



THE ROLE OF GENE XPERT FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS BACILLI IN PLEURAL BIOPSY SAMPLE OF SUSPECTED TUBERCULAR PLEURAL EFFUSION PATIENTS

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ABSTRACT

BACKGROUND - Microscopy of the pleural fluid for acid-fast bacilli positive in less than 5% of tubercular pleural effusion cases, due to the paucibacillary nature of the disease. Detection of Mycobacterium tuberculosis in the pleural fluid, pleural biopsy tissue either by microscopy, Gene Xpert or culture and demonstration of granulomatous lesion with caseation, is the gold standard for the diagnosis of tubercular pleural effusion.

MATERIALS & METHODS - Suspected tubercular pleural effusion patients aged 16 years or more were en-rolled for this study from the Outpatient department of MGM Medical College Indore after informing them about the study in duration of July 2019 to February 2020.

Pleural biopsy sample was tested for tuberculosis by smear microscopy, histopathology and Gene Xpert. A sample which is found to be positive by any of two tests (smear microscopy, histopathology) were considered as final positive as well as was considered as composite reference standard (gold standard) and was compared with Gene Xpert.

RESULTS - Closed percutaneous pleural biopsy was performed in 70 patients. It yielded granulomatous lesion in 45 patients & In 21 cases detected MTB by Gene Xpert Method. Among these 70 patients 3 patients diagnosed Rifampicin resistant by Gene Xpert Method.

CONCLUSION - The Gene Xpert method performs well in pleural biopsy sample for rapid and accurate diagnosis of tubercular pleural effusion and also effective in diagnosis of rifampicin drug resistant cases.

KEYWORDS : Pleural Biopsy, Gene Xpert, Tubercular pleural effusion

BACKGROUND

Pleural effusion is an abnormal collection of fluid in the pleural space resulting from excess fluid production or decreased absorption or both.

The tubercular pleural effusion is thought to result from rupture of a subpleural caseous focus in the lung into the pleural space. Detection of Mycobacterium tuberculosis in the pleural fluid, pleural biopsy tissue either by microscopy, Gene Xpert or culture and demonstration of granulomatous lesion with caseation, is the gold standard for the diagnosis of tubercular pleural effusion.

Tubercular pleural effusion is a type IV hypersensitivity reaction to mycobacterial protein and the mycobacterial load in the pleural fluid is usually low.

Microscopy of the pleural fluid for acid-fast bacilli positive in less than 5% of tubercular pleural effusion cases, due to the paucibacillary nature of the disease.

According to Tomlinson et al & Suri et al studies, diagnostic yield of closed pleural biopsy in tubercular pleural effusion ranges from 60 to 95%.

The Gene Xpert MTB/RIF assay is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of tuberculosis and simultaneous assessment of rifampicin resistance within 2 hours.

Several studies have evaluated the performance of Gene Xpert MTB/RIF assay using pleural fluid. Overall, these studies show sensitivity of 46.4% and specificity 99.1%. Some studies have evaluated the performance of Gene Xpert MTB/RIF assay using tissue biopsy sample. Overall, these studies show sensitivity of 81.2% and the specificity 98.1%. Jinghui et al reported, overall sensitivity and specificity of Gene Xpert MTB/RIF assay in pleural biopsy specimens of 85.5%, 97.2%, respectively.

As there are limited studies about the use and efficacy of the

Gene Xpert MTB/RIF assay in pleural biopsy, there is a need for more research on this novel approach in pleural biopsies.

MATERIALS & METHODS

This study was a cross-sectional study. Suspected tubercular pleural effusion patients aged 16 years or more were en-rolled for this study from the Outpatient department of MGM Medical College Indore after informing them about the study.

Informed written consent was taken from all enrolled patients. Sample size & Duration: - In this study total 70 patients enrolled for duration of July 2019 to February 2020.

Pleural biopsy sample was tested for tuberculosis by smear microscopy, histopathology and Gene Xpert. A sample which is found to be positive by any of two tests (smear microscopy, histopathology) were considered as final positive as well as was considered as composite reference standard and was compared with Gene Xpert.

INCLUSION CRITERIA:-

1) Patients aged more than 16 years, with clinically suspected tubercular pleural effusion.

EXCLUSION CRITERIA:-

- 1) Sputum AFB positive cases
- 2) Contraindications to pleural biopsy procedure like:
 - a. Dry Tap
 - b. Uremia
 - c. Empyema
 - d. An Uncooperative Patient
 - e. Uncorrectable coagulation defects like haemophilia, low platelets or platelet function abnormalities, abnormal PT and PTT. [Absolute contraindication]

A diagnosis of tubercular pleural effusion was established when any of the following criteria or composite reference standard were met:

- 1) Identification of bacilli in pleural fluid, or pleural biopsy specimens by direct smear or Gene Xpert method.
- 2) Presence of granuloma in pleural biopsy tissue.
- 3) Lymphocytic exudate with adenosine deaminase (ADA) levels >40 U/l in the absence of any other obvious cause of pleural effusion.

