A Prospective Study for comparison of diagnostic utility of Gene XPERT MTB/RIF Assay, adenosine deaminase and cytology in Tuberculous Pleural Effusion

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ABSTRACT

Introduction: Tubercular pleural effusion is the second most common extrapulmonary form of tuberculosis in India. It poses several health challenges in a developing country like India which has limited resources, for developing appropriate methods to diagnose.

Aim: The objective of the study was to determine the role of cartridge-based nucleic acid amplification test (CBNAAT) in the diagnosis of tubercular pleural effusion (TPE) and compare it with the diagnostic utility of Adenosine Deaminase (ADA) and lymphocyte counts in pleural fluid.

Methods: Total 100 patients were selected from July 2019 to December 2019. Pleural fluid was collected in sterile container. Gene/xpert, Adenosine deaminase, total leukocytes count and lymphocytes percentage were evaluated.

Results: It was found that high leukocytes count and lymphocyte predominance was present in >80% of the patient. Mean ADA was 68.7 U/L ± 13.2 (SD). CBNAAT was positive in 30 patients. Acid fast bacilli stain was negative in the entire patient.

Conclusion: The usefulness of Xpert MTB/RIF to diagnose pleural TB is limited by its poor sensitivity. A high ADA ≥ 40 U/L in combination with Light’s criteria to define exudates, with lymphocyte predominance is sufficient evidence to diagnose tuberculous pleural effusion and initiate anti-tubercular therapy, thereby deferring the need to perform an invasive pleural biopsy.

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1. Introduction

Tuberculosis (TB) is one of the deadliest infectious diseases caused by Mycobacterium tuberculosis. It is one of the top 10 causes of death worldwide. Approximately a third of the world’s populations who are infected with Mycobacterium tuberculosis are at risk of developing TB disease.

Pulmonary TB is the most common form of TB, with extrapulmonary tuberculosis (EPTB) in the form of pleural effusion, accounting for ~15% of cases. It may increases to 50% in high HIV prevalence settings.¹

Mycobacteria infect the pleura and pleural space. This initiates the delayed hypersensitivity reaction resulting in the increase in fluid formation and decreases its removal.² There is increase in neutrophilic infiltrate initially followed by lymphocyte driven immune reaction and granuloma formation. This further increases the release of adenosine deaminase (ADA).

Diagnosis of EPTB is a challenge due to the pauci-bacillary and non-uniform distribution of microorganisms and the variable clinical presentation. Clinical guidelines for diagnosis alone can lead to over-diagnosis and treatment which can increase the resistant strain and also the mortality and morbidity of the patient.
The gold standard for diagnosis of tuberculous pleural effusion (TPE) depends on the demonstration of tubercle bacilli in pleural fluid either by culture or AFB positive and granuloma formation in pleural biopsy specimen and fluid. Each test has its own limitations.

Due to paucibacillary nature of pleural fluid, direct lung involvement may not occur, and is found to be positive in less than 5% of cases.³ Culture of pleural fluid also has low sensitivity (24–58%) and time consuming as it takes approximate 2 to 8 weeks.⁴⁻⁵

Thoracoscopic pleural biopsy is an invasive, time consuming procedure and is associated with risk, with a sensitivity ranging from 93 to 100%.⁶⁻⁹ Rapid identification is essential for early treatment initiation and improved patient outcome

To overcome above limitations newer methods has been developed. One among many is GeneXpert MTB/RIF assay, a fully automated quantitative real-time hemi-nested PCR which can detect Mycobacterium tuberculosis complex directly from clinical samples and also rifampicin susceptibility in less than 2 hours. It is recently endorsed by the WHO as a rapid test for both smear-positive and smear-negative (paucibacillary) respiratory samples.¹⁰,¹¹

It is not prone to cross-contamination, requires minimal biosafety facilities, can be performed by technicians with little training; however, a recent meta-analysis reported the pooled sensitivity and specificity of GeneXpert in TPE as 46.4% and 99.1%, respectively, compared with those of pleural fluid mycobacterial culture.¹² However, there are limited data about the Xpert MTB/RIF assay using pleural fluid.

