Diagnostic Accuracy of Ziehl-Neelsen Smear Microscopy in Comparison with GeneXpert in Pulmonary Tuberculosis: A Multi-Center Study in Kandahar Province, Afghanistan

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MA, NR and HS designed the study. Authors MA, MHS and HS analyzed the data and prepared the initial manuscript. Author AWW advised the whole research. All authors discussed the results and critically commented on the manuscript at all stages. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Tuberculosis (TB) is a major global health problem. The early and accurate diagnosis is crucial for disease management and to control disease transmission and the emergence of drug resistance TB.
Objectives: This study was carried out to determine the diagnostic accuracy of Ziehl Neelsen (ZN) smear microscopy in comparison with GeneXpert MTB/RIF in pulmonary tuberculosis in Kandahar province, Afghanistan.
Methods: This was a facility-based cross-sectional study. We scrutinized TB registers of three health facilities to include patients who had their sputum tested by both ZN smear microscopy and

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GeneXpert MTB/RIF. We extracted 734 patients' data registered during January 2019 - June 2020 in a structured form. Kappa value was analyzed using SPSS version 19 software at 95% Confidence Interval (CI). We calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of ZN smear microscopy against GeneXpert MTB/RIF.

Results: In comparison with GeneXpert as a reference test, ZN smear microscopy has 67.7% (95% CI:63.44%-70.74%) sensitivity and 100% (95% CI:98.74%-100.00%) specificity. The positive predictive value (PPV) and negative predictive value (NPV) were 100% and 99.89% (95% CI:99.88%-99.90%), respectively. The agreement between ZN smear microscopy and GeneXpert MTB/RIF results was moderate (70.4%) and the Kappa value was 0.45 (95% CI:0.38-0.59).

Conclusion: This study has found high specificity but moderate sensitivity for the diagnosis of pulmonary TB using sputum ZN smear microscopy test. Hence, GeneXpert MTB/RIF test is more accurate and reliable for the diagnosis of pulmonary TB.

ABBREVIATIONS

TP   :   *True Positive*
FP   :   *False Positive*
FN   :   *False Negative*
TN   :   *True Negative*

1. INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease that is predominantly affecting the lungs [1,2]. Globally, TB is still one of the top 10 causes of death and the leading cause of death from a single infectious disease. In 2019, there were an estimated 10.0 million (range, 8.9–11.0 million) cases and 1.4 million deaths. Geographically, most TB cases occurred in South-East Asia (44%), followed by Africa (25%) and the Western Pacific (18%). Afghanistan had 47406 new cases in 2017 and ranked 22nd among the top 30 high TB burden countries [3].

Various methods have been introduced for the diagnosis of tuberculosis [4,5]. World Health Organization recommends mycobacterial culture in high-resource settings. However, in low-resource settings, Ziehl Neelsen (ZN) smear microscopy is still recommended and is the preferred method for the diagnosis of pulmonary tuberculosis [4]. In Afghanistan, health facilities usually rely on results of ZN smear microscopy in conjugation with clinical symptoms and radiographic evidence to diagnose TB. Although ZN smear microscopy is a rapid and inexpensive method, studies have reported poor sensitivity [5,6].

GeneXpert mycobacterium tuberculosis (MTB)/ rifampicin (RIF) assay is considered a breakthrough for tuberculosis diagnostics with special features, such as accurate and rapid detection of TB patients in low-resource settings. Additionally, it has the potential to detect Rifampicin resistance. Recent studies have reported comparable sensitivity to mycobacterial culture [7,8-11].

Although National Tuberculosis Guideline (NTG) recommends GeneXpert test for the diagnosis of pulmonary TB, it is not widely famous for the diagnosis of pulmonary TB in Kandahar province. Many clinicians rely on results of ZN smear microscopy in conjugation with clinical symptoms and radiographic evidence to diagnose TB. A miss or wrong diagnose of smear positive pulmonary TB can have both health and cost related implications. Therefore, we aimed to determine the diagnostic accuracy of ZN smear microscopy taking GeneXpert as a reference test in pulmonary tuberculosis in Kandahar province, Afghanistan.

2. MATERIALS AND METHODS

2.1 Study Setting and Period

This is a cross-sectional analysis of a retrospective cohort involving suspected pulmonary tuberculosis patients treated at public hospitals in Kandahar province from January 2019 to June 2020. Briefly, the study was conducted in three hospitals in Kandahar province with sputum ZN smear microscopy and GeneXpert MTB/RIF tests available. The study sites included Provincial Tuberculosis Center (PTC), Mirwais Regional Hospital, and Médecins Sans Frontières (MSF) tuberculosis center.

