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Utility of MTB/RIF Assay in Diagnosing Smear Negative Pulmonary Tuberculosis in Low Income Countries

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ABSTRACT

Objective: To determine the effectiveness of the GeneXpert MTB/RIF assay in diagnosing smear-negative pulmonary tuberculosis and to assess its diagnostic yield in comparison with Ziehl–Neelsen smear microscopy in a low-resource setting

Study Design: Cross sectional study.

Place and Duration of Study: Pulmonology Department, PNS Shifa Hospital Karachi, Pakistan from Jan to Dec 2023.

Methodology: We examined the efficacy of GeneXpert in pulmonary (n=210) pulmonary specimens with Ziehl Neelsen (ZN) staining. Patients older than 12 with suspected TB were included in our study. Samples were sent for routine AFB smear and GeneXpert MTB/RIF by convenient sampling method. The individuals' demographic information and clinical history were obtained from their hospital records.

Results: A total of 210 patients suspected of pulmonary tuberculosis were included in the study. Among them, 153 (72.9%) were males and 57 (27.1%) were females. Median age was 36.0 (32.5) year range from 12 to 83 years. Smear microscopy for AFB/Ziehl Neelsen was positive in 13 (6.1%) sputum samples and GeneXpert MTB/RIF was positive in 121 (57.61%) samples. Two of the samples came positive for RIF resistance.

Conclusions: GeneXpert is the most effective quick diagnostic tool available since it can identify MTB and rifampicin resistance genes at the same time. GeneXpert's reliability and accuracy were shown to be superior to AFB staining. As a result, a negative GeneXpert test can rule out tuberculosis.

When compared to smear microscopy, GeneXpert is costly and requires advanced apparatus.

Keywords: Acid-Fast Bacillus, GeneXpert Assay, Mycobacterium tuberculosis (MTB), Resistance to Rifampicin (RIF) Ziehl Neelsen Staining.

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INTRODUCTION

Tuberculosis (TB) is an airborne infection caused by Mycobacterium tuberculosis (MTB) that often attacks the lungs. It remains a major contributor to deaths in low-income communities due to low living standards, malnourishment, crowded living, and lack of proper healthcare. The most necessary step is rapid detection to prevent transmission. According to WHO, Pakistan ranks 5th among TB high-burden nations, accounting for 61% of the regional burden with 608,000 cases in 2022.[1] Pakistan also ranks 4th in multidrug-resistant TB (MDR-TB) incidence. Following factors have contributed to drug-resistant tuberculosis: delayed diagnosis, poor compliance, inadequate treatment, and lack of support for high-risk people.[2]

Early case diagnosis is critical, but sputum MTB culture, the gold standard, is unavailable in low-income communities and time-consuming.[3,4] Instead, diagnosis relies on sputum smear for Acid Fast Bacilli (AFB) and radiological findings. CT frequently suggests pulmonary TB through miliary patterns, cavitation with centrilobular nodules, consolidations, and pleural effusion.[5,6] However, AFB smear has poor sensitivity—samples below 4.0 log10 cfu/ml yield negative results[7] and cannot test drug resistance. This limitation is dangerous as MDR-TB and XDR-TB spread quickly and require prompt second-line treatment. The Xpert MTB/RIF assay, a cartridge-based real-time PCR, detects MTB and rifampicin resistance within two hours. Compared to smear microscopy, it boosts detection by 45% in high-risk populations.[8,9] However, it has limitations: short cartridge shelf-life, need for stable electricity, temperature restrictions, high maintenance costs, and occasional inaccurate rifampicin resistance results.[10] The threat of tuberculosis is egregious, and the most important cause is the enormous number of

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undiagnosed cases. The illness can only be managed through efficient programs and early diagnosis, which remain challenging in resource-constrained settings like Pakistan.

METHODOLOGY

This cross sectional study was carried out over a period of a year [January 2023-December 2023] at Pakistan Naval Station [PNS] Shifa Hospital, Karachi after approval from PN Shifa Ethical Committee ERC/2024/MED.

The study design consisted of samples from 210 suspected TB patients [age >12 years] based on clinical and radiological findings presented to PNS Shifa Hospital, Karachi, Pakistan for routine AFB Culture and GeneXpert MTB/RIF by convenient sampling methods.

Inclusion Criteria: Clinical signs and symptoms, such as cough and/or protracted fever lasting more than two weeks, age >12 years, abnormal chest radiograph, availability of sputum AFB smear, GeneXpert MTB/RIF assay and received treatment at PNS Shifa Hospital.

