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Sensitivity and Specificity Assessment of Histopathology and GeneXpert in Diagnosing Extrapulmonary Tuberculosis at Gulab Devi Hospital, Lahore, Pakistan: A Retrospective Study

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ABSTRACT

Extrapulmonary Tuberculosis (EPTB), including Spinal Tuberculosis (STB), poses diagnostic challenges due to diverse clinical presentations and limitations of conventional diagnostic methods. **Objective:** To assess the sensitivity and specificity of Gene Xpert and histopathology in diagnosing EPTB at Gulab Devi Chest Hospital, Lahore, Pakistan. **Methods:** Data from patients diagnosed with EPTB between May 15th and October 15th, 2024, were analyzed. Gene Xpert and histopathology results were compared with Culture as the gold standard. **Results:** The study included 299 individuals, 61.2% of whom were females and 38.8% of whom were males. Gene Xpert demonstrated a sensitivity of 90.48% and specificity of 59.75%, while histopathology exhibited a sensitivity of 88.89% and specificity of 60.17%. **Conclusions:** Gene Xpert and histopathology were valuable tools for diagnosing EPTB, complementing traditional methods.

INTRODUCTION

Tuberculosis (TB), caused by the Mycobacterium tuberculosis complex, primarily affects the lungs but can also spread to other body parts [1]. Typical symptoms include chronic cough, hemoptysis, fever, night sweats, and weight loss. According to the Global Tuberculosis Report of 2023, 7.5 million people were newly diagnosed with TB globally in 2022, marking the highest number since the WHO began global TB monitoring in 1995. This figure surpasses the pre-COVID baseline of 7.1 million in 2019 and shows an increase from 5.8 million in 2020 and 6.4 million in 2021. The 2022 figure likely includes a backlog of TB cases from previous years, whose diagnosis and treatment were delayed due to COVID-related disruptions in health services [2]. Pakistan ranks as the 5th highest TB burden country, contributing to nearly 5.8% of new TB cases worldwide annually [3]. Extrapulmonary tuberculosis refers to a range of conditions affecting various anatomical locations aside from the lungs, commonly including lymph nodes, pleura, urogenital tract, bones and joints, meninges, Central Nervous System (CNS), intestines, peritoneum, pericardium, and skin [4]. Despite its significant impact on morbidity, mortality, and adverse outcomes, extrapulmonary tuberculosis is often neglected due to its minimal role in tuberculosis transmission. Clinicians encounter multiple hurdles in diagnosing extrapulmonary tuberculosis, such as its diverse clinical presentations and challenges in obtaining samples from deep-seated tissues [5]. The criteria used to diagnose tuberculosis in lymph nodes are not very specific. The lack of distinct findings does not completely rule out the

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possibility of the disease. Ziehl Neelsen staining, a conventional method, for detecting and monitoring tuberculosis is commonly used but it has low sensitivity typically ranging from 20% to 43%. Mycobacterial culture, which is considered the gold standard for diagnosing tuberculosis is labor intensive and also requires specialized safety measures in the laboratory [5]. Meanwhile, serological techniques have demonstrated notable sensitivity and specificity in TB diagnosis. However, the widespread adoption of newer molecular techniques like Polymerase Chain Reaction (PCR) in developing countries is hindered by cost constraints [5]. Diagnosing extrapulmonary tuberculosis poses challenges because these areas typically have levels of bacteria compared to lung samples, often requiring invasive procedures to collect samples [6]. The GeneXpert assay, an advancement, in tuberculosis diagnosis uses a method called heminested real time PCR to amplify specific genetic material from M. Tuberculosis. It can also detect resistance to rifampin by focusing on an area of the gene using molecular beacons [6]. It's entirely automated, handling processes such as breaking down bacteria extracting genetic material amplifying it and detecting the target sequences. A study conducted in Morocco analyzed 714 patient samples, with 285 from the lungs and 429 from other body areas. The detection rates were 12.88% for microscopy (18.9% for lung samples and 9.71% for extrapulmonary samples), 20.59% for GeneXpert MTB/RIF (23.85% for lung samples and 18.41% for extrapulmonary samples), and 15.82% for culture (20.35% for lung samples and 12.82% for extrapulmonary samples). The GeneXpert MTB/RIF demonstrated a sensitivity of approximately 78.2% and specificity of 90.4% for both types of samples. For extrapulmonary samples specifically, the sensitivity was 79.3% and the specificity was 90.3% [7]. Endorsed by the World Health Organization (WHO) since December 2010 the GeneXpert test is recommended as a method over sputum smear microscopy in regions, with high occurrences of HIV related tuberculosis and drug resistant strains [8, 9]. In the Pakistani context, various diagnostic investigations, including X-rays, Acid- Fast Bacilli (AFB) cultures, histopathological analyses, and Gene Xpert, are commonly employed.