2.2 Study Design

This is a hospital-based cross-sectional study.

2.3 Study Population

For this study, we included patients who had sputum samples tested by both ZN smear

microscopy and GeneXpert MTB/RIF methods. We excluded patients with incomplete files and those with missing either ZN smear microscopy or GeneXpert tests result.

2.4 Data Collection Methods

We extracted patient's data including, socio-demographic characteristics and results of both sputum smear microscopy and GeneXpert tests from TB registers.

In Kandahar, health facilities usually rely on results of ZN smear microscopy in conjugation with clinical symptoms and radiographic evidence to diagnose TB. In selected health facilities, all suspected pulmonary TB patients were initially subjected to sputum smear microscopic examination by fluorescence microscope. They were classified as sputum smear-negative (smear contains no acid-fast bacilli (AFB) in 100 fields) and sputum smear-positive. Sputum smear-positive cases were further graded as follows: "Scanty (1–9 AFB in 100 fields), 1+(10–99 AFB in 100 fields), 2+(1–9 AFB/field in at least 50 fields), and 3+(>10 AFB/field in at least 20 fields)" [12].

Later due to the low sensitivity of sputum smear microscopy and National Tuberculosis Guidelines [13], and as well as to detect Rifampicin resistance cases among smear positive pulmonary TB patients, all suspected pulmonary TB patients were subjected to be tested by Cepheid's GeneXpert MTB/RIF method. In this method, pulmonary Tuberculosis cases were classified as sputum positive and sputum negative.

2.5 Statistical Analysis

Patients who tested positive on GeneXpert MTB/RIF and those tested negative on GeneXpert MTB/RIF were defined as true positive and true negative, respectively. The diagnostic accuracy of ZN smear microscopy was evaluated using GeneXpert MTB/RIF test as a reference category. We used descriptive statistics such as percentage for true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Kappa value was estimated using SPSS version 19 software at 95% Confidence Interval (CI). We calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio of ZN smear microscopy against GeneXpert MTB/RIF [14,15].

3. RESULTS

A total of 734 patients were included in the analysis. Among these patients, 373 (50.8%) were male and 385 (52.2%) were from the urban residence. The mean (+SD) age of study participants was 38 (±7.1). It was found that 22.6% (166) were from age group 10-20, 203 (27.7%) from 21-30, and 240 (32.7%) were of age above 50 years. As shown in Table 1, about 76.6% (563) had no formal education, 100 (13.6%) had primary education, 67 (9.1%) had attained secondary education, and only 3 (0.4%) had higher education. About half (54.9%) were married. Around half (54.8%) of study patients were living with families having more than ten members (Table 1).

Among the sputum smear microscopy specimens on the fluorescence microscope, 227 (30.9%) were smear positive while 507 (69.1%) were smear negative. Of all the patients, 444 (60.5%) were sputum positive on GeneXpert MTB/RIF assay, yielding a 29.6% increase in sputum positivity compared to ZN smear microscopy. Of these 444 patients, 41 (9.3%) cases were detected as rifampicin resistance (Table 2).

Taking GeneXpert MTB/RIF as a reference test, we compared the performance of ZN sputum smear microscopy. Against GeneXpert MTB/RIF assay, ZN sputum smear microscopy has shown 67.7%, 95% CI (63.44%-70.74%) sensitivity and 100%, 95% CI (98.74%-100.00%) specificity (Tables 3 and 4).

According to GeneXpert MTB/RIF, ZN smear microscopy PPV was 100% and the NPV was 99.89 % (95% CI:99.88%-99.90%). The agreement for the results between ZN smear microscopy and GeneXpert was moderate (70.4%) and the Kappa value was 0.45 (95% CI:0.38-0.59) (Table 4).

4. DISCUSSION

This study was designed to determine the diagnostic accuracy of ZN smear microscopy in comparison with GeneXpert MTB/RIF in pulmonary TB in Kandahar province. WHO has recommended mycobacterial culture as the gold standard test for the diagnosis of TB. However, ZN smear microscopy and GeneXpert MTB/RIF are the most widely used tests in low resource settings. From this study, we found that the positive rate was 30.9% by ZN smear microscopy, and this rate was lower than that (60.5%) by GeneXpert MTB/RIF. The sensitivity

and specificity of ZN smear microscopy was 67.7% and 100%, respectively. Additionally, we found that there was a moderate agreement (70.4%) between the two tests.

