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Role of Gene Xpert in Diagnosis of Tuberculous Pleural Effusion

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Abstract: The diagnosis of tuberculous pleural effusion (TPE) depends on the demonstration of tubercle bacilli in pleural fluid, a pleural biopsy specimen, or the demonstration of granulomas in the pleura. Since pleural tissue sampling is more difficult than simple thoracocentesis, pleural fluid markers of TPE like Adenosine deaminase (ADA) have been extensively evaluated as an attractive alternative to pleural biopsy. Gene Xpert Mycobacterium tuberculosis (MTB) assay is an automated system which employs real-time PCR and molecular probes to determine the presence of M. tuberculosis complex DNA rapidly and with high accuracy. The aim of this study was to investigate the utility of the Xpert MTB assay for diagnosing pleural TB from pleural fluid. The study population consisted of 146 probable patients of tuberculous pleural effusion admitted in Chest & TB ward of SHKM GMC, Nalhar. Pleural fluid was obtained during thoracocentesis. The sample was divided equally into three parts and sent for Gene Xpert analysis, AFB microscopy and for Bactec Mycobacteria growth indicator tube (MGIT-960) culture. Analysis was carried out by comparing the tuberculous pleural effusion positive by Bactec MGIT-960 culture with that of gene Xpert result. 146 pleural fluid samples from patients with probable tuberculous pleural effusion were tested by the Xpert MTB assay and the Bactec MGIT-960 culture system. Of these, 40 were positive in the MGIT-960 culture, and 21, in the Xpert MTB assay. The sensitivity and specificity of the Xpert MTB compared with the MGIT culture were 52.5% and 100%, respectively, Positive Predicted Value (PPV) of 100%, Negative Predicted Value (NPV) 84.8% and negative Likelihood Ratio (LR) of .47. The results of present study show that Gene Xpert MTB assay could come to play a significant role in routine tubercular pleural effusion diagnosis, where resources of pleural fluid culture are limited and time consuming and case burden is high. Results of Gene Xpert MTB are available in same day with acceptable sensitivity and high specificity.
Keywords: Tuberculous pleural effusion, gene expert, Bactec MGIT-960 culture.

INTRODUCTION

Tuberculosis (TB) remains one of the major public health concerns in India. Our country is ranked first among the high-burden countries and contributed 24% of the estimated global TB cases and about 20% of global TB-related deaths in 2013[1]. TB is classified as pulmonary and extrapulmonary. Pleural TB is responsible for 30% to 80% of all pleural effusions (PE) encountered in India. PE in TB usually have lymphocytic and exudative characteristics.

The diagnosis of tuberculous pleural effusion (TPE) depends on the demonstration of tubercle bacilli in pleural fluid, a pleural biopsy specimen, or the demonstration of granulomas in the pleura [2]. Due to the paucity of Mycobacterium tuberculosis in the PE, the performance of a pleural biopsy has historically been considered the most reliable method to confirm the diagnosis when tuberculous etiology is suspected.

However, since pleural tissue sampling is more difficult than simple thoracocentesis, pleural fluid markers of TPE have been extensively evaluated as an attractive alternative to pleural biopsy [3].

The definitive diagnosis of TPE depends on the demonstration of Mycobacterium tuberculosis in pleural fluid, sputum or pleural biopsy specimen, and

can also be established with reasonable certainty by demonstration of granuloma in the parietal pleura. Microscopy of the pleural fluid for acid fast bacilli is positive in fewer than 5% of cases. Closed pleural biopsy demonstrates granulomas in approximately 80% of cases, and its culture yields Mycobacterium tuberculosis in about 55%. Thoracoscopy offers a near 100% positive diagnostic yield on histology and 76% positive on culture. However, historically, since pleural biopsy is more invasive and hazardous than thoracocentesis [4], and histological examination has its limitations as it cannot differentiate between TB and other related diseases like sarcoidosis or NTM infections. Several other tests that are also being employed for the diagnosis of extrapulmonary tuberculosis (EPTB) include serological assays like adenosine deaminase(ADA) enzyme, Mantoux test, and Polymerase chain reaction (PCR) assays; however, the specificities and sensitivities of these tests are variable. Also, these tests require a number of manual steps, and some have a relatively long turnaround time. Thus to overcome these difficulties, alternative diagnostic approaches have been extensively evaluated.