RESULTS

The mean age of the enrolled patients was 34.2(± 11.4) years with range of 16 years to 66 years.

The male to female ratio of the enrolled patients was approximately 2.5:1.

Half of the enrolled patients were educated only up to high school.

The main presenting respiratory symptoms of our enrolled patients were cough (60%), pleuritic chest pain (52%), and shortness of breath(9%), constitutional symptoms including fever (45%),were loss of appetite (36%), loss of weight (36%), also common among the participants.

More than half of the patient presented after two month of symptoms.

Majority of the patients (78 %) were found to be normal weight with a BMI between 18.5 to 22.9, Only two patients were classified as overweight with BMI of more than 23.

| Mantoux Test | Positive | Negative |
|-------------------------------------|----------|----------|
| No. Of Patient (Total= 70 Patients) | 49 | 21 |

Table 1 - Pleural fluid ADA profile of the patients

Thirty six patients has pleural fluid ADA level between 40 u/l to 70 u/l and remaining thirty four patients has ADA above 70u/l. In Pleural fluid Gene Xpert was found to be positive for Mycobacterium tuberculosis in only nine patients.

In sixty five percent of enrolled patients had granulomatous lesions in pleural biopsy samples.

In pleural biopsy direct smear microscopy, only ten percent was positive for AFB.

In pleural biopsy samples of 70 enrolled patients gene xpert detect mycobacterium tuberculosis bacillus in 21 cases, among these detected rifampicin resistant were found in 3 patients.

Table 1: Pleural biopsy Gene Xpert of the Patients

| Pleural biopsy Gene Xpert | Negative | Positive Rif. Sensitive | Positive Rif. Resistant |
|---------------------------|----------|-------------------------|-------------------------|
| No. of Patients | 49 | 18 | 3 |

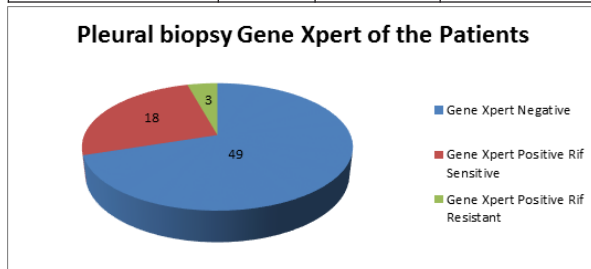


Figure 2 Pleural biopsy Gene Xpert of the Patients

DISCUSSION

Seventy percent of our patients had positive Mantoux skin test of more than 10 mm. Valdes et al reported that Mantoux test was positive in 66.5% patients with tubercular pleural effusion.

In our patients, 36 patients had pleural fluid ADA level between 40 u/l to 70 u/l and remaining 34 patients had pleural fluid ADA level more than seventy. Kate et al (2015) study reported that 93.33% of patients had a level more than 40 IU/L. Pleural fluid Gene Xpert was positive in only nine of our cases of pleural effusion. Denkinger et al in a meta-analysis of

studies of Gene Xpert in pleural fluid reported a pooled sensitivity of 46.4% against culture and 21.4% against composite reference standard. Gene Xpert pooled specificity was consistently 98.7% against composite reference standard across different sample types.

In majority of our patients AFB were not seen in pleural biopsy direct smear microscopy as only ten percent were positive for AFB. Jinghui et al found in pleural biopsy specimens, AFB microscopy was positive in 12 percent.

Sixty five percent of our patients had granulomatous lesions in pleural biopsy samples. Suri et al reported that three serial pleural biopsies increase the yield from 60% to 93%.

In our study, pleural biopsy Gene Xpert assay was found positive in 30 percent cases of pleural biopsy. Among these, 3 cases were rifampicin resistant. Gene Xpert of pleural biopsy sample is a better diagnostic modality as compared to the direct smear examination of pleural biopsy for diagnosis of tubercular pleural effusion. Also, Gene Xpert assay of pleural biopsy sample is a better diagnostic modality as compared to the Gene Xpert assay of pleural fluid for diagnosis of tubercular pleural effusion. The sensitivity and specificity of the Gene Xpert MTB/RIF assay in pleural biopsy against our Composite Reference Standard were 30 % and 100% respectively. Jinghui et al found in pleural biopsy specimens, Gene Xpert assay was positive in 49 patients out off 126 patients and the overall sensitivity, specificity, PPV and NPV of Xpert assay using biopsy specimens for pleural tuberculosis diagnosis were 85.5%, 97.2%, 95.9% and 89.6%, respectively.

CONCLUSION

This study demonstrates that Gene Xpert MTB/RIF assay performs well in biopsy specimens for rapid and accurate diagnosis of pleural tuberculosis. Pleural biopsy Gene Xpert assay was found positive in 30 % cases; among these, 3 cases were rifampicin resistant.

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