Comparative Evaluation Of GENE-XPERT MTB/RIF And ADA In Pleural Fluid For The Diagnosis Of Tuberculosis

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Abstract

India accounts for 23% of global TB burden. Of these 38% are of Extra Pulmonary Tuberculosis.Lymph node TB is the most common EPTB, pleural effusion is second most common type of EPTB in India. The bacteriological confirmation to diagnose EPTB is more difficult due to its pauci-bacillary nature.This study aims assessing the comparison of ADA with Gene-Xpert MTB RIF in pleural fluid in the diagnosis of tubercular pleural effusion. Study was a cross-sectional studycarried out at the Department of Respiratory Medicine and Department of Internal Medicine, Muzaffarnagar Medical College, and School of Medical Science and Research,Greater Noida, Gautam Budh Nagar,UP from January 2018 to March 2019.Paired and unpaired Student's *t*-test, Chi square test, $P < 0.05$ was considered statistically significant;SPSS 18.0 software was used for analysis. The detection of MTB and rifampicin resistance using the Gene-Xpert MTB/RIF with pleural fluid ADA assay was assessed in 66 specimens. With ADA cut off as 40IU/L, 60 cases had an ADA > 40IU/L and 6 cases had ADA < 40IU/L. Pleural fluid Gene-Xpert detected MTb in 22 samples while in 44 samples MTB not detected.All pleural fluid MTb detected by Gene-Xpert were rifampicin sensitive. Gene-Xpert detected 2 tubercular pleural effusion in which ADA level was less than 40.Study shows that there was no statistically significance in diagnostic value between Gene-Xpert and ADA pleural fluid however Gene-Xpert MTB/RIF is useful method for rapid detection of MTB and Rifampicin resistance simultaneously. Estimation of ADA in pleural fluid is rapid inexpensive laboratory investigation where the diagnosis is uncertain.We found no significant connection between CBNAAT positivity and ADA level. In developing country lymphocyte predominant effusions with high ADA levels (> 40 U/L) is better option but definitely combined use of ADA and Gene-Xpert is better than using individually for the diagnosis of Tubercular pleural effusion

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Introduction

After lymph node TB, tubercular pleural effusion is second most common type of EPTB and accounts>20% of all extrapulmonary TB cases.[1] Demonstration of AFB in a pleural fluid smear is positive in less than 10%, while the culture of mycobacteria from the pleural fluid is seen in 10-70% cases and time-consuming, taking 6-8weeks[2] and demonstrating granulomas in pleural biopsy specimen is being invasive less preferred.Levels of ADA greater than 40 U/L has a sensitivity of more than 90% and a specificity of about 85% for the presence of tuberculosis.[3] In lymphocyte-predominant effusions, the specificity of ADA for tuberculosis increases to more than 95%. Tubercular pleural fluid is typically clear or straw-coloured, some time cloudy or sero-sanguinous and characterized by a protein content >30 g/L and glucose concentration below the serum glucose concentration.[4] The Gene-Xpert (real-time PCR) recommended by WHO for the diagnosis of EPTB,gives result within 2 hours with simultaneous detection of rifampicin resistance

Aim

To compare pleural fluid ADA with Gene-Xpert RIF/MTB in tubercular Pleural effusion

Materials And Methods

Ethical clearance was taken from Institutional Ethical Committee. No individual consent was required as patient records were collected and no patient identification was used. No additional specimen for GENEXPERT and ADA was taken for the purpose of this study

This study was a cross sectional prospective study conducted in the Department of Respiratory Medicine and Department of Intrernal Medicine, Muzaffarnagar Medical College and Department of Respiratory Medicine, School of Medical Science and Research,Greater Noida, Gautam Budh Nagar,Uttar Pradesh from January 2018 to March 2019 .

Inclusion criteria

1. All patients aged >18 with a history compatible with tuberculosis including symptoms of cough for >2 weeks, weight loss, fatigue, and loss of appetite and radiological evidence of pleural effusion on a chest X-ray.
2. Diagnostic thoracocentesis was performed from IPD and OPD and pleural fluid was sent for biochemical, cytological and ADA examination. 10 mL of pleural fluid was sent for CB-NAAT

Exclusion criteria

1. Those specimens which had only one method, either GeneXpert or ADA were excluded from the study.
2. Transudative pleural effusions

Results

Among total no of 66 patients , in 60 patient pl fluid ADA level was more than 40 and in 6 patients pleural fluid level ADA was less than 40.

