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GENEXPERT PCR VERSUS MICROSCOPY IN DETECTING PULMONARY MYCOBACTERIUM TUBERCULOSIS: A DIAGNOSTIC ACCURACY STUDY

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Abstract

Objectives: *The main objective of this study was to establish and compare the diagnostic performance of ZN microscopy and GeneXpert PCR.*

Study Design: *The current study was comparative cross-sectional study.*

Place and Duration of Study Duration: *The current study was conducted at Provincial Reference Laboratory Hayatabad Medical Complex with in the duration of six months from November 2024 to May 2025.*

Material and Methods: *80 Sputum samples were collected from though patients by using non probability consecutive sampling technique who present clinical sign and symptoms of Tuberculosis including both gender male and females who's aged were 1 to 80 years while those patients who have other lungs infection or lung diseases were excluded from the current study. Each patient gave two sputum specimens for examination. One sample was tested for acid-fast bacilli using the ZN staining method, while the second was subjected to the GeneXpert MTB/RIF assay. Comparing ZN microscopy's results with GeneXpert, the reference standard, underpinned evaluation of the performance of ZN microscopy's prediction.*

Results: *Of this sample of 80 patients, 43 (53.75%) were given the diagnosis of TB. GeneXpert detected each and every single case of TB hence 100% sensitivity and specificity. By contrast, 15 cases were found by ZN microscopy with a sensitivity of 45.45%, specificity 100%. The number for the positive predictive value was 100%, and the negative predictive value was 72% for ZN microscopy. The McNemar test indicated that the two diagnostic methods were significantly different ($p = 0.000$).*

Conclusion: *GeneXpert PCR demonstrates increased diagnostic potential over ZN microscopy in pulmonary TB, notably increased sensitivity and NPV. Although with limitations, ZN microscopy is still useful in resource-limited domains but the introduction of GeneXpert into TB diagnostics could result into early diagnosis, faster response and better control of TB transmission.*

INTRODUCTION

Pathogens causing tuberculosis stand as one of the serious public health difficulties which the twenty-first century faces worldwide. Many lives end each year from TB infections even though curative treatments exist principally in low- and middle-income nations (1).The success of TB control requires both early identification of TB cases and immediate start of treatment. Elective diagnosis of TB proves complex especially when operating in areas with limited resources (2). Verification through laboratory tests becomes essential in making an accurate diagnosis of TB because its clinical manifestations show non-specific features(3). The Ziehl-Neelsen (ZN) microscopy examination of sputum stains stand as the historic main approach to diagnose TB through acid-fast bacilli (AFB) detection (4). TB control programs require urgently new diagnostic tools able to simultaneously identify TB and evaluate drug resistance because they face increasing numbers of patients whose TB strains resist first-line medications such as rifampicin(5).The widespread recognition has been achieved by the GeneXpert MTB/RIF assay because researchers developed it to overcome the limitations present in earlier methods (6). Cepheid established the GeneXpert system which serves as an automated NAAT test through cartridges to determine Mycobacterium tuberculosis DNA content and rifampicin resistance peak mutations (7). Real-time PCR technology powers the diagnostic test which generates results during two hours. Since 2010 the World Health Organization granted GeneXpert status as the first test that doctors should use to diagnose MDR-TB and HIV-associated TB patients(8). Various studies confirm GeneXpert better performance than microscopy regarding detection in cases where smears exhibit negative results and among immunocompromised patients (9). The GeneXpert system produces diagnostic outputs and resistance information at once which makes it an extremely useful tool for tuberculosis control programs (10). The study findings will help advance worldwide tuberculosis elimination efforts through proven recommendations about diagnostic instruments together with implementation and optimization methods.