The most widely used diagnostic marker for TPE is the pleural fluid adenosine deaminase (ADA) level. ADA testing also gives same day result. It is relatively easy, inexpensive and rapid, with pooled sensitivity and specificity estimates of 92% and 90%, respectively, across different prevalence settings depending on the cut-point used.¹³ Since it is biomarkers of the inflammatory process in the pleural space and thereby do not confirm the etiologic agent.

So combinations of tests seem to perform better than any single test, especially combinations that include adenosine deaminase, Gene Xpert, total leukocytes count and lymphocytes in pleural fluid.

Therefore, the present study was done to evaluate and compare the role of Adenosine deaminase, Gene Xpert, total leukocytes count and lymphocytes in diagnosing tuberculosis pleural effusion.

2. Material and Methods
The study population consisted of 100 suspected patients of tuberculous pleural effusion admitted in Chest & TB ward of Government Medical College, KOTA from July 2019 to December 2019.

Pleural fluid samples were collected for routine microbiology, biochemical and cytological analysis. The samples were processed as follow:

Adenosine deaminase activity in pleural fluid was determined by colorimetric technique using the user defined method on a Roche Cobas Integra (Roche Diagnostics Ltd, Switzerland). Pleural fluid ADA levels greater than 30 U/L, were reported as suggestive of pleural TB.¹⁴,¹⁵

Gene/xpert- 1 ml aliquot of raw pleural fluid and a 1 ml aliquot of concentrated pleural fluid (Prepared by centrifugation of 10-20 ml pleural fluid at 3000×g for 15 min, with the supernatant discarded and the pellet made up to 1 ml with phosphate buffer solution) from each patient was diluted with 2 ml of the Xpert MTB/RIF sample buffer. It was mixed vigorously and incubated at room temperature for 15 min, finally run on the GeneXpert machine.

For Cytology the slides were made from centrifuged deposits and stained with Giemsa. The total count was done by using modified neaubaur chamber and differential count was done on giemsa stained slides. Acid fast bacilli staining results were also documented.

All patients were started on anti-tubercular therapy. They were followed up till the completion of treatment. Response to treatment was assessed on the basis of Chest X-ray and clinical findings.

2.1. Inclusion criteria
1. Patients of all age and sex with suspected tubercular pleural effusion (i.e. sign and symptoms -an acute febrile illness characterised by cough and pleuritic chest pain, night sweats, chills, weakness, dyspnoea, haemoptysis and weight loss)
2. Chest X ray findings of pleural effusion.

2.2. Exclusion criteria
Patients on anti-tubercular therapy
Diagnosed case of carcinoma of any site.
Transudative pleural effusion

3. Objectives
1. To determine the role of genexpert in the diagnosis of tubercular pleural effusion.
2. To study the association between pleural fluid CBNAAT, ADA and lymphocytes percentage.

4. Results
There were 65 male and 35 female patients, with a mean age of 40 years. Cough and low grade fever was the most common symptoms followed by weight loss and loss of appetite. CBNAAT was positive in 30 patients. ADA was
increased (>30IU) in 85 patients with a mean value of 651U. 15 patients had value less than 30. The lymphocytic predominance was found in 95 patients whereas in 5 patients neutrophils predominance was seen. The mean value for total leukocytes count was 720 cell/μL. It was found that 12 patients with low ADA value has increased total leukocytes count and lymphocytic predominant. All the CBNAAT positive patients had increased ADA value and 100% lymphocytes, increased total leukocytes count. AFB staining was negative in all the cases. The culture was done in a few cases\textsuperscript{16} and was negative.

In the current study, increased ADA and lymphocytes predominance was seen in most of the patients. CBNAAT was less sensitive due to paucibacillary nature of the disease process.

5. Discussion

TB remains one of the most frequent causes of pleural effusions in developing countries like India. The gold standard for the diagnosis of tuberculous pleuritis remains the detection of M. tuberculosis in pleural fluid or pleural biopsy specimens, either by microscopy and/or culture, or the histological demonstration of caseating granulomas in the pleura along with AFB.

The results of present study show that Gene Xpert MTB assay play significant role in routine tuberculous pleural effusion diagnosis. The result is available in same day with high specificity. But it cannot be used alone for the diagnosis of TPE, given its low sensitivity. So it cannot be used alone.

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.\textsuperscript{17} It 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 microorganisms. Hence it should not be used to monitor patients on treatment.

In our study, pleural fluid CBNAAT was found to be positive in only 30% of cases.