Though ADA in body fluids like pleural fluid has got high sensitivity[3], culture is the "gold standard" for detection of Mycobacterium tuberculosis (MTB) but it is slow and may take up to 2 to 8 weeks. Although microscopic examination for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and specificity and is unable to differentiate between tuberculous and non-tuberculous mycobacteria (NTM). Thus, rapid identification is essential for early treatment initiation, improved patient outcome as well as for more effective public health intervention and it relies mainly on Nucleic acid amplification techniques (NAAT). Recently introduced GeneXpert MTB/RIF assay, which can detect Mycobacterium tuberculosis complex directly from clinical samples has been shown to be rapid, yielding results in less than 2 hours. The assay based on nested real-time PCR and molecular beacon technology is not prone to cross-contamination, requires minimal biosafety facilities, can be performed by technicians with little training; and has high sensitivity in smear-negative pulmonary TB (particularly relevant in HIV positive individuals). Based on an updated Cochrane systematic review, GeneXpert MTB/RIF (hence forth referred to as GeneXpert) assay has an overall sensitivity of 88% and a pooled specificity of 98% as compared to culture. These characteristics make it a potentially useful technique in the diagnosis of EPTB as well. The information provided by GeneXpert assay helps in selecting treatment regimens and reaching infection control decisions quickly [5]. The assay can be used for other specimens than sputum, such as cerebrospinal fluid or fluids from various sites, but data are still scarce [4]. Therefore, the aim of this study was to investigate the utility of the Xpert MTB assay for diagnosing pleural TB from pleural fluid in patients with strong clinical suspicion of TB.

OBJECTIVE

To determine the diagnostic accuracy of Gene Xpert MTB in diagnosis of tuberculous pleural effusion

MATERIALS AND METHODS

Study Area

The study was conducted at SHKM Government Medical College, Nalhar, Nuh, Haryana, a tertiary care teaching hospital.

Study Population

The study population consisted of 146 probable patients of tuberculous pleural effusion admitted in Chest & TB ward of SHKM Government Medical College, Nalhar included if both the following clinical criteria were met:

- Fever, cough, pleuritic pain, malaise, anorexia;
- Chest X ray findings of pleural effusion.

Study design

Prospective study.

Exclusion criteria

- Patients with history of previous thoracocentesis and bleeding diathesis.
- Patients not giving consent to participate in the study.

Pleural fluid was obtained during thoracocentesis. The sample was divided equally into three parts:

- One part was used for the Xpert test and sent to microbiology department. For the test procedure, the sample was poured into a single-use disposable cartridge (after centrifugation and discarding the supernatant) that is placed in the Xpert module, with the results produced in less than 2 hours. The system automatically interpreted all results from measured fluorescent signals, with calculation algorithms, into the following categories: invalid, if PCR inhibitors are detected with amplification failure; negative or positive[6].
- The second was tested by acid fast bacillus (AFB) microscopy (Ziehl-Nielson [ZN] staining). Mycobacteria isolated from the pleural fluid by smear will be diagnosed to have tuberculous pleural effusion.
- Third part was sent for Bactec MGIT-960 culture.

Closed pleural biopsy with Abram's needle in standard way was done to obtain a confirmed diagnosis of tuberculous pleural effusion[7]. Specimen was examined histopathologically. Patients who had granulomas on pleural biopsy or those in whom mycobacteria were isolated from the pleural biopsy

were diagnosed to have tuberculous pleural effusion.[3] But due to uncooperative patients and nonavailability of consent from some of them for biopsy, biopsy was not possible in all of them. So, tuberculous pleural effusion was diagnosed by any one of the following:

- Mycobacteria isolated from the pleural fluid by smear
- Or Bactec MGIT-960 culture positive

Analysis was carried out for comparing the tuberculous pleural effusion positive by Bactec MGIT-960 culture with that of gene Xpert result and note was made.

DATA ANALYSIS

The collected data was entered in Excel and analyzed using SPSS version 20. The result of the diagnostic tests was expressed as sensitivity, specificity, predictive values (positive and negative) and likelihood ratio.

RESULTS

Out of 150 patients of probable tubercular pleural effusion, 4 patients had dry tap. 146 pleural fluid samples were tested by the Xpert MTB assay and the Bactec MGIT-960 culture system. In the study, males and females were 82 and 64 respectively. Clinical features with which the patient presented are described in Table 1. Chest radiology represented right sided pleural effusion in 79%, left sided plerural effusion in 18% and bilateral effusion in 3% cases.

Of these 146 probable cases, 40 were positive in the MGIT-960 culture, and 21 in the Xpert MTB assay. The sensitivity and specificity of the Xpert MTB compared with the MGIT culture were 52.5% and 100%, respectively, Positive predictive value (PPV) of 100%, Negative predictive value (NPV) 84.8% and likelihood ratio of a negative test (LR^-) of 0.47 (table 2, 3,4).