Material and Methods:

The present comparative cross-sectional diagnostic accuracy study was performed at TB Center of Hayatabad Medical Complex (Peshawar, Pakistan) during six months, between November 2024 and May 2025. Eighty people suspected to be pulmonary tuberculosis were recruited through non-probability consecutive sampling method. The sample was determined using the sensitivity of microscopy and its specificity which would be expected taking into consideration the 95 % of confidence interval and hence the prevalence of pulmonary TB. The study enrolled patients consecutively over the study period and who fitted in the inclusion criteria. Sample size was calculated by using formula ($n= Z^2 \times P \times (1 - P) / d^2$) for evaluating the diagnostic accuracy. Each of the enrolled subjects had two samples of sputum obtained two hours apart and before 11 a. m. in clean sterile containers that followed the standard biosafety practices. The processing of one of the sputum specimens was done on a microscope examination with Ziehl-Neelsen (ZN) stain, and the other sputum specimen was done with the GeneXpert MTB/RIF assay. The ZN staining was carried out in the following scheme: the smear was made, air-dried, fixed in heat, stained first with carbol fuchsine then rinsed in acid alcohol and lastly stained with methylene blue. A smear was stained and observed using an oil immersion lens under a microscope at 1000x magnification and AFB staining was graded by WHO/IUATLD criteria. The GeneXpert MTB/RIF was also performed using the manufacturer protocol. The sample reagent was added to the sputum sample ratio 2:1, heat incubated in a 15-minute period and then loaded into a GeneXpert cartridge. GeneXpert machine was used to insert the cartridge and thus a nucleic acid amplification was carried out automatically and the results obtained in terms of Mycobacterium tuberculosis detection and resistance to rifampicin within a period of two hours. Accordingly, sensitivity, specificity and general total diagnostic accuracy was calculated. The content of research was examined by the institutional Bioethical Committee (IBC) and found in accordance with ethical principles and policies followed by university with (Reference Number 1870 /ORIC/SUIT).

Inclusion Criteria:
1 to 80 years old males and females who had symptoms suggestive of pulmonary tuberculosis, such as persistent cough, weight loss, fever and night sweats and those who did not receive anti tuberculosis abuses in the past two months formed the inclusion criteria.

Exclusion criteria:
Exclusions included patients already undergoing anti-tuberculosis treatment for over two weeks, patients with extra pulmonary tuberculosis, patients known to resistant to TB drugs and patients who failed to yield a reasonable number of sputum samples.

Results:

Figure no.1

In current study total 80 sputum's samples from both and male and females having age ranges from 1 to 80 years were tested for tuberculosis among these 80 suspected patients the total positive cases of tuberculosis were (n=43). In these 43 positive cases of tuberculosis male (n=28) were more prevalent than female (n=15) as shown in figure no.1 and the most effected age group by Mycobacterium tuberculosis were Group III whose age were between 40 to 60 years as shown in figure 1.
Figure No.1 showing the distribution of TB cases in males and females and distribution of Tb cases in different age groups

In the current study total 80 sputum samples were tested for tuberculosis infection by both AFB Microscopy and GeneXpert PCR techniques. Among these 80 sputum samples n=33 samples were identified positive and n=47 samples were identified negative for tuberculosis infection by PCR as shown in figure no 3 while n=15 samples were identified positive while the rest of n= 65 samples were identified negative by microscopy as shown in figure no 3.

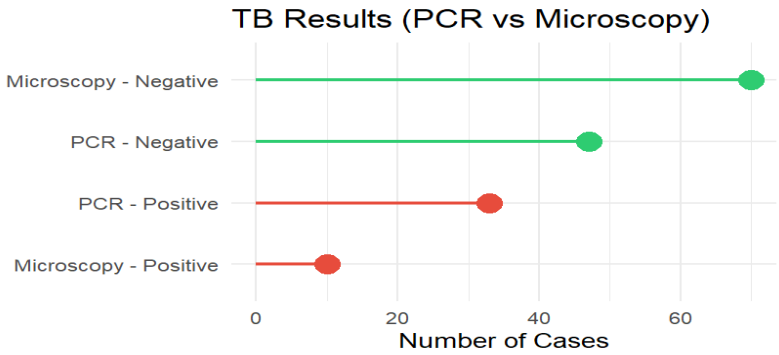

Figure no. 2

In the current study by comparing the both the techniques GeneXpert PCR is superior as far as TB diagnosis ability is concerned than AFB Microscopy. Overall total 80 cases GeneXpert PCR correctly identified all 33 TB-positive cases with no false negatives, demonstrating a perfect true positive rate, whereas AFB Microscopy only identified 15 cases, which resulted in 18 missed true positives reflective of lower sensitivity. In cases of TB absence, true negatives were correctly identified in all 47 instances by both GeneXpert PCR and AFB Microscopy as being equally specific. However, AFB Microscopy detected only 15 of the 33 TB-positive cases, indicating 18 cases as false negatives and hence done with a sensitivity of 45.45% and NPV 72% while PCR showed 100% sensitivity, 100% specificity, 100% NPV, and 100% PPV as shown in table no. I.

