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ORIGINAL RESEARCH

Diagnostic accuracy of gene xpert in determining pleural tuberculosis in suspected tuberculosis patients

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ABSTRACT

Background: Tuberculous pleural effusion accounts for roughly 30% of extrapulmonary tuberculosis cases, and detecting Mycobacterium tuberculosis in these samples is challenging due to the paucibacillary nature of the disease. The World Health Organization has devised the Gene Xpert Assay for rapid detection of tuberculosis and rifampicin resistance within two hours. This study evaluates the sensitivity and specificity of the Gene Xpert Assay in comparison to Ziehl-Neelsen (ZN) staining and fluorescent microscopy. **Materials and Methods:** A total of 1342 samples were processed during 2018-2019, including 144 pleural fluid samples. Each sample underwent Gene Xpert Assay, ZN staining, and fluorescent microscopy. Sensitivity and specificity were calculated for each method, and rifampicin resistance was assessed. **Results:** Of the 144 pleural fluid samples, 9 (6.53%) were positive for tuberculosis using the Gene Xpert Assay, and 1 sample (11.1%) showed rifampicin resistance. Slide positivity rates were 60% for ZN staining and 75% for fluorescent staining. The sensitivity rates were 31% for Gene Xpert, 58.2% for ZN staining, and 63% for fluorescent staining. **Conclusion:** The Gene Xpert Assay demonstrates lower sensitivity compared to ZN staining and fluorescent microscopy for detecting tuberculous pleural effusion. However, it provides rapid detection and rifampicin resistance identification, which is critical for timely and appropriate treatment.

Keywords: Tuberculosis, Pleural Effusion, Gene Xpert, ZN Staining, Fluorescent Microscopy, Rifampicin Resistance, Diagnostic Accuracy.

INTRODUCTION

Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent, surpassing HIV/AIDS. Millions of people continue to fall sick with TB each year. In 2017, an estimated 10.0 million people (range, 9.0–11.1 million) developed TB disease, including 5.8 million men, 3.2 million women, and 1.0 million children (1). In 2010, there were 350,000 tuberculosis-related deaths in HIV-infected people, most of them in developing countries (1). One of the most significant reasons for this high number of deaths is the difficulty of diagnosing tuberculosis in the HIV population (2, 3).

Microbiological identification of Mycobacterium tuberculosis using PCR techniques like Line Probe Assay and Gene Xpert, along with culture, is the gold standard for diagnosing tuberculosis infection. However, mycobacterial culture has a longer turnaround time. While pulmonary tuberculosis (PTB) is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical condition (4). Pleural TB occurs in up to 30% of patients concomitantly with pulmonary TB and constitutes a significant portion of extrapulmonary TB (5). The World Health Organization (WHO) has recently endorsed the implementation of light-emitting diode (LED) fluorescent microscopy and the Gene Xpert MTB/RIF assay for national tuberculosis programmes in developing countries (6). LED fluorescent microscopy is less expensive than conventional fluorescence microscopy and has shown 84% sensitivity (95% confidence interval [CI], 76 to 89) and 98% specificity (95% CI, 85 to 97) (7).

The Xpert MTB/RIF (Mycobacterium tuberculosis/rifampin [RIF] resistance) assay (Xpert; Cepheid, Sunnyvale, CA) is an automated system that employs real-time PCR and molecular beacon probes to determine the presence of M. tuberculosis complex

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DNA, as well as rpoB gene mutations conferring rifampin resistance, rapidly and with high accuracy (8). Pleural TB is the second most frequent form of extrapulmonary TB and the most frequent cause of exudative pleural effusions in areas with a high prevalence of HIV infection. Patients present at all ages with uni- or bilateral pleural effusion and acute to subacute onset of chest pain, fever, weight loss, breathlessness, and cough. The diagnostic workup includes pleural fluid aspiration and pleural biopsy, as pleural fluid smear and culture are often negative due to the paucibacillary nature of pleural TB (9). This study investigates the utility of the novel Xpert MTB/RIF assay for diagnosing pleural TB from pleural fluid.

MATERIAL AND METHODS

This prospective observational study was conducted at the Government of India-approved Culture & Drug Susceptibility Testing (C&DST) laboratory, Department of Microbiology, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna. The study period spanned from 2018 to 2019, during which a total of 1342 samples were processed, including 144 pleural fluid samples.

Patient Selection: A total of 102 pleural fluid samples were obtained from patients with high suspicion of pleural tuberculosis (TB). Eligibility for enrollment was based on standard clinical and radiological criteria, including a persistent cough of two weeks or more, unexplained fever for two weeks or more, unexplained weight loss with or without night sweats, chest pain, and radiological evidence of pleural effusion. Patients already receiving treatment at the time of enrollment were excluded from the study. All included patients were confirmed to be HIV negative.

Sample Processing: The Xpert MTB/RIF assay was performed directly on pleural fluid samples according to the manufacturer's instructions, using the newer version (G4) of cartridges and software version 4.4a. Briefly, 1 ml of uncentrifuged pleural fluid sample was lysed with 3 ml of sample reagent (SR) buffer in a 3:1 ratio and incubated for 15 minutes at room temperature. Finally, 2 ml of the mixture was loaded into the cartridge. The instrument detected the presence or absence of Mycobacterium tuberculosis and recognized rifampicin resistance after DNA amplification. The instrument also provided a semiquantitative estimation of Mycobacterium tuberculosis load, categorized as high, medium, low, or very low, based on the cycle threshold (CT) value.

