such as gender, marital status, educational status and SM for AFB/Gene Xpert and culture. The inferential statistics was applied for the inference of data. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by using 2x2 contingency table as: Sensitivity = TP/(TP+FN) x 100; Specificity = TN/(FP+TN) x 100; PPV = TP/(TP+FP) x 100; NPV = TN/(FN+TN) x 100, where TP= True positive, FP = False positive, TN = True negative, FN = False negative.

Results

A total of 87 patients were included in the study to determine the comparative sensitivity of SM for AFB versus Gene Xpert MTB/RIF assay on endobronchial washings of the Sputum smear negative/Sputum scarce patients with suspected PTB keeping culture as gold standard.

Mean age of the patients was 47.02±14.65 years ranging from 20 to 75 years. Forty four (50.6%) patients were males and 43 (49.4%) were females. Seven (8.0%) patients were single and 80 (92.0%) were married. Eleven (12.6%) were illiterate, 16 (18.4%) had primary, 7 (8.0%) had middle, 23 (26.4%) had secondary, 24 (27.6%) had higher secondary and 6 (6.9%) had graduation level of education.

Seventy four (85.1%) patients were culture positive and 13 (14.9%) were culture negative, AFB SM revealed 61 (70.1%) cases as positive and 26 (29.9%) cases were negative while Gene Xpert was positive in 71 (81.6%) patients and negative in 16 (18.4%) cases. Twenty nine (33.3%) patients were below 40 years of age group and 58 (66.7%) were of age 40 years and above.

In frequency distribution of gender with respect to culture, 34 (39.1%) male patients were positive while from female patients 40 (46.0%) were positive. This finding is statistically significant (p=0.039). In frequency distribution of gender with respect to SM, 31 (35.6%) male patients were positive and 30 (34.5%) female patients were positive. This finding is statistically insignificant (p=0.944). In frequency distribution of gender with respect to Gene Xpert, from male patients 34 (39.1%) were positive while from female patients 37 (42.5%) were positive. This finding is statistically insignificant (p=0.291).

In frequency distribution of gender with respect to marital status, from male patients 0 (0%) were single and 44 (50.6%) were married while from female patients 7 (8.0%) were single and 36 (41.4%) were married. This finding is statistically significant (p=0.005).

In frequency distribution of gender with respect to education level, from male patients 6 (6.9%) were illiterate, 8 (9.2%) had primary, 4 (4.6%) had middle, 9 (10.3%) had secondary, 12 (13.8%) had higher secondary and 5 (5.7%) had graduate level of education while from female patients 5 (5.7%) were illiterate, 8 (9.2%) had primary, 3 (3.4%) had middle, 14 (16.1%) had secondary, 12 (13.8%) had higher secondary and 1 (1.1%) had graduate level of education. This finding is statistically insignificant (p=0.553).

In frequency distribution of age group with respect to culture as gold standard, from age group of below 40 years 25 (28.7%) were positive and 4 (4.6%) were negative while from age group of 40 years and above 49 (56.3%) were positive and 9 (10.3%) were negative. This

		+ Culture -	
Gene Xpert	+	a TP	b FP
	-	c FN	d TN

		+ Culture -	
SM for AFB	+	a TP	b FP
	-	c FN	d TN

Contingency table for the calculation of sensitivity and specificity of Gene Xpert and Smear microscopy.
TP = True positives, FP = False positives, TN = True negatives, FN = False negatives

finding is statistically insignificant (p=0.832).

In frequency distribution of age group with respect to AFB microscopy, from age group of below 40 years 22 (25.3%) were positive and 7 (8.0%) were negative while from age group of 40 years and above 39 (44.8%) were positive and 19 (21.8%) were negative. This finding is statistically insignificant (p=0.408).

In frequency distribution of age group with respect to Gene Xpert, from age group of below 40 years 25 (28.7%) were positive and 4 (4.6%) were negative while from age group of 40 years and above 46 (52.9%) were positive and 12 (13.8%) were negative. This finding is statistically insignificant (p=0.434).

In frequency distribution of age group with respect to marital status, from age group of below 40 years 7 (8.0%) were single and 22 (25.3%) were married while from age group of 40 years and above, 0 (0%) were single and 58 (66.7%) were married. This finding is statistically significant (p=0.000).

In frequency distribution of age group with respect to education level, from age group of below 40 years, 0 (0%) were illiterate, 3 (3.4%) had primary, 1 (1.1%) had middle, 12 (13.8%) had secondary, 13 (14.9%) had higher secondary and 0 (0%) had graduate level of education while from age group of 40 years and above, 11 (12.6%) were illiterate, 13 (14.9%) had primary, 6 (6.9%) had middle, 11 (12.6%) had secondary, 11 (12.6%) had higher secondary and 6 (6.9%) had graduate level of education. This finding is statistically insignificant (p=0.002).