Table no. I Indicating the sensitivity and Specificity of Tb GeneXpert PCR and Microscopy (n=Number of samples, TP=True positive, FN= Fall Negative, FP= False Positive, TN=True Negative, NPV= Negative Predictive value and PPV= Positive Predictive value)

Test Method	TB Positive (TP)	TB False Negative (FN)	TB False Positive (FP)	TB Negative (TN)	Total	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
GeneXpert PCR	33	0	0	47	n=80	100	100	100	100
AFB Microscopy	15	18	0	47	n=80	45.45	100	72	100

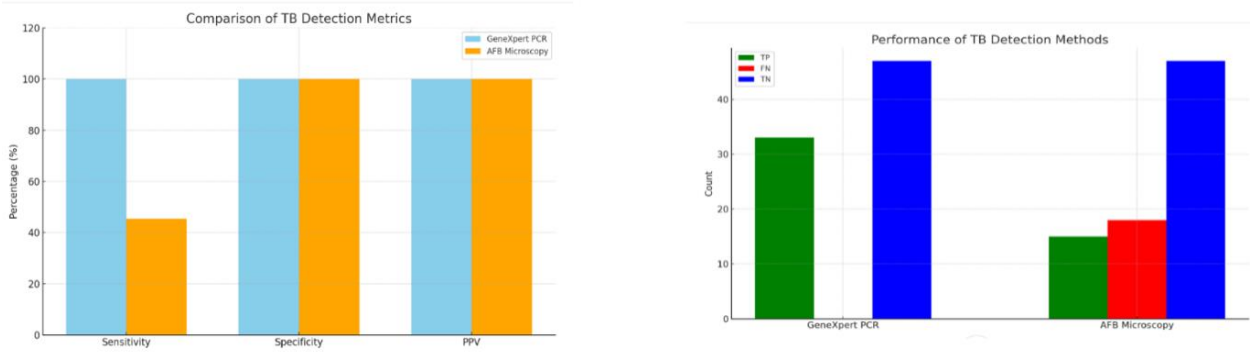

Figure no. 3

Figure no. 3 data show that while Gene Expert PCR scored a perfect 100%, sensitivity, specificity, and PPV, the AFB Microscopy scored 45.45% in testing sensitivity, yet scored 100% in not only specificity but also PPV. GeneXpert PCR is distinguished by the high accuracy and certainty provided in diagnosing TB and as such is the method of choice—particularly in the clinical setting where accurate TB diagnosis is essential.

McNemar Test for comparison of Sensitivity and Specificity of GeneXpert PCR and AFB Microscopy: In the current study we applied the McNemar Test to find out the significance difference in the accuracy of both techniques GeneXpert and AFB microscopy in the detection of tuberculosis. The P-value (Level of Sig) for the McNemar's Test was 0.000 which indicated that there is statistically significant different between the PCR and microscopy as shown in table no II.

Table no. II Indicate the McNemar Test Statistics.

Test Statistics[a]	
	Tb Report by PCR & TB Report by Microscopy
N	80
Exact Sig. (2-tailed)	.000[b]
a. McNemar Test	
b. Binomial distribution used.	

