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Original Research Article

The advantage of PCR for MTB in comparison to ADA in diagnosing tubercular pleural effusion

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ABSTRACT

Background: Tuberculosis continues to be an important health problem globally. The bacteriological confirmation of diagnosis in extrapulmonary tuberculosis patients is more difficult because most of the cases of extrapulmonary tuberculosis are paucibacillary in nature. In this study we have compared the pleural fluid ADA levels with PCR for MTB in pleural fluid to confirm the diagnosis of tuberculosis in the pleural fluid.
Methods: The study was done over two years and a total of 106 patients with a clinico-radiological diagnosis of pleural effusion were enrolled for the study. The pleural fluid was aspirated and examined for total cell count, differential cell count, protein, sugar, ADA and PCR for MTB.A CT Thorax was done in all the 106 patients of pleural effusion and underlying consolidation along with pleural effusion was found in 60 patients.
Results: The pleural fluid was exudative in nature in all the patients. 90 patients (84.9%) had lymphocyte predominant pleural effusion while 16 patients (15.1%) had neutrophil predominant pleural effusion. The overall sensitivity of ADA in all the cases of pleural effusion was 85.2% while the overall sensitivity of PCR for MTB in all the cases of pleural effusion was 51.1%. However, in the 60 patients of pleural effusion with underlying lung consolidation, the overall sensitivity of ADA was 69.1% while the overall sensitivity of PCR for MTB was 92.8% for diagnosing tubercular pleural effusion.
Conclusions: PCR for MTB is a useful test along with ADA for diagnosing tubercular pleural effusion. PCR for MTB is especially useful in the diagnosis of tubercular pleural effusion in patients with underlying lung consolidation.

Keywords: ADA, PCR for MTB, Pleural effusion, Tuberculosis

INTRODUCTION

Tuberculosis continues to be an important health problem globally. Inspite of the best efforts by WHO, globally there were 10.4 million new tuberculosis cases in 2015 in comparison to the 9.6 million new tuberculosis cases in 2014.[1,2] In India also, there were 28 lakh new tuberculosis cases in 2015 in comparison to 22 lakh new tuberculosis cases in 2014.[1,2] In 2015, 82% of the total tuberculosis cases globally were of pulmonary tuberculosis while the remaining 18% cases were of extrapulmonary tuberculosis.[1] In India, about 20% of the total tuberculosis cases in HIV negative patients are of extrapulmonary tuberculosis.[3]

However, in HIV positive patients the extrapulmonary tuberculosis cases may account for upto 50% of the total tuberculosis patients.[4] In India, pleural effusion due to tuberculosis is the commonest type of extrapulmonary tuberculosis followed by lymph node tuberculosis.[5]

Only 64% of the total notified cases of pulmonary tuberculosis were bacteriologically confirmed in 2015.[1] The bacteriological confirmation of diagnosis in extrapulmonary tuberculosis patients is even more difficult because most of the cases of extrapulmonary tuberculosis are paucibacillary in nature. The diagnosis of extrapulmonary tuberculosis mainly relies on acid fast bacilli (AFB) smear examination on microscopy and on cartridge based nucleic acid amplification test (CB-NAAT) which is a polymerase chain reaction(PCR) based rapid molecular test. Other methods used to diagnose extrapulmonary tuberculosis include culture of mycobacterium tuberculosis (MTB) and histopathological examination of specific tissue specimens.

Here, we are comparing pleural fluid adenosine deaminase (ADA) levels with PCR for MTB in pleural fluid to confirm the diagnosis of tuberculosis in the pleural fluid. ADA is an enzyme involved in purine metabolism. It catalyses the conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine. Deoxyadenosine produced by breakdown of DNA is toxic to lymphocytes and hence is essential to convert it to deoxyinosine through adenosine deaminase enzyme. PCR amplifies specific segment of the MTB DNA thereby detecting the bacilli in the pleural fluid.

