

# Diagnostic accuracy of pleural fluid adenosine deaminase in tubercular pleural effusion

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## Abstract

**Background:** In India, tuberculosis is an endemic disease. Delay in diagnosis results in poor prognosis and fast spread of the disease. The objective of the present study is to look for an effective and acceptable diagnostic test, which may be helpful to initiate early treatment to improve prognosis and reduce spread. The presence of ADA in pleural fluids reflects the cellular immune response in the pleural cavity and in particularly, the activation of T lymphocytes. Objective of study include evaluating efficacy of combined use of ADA activity diagnosing tuberculous pleural effusion. **Methods:** Biochemistry, cytology, and microbiology studies were performed on 164 consecutive pleural fluids. ADA were determined on all exudative effusions. **Results:** Pleural fluid ADA activity at a level of  $\geq 40$  U/L, the sensitivity=95.5%, specificity=93.4%, positive prediction value=94.4%, negative prediction value=94.7% and efficacy= 94.5 %. It was statistically significant (p value<0.001). **Conclusion:** ADA is a highly sensitive diagnostic marker of tubercular pleural effusion.

**KeyWord:** tuberculosis is an endemic disease, microbiology, diagnostic test

## Introduction

Pleural effusion refers to an excessive fluid accumulation in the pleural cavity. Normally, the pleural cavity contains about 10 ml of fluid on each side [1]. Pleural effusion occurs due to an imbalance between the production of the fluid and its resorption. It can be an initial manifestation of any cardiac disorder or any pulmonary disorder.

Pleural effusion is indicative of an underlying disorder and it is not by itself an individual disease entity. In the case of diagnosis of pleural effusion, there should be an effort to diagnose the causative disorder. According to the criteria laid out by Light, [2] pleural effusion is broadly divided into transudative and exudative. Transudative effusion is usually caused by congestive heart failure or a hypoalbuminemic state. Exudative effusion occurs due to pleuro-pulmonary infection, local or metastatic malignancy, pulmonary thromboembolism or local trauma etc. Tuberculosis is the most common

infectious cause of death around the globe [3]. Pleural tuberculosis is the second most common extra pulmonary manifestations of tuberculosis, next only to tubercular lymphadenitis [4].

In India and many other countries, the most common cause of pleural effusion is tuberculosis, in the absence of any demonstrable pulmonary disease. If left untreated, the tubercular pleural effusion develops into active tuberculosis [5] and thus it is very crucial to diagnose it early and institute an appropriate treatment. The definitive diagnosis is at times difficult because the sole site of the infection is pleura, in more than half of the patients [5].

The definitive diagnosis of tubercular pleural effusion is done by the demonstration of tubercle bacilli either in pleural fluid or in a pleural biopsy specimen/sputum, or by the demonstration of pleural granulomas. Because there is a paucity of the tubercle bacilli in the pleural fluid, whenever a tubercular pleural effusion is a possibility, the pleural biopsy is believed to be crucial for an accurate diagnosis. But because the pleural

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biopsy is a difficult process, and moreover, sampling of pleural fluid is an easier alternative, there has been ample effort being done in study of pleural fluid markers of tubercular pleural effusion. The enzyme ADA is involved in purine catabolism. It deaminates adenosine to inosine and deoxyadenosine to deoxyinosine. It also regulates the proliferation and differentiation of lymphocytes, more importantly the T-lymphocytes. Compared to the erythrocytes, its concentration is ten times higher in the lymphocytes. When T cells get activated due to the entry of the tubercle bacilli, they secrete ADA in the medium. ADA thus serves as a marker of the Tcell mediated immunity Pleural fluid adenosine deaminase level is a chemical biomarker which is cost effective and therefore it is an attractive screening tool particularly, in areas endemic to tuberculosis. We have planned this study to evaluate efficacy of ADA activity for diagnosing tuberculous pleural effusion.