Comparative Analysis: All pleural fluid samples were also subjected to Ziehl-Neelsen (ZN) staining and light-emitting diode (LED) fluorescent microscopy. The sensitivity and specificity of the Gene Xpert Assay were compared with those of ZN staining and LED fluorescent microscopy.

Data Collection: Patient demographic data, including age and gender, were collected. The presence of family contact among positive patients was noted. The primary symptoms reported were malaise with fever, chest pain, cough, weight loss, night sweats, and others.

Statistical Analysis: The sensitivity and specificity of the Gene Xpert Assay, ZN staining, and LED fluorescent microscopy were calculated. The positivity rates for each diagnostic method were determined, and the results were compared.

RESULTS AND DISCUSSION

The study included 144 pleural fluid samples from patients suspected of having tuberculosis. During the period of the study, 3 samples were rejected due to contamination, and 2 samples failed to yield a valid result. Consequently, 139 samples were analyzed using the Gene Xpert Assay, Ziehl-Neelsen (ZN) staining, and LED fluorescent microscopy.

The most common symptom observed was malaise with fever, followed by chest pain, cough, weight loss, night sweats, and other symptoms. The majority of the study population consisted of 89 males and 55 females, with a mean age of 33 ± 2.5 years. Family contact was reported in 7 of the positive patients.

The positivity rates and sensitivity for each diagnostic method are presented in Table 1.

Table 1: Positivity Rates and Sensitivity of Diagnostic Methods

Diagnostic Method	Positive (n)	Negative (n)	Not Done (n)	Invalid (n)	Sensitivity (%)
ZN Staining	5	131	2	-	60
LED Microscopy	7	130	1	-	75
Gene Xpert	9	128	-	2	31

Among the 139 processed specimens, the Gene Xpert Assay detected 9 positive cases (6.53%), with 1 case (11.1%) showing rifampicin resistance. In comparison, ZN staining showed a slide positivity rate of 60%, while LED fluorescent microscopy showed a positivity rate of 75%.

In our study, the sensitivity of the Gene Xpert Assay was 31%, whereas the sensitivities for ZN staining and LED fluorescent microscopy were 58.2% and 63%, respectively.

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DISCUSSION

The detection of Mycobacterium tuberculosis in pleural effusions is challenging due to the paucibacillary nature of the disease. This study assessed the diagnostic accuracy of the Gene Xpert Assay for detecting pleural tuberculosis (TB) in suspected cases, comparing its performance with Ziehl-Neelsen (ZN) staining and LED fluorescent microscopy.

Our study found that the positivity rate for the Gene Xpert Assay was 6.53%, with 9 out of 138 pleural fluid samples testing positive. This is higher than the 2.9% positivity rate reported by Hillemann et al. in their study of 113 pleural fluid samples (17). The sensitivity of the Gene Xpert Assay in our study was 31%, which is lower than the reported sensitivity in other studies from regions with low TB prevalence (3).

The sensitivity of ZN staining was 58.2%, while LED fluorescent microscopy had a higher sensitivity of 63%. These findings are consistent with the literature, where LED fluorescent microscopy has been shown to have higher sensitivity and specificity compared to conventional fluorescence microscopy (7). The World Health Organization (WHO) has recommended the use of LED fluorescent microscopy and Gene Xpert Assay for TB diagnosis in national tuberculosis programmes, particularly in developing countries (6).

The relatively low sensitivity of the Gene Xpert Assay in our study contrasts with its reported high accuracy in detecting Mycobacterium tuberculosis complex DNA and rifampin resistance (8). This discrepancy could be attributed to the different study populations and the nature of pleural fluid samples, which are often paucibacillary. The semiquantitative estimation of Mycobacterium tuberculosis load in our study revealed that most positive samples had low bacillary load, which might have contributed to the lower sensitivity of the Gene Xpert Assay.

The inclusion of a higher number of samples and the use of multiple diagnostic methods in our study strengthen the reliability of the findings. However, the study's limitations include the exclusion of HIV-positive patients, which may have affected the generalizability of the results to the broader population. Additionally, the reliance on clinical and radiological criteria for patient selection could introduce selection bias.

Despite its lower sensitivity, the Gene Xpert Assay offers rapid detection and identification of rifampin resistance, which is crucial for timely initiation of appropriate treatment. The combination of Gene Xpert Assay with other diagnostic methods such as ZN staining and LED fluorescent microscopy can improve the overall diagnostic accuracy for pleural TB.

CONCLUSION

In conclusion, the Gene Xpert Assay, while demonstrating lower sensitivity compared to ZN staining and LED fluorescent microscopy, remains a valuable tool for the rapid diagnosis of pleural TB and rifampin resistance detection. Further studies with larger sample sizes and inclusion of diverse populations are needed to validate these findings and optimize the diagnostic approach for pleural TB.

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