Exclusion Criteria: The diagnosis of extrapulmonary tuberculosis was exclusion criteria.

The hospital records were used to collect demographic data such as age, sexual category, ethnicity, history of lung illnesses and HIV sero-status. Reticulonodular lesions, patchy alveolar infiltration, consolidation, pleural effusion and cavitary lesions were the most common abnormal findings on chest X-ray. Sputum samples were gathered and processed for ZN Staining and GeneXpert MTB/RIF. The test procedures consisted for pulmonary samples subjected to Ziehl Neelsen (ZN) staining and GeneXpert MTB/RIF (Cepheid, Sunnyvale, US) assay. All equipment and stains used in this study were issued by WHO T.B Control Program in collaboration with Government of Sindh.

For sputum specimens, a direct smear was made and heat fixed. The Ziehl Neelsen staining procedure was used to stain acid fast bacilli (AFB). Ziehl Neelsen staining is systemically done by addition of Carbol Fuschin [basic dye] for 5 minutes along with heat fixation. After washing the slides with moderate streams of water, it is decolorised by addition of 20% H2SO4 for 1 minute. Lastly, Methylene Blue is added as a counter stain for 1 minute. This process ends up giving a acidophilic slide containing thread like rods in a blue background.This is then visualised under 40X and 100X microscope under oil immersion. Interpretation and reporting of sputum was done according to the recommendations of the Revised National Tuberculosis Control Programme (RNTCP) and the National TB Reference Laboratory, Islamabad.

A GeneXpert kit [sterilised and disposable] contains: [1] Buffer such as NaOH and isopropanol [2] Dropper [3] Cartilage. Sample is prepared by adding 2mL sample with 4mL buffer[NaOH and isopropanol]. It was then mixed in a vortex. After 5-10 minutes, 2mL of prepared suspension is added into cartilage which is loaded into GeneXpert instrument. The lab results are then used for analysis.After 2 hours, the results are noted as "Detected" or "Non Detected".

To statistically analyze the collected data, Statistical Package for Social Sciences (SPSS) software version 22 was used. Quantitative variable (age) was presented as median interquartile range (IQR) after checking normality of data by Shapiro-Wilk test which showed that it was not distributed normally. Qualitative variables were presented as frequency and percentages.

RESULTS

A total of 210 patients suspected of pulmonary tuberculosis were included in the study. Among them, 153 (72.9%) were males and 57 (27.1%) were females. Median age was 36.0 (32.5) year range from 12 to 83 years. Out of 210 samples, ZN smear microscopy detected acid fast bacilli in 13 patients (6.2%), while 197 patients (93.8%) were smear negative. However, the Gene X perty MTB/RIF assay detected mycobacterium tuberculosis in 121 patients (57.6%) where 89 patients (42.4%) tasted negative shown in Table. Additionally, among the patients tested with Gene X perty MTB/RIF, 2 cases (0.95%) were identified as rifampicin resistant tuberculosis. Furthermore, two cases were later confirmed to have non tuberculous mycobacterial (NTM) infection. One case could not be further investigated due to inability to obtain a repeat sample.

Table-: Patients Positive for Ziehl-Neelsen and GeneXpert (n=210)

Results	Ziehl Neelsen / Acid-Fast Bacilli Microscopy n[%]	GeneXpert Mycobacterium tuberculosis / Resistance to Rifampicin n[%]
Positive	13(6.1%)	121(57.61%)
Negative	197(93.8)	89(42.3)
Total	210	210

DISCUSSION

Early TB diagnosis is imperative for stopping the spread and transmission chain. People who appear with an unexplained cough lasting more than two weeks or with chest radiography results indicative of tuberculosis should be tested for the disease. The ever-increasing prevalence of tuberculosis in developing nations is a severe hazard to human life, necessitating the deployment of extremely sensitive and specialized approaches for early diagnosis of MTB. Although positive MTB culture is the gold standard for diagnosing active pulmonary TB, its use is hindered by the lengthy processing period. It has a great sensitivity and can identify MTB when there are 10 viable bacilli / mL of sputum.[11] However, culture necessitates a longer period of four weeks to a month by which time the patients condition may already have worsened/spread of disease. Furthermore, culture necessitates the use of a biosafety level 3 facility.[12] In contrast, Smear microscopy is a quick and inexpensive way to identify AFB, but it requires at least 5,000-10,000 bacilli / mL sputum to obtain a affirmative result. Smear microscopy will produce a negative result if there are fewer bacilli. As a result, it is less sensitive. Since the infectious dosage of tuberculosis is less than 10 bacilli, smear microscopy can easily miss such infections. In middle or low income countries (including Pakistan), smear-negative can often misdiagnose for tuberculosis.