Total	ADA > 40	ADA < 40
66	60	6

Table 1(Original): Tubercular pleural effusion detected by ADA level.
All pleural effusion patients were subjected to GeneXpert MTB/ RIF assay.

Total	Gene-Xpert Mtb detected	Gene-Xpert Mtb NOT detected
66	22	44

Table 2(Original): Tubercular pleural effusion detected by Gene-Xpert

Out of the 66 samples examined, 22 samples (33.33%) were MTB detected by GeneXpert assay while in 44 sample (66.66%) Mtb were NOT detected by Gene-Xpert.

	ADA > 40	ADA < 40	Total
Gene-Xpert Mtb	20	2	22

detected			
Gene-Xpert Mtb NOT detected	40	4	44
Total	60	6	66

Table 3(Original): Among the patients pleural fluid level ADA more than 40,20 patients were GeneXpert MTB positive and 40 were GeneXpert MTB negative. Among the 6 patients having pleural fluid level ADA < 40, 2 patients were GeneXpert positive and 04 patients were GeneXpert negative.

No clinical isolates of the entire patients were resistant to Rifampicin.

Student t test was done to determine the association between CBNAAT and pleural fluid ADA. A p value of < 0.05 was considered significant. The statistical software, namely SPSS 18.0 was used for the analysis of the data .The chi square statistic with Yates correction is 0.2062. The p- value is .642973. There was no statistically significance in diagnostic value between GeneXpert and ADA in pleural fluid samples ($p< 0.05$).

Discussion

TB remains one of the most frequent causes of pleural effusions in developing countries like India.The sensitivity of CBNAAT depends on the type of sample on which it is performed. Considering the cost and resources involved in performing the test, its judicious use lies in the hands of the treating physicians.

The role of ADA in diagnosing TPE has chemical and cytological characteristics. Owing to its high negative predictive value (NPV) and positive predictive value (PPV) in areas of high prevalence, it remains a test of great clinical utility. It seldom rises beyond 40 U/L in non-tubercular effusions, thereby increasing its specificity. That is, ADA < 40 excluded tuberculosis in 99% of case.[5]

We studied the association between pleural fluid CBNAAT and ADA and found no significant connection between CBNAAT positivity and ADA level. In our study, pleural fluid CBNAAT detected Mtb in only 33% of cases, and rifampicin resistance was not detected in any case.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, due to 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.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.

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.[6]

Shukla et al found that sensitivity of GENE-XPERT 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 GENE-XPERT.[7]

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.That is,ADA<40 excluded tuberculosis in 90% of cases.[8]

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. 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.

Thus it would be possible to establish the diagnosis of TPE with use of GENEXPERT, 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.[9]

Conclusion

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 pleural biopsy. Pleural biopsy being invasive is not feasible and not put into routine clinical practice.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 tubercular pleural effusion.Sensitivity of ADA when combined with lymphocyte predominant exudates, in high prevalence areas has stood valuable test in deciding the initiationof ATT. It is inexpensive, easily available and has got reliable sensitivity and negative predictive value.

Low sensitivity of pleural fluid CBNAAT limits its clinical usefulness. CBNAAT is very specific as positive test indicate tuberculous lesion but negative result does not rule out the same. CBNAAT due to its high specificity, can potentially obviate the need for an invasive procedure in at least one fourth of patients with TPE. Although we should keep in mind the added benefit of detecting Rifampicin resistant cases through Gene expert. A combined use of ADA and Gene-Xpert is better than using individually for the diagnosis of Mycobacterium tuberculosis aetiology in pleural fluid.But GeneXpert in suspected MDR tubercular pleural effusion does not eliminate the need of culture to detect resistance to drugs other than Rifampicin and therefore test results must always be confirmed by culture and DST due to rising incidence of XDR cases.

Limitations: As number of pleural fluid samples present in this study is less, further studies with more number of samples need to be done.

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