

Diagnostic accuracy of GeneXpert and sputum Zeil Nelson Staining in predicting tuberculosis taking Sputum Culture as Gold standard

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SWHS RU ZI conceived idea, SKR JA JKA drafted the study, SO SN collected data, RU ZI did statistical analysis & interpretation of data, SWHS RU SKR JKA ZI critical reviewed manuscript, All approved final version to be published.

Declaration of conflicting interests

The Authors declares that there is no conflict of interest.

Abstract

Background: Tuberculosis (TB) is a highly prevalent infectious disease that can involve any part of the body; the most favored site is lung. Tests used for its diagnosis include sputum Zeil Nelson staining, Gene Xpert, and culture.

Objective: Objective of this study was to determine the diagnostic accuracy of GeneXpert and sputum-ZN staining in predicting tuberculosis taking sputum culture as Gold standard.

Methodology: This cross sectional validation study was conducted in March 15/2015 the Pulmonology department, Military Hospital Rawalpindi from September 14/2015 to 14/09/2015. Patients of both gender aged 18-70 years and pulmonary TB (PTB) suspect were included in the study through consecutive non-probability sampling technique. The sample size was calculated through WHO software, assuming 25% TB prevalence, 95% confidence interval and 10% margin of error. Diagnosed PTB Patients, those on anti-tuberculosis (ATT) treatment, patients with co-morbid like congestive heart failure, Diabetes mellitus, HIV positive and patients on steroids were excluded from the study.

Results: Out of 265 patients, 183 (69.1%) were male and 82 (30.9%) female. Mean age was 45.65±14.91 years. Sensitivity and Specificity of Sputum smear microscopy was 68.48% and 74.07% respectively. While PPV, NPV and diagnostic accuracy of sputum smear microscopy was 85.71%, 50.85% and 70.19% respectively. Sensitivity and Specificity of Gene Xpert was 97.83% and 92.59%. However Positive Predictive Value, Negative predictive Value and diagnostic accuracy of Gene Xpert test was 96.77%, 94.94% and 96.23% respectively.

Conclusion: GeneXpert is more accurate and reliable than sputum microscopy in predicting pulmonary TB.

Key words: Pulmonary TB; Gene Xpert; Sputum culture; sputum microscopy

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Introduction

Tuberculosis is a highly prevalent infectious disease with global burden of 9.4 million annual cases and two million annual deaths. Eighty percent (80%) of this global burden is shared by 22 low

income countries, including Pakistan. Pakistan ranked 5th among countries with high burden of Tuberculosis¹. PTB prevalence in Pakistan is 350 cases per 100 000 population which makes 25%². Tuberculosis can virtually involve any part of the body; the most favored site is being the Lungs.

Pulmonary tuberculosis can be diagnosed with various diagnostic including sputum ZN staining, Gene Xpert, and culture. A large population of patients remains sputum smear negative in spite of having pulmonary tuberculosis, which causes spread of tuberculosis resulting in huge mortality and morbidity of the disease which is completely curable once diagnosed early.

Sputum ZN stain is most commonly used test for PTB diagnosis. It detects tuberculosis within hours but it is less sensitive for diagnosing active Pulmonary Tuberculosis. Its sensitivity is 23% and specificity 100%³. Sputum culture is gold standard for diagnosing pulmonary tuberculosis (sensitivity 80 to 93 percent and specificity 100 percent) and it can be carried out in solid or liquid based mediums. Liquid based medium (BACTEC) takes 3 weeks for the detection of Mycobacteria while solid medium takes 4 to 6 weeks to grow AFB⁴.

Gene-Xpert is a new development in diagnosing PTB. It is real-time PCR based rapid molecular assay and simultaneously detects Rifampicin resistance. It can give results within 02 hours and is more sensitive in detecting mycobacterium tuberculosis than sputum smear ZN staining. Maynard-Smith L et al.⁵ stated that sensitivity of Gene Xpert is 92% and specificity is 100% while Sensitivity of sputum smear for pulmonary tuberculosis is 43%⁶ and specificity 100%. It can also detect those cases of pulmonary tuberculosis which are missed by sputum ZN staining.

WHO recommends the Gene Xpert® MTB/RIF assay due to its high yield especially in cases of smear-negative specimen⁶. By early detection of tuberculosis in sputum negative patient, Gene Xpert can prevent the spread of tuberculosis and reduces burden of tuberculosis. Gene xpert has significant mortality and morbidity benefits as it can reduce spread of mycobacterium by early detection and prompt treatment of tuberculosis.

Operational Definitions:

Tuberculosis suspect:

“A patient who is having history of fever 38 degree, cough and weight loss for greater than 3kg in a period of one month and having chest x-ray opacities in upper lobes but still under diagnostic workup”.