This research seeked to assess the sensitivity and specificity of histopathology and Gene Xpert in diagnosing extrapulmonary tuberculosis, shedding light on their roles in guiding definitive therapy planning.

METHODS

This comparative analytical study, utilizing pre-existing data gathered from patients diagnosed with Extrapulmonary Tuberculosis (EPTB), was conducted from May 15th to October 15th, 2024. The study was conducted at Gulab Devi Chest Hospital in Lahore, Pakistan. The study was conducted after receiving IRB approval from Al- Aleem Medical College, Gulab Devi Teaching hospital (Ref: AAMC/IRB/EA402024). A total of 299 patients, whose culture, histopathology, and Gene Xpert were done to diagnose EPTB were included using purposive sampling technique. The study included individuals of both genders aged >10 to <70 years, specifically focusing on suspected EPTB cases. Excluded were individuals with incomplete records or missing data, those previously diagnosed with tuberculosis, and patients suspected of harboring Multidrug-Resistant Tuberculosis (MDR TB). After taking the specimen from cervical lymph nodes, they were preserved with normal saline and formalin solutions. The saline-preserved samples were then sent for culture, AFB smear, and GeneXpert analysis. These samples were processed in a manual sterile tissue homogenizer and centrifuged, while the formalin samples were sent for histopathological examination. Two slopes of solid LJ medium and one mycobacterial growth indicator tube were used for culture analysis. The histopathology samples were stained with hematoxylin and eosin and examined under a microscope for the presence or absence of granulomas. The GeneXpert analysis was performed according to the manufacturer's protocol. All laboratory investigations were obtained from the patient records, including standard AFB culture, histopathology, and GeneXpert analyses. To ensure accuracy and reliability, reports from these investigations were confirmed by the respective senior pathologist responsible for overseeing and validating the results. The GeneXpert assay was a molecular diagnostic test designed for the rapid and accurate detection of Mycobacterium Tuberculosis Complex (MTB) and resistance to rifampicin (a marker for multidrug-resistant tuberculosis) [10]. This assay utilizes a technology known as heminested real-time PCR, which amplifies specific genetic sequences of MTB bacteria present in clinical samples. The process involves automated steps including bacterial lysis, nucleic acid extraction, amplification of the target DNA, and detection using molecular beacons. Diagnostic accuracy was assessed by comparing GeneXpert and histopathology results against AFB culture as the gold standard. Similar to the approach by Uddin MK *et al.*, the sensitivity and specificity of diagnostic methods were initially evaluated by directly comparing results obtained from Gene Xpert and histopathological examinations with the reference standard, AFB culture [11]. Sensitivity was assessed by comparing the true positive results from Gene Xpert and histopathology against AFB culture-confirmed cases. Specificity was determined by comparing the number of true negative results from Gene Xpert and histopathology against AFB culture-negative cases. This comparative analysis provided a comprehensive understanding of GeneXpert and histopathology's diagnostic performance concerning AFB culture as the gold standard. All procedures strictly adhered to ethical guidelines and principles, ensuring patient confidentiality and welfare throughout the study.

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RESULTS

The provided data reveals the gender distribution in a dataset of 299 individuals, where 61.2% were females and 38.8% were males (Table 1).