Table 1. Socio-demographic characteristics of the patients

Variable	Total n (%)
Age groups	
10-20	166 (22.6)
21-30	203 (27.7)
31-40	87 (11.9)
41-50	38 (5.2)
> 50	240 (32.7)
Gender	
Male	373 (50.8)
Female	361 (49.2)
Residence	
Rural	349 (47.5)
Urban	385 (52.5)
Education level	
No formal education	563 (76.6)
Primary	100 (13.6)
Secondary	67 (9.1)
Higher Education	3 (0.4)
Marital Status (n=734)	
Married	403 (54.9)
Single	198 (27)
Widow	38 (5.2)
Household members	
1-5	42 (6.6)
6-10	247 (38.7)
>10	350 (54.8)

Table 2. ZN smear microscopy and genexpert MTB/RIF results of study participants

Result	Frequency (%)
ZN Smear Microscopy (n=734)	
Positive	227 (30.9)
Negative	507 (69.1)
GeneXpert MTB/RIF (n=734)	
Positive	444 (60.5)
Negative	290 (39.5)
GeneXpert Positive (n=444)	
RIF/Drug sensitive	403 (90.7)
RIF/Drug resistant	41 (9.3)

Table 3. Results of genexpert among suspected pulmonary Tuberculosis patients

		Disease		Total
		Positive	Negative	
Test	**Positive**	TP=404	FP=0	404
	Negative	FN=217	TN=290	507

Table 4. Measures of diagnostic accuracy of ZN smear microscopy

Indicators	Value (95% CI)
Sensitivity	67.17% (63.44%-70.74%)
Specificity	100% (98.74%-100.00%)
Positive Predicative Value (PPV)	100%
Negative Predicative Value (NPV)	99.89% (99.88%-99.90%)
Positive Likelihood ratio	-Infinity
Negative likelihood ratio	0.32 (0.29-0.37)
Accuracy	77.18% (99.40%-100.00%)
Kappa Value	0.45 (95% CI:0.38-0.59)

In this study, the finding of sensitivity for ZN smear microscopy is lower than that from previous studies (82.1-90.3) [5,7,6]. Our finding of specificity is similar to the results (93.8%-100%) of previous studies from other developing countries [5,7,6,16]. The variations reported in studies may be explained by differences in specimen collection, standard and quality of laboratories, and specimen testing times.

ZN smear microscopy missed 217 (29.6%) cases in comparison with cases detected by GeneXpert MTB/RIF. This finding of lower sensitivity in ZN smear microscopy is reasonable due to higher threshold detection in ZN smear microscopy (5000 bacilli/ml) in comparison with GeneXpert MTB/RIF (136 bacilli/ml) in specimens. A missed diagnosis of smear-negative pulmonary TB can have both health-related and financial implications for individuals and the health system.

In this study, we found that agreement between the two test results was moderate (70.4%, Kappa value, 0.45). 217 (29.6%) cases were positive with GeneXpert MTB/RIF assay but were negative with ZN smear microscopy [17,18]. This variability can be expected as GeneXpert MTB/RIF detects at lower bacillary load and can also be influenced by other factors, such as specimen collection and processing.

As our objective was to determine the diagnostic accuracy of ZN smear microscopy, the denominator linked to cases was not a gold standard test in the diagnosis of pulmonary TB. Additionally, we did not address any particular individual or system-related factors affecting the diagnostic accuracy of ZN smear microscopy.

Despite the aforementioned limitations, the significance of this research lies in the points below. First, it provides a clearer image of the diversity in two assays for the diagnosis of pulmonary TB in a relevant population of low-income and a high burden of TB. Second, the provision of such a large sample may prove worthwhile in comparison of the two assays. Hence, we believe this research will provide important findings related to these two assays for the diagnosis of pulmonary TB.

5. CONCLUSION

This study has found that GeneXpert outperforms ZN smear due to its high sensitivity and specificity. Hence, this study recommends that all suspected pulmonary TB cases should be diagnosed by GeneXpert MTB/RIF test in Kandahar province.

CONSENT AND ETHICAL APPROVAL

Ethical approval of the study was obtained from the Ethics and Research committee, Faculty of Medicine, Kandahar university. We obtained administrative approval from the Kandahar public health directorate. Informed consent of patients was not sought, as we collected and analyzed routine program data. Unique identifiers were removed from data analysis to ensure confidentiality of the study participants.

DATA AVAILABILITY

The primary data used to support the findings of this study are available from the corresponding author upon request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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