Discussion:

In our finding out of the 80 patients suspected of tuberculosis, 43 (53.75%) were found positive and the prevalence of cases among male (n=28) was more than that in female (n=15). This finding explains earlier reports of predisposition of TB in males as showing in figure no. 1. In the current study finding, a group of people between the age of 40-60 was affected most, which means that middle-aged people can be considered at risk. We found that, the accuracy of GeneXpert PCR was far better than AFB microscopy. The GeneXpert found 33 positive samples and in the AFB microscopy 15 positive were found leaving behind 18 cases of the real TB. The level of sensitivity of GeneXpert was 100 percent compared to microscopy of 45.45 percent. According to Maison et, al 2022, an estimated 57 percent of the world TB cases affected men and this trend was explained by more risk factors in men, including smoking, exposure to workplace hazards, and late presentation of ill health.(11)This coincides with the results of Acharya et al. (2020) who, in turn, saw that TB can affect the greatest number of people at the age of 35-60 years, as it is in such individuals that the cumulative effect of the infection and a decrease in the level of immunity takes place(12). Li et al. (2019) found out the similar age distribution pattern in Pakistan, which implies that awareness and screening programs focused on it should be organized in order to cover the most productive age segment. The accuracy of the diagnosis is an important TB control measure and our findings revealed that GeneXpert PCR was much more accurate than the AFB microscopy. GeneXpert detected 33 positive samples, whereas under the AFB microscopy, 15 positive samples were detected leaving behind 18 cases of true TB. The GeneXpert sensitivity level was 100 percent as opposed to 45.45 percent in microscopy. Such findings follow the evidence of Mirghani et al. (2024) who proved that a much higher TB sensitivity rate of GeneXpert in detecting TB especially in smear-negative and HIV-coinfected people (13) (14). Further, Gota et al. (2023) described that microscopy could not detect early-stage or low-bacillary-load infections because the examination needed at least 10,000 bacilli/mL in the sample(15).

In our study, the specificity of diagnosing both tests were 100 percent, which implies that both methods are equally good in excluding non-TB cases. Nevertheless, GeneXpert gave a perfect negative and positive predictive value (NPV and PPV) of 100 percent whereas AFB microscopy NPV was 72 percent. This difference indicates that a negative outcome of the microscopy may imply a false negative result in almost a third of patients. The same issues were addressed by Lawn and Jain et al (2021) who highlighted the role of high NPV in TB diagnosis to avoid missed diagnoses during early stages of the disease. Working with statistically significant differences, the difference in diagnostic accuracy between two methods was proved using the McNemar test ($p = 0.000$)(16). This importance indicates the accuracy of GeneXpert relative to AFB microscopy and is in agreement with published results like those of Li et al. (2025), which had equally shown a far superior diagnostic accuracy of the former as per the comparative pairing of results(17). Fast turnaround time and automation of the GeneXpert process is one of the strengths of the asset since it does not depend on users to operate and hence less prone to human error. Bajracharya et al. (2022) emphasized the fact that GeneXpert also determines rifampicin resistance, which is an important characteristic in high-proportion multidrug-resistant TB (MDR-TB) regions. In our setting, although, we did not highly consider examining the resistance to rifampicin, the fact that GeneXpert will give this information makes it favorable in clinics(18).

There is a significant public health implication on the findings of this study. Cases of TB that do not get diagnosed can be high in terms of the number when AFB microscopy is used as a sole mode of diagnosis more so in low-resource settings. There are also missed diagnoses, which slow down further treatment, raise the contagion level, and lead to adverse patient outcomes. Since TB control programs are directly affected by the results of early and correct diagnosis, the replacement, or augmentation of microscopy with GeneXpert in diagnostic algorithms would be of great benefit of case detection and case management. This research has a few weaknesses notwithstanding its advantages. The sample size (n=80) is somewhat small which can restrict the studies generalization. There was also a lack of confirmation of culture-based methods, which were the paramount methods of TB diagnosis, and that could have served as the additional layer of validation. However, the study provides a bright idea of why GeneXpert PCR is a better alternative to others that offer poor sensitivity as well as predictive accuracy

Conclusion:

GeneXpert PCR outperforms AFB Microscopy by all the major diagnostic stages, especially with regard to sensitivity and NPV – critical factors in TB diagnoses reduction. Because of its ability to miss cases yet claim perfect specificity and PPV, AFB microscopy fails to compete with GeneXpert accuracy and clinical safety in TB screening and diagnosis even though it has perfect specificity and PPV.

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Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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