In a well-structured meta-analysis of 24 studies from India, it was seen that the sensitivity of CBNAAT in TPE was between 22.7–51.4% using a composite reference standard (CRS) and pleural fluid culture as the reference standard.\textsuperscript{18}

In another meta-analysis, determining the role of genexpert in the diagnosis of EPTB, a total of 18 studies were analyzed with 4461 samples. Pooled sensitivity for pleural fluid was 46.4% against culture and 21.4% against CRS.\textsuperscript{19}

Meldau et al, compared the diagnostic utility of ADA, genexpert and gamma interferon (IFN gamma). They proposed IFN to be as sensitive as ADA in high prevalence settings, and finally, concluded that either of the two could be used to guide therapy, as routine pleural biopsy may be challenging in high prevalent, resource-limited countries.\textsuperscript{20} Shukla et al found that sensitivity of genexpert in TPE was 20.58\% in their study. Rifampicin resistance was detected in 21\% of cases. They found a positive correlation with high ADA values, pleural fluid lymphocyte counts and MTB detection by genexpert.\textsuperscript{16}

We conclude that a high adenosine deaminase (ADA) (> 40 U/L) combined with Light’s criteria to define exudates in a lymphocyte predominant effusion constitutes enough evidence to diagnose TPE, able to avoid pleural biopsy and initiate anti-tubercular therapy.\textsuperscript{21,22}

That is, ADA < 40 excluded tuberculosis in 90\% of cases.

When interpreting ADA levels, the clinician must additionally be aware of situations which may increase the likelihood of both the false-negative and false-positive ADA results. In the early phase of the disease low levels of ADA in the pleural fluid may be found, giving rise to a false negative result.

Conversely, raised ADA levels may be observed in a number of conditions potentially leading to a false positive diagnosis of TB. These include rheumatoid effusion, empyema due to other bacteria, mesothelioma, lung cancer, parapneumonic effusion, and haematological malignancies.\textsuperscript{23,24}

Also the diagnostic usefulness of ADA depends not only on its sensitivity and specificity, but also on the local prevalence of TB. In populations with a high prevalence of TB and clinical suspicion of TB effusion, elevated ADA level might be considered as a confirmatory test justifying treatment initiation. Specificity of ADA in low prevalence areas has also been estimated. In a study done in north-western Europe, 338 patients with effusions were analysed, and when an ADA cut-off of 35 U/L was used in combination with lymphocytic effusions, sensitivity was 90.9\% and specificity was 98.9\%.\textsuperscript{25}

Thus it would be possible to establish the diagnosis of TPE with use of gene xpert, and ADA without the need for a pleural biopsy. Pleural biopsy should be reserved for patients with a low pleural fluid ADA, negative cytology and a high suspicion of a neoplasm or those suspected to have multiple drug- resistant TB.\textsuperscript{26}

When a lymphocyte neutrophil ratio of 0.75 or greater is used in combination with ADA, the sensitivity, specificity, positive predictive value, negative predictive value, and efficiency for the identification of TB were reported at 88\%, 95\%, 95\%, 88\%, and 92\%, respectively.\textsuperscript{27} CBNAAT is very specific as positive test indicate tuberculous lesion but negative result does not rule out the same. Out of 100 patients 95 patients responded to treatment. After 6months chest X-ray and clinical condition were evaluated. The response was not satisfactory among 5 patients. It may be due to irregular treatment or might have developed drug resistant.
6. Conclusion

In India lymphocyte predominant effusions with high ADA levels (> 40 U/L) and clinical suspicious of TB, where alternate diagnosis seems unlikely are treated as TPE in India. Pleural biopsy being invasive is not feasible at all health care centres, it is not put into routine clinical practice. Sensitivity of ADA when combined with lymphocyte predominant exudates, in high prevalence areas has stood limited test in deciding the initiation of ATT. Low sensitivity of pleural fluid CBNAAT as shown in our study limits its clinical usefulness. But given its high specificity, it can potentially obviate the need for an invasive procedure in at least one fourth of patients with TPE.

The results of our study demonstrate that in regions with a high prevalence of tuberculosis, it is possible to establish the diagnosis of TPE from clinical data and pleural fluid analysis, with good diagnostic accuracy.

7. Source of Funding

None.

8. Conflict of Interest

None.

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