Table 1: Results of Gene Xpert, Histopathology and Culture

Test	Category	N (%)
Culture	Negative	236 (78.9%)
	Positive	63 (21.1%)
H/P	Negative	149 (49.8%)
	Positive	150 (50.2%)
Gene Xpert	Detected	152 (50.8%)
	Not Detected	147 (49.2%)

Table 2 summarized the performance of Gene Xpert and Histopathology (H/P) in comparison to Culture, highlighting their sensitivity and specificity. Gene Xpert demonstrated a sensitivity of 90.48% and specificity of 59.75%. In contrast, H/P exhibited a sensitivity of 88.89% and a specificity of 60.17%.

Table 2: Diagnostic Performance of Gene Xpert and Histopathology

Variables		Culture		Sensitivity and Specificity	
		Negative	Positive		
Gene Xpert	Detected	95	57	Sensitivity = TP / (TP + FN) × 100	57/(57+06)×100= 90.48%
	Not Detected	141	06	Specificity = TN / (TN + FP) × 100	141/(141+95)×100= 59.75%
H/P	Positive	94	56	Sensitivity = TP / (TP + FN) × 100	56/(56+07)×100= 88.89%
	Negative	142	07	Specificity = TN / (TN + FP) ×100	142/(142+94)×100 =60.17%

DISCUSSION

Diagnosing Tuberculosis (TB) presents significant challenges due to the limitations of available diagnostic methods [12]. Microscopy, culture, and drug sensitivity tests each have their drawbacks in terms of accuracy, effectiveness, and resource requirements. The GeneXpert test has emerged as a pivotal advancement in TB management, surpassing microscopy and recommended as a crucial step [13, 14]. However, clinicians must integrate clinical symptoms, radiological findings, and supplementary tests such as culture and tissue examination to comprehensively assess TB diagnosis. Culture, often considered the gold standard for TB diagnosis, is hindered by its limited sensitivity, especially for extrapulmonary samples [13, 15]. Even with definitive histopathological evidence, some specimens may yield negative culture results, highlighting the necessity for alternative diagnostic approaches [16]. While initially validated for respiratory specimens, GeneXpert has demonstrated promise in diagnosing extrapulmonary TB, mitigating the shortcomings of traditional culture methods. Its efficacy across diverse extrapulmonary sites has been evidenced, with sensitivities ranging from 50% to 100% for lymph nodes, 62.8% for cerebrospinal fluid, and 82.65% for joint TB [17-20]. These findings underscore GeneXpert's versatility in diagnosing extrapulmonary TB. Current study's findings align closely with existing literature, validating both GeneXpert and histopathology's utility in diagnosing extrapulmonary TB, particularly Spinal TB (STB) [21-24]. GeneXpert exhibited a sensitivity of 90.48% and specificity of 59.75%, while histopathology demonstrated a sensitivity of 88.89% and specificity of 60.17%. These results reinforce the importance of employing multiple diagnostic modalities and integrating clinicoradiological presentations for accurate TB diagnosis, facilitating optimal patient management and TB control efforts. This study has limitations that must be considered. Its retrospective design limits control over confounding variables and introduces potential biases in data collection and analysis. Conducted at a single center, the findings may not be generalizable to other settings or populations. The study lacks longitudinal data, limiting the understanding of long-term outcomes and effectiveness.

CONCLUSIONS

In conclusion, current study highlighted the importance of utilizing effective diagnostic methods such as Gene Xpert and histopathology for diagnosing EPTB. Both Gene Xpert and histopathology demonstrate high sensitivity and reasonable specificity, affirming their utility as diagnostic tools. Gene Xpert's rapid and accurate identification of TB, coupled with histopathology's ability to characterize tissue, enhances diagnostic precision. Continued exploration and evaluation of these technologies were essential for improving TB diagnosis and treatment, particularly in high-prevalence regions like Pakistan.

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Authors Contribution

Conceptualization: RM, MA
Methodology: MS
Formal analysis: MZ, AA
Writing, review and editing: HK

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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