METHODS

A prospective study was conducted in the department of Pulmonary medicine at Era's Lucknow medical college and hospital, Lucknow over a period of two years. Patients admitted in the department with a clinico-radiological diagnosis of pleural effusion on the basis of clinical examination and chest X-ray were subjected to thoracocentesis after confirming that the bleeding time, coagulation time, INR (international normalized ratio) and platelets are within normal limits. In some cases of mild pleural effusion an ultrasound thorax was also done to confirm the diagnosis. After thoracocentesis, the fluid aspirated was sent for total cell count, differential cell count, protein, sugar and lactate dehydrogenase measurement. Simultaneously serum protein and serum LDH were also measured to determine the transudative or exudative nature of the pleural fluid according to Light's criteria.[6] Only the pleural effusions which were non-purulent and were exudative in nature were included in the study. Hence a total of 106 patients were enrolled for the study and their pleural fluid was also sent for AFB smear examination, gram stain and culture pyogenic, ADA and PCR for MTB. Ultrasound guided thoracocentesis was done in cases of mild pleural effusion. A computed tomography (CT) thorax was done in all the patients to look for underlying lung consolidation in these patients with pleural effusion. The patients were between the age group of (17-68) years of which 75 were males and 31 were females. The patients with chronic kidney disease, chronic liver disease, any malignancy, patients on treatment for any malignancy and HIV, HCV or HBsAg positive patients were excluded from the study. If the patient had expectoration of sputum then sputum was examined for AFB smear, gram stain and culture pyogenic.

ADA is measured in pleural fluid by colorimetric or fluorescent immunoassays. These tests are enzyme linked and the enzymes commonly used for these colorimetric and fluorescent tests are horseradish peroxidase and calf intestine alkaline phosphatase. The fluorescent test is slightly more sensitive than the colorimetric test with an ability to give more accurate higher measurements. In real time PCR, the MTB DNA was extracted from the centrifuged pleural fluid sample. This DNA was amplified followed by analyses for IS6110 DNA sequence to detect the presence of Mycobacterium tuberculosis in the pleural fluid. The results of real time PCR were obtained in about two to three hours. Informed consent was taken from all the patients enrolled in the study. The approval for the study was obtained from the institute's ethical committee.

RESULTS

The most frequent age group affected was (21-30) years of age followed by (41-50) years of age (Table 1).

Table 1: Demographics of the study population (n=106).

Age	Number	Percentage
11-20 years	03	2.8
21-30 years	35	33.0
31-40 years	18	17.0
41-50 years	30	28.3
51-60 years	11	10.4
61-70 years	09	8.5
Sex		
Male	75	70.8
Female	31	29.2

Chest pain on the side of pleural effusion was the most common symptom and was present in all the patients while fever was present in 102 patients (96.2%). Cough was present in 81 patients (76.4%) and dyspnea was seen in only 58 patients (54.7%). The pleural fluid was exudative in nature in all the patients. 90 patients (84.9%) had lymphocyte predominant pleural effusion while 16 patients (15.1%) had neutrophil predominant pleural effusion. In all the 106 patients, the pleural fluid was sent for ADA and PCR for MTB. The mean ADA levels in patients with lymphocyte predominant pleural effusion was 53.8±18.2U/L and in patients with neutrophil predominant pleural effusion was 26.4±5.3U/L. Pleural fluid for AFB smear was negative in all the patients. All the 16 patients with neutrophil predominant pleural effusion had underlying lung consolidation and their ADA was <40U/L while PCR for MTB was positive in 2 of these 16 patients (Table 2). Hence these two patients were put on antituberculosis treatment to which they responded.

These two patients had diabetes mellitus along with consolidation in superior segment of right lower lobe and their sputum was also positive for AFB. Out of these 16 patients only 6 patients showed culture pyogenic positive results. The most common bacterial growth seen was of Streptococcus pneumoniae followed by Staphylococcus aureus and Enterococcus in one patient.

Table 2: Type of pleural effusion and the test results of ADA and PCR for MTB in the study population (n=106).