## Materials and Methods

This study was a hospital based prospective study carried out in the department of Pulmonary Medicine, JIPMER, Puducherry during the period between June 2012 to June 2014. This study was approved by Institute ethics sub-committee (human studies).

### Patient selection

#### Inclusion criteria:

1. All exudative pleural effusion cases

#### Exclusion criteria:

1. Patients with transudative pleural effusion.
2. Patients with malignant pleural effusion.
3. Patients with immunodeficient states like HIV, those on chemotherapy.
4. Patients with empyema and hemothorax.

**Brief Procedure:** According to a predetermined pro forma, demographic data collected, a thorough clinical history was taken and clinical examination done. The patients underwent the following investigations for the evaluation of the pleural effusion –sputum for AFB smear and mycobacterium culture, hemogram, random blood sugar, ESR, liver function test, renal function test, tuberculin skin testing, pleural biopsy, pleural fluid-glucose, protein, albumin, gram staining, auraminerhodamine staining, LDH, ADA, cytology and cell count.

A total of 244 patients who were clinically/radiologically examined and diagnosed to have pleural effusion were taken up for the study after

written consent and further evaluated. As per Light's criteria (pleural fluid protein/serum protein  $>0.5$ ; fluid LDH/serum LDH  $> 0.6$ ), 238 patients diagnosed as exudative effusion and 6 patients were diagnosed as transudative pleural effusion.

Among these 238 exudative pleural effusion patients on further evaluation, 44(18.5%) patients had malignant pleural effusion and 22(9.2%) had empyema thoracic and 8(3.3%) had pleural effusion with HIV infection. These patients were excluded from the study. So total 164 patients included in our study. Among the 164 exudative pleural effusion patients, 76 patients had non tubercular pleural effusion [includes 72 patients with par pneumonic, 3 with pancreatic pleural effusion and 1 with chylothorax] and 88 patients were tubercular pleural effusion as per criteria mentioned below

Patients were diagnosed as a case of tubercular pleural effusion based on presence of first or more than one of the following criteria [6].

1. Bacteriological confirmation of presence of Mycobacterium tuberculosis in pleural fluid or in sputum (direct smear or culture or histological finding),
2. Histopathologically proven cases of tuberculosis,
3. Radiological findings consistent with TB,
4. Clinical presentation consistent with TB with exclusion of other clinical considerations,
5. Definite clinical and radiological improvement in 6 – 8 weeks of administration of anti- tubercular treatment,
6. Positive reaction ( $> 10$  mm induration) to the 1 tuberculin unit (TU) purified protein derivative (PPD),
7. Pleural fluid adenosine deaminase levels of  $> 40$ U/L.

Chest X ray-PA view was taken for all 88 patients with tubercular pleural effusion. Quantity of fluid, side of involvement, hilar/mediastinal adenopathy, parenchymal involvement, cavitation and other radiological abnormalities were noted. Effusions occupying more than two thirds of the hemithorax were considered massive, one-third to two thirds of the hemithorax as moderate and less than one third of the hemithorax as minimal.

All patients' with pleural effusion underwent diagnostic thoracentesis. After site for thoracentesis was identified, the skin surrounding the site was cleansed thoroughly with an antiseptic solution over an area of 4 inches in all directions from the proposed thoracentesis

site. The sterile drape with the center hole was then placed to the patient's back, and another sterile drape is placed on the bed. The skin was anaesthetized using a 25-gauge needle by injecting 2 % lidocaine, then a 22-gauge needle attached to a 50- to 60-mL syringe containing 1 mL of heparin is inserted. Heparin is added into the syringe to prevent clotting of the pleural fluid. Pleural fluid was examined for acid-fast bacilli with Auramine-Rhodamine staining according to standard confirmed procedures. LED fluorescent microscopy was used for reporting. The results of staining were reported after viewing 100 fields

Pleural fluid culture and sputum culture for Mycobacterium were done by inoculating processed sputum/ pleural fluid specimens on to Lowenstein-Jensen media. Samples (0.25 ml) of each specimen were inoculated onto each of two slants of glycerol-free LJ medium supplemented with sodium pyruvate and one slant of LJ medium containing nalidixic acid (35 mg/liter), vancomycin (20 mg/liter), polymyxin B (1,600 U/ml). All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures were examined weekly, up to 8 weeks on a specified day of the week. The colony with doubtful morphology, the acid-fastness was confirmed by Ziehl-Neelsen (ZN) staining.