In comparative sensitivity of Gene Xpert with respect to culture as gold standard, Gene Xpert revealed sensitivity of 93.24% and specificity of 84.62%. PPV was 97.18%, NPV was 68.75% and the accuracy was found to be 91.95% while SM had sensitivity of 74.32% and specificity of 53.85%. PPV was 90.16%, NPV was 26.92% and the accuracy was 71.26% with respect to culture as gold standard (Table 1, 2).

Discussion:

World Health Organization recommends bacteriological confirmation of PTB by the detection of acid-fast bacilli (AFB) in respiratory specimens. However about 40-60% of patients with clinically or radiologically suspected PTB may fail to produce sputum, or when it is available, AFB may be negative on repeated smear examinations. It means a large population of patients remain smear negative despite having PTB, which causes unnecessary delay in diagnosis and in prompt management, resulting in huge mortality and morbidity for the disease which is completely curable once diagnosed earlier. These sputum smear negative patients and those who fail to produce any sputum can be diagnosed by flexible fiber optic bronchoscopy (FOB).[12]

Several studies around the world have proven the value of FOB whose prebronchoscopic sputum specimens were negative both for smear and PCR analyses. Furthermore, while mycobacterial culture remains the gold standard for laboratory diagnosis of TB, it requires 2-6 weeks to confirm a diagnosis. This results in delays in initiating appropriate treatment while waiting for this confirmation, except for cases where there is strong enough clinical suspicion to initiate a presumptive anti-TB therapy.[13]

Since December 2010, WHO has recommended the use of Gene Xpert MTB/RIF assay due to its high-quality performance compared to microscopy, and especially in cases of smear-negative specimens.[14] Few studies around the world have compared the diagnostic accuracy of SM for AFB to Gene Xpert MTB/RIF assays on bronchial washings.[15] A Pakistani study demonstrated positive BAL SM in 24% of their subjects and 32.4% positive Gene Xpert. Our study shows positive Gene Xpert in 71(81.6%) cases while AFB SM was positive in 61 (70.1%) cases. The difference may be due to selection of patients with different extents of disease. The mean age of their study participants was 45.75±17.73 years while it was 47.02±14.65 years in our study.[15]

In comparative sensitivity of Gene Xpert with respect to culture as gold standard, Gene Xpert revealed sensitivity of 93.24% and specificity of 84.62% while AFB SM revealed sensitivity of 74.32% and specificity of 53.85%. Raja SK et al reported sensitivities of 94.74% and 75.44% and specificities of 80.43% and 86.59% respectively for Gene Xpert and AFB SM. Their findings are quite similar to our findings.[15] We observed in our study that Gene Xpert is

Table 1. Comparative sensitivity of Gene Xpert with respect to culture as gold standard

		Culture				
		Positive	**Negative**	**Total**	Sensitivity	93.24
Gene Xpert	Positive	69	2	71	Specificity	84.62
	Negative	5	11	16	PPV	97.18
Total		74	13	87	NPV	68.75
					Accuracy	91.95

Table 2. Comparative sensitivity of AFB microscopy with respect to culture as gold standard

		Culture				
		Positive	Negative	Total	Sensitivity	74.32
					Specificity	53.85
AFB microscopy	Positive	55	6	61	PPV	90.16
	Negative	19	7	26	NPV	26.92
Total		74	13	87	Accuracy	71.26

significantly more sensitive test than SM for the diagnosis of PTB.

We observed the PPV of Gene Xpert as 97.18%, hence this test can be confidently used for diagnosis of PTB in a majority of cases while relative lower NPV of 68.75% in our study is indicative of lesser value in confidently ruling out PTB. The literature suggests that Gene Xpert can be positive in patients with dead and non functional AFB. These are the ambiguous areas where culture is required to get the more accurate diagnosis by demonstrating growth of bacilli.[16]

Our study revealed the PPV of SM to be 90.16% and NPV to be 26.92% with respect to culture as gold standard. The low NPV of SM is again an indication of low reliance on this test in ruling out active PTB.

Limitations of our study was as CT was not performed in all patients hence the investigators may have not received proper samples in some patients. This might have affected the diagnostic yield. The strength of our study was both Gene Xpert and AFB SM were compared with the gold standard test.

Conclusion

Gene Xpert is more sensitive than SM to detect MTB and rifampicin resistance in endobronchial washings of patients with smear negative or sputum sparce disease. Selection of appropriate patients for this investigation can increase the diagnostic yield in suspected PTB cases.

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