	Number	Percentage	ADA		PCR for MTB	
			>40 U/L	<40 U/L	Positive	Negative
Neutrophil predominant fluid	16	15.1	0	16	02	14
Lymphocyte predominant fluid	90	84.9	75	15	43	47

In 90 patients with lymphocyte predominant pleural fluid, ADA was >40U/L in 75 patients. PCR for MTB was positive in only 35 of these 75 patients (Table 2). 29 of these 35 patients with ADA >40U/L and PCR for MTB test positive had underlying lung consolidation. Hence six patients with positive PCR for MTB test and forty-six patients with ADA >40U/L had no underlying lung consolidation. The 75 patients with lymphocyte predominant pleural fluid and high ADA values were put on antituberculosis treatment and they responded to treatment. In the remaining 15 patients with lymphocyte predominant pleural fluid, ADA was <40U/L and all these 15 patients had underlying lung consolidation. PCR for MTB was positive in 8 of these 15 patients (Table 2).

All these 8 patients were of diabetes mellitus with concomitant consolidation in the lung parenchyma. Hence these patients were put on antituberculosis treatment and they responded to treatment. The remaining seven patients with ADA <40U/L and PCR for MTB result being negative were put on antibiotics. The culture pyogenic result in all these seven patients was negative. Four patients responded to antibiotic treatment while three patients showed clinico-radiological deterioration even after ten days of antibiotic treatment. These three patients had no features of connective tissue disorder and malignant cells were also negative in three consecutive pleural fluid samples, but these patients were mantoux positive with an induration of >12mm. Hence, these three patients were initiated on antituberculosis treatment and they responded to treatment.

Overall, out of the total 106 patients of pleural effusion,75 patients had high ADA levels >40U/L while 45 patients had a positive PCR for MTB test. A total of 88 patients were given antituberculosis treatment on the basis of high ADA level or a positive PCR for MTB test or a positive mantoux test. The remaining 18 patients were successfully treated with antibiotics due to a pyogenic infection in these patients. CT thorax of the 106 patients with pleural effusion revealed underlying lung consolidation in 60 patients. ADA >40U/L was seen in 29 patients while PCR for MTB was positive in 39 patients out of these 60 patients (Table 3). In three patients of pleural effusion with underlying lung consolidation both ADA and PCR for MTB tests were negative, and these patients were given antituberculosis treatment on the basis of history and a positive mantoux test.

Table 3: Type of pleural effusion and the test results of ADA and PCR for MTB in patients of pleural effusion with underlying lung consolidation (n=60).

	Total number	Lung consolidation present	ADA		PCR for MTB	
			>40 U/L	<40 U/L	Positive	Negative
Neutrophil predominant fluid	16	16	0	16	02	14
Lymphocyte predominant fluid	90	44	29	15	37	07

The overall sensitivity of ADA in all the 106 cases of pleural effusion was 85.2% while the overall sensitivity of PCR for MTB in all the cases of pleural effusion was 51.1%. The increased sensitivity of ADA in comparison to PCR for MTB was statistically significant with a p value of <0.001 (Table 4). The overall negative predictive value (NPV) of ADA in all the cases of pleural effusion was 58.1% while the overall NPV of PCR for MTB was 29.5%. The increased NPV of ADA in comparison to PCR for MTB was also statistically significant with a p value of 0.001 (Table 4). However, in the 60 patients of pleural effusion with underlying lung consolidation, the overall sensitivity of ADA was 69.1% while the overall sensitivity of PCR for MTB was 92.8% for diagnosing tubercular pleural effusion. In these 60 patients with underlying lung consolidation, the increased sensitivity of

PCR for MTB in comparison to ADA was statistically significant with a p value of 0.013 (Table 5). The overall NPV of ADA in these patients with underlying lung consolidation was 58.1% while the overall NPV of PCR for MTB in these patients was 85.7% and the increased NPV of PCR for MTB in comparison to ADA here was also statistically significant with a p value of 0.010 (Table 5). No false positive results were seen either with ADA or PCR for MTB in all the cases of pleural effusion and therefore the specificity of both the tests is high for diagnosing tubercular pleural effusion.

Table 4: Overall sensitivity and NPV of ADA and PCR for MTB in diagnosing tubercular pleural effusion (n=106).

	Number	Sensitivity	NPV
ADA >40 U/L	75	85.2%	58.1%
PCR for MTB positive	45	51.1%	29.5%
Z value		4.010	3.224
p value		<0.001	0.001

Table 5: Overall sensitivity and NPV of ADA and PCR for MTB in diagnosing tubercular pleural effusion in patients with underlying lung consolidation (n=60).