36 patients having exudative pleural effusion have given consent for pleural biopsy. Pleural biopsy was done with an Abram's or a Cope needle. Among these 36 patients on whom pleural biopsy was done, on histopathological examination 11 were reported as tuberculosis, 14 as malignancy and remaining 11 were inconclusive. Effusion was called as malignant when

pleural fluid cytology or pleural biopsy showed evidence of malignancy or if the patient had proved metastatic malignancy with no other detectable cause of effusion. The method described by Giusti was used for the determination of the pleural fluid ADA levels. Berthelot reaction forms the basis of this calorimetric method in which the ammonia produced due to the reaction of ADA with adenosine is estimated. One unit of ADA is defined as the amount of enzyme required to release 1  $\mu$ mol ammonia per minute from adenosine under standard assay conditions. The enzyme is stable for at least 24 h at 25°C, for 7 days at 4°C, and for 3 months at -20°C.

### Methods of Analysis

Patients demographic, clinical and laboratory parameters were collected in a proforma and data was entered in excel spread sheet. All categorical data was presented as frequencies/percentage. Continuous variables are expressed as mean (SD) while categorical variables are expressed as number and group percentages. Differences in ADA levels between groups were analyzed using unpaired Student t test and one way Anova. Correlation between ADA and specified variables was quantified using pearson correlation coefficient. This was performed for the whole study population (n = 164) as well as individually for the TPE and non TPE groups. All statistical analyses were performed using IBM SPSS Statistics version 20. A 2 tailed p value of <0.05 was taken to be statistically significant. In all 164 cases included in the study, the sensitivity, specificity, positive predictive value and negative predictive value using pleural fluid ADA for diagnosing tubercular pleural effusion were calculated.

### Results

**Table 1: Gender distribution in tubercular pleural effusion study patient**

Gender	Number of patients	% of patients
Male	66	75
Female	22	25
<b>Total</b>	<b>88</b>	<b>100</b>

Among 88 tubercular pleural effusion patients 66(75%) were males and 22 (25%) were female.

Among 88 of tubercular pleural effusion patients, 84 had pleural fluid ADA  $\geq$ 40 and 4 patients had ADA <40. Among 76 patients of non-tubercular pleural effusion, 5 had pleural fluid ADA  $\geq$ 40 and 71 patients had ADA <40. Hence in exudative pleural effusion cases, pleural fluid ADA  $\geq$ 40 for diagnosis of tubercular pleural effusion had

Sensitivity = 95.5%

Specificity = 93.4%

Positive Predictive Value = 94.4%

Negative Predictive Value = 94.7%, Efficacy = 94.5 %

**Table 2: Distribution of tubercular pleural effusion study subjects in relation to age**

Age	Number of patients	% of patients
11-20	8	9.1
21-30	22	25
31-40	15	17
41-50	19	21.6
51-60	14	15.9
61 and above	10	11.4
<b>Total</b>	<b>88</b>	<b>100</b>

Among 88 tubercular pleural effusion patients, mean age was 40.7 years with standard deviation of  $\pm 15.8$  years (range 14 to 76 years)

**Table 3: Yield of pleural biopsy in study subjects**

Biopsy results	Number of patients	Percent
Histopathological examination suggestive of tuberculosis	11	68.7
Histopathological examination not suggestive of tuberculosis	5	31.3
<b>Total</b>	<b>16</b>	<b>100</b>