PTB:

“A patient is labeled as a case of PTB when sputum yields Mycobacterium tuberculosis which is seen as rod shaped bacilli under light microscopy”

GeneXpert MTB/RIF test:

Gene Xpert is Rapid molecular assay, which permits rapid TB diagnosis through Detection of the DNA of the

MTB.

Sensitivity:

The strength of Gene Xpert/ZN staining in detecting a positive case of TB when it is actually positive on culture based test

Specificity:

The strength of Gene Xpert/ZN staining in detecting a negative case of TB when actually it is negative on culture based test

Positive predictive value:

The probability of detecting a positive case of TB with Gene Xpert/ZN staining when actually it also positive on culture based test

Negative predictive value:

The probability of detecting a negative case of TB with GeneXpert/ZN staining when actually it also negative on culture based test.

True positive:

True positive test is a positive case of TB with Gene Xpert/ZN staining when actually it is also positive on culture based test.

False positive:

False positive test is a positive case of TB with Gene Xpert/ZN staining when actually it is negative on culture based test.

False negative:

False negative test is a negative case of TB with Gene Xpert/ZN staining when actually it is positive on culture based test.

Methodology

This was a Cross sectional validation study conducted at Pulmonology wards/OPD of Military Hospital, Rawalpindi from March 2015 to September 2015. A total of 265 patients were included in the study through consecutive non-probability sampling technique. Sample size was calculated Using WHO sample size calculator assuming 25% prevalence of PTB, Gene Xpert sensitivity and specificity of 92% and 100% respectively. Sensitivity and specificity of sputum ZN staining of 43.6% and 100%. Margin of error 10%, Confidence level 95%. Patients of either gender aged 18-70 years and pulmonary TB (PTB) suspect were included in the study. Diagnosed cases of PTB, those on anti-tuberculosis (ATT) treatment and those patients with co-morbid like congestive heart failure, Diabetes mellitus, HIV positive and patients on steroids were excluded from the study.

Patients, who fulfilled the diagnostic criteria and

presenting to pulmonology OPD/ward in military hospital Rawalpindi, were enrolled in the study after taking a written informed consent. Demographic characteristic was recorded. Three Early morning sample of sputum (each for sputum ZN staining, Gene Xpert and sputum culture) were taken in a sterile bottle after rinsing mouth thoroughly with water to avoid contamination. Collected sample were sent for sputum ZN staining for microscopy, Gene xpert and sputum AFB culture. All patients were reviewed after result of sputum culture after 6 weeks.

Statistical analyses were carried out with SPSS-15. Frequencies and percentages were calculated for categorical variables like gender, sensitivity, specificity. Mean±standard deviations (SD) were calculated for continuous variables like age, duration of illness. All results were presented in tables.

Results

Mean age of patients was 45.65±14.91 years (20-60 years) (Table-1). There were 183(69.1%) male and

82(30.9%) female patients (Table-2). 147(55.5%) patients were positive for Sputum Smear Microscopy (Table-3), 186(70.2%) for GENE Xpert MTB/RIF (Table-4) and 184(69.4%) patients for Sputum culture (Table-5).

Sputum Smear microscopy diagnostic accuracy was calculated by taking Sputum Culture findings as Gold standard. As per findings Sensitivity and Specificity of Sputum smear microscopy was 68.48% and 74.07%. While Positive predictive, Negative Predictive value and Diagnostic accuracy of Sputum Smear microscopy was 85.71%, 50.85% and 70.19% respectively. (Table-6)

Sensitivity and Specificity of Gene XPERT when compared with Sputum Culture (Gold Standard) was 97.83% and 92.59%. However Positive Predictive, Negative Predictive value and Diagnostic accuracy of Gene XPERT test was 96.77%, 94.94% and 96.23% respectively. (Table-7)

Discussion

No straightforward rapid tests has been available to accurately detect tuberculosis and its drug resistant

Table-1 Age Distribution of Patients

N0	265
Mean	45.65
SD	14.91
Minimum	20
Maximum	70

Table-2 Gender Distribution of Patients

	Frequency	Percentage
Male	183	69.1%
Female	82	30.9%
Total	265	100%

Table-3 Results of Sputum Smear Microscopy

	Frequency	Percentage
Male	183	69.1%
Female	82	30.9%
Total	265	100%

Table-4 RESULTS of Gene Xpert MTB/RIF

	Frequency	Percentage
Positive	186	70.2%
Negative	79	29.8%
Total	265	100%

Table-5 Results of Sputum Culture

	Frequency	Percentage
Positive	184	69.4%
Negative	81	30.6%
Total	265	100%

Table-6 Diagnostic Accuracy of Sputum Smear Microscopy

Sputum Smear Microscopy		SPUTUM CULTURE		Total
		Positive	Negative	
	Positive	126	21	147
	Negative	58	60	118
Total		184	81	265