	Number	Sensitivity	NPV
ADA >40 U/L	29	69.1%	58.1%
PCR for MTB positive	39	92.8%	85.7%
Z value		2.488	2.569
p value		0.013	0.010

DISCUSSION

ADA has two isoforms ADA1 and ADA2. ADA1 is widely present in various cells of the body but is particularly found in lymphocytes and macrophages. ADA2 although first isolated from human spleen, is mainly found in serum and plasma in the human body. ADA is infact a marker of lymphocyte proliferation. Increased pleural fluid ADA level has a high sensitivity and specificity for diagnosing tubercular pleural effusion.[6] Increased ADA level in red blood cells is seen in cases of hereditary hemolytic anemia and in case of acquired immunodeficiency syndrome.[7,8] CB-NAAT which was earlier employed mainly for diagnosing multidrug resistant tuberculosis has now been approved by the revised national tuberculosis control programme (RNTCP) for diagnosing extrapulmonary tuberculosis.[3] The sensitivity of nucleic acid amplification test, Xpert MTB/RIF ranged from (70-100)% in most of the extrapulmonary samples but was much lower around (40-50)% in the pleural fluid.[9-11] In our study also the overall sensitivity of PCR for MTB for diagnosis of tubercular pleural effusion has been found to be 51.1% which is low. CB-NAAT has also been shown to be useful in diagnosing abdominal tuberculosis and meningeal tuberculosis.[12,13]

A cut off value of >40U/L is usually used to strongly suspect tubercular etiology in a pleural effusion.[14,15] The sensitivity of ADA for diagnosing tubercular pleural effusion has been high and has been reported to be around 90%.[15,16] In our study also the sensitivity of ADA for diagnosis of tubercular pleural effusion has been found to be 85.2% which is comparable to other studies. ADA levels <16U/L almost excludes the diagnosis of tubercular pleural effusion and ADA levels >49U/L almost confirms the diagnosis of tubercular pleural effusion.[17] ADA levels have been found to be falsely high in empyema and malignancies while falsely low value of ADA has been reported in cases with chronic kidney disease, hence these patients were excluded in our study.[18,19]

Comparing the overall sensitivity of ADA and PCR for MTB in our study, it can be inferred that the overall sensitivity of ADA is much more than that of PCR for MTB for diagnosing tubercular pleural effusion. In our study, the NPV of PCR for MTB was low in comparison to ADA which has been found in other studies also.[20] Hence, a negative result of PCR for MTB is not useful in excluding the diagnosis of tuberculosis in a pleural effusion.

However, when we compared the sensitivity of these two tests in patients of pleural effusion with underlying lung consolidation it was seen that the sensitivity of PCR for MTB was much more than that of ADA for diagnosing tubercular pleural effusion thereby increasing the diagnostic value of PCR for MTB in these patients. It may be because the bacillary load in the pleural effusion in patients with underlying lung consolidation is more in comparison to the pleural effusion patients with no

underlying lung consolidation. Similarly, it has been found that the sensitivity of Xpert MTB/RIF is more in the pleural tissue taken through pleural biopsy in comparison to the pleural fluid samples.[21] The NPV of PCR for MTB increased in patients of pleural effusion with underlying lung consolidation thereby increasing the accuracy of PCR for MTB in excluding the diagnosis of tuberculosis in these cases of pleural effusion. The sensitivity of AFB smear examination in the pleural fluid is around 20% and this is much lower than the sensitivity of ADA or PCR for MTB for diagnosing tubercular pleural effusion.[22]

CONCLUSION

PCR for MTB is a useful test along with ADA for diagnosing tubercular pleural effusion. PCR for MTB is especially useful in the diagnosis of tubercular pleural effusion in patients with underlying lung consolidation. Both the tests are rapid tests in comparison to the culture MTB test and they have a much higher sensitivity than the AFB smear examination. ADA and PCR for MTB are the tests with high specificity for diagnosing tubercular pleural effusion. The two tests when used together increase the detection of tubercular pleural effusion at the earliest and thus prevent the development of complications of untreated long standing pleural effusion.

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