When left untreated, AFB smear microscopy positive TB patients are the primary source of TB transmission to healthy persons. The investigations also indicated that AFB smear negative microscopy TB suspects cause around 17% of TB disease transmission, and so the possibility of disease transmission by AFB-negative patients to healthy individuals could not be overlooked.[13] In the past, pulmonologists heavily relied on chest X-rays for diagnosing pulmonary tuberculosis, particularly in rural areas where access to advanced testing facilities was limited and still do so in many low income states Due to the scarcity of resources, chest X-rays served as a primary tool to detect abnormalities in the lungs indicative of tuberculosis infection.[14] However, it's essential to acknowledge the limitations of this approach, as chest X-rays may not always provide conclusive evidence, leading to potential misdiagnosis or delayed treatment initiation.[15] As we continue to advance in medical technology and diagnostics, the landscape of tuberculosis detection has evolved, offering pulmonologists a broader range of tests such as sputum culture, nucleic acid amplification tests (NAATs) such as MTB GeneXpert and chest CT scans, enhancing the accuracy and efficiency of diagnosis. Nonetheless, the significance of chest X-rays in resource-constrained settings cannot be overlooked, highlighting the importance of adapting diagnostic strategies to suit the context and available resources. Because of this foundation, GeneXpert assay is a dependable alternative. It can help with smear - negative and certain culture - negative TB diagnosis. The GeneXpert assay yield is increased by the mucopurulent sputum.[16]

Rifampicin resistance is also detected by GeneXpert assay. Since Rifampacin resistance was relatively low in this investigation, the genuine evaluation of GeneXpert assay to identify Rifampacin resistance was limited. Two cases of Rifampicin resistance was found who were referred to Multi-drug Resistant Tuberculosis Centre. This study's shortcoming is that RIF-resistant and susceptible patients identified by GeneXpert assay were not verified further by culture sensitivity in all cases. Lack of culture sensitivity facility in our centre prompted us to direct patients to other centres. Two cases in which AFB was positive and MTB was negative with strong suggestion of non TB mycobacteria, were prompted to get their culture sensitivity done which confirmed NTM tuberculosis signifying that GeneXpert can help in identifying NTM early on in the disease.[17]

In our study, Positivity rate was higher in GeneXpert MTB/RIF in sputum specimens (57.61%) in contrast to conventional ZN staining detection (6.1%). The high frequency of MTB GeneXpert seems to give a much faster and error-free result which can help physicians reach better diagnostic speed especially in low income countries such as Pakistan where the transmission of tuberculosis is rapid. The study's findings clearly imply that GeneXpert assay is a beneficial addition to the TB arsenal as a quick test that may be used with clinical findings for the initial PTB therapy options as the main diagnostic test in low; income countries and public settings. WHO supports the use of GeneXpert MTB / RIF for the fast diagnosis of tuberculosis as well as the identification of rif resistance in HIV infected persons at risk of tuberculosis (WHO and STOP TB Department, 2010).[1]

Subsequently, the GeneXpert MTB/ RIF assay is accurate and precise enough to be used as a first test for TB. The assay may also be useful as a follow-up

test after microscopy for patients who have previously been confirmed to be smear-negative.[18] In high TB burden nations, use of GeneXpert MTB/RIF test in routine and peripheral health care settings, as well as at the point-of-care, could be advantageous in giving evidence on the true prevalence of TB and rifampicin resistance. Furthermore, early detection of pulmonary tuberculosis will result in earlier suitable treatment and opportunity to prevent TB transmission, particularly in high-burden TB countries.

CONCLUSION

In low income countries like Pakistan culture is not laws available and When compared to smear microscopy and culture, GeneXpert is proven to be a faster test for the detection of TB and rifampicin resistance. A smear- positive but GeneX pert-negative result raises the possibility of NTM infection. Thus, smear microscopy combined with GeneXpert will be extremely useful in discriminating MTB, RIF resistant TB, and NTM infections in resource-limited countries where TB culture facilities are unavailable.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SA & SLO: Data acquisition, data analysis, critical review, approval of the final version to be published.

SOA & MB: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ST, LS & RZ: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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