SENSITIVITY= 68.48%

SPECIFICITY= 74.07%

POSITIVE PREDICTIVE VALUE= 85.71%

NEGATIVE PREDICTIVE VALUE= 50.85%

DIAGNOSTIC ACCURACY= 70.19%

Table-7 Diagnostic Accuracy of Gene Xpert MTB

GeneXpert MTB		SPUTUM CULTURE		Total
		Positive	Negative	
	Positive	180	6	186
	Negative	40	75	79
Total		184	81	265

SENSITIVITY= 97.83%

SPECIFICITY= 92.59%

POSITIVE PREDICTIVE VALUE= 96.77%

NEGATIVE PREDICTIVE VALUE= 94.94%

DIAGNOSTIC ACCURACY= 96.23%

forms, and this absence has been a major obstacle to improvement of tuberculosis care and reduction of the global burden of disease. Microscopy alone, although inexpensive, misses many patients and detects only those with relatively advanced disease.^{7,8}

Presently, only 28% of expected incident cases of tuberculosis are detected and reported as smear positive.² Undetected cases of disease increase morbidity, mortality, and disease transmission^{9,10}. In many countries, epidemic HIV infection has further reduced the sensitivity of microscopy and increased the necessity of rapid diagnosis of tuberculosis. The mortality of untreated or mistreated tuberculosis in people with advanced HIV is high.^{11,12}

Early identification of cases and their good treatment is essential to limit its transmission and achieve

successful TB control. Gold standards test for TB diagnosis is culture. However due to lack of access to culture facilities and the long turn-around time involved with culture, most laboratories use direct Ziehl-Neelson (ZN) microscopy for detection of acid fast bacilli (AFB) as their main diagnostic tool.

In recognition of these issues, substantial efforts are being made to strengthen laboratory capacity to diagnose smear-negative and multidrug-resistant tuberculosis, including increased use of solid and liquid culture, conventional drug-susceptibility testing, and line-probe assays. Unfortunately, these tests require extensive laboratory infrastructure and cannot be done outside of reference facilities.^{13,14}

Recently, a real-time PCR assay for Mycobacterium tuberculosis that simultaneously detects rifampicin

resistance was developed on the GeneXpert platform, which integrates sample processing and greatly simplifies testing.¹⁵ This assay, Xpert MTB/RIF, showed excellent performance in a multicenter study undertaken in reference laboratories. In the study, one-off direct MTB/RIF testing detected 922% of cases of pulmonary tuberculosis, including 725% of those with smear-negative disease, which was equivalent to that reported for solid culture.¹⁶

As per results of our study, sensitivity, specificity, PPV, NPV and diagnostic accuracy of GENE XPERT was 97.83%, 92.59%, 96.77%, 94.94% and 96.23%. On the other hand sensitivity, specificity, PPV, NPV and diagnostic accuracy of Sputum smear microscopy was 68.48%, 74.07%, 85.71%, 50.85% and 70.19% respectively. GENE XPERT showed more sensitivity and specificity as compared to sputum smear microscopy.

Andrea Rachow in his study validated the diagnostic accuracy of the Xpert MTB/RIF Assay. As per his findings Xpert MTB/RIF Assay achieved 88.4% (95%CI = 78.4% to 94.9%) sensitivity among patients with a positive culture and 99% (95%CI = 94.7% to 100.0%) specificity in patients who had no TB.¹⁷ Results of our study are consistent with the findings reported by Andrea Rachow. However sensitivity of GENE XPERT in our study was high i.e. 97.83% as compared to that of 88.4% and specificity was lower i.e. 92.59% vs. 99%.

A recent study from China evaluated the sensitivity and specificity of Xpert MTB/RIF which showed, the sensitivity and specificity to be 94.4% and 90.2%, respectively¹⁸. Our study findings of sensitivity and specificity of Gene-Xpert are consistent with the results of study by Ou X et al.

Previous studies of the MTB/RIF test that assessed either sputum samples or concentrated, decontaminated sputum pellets, have consistently reported test sensitivity of 72–75% in cases of smear-negative tuberculosis and 98–100% in cases of smear-positive tuberculosis.¹⁹⁻²²

Zeka in his study reported sensitivities for pulmonary specimens, to be 100% and 68.6% for smear-positive and smear-negative specimens, respectively.²³ Our findings on the performance of the Xpert MTB/RIF assay correlate well with those reported by others regarding the effectiveness of the assay in accurately detecting the presence of MTBC bacilli in AFB-negative specimens. In routine practice, the MTB/RIF test was quite faster (3 to 24 h) than culture, which require 6-8 weeks. Also the MTB/RIF test is less dependent on the user's skills, and routine staff with minimal training can use the test.

Conclusion

Hence it has been concluded through results of this

study is that GeneXpert is more accurate and reliable than sputum smear microscopy in predicting pulmonary TB. Now we are able to implement the use of GeneXpert for diagnosis of PTB instead of wasting time and cost on other diagnostic methods.

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