Table-1: **Presenting features of subjects**

Clinical feature	Present	Percentages
Fever	134	92%
Chest pain	105	72%
Cough	99	68%
Dyspnea	76	52%

Table-2: **Sensitivity and specificity of Xpert MTB assay in comparison with MGIT culture on the pleural fluid samples**

Xpert MTB assay result	Sensitivity		Specificity	
	No. positive/ no. tested	%	No. negative/ no. tested	%
Overall	21/40	52.5	106/106	100
Smear positive, Culture positive	3/3	100	143/143	100
Smear negative, Culture positive	18/37	48.6	109/109	100

Table-3: **Predictive value of Xpert MTB assay in comparison with MGIT culture on the pleural fluid samples**

Xpert MTB assay result	Predictive value	
	Positive Predictive value %	Negative Predictive value %
Overall	100	84.8
Smear positive, Culture positive	100	100
Smear negative, Culture positive	100	84.8

Table-4: **Likelihood Ratio of Xpert MTB assay in comparison with MGIT culture on the pleural fluid samples**

Xpert MTB assay result	Likelihood Ratio Negative
Overall	0.47
Smear positive, Culture positive	0.00
Smear negative, Culture positive	0.51

DISCUSSION

Demonstration of tubercle bacilli as well as caseating granuloma are the gold standard for the diagnosis of tuberculosis[8,9]. Inspite of all the efforts, it is negative in many cases of tuberculous pleural effusion due to its paucibacillary nature and etiology of pleural fluid remain undiagnosed or misdiagnosed. Pleural fluid analysis by cytology, biochemistry and ADA is not always diagnostic, similar picture may be present in some other diseases thus, creating diagnostic confusion.

Globally the use of GeneXpert assay has resulted in an increase in the number of positive results by 16.5% and this increase has been more important for the extra-pulmonary specimens especially the body fluids[10].

Our study was planned to know the role of Gene Xpert in the diagnosis of tuberculous pleural effusion as not many Indian studies are available in the literature. The results showed MTB detection in 21 (52.5%) cases. Vadwai *et al.* [11] in 2011 did a similar study with a total of 25 pleural fluid samples in which Gene Xpert MTB/RIF was positive in 5(50%). Similarly, Gene Xpert results with a study by Tortolli *et al.* [12] had 44% sensitivity for MTB detection, while Coleman *et al.*[13] had sensitivity of 66%. The specificity of the Xpert MTB compared with the MGIT culture was 100%, PPV of 100%, NPV 84.8% and negative LR of 0.47.

Friedrich *et al.* [14] published one of the earliest study on the use Gene expert in diagnosing tuberculous pleural effusion. They studied 20 patients and found the sensitivity and specificity of this test for the diagnosis of TPE to be 25% and 100% respectively. Since then, studies from different parts of the world have confirmed with such results. Analysing of various studies result shows sensitivity between 3% to 64% and specificity ranging from 97% to 100%[1].

Particularly their high NPV value, make them a useful bedside screening tool, especially in the ambulatory setting such as outpatient clinics or in the emergency room. In patients with negative Gene Xpert MTB test, tuberculous pleural effusion can be ruled out with a high degree of certainity. Conversely, the satisfactory PPV of this test substantiates the immediate start of appropriate antibiotic therapy in cases of positive testing, without any delay.

Although GeneXpert assay is considered a breakthrough in the diagnosis of TB and EPTB, one of the major limitations of this technique is that it cannot distinguish between viable and non-viable micro-organisms while detecting MTB DNA. Hence it should not be used to monitor patients or efficacy of the treatment[5]. A major limitation of this study is not having comparative data with histopathology. This molecular technique of GeneXpert assay is relatively less expensive than traditional culture methods; however it makes an important contribution to the modern day detection of TB as it has higher sensitivity than smear and provides a more rapid diagnosis than culture and histology. Our findings support the routine use of Gene Xpert assay for the diagnosis of EPTB in pleural fluid as time factor has a very crucial role in its laboratory diagnosis. Gene Xpert assay has the potential to significantly improve and escalate the diagnosis of smear-negative pleural fluid specimens at both hospital as well as point-of-care settings in regions with high TB burden and aids in prompt initiation of appropriate therapy and thus improving the overall quality of TB care.

CONCLUSION

The results of present study show that Gene Xpert MTB assay could come to play a significant role in routine tuberculous pleural effusion diagnosis, where resources of pleural fluid culture are limited, case burden is high and is time consuming, as results of Gene Xpert MTB are available in same day with acceptable sensitivity and high specificity. There is an indication for high specificity, which must be verified with larger studies, including more patients with a pleural effusion due to other causes than TB. Before this is attempted, the methods for collection, storage, and preparation of pleural fluid samples need to be optimized in order to increase the sensitivity of the Xpert assay on pleural fluid.

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