**Table 4: Sensitivity and specificity of pleural fluid ADA $\geq 40$** 

ADA level	Tubercular pleural effusion	Non-tubercular pleural effusion	Total
ADA $\geq 40$ IU	84	05	89
ADA $< 40$ IU	04	71	75
Total	88	76	164

**Table 5: Comparison of pleural fluid ADA value with sputum results in study subjects.**

Mycobacterium tuberculosis in sputum (direct smear and/or culture)	Number of patients	Pleural fluid ADA mean $\pm$ Standard deviation	p- value
positive	31	77.41 $\pm$ 31.13	0.628
Negative	57	74.44 $\pm$ 25.27	

In our study, among 34 patients with both parenchymal lesion and pleural effusion in chest x-ray, pleural fluid ADA mean $\pm$ Standard deviation was 72.55 $\pm$ 24.52 and among 54 patients with pleural effusion only in chest x ray, pleural fluid ADA mean $\pm$ Standard deviation was 77.34 $\pm$ 29.04. Pleural fluid ADA value of both groups was comparable ( $p > 0.05$ ) (TABLE 5).

**Table 6: Comparison of pleural fluid ADA value with chest X ray finding in study subjects.**

Chest x ray finding	Number of patients	Pleural fluid ADA mean $\pm$ Standard deviation	p- value
Suggestive of parenchymal lesion and pleural effusion	34	72.55 $\pm$ 24.52	0.42
Suggestive of pleural effusion only	54	77.34 $\pm$ 29.04	

In our study, among 31 patients positive for mycobacteria sputum culture and/or smear, Pleural fluid ADA mean $\pm$ Standard deviation was 77.41 $\pm$ 31.13 and among 57 patients negative for mycobacterium sputum culture and

smear, pleural fluid ADA mean  $\pm$ Standard deviation was  $74.44 \pm 25.27$ . Pleural fluid ADA value of both group were comparable ( $p > 0.05$ ) (TABLE 6).

**Table 7: Comparison of pleural fluid ADA value with pleural biopsy results**

Biopsy results	Number of patients	Pleural fluid ADA mean $\pm$ Standard deviation	p-value
Histopathological examination suggestive of tuberculosis	11	81.88 $\pm$ 13.13	0.41
Histopathological examination not suggestive of tuberculosis	5	88.1 $\pm$ 13.47	

## Discussion

In our study, all 88 patients with tubercular pleural effusion had unilateral effusion. Right sided in 50 patients (56.82%), and left sided in 38 patients (43.18%). In a study done by Valdes L et al out of 254 tubercular pleural effusion patients, 142 (55.9%) were right sided and 108 (42.5%) were left sided [7].

In our study, among 88 patients of tubercular pleural effusions, chest x-ray showed both pleural effusion and parenchymal lesion in 34 (38.6%) patients and pleural effusion only in remaining 54 [61.4%] patients. In a study done by Berger HW et al [8] 18 (37.5%) of 48 patients had parenchymal lesion and pleural effusion. In a study by Valdes L et al [7] in 254 tubercular pleural effusion patients, In 48 patients (18.9%), pleural effusion was associated with parenchymal involvement.

### Pleural biopsy

In our study, out of 88 patients with tubercular pleural effusion, 16 underwent closed needle biopsy of the parietal pleura. Among these 16 patients, histopathological examination of the biopsy revealed caseation granuloma in 11 (68.7%) and remaining 5 were inconclusive. Acid-fast stain were positive for mycobacteria in 2 (12%) cases. In a study by Valdes L et al [7], out of 248 patients of tubercular pleural effusion, closed biopsy of the pleura in 248 patients showed granulomas in 198 patients (80%), the acid-fast stain positive for mycobacteria in 64 (25.8%), and culture of the biopsy positive for mycobacteria in 140 (56%).

### Pleural fluid evaluation

In our study, among 88 patients of tubercular pleural effusion, pleural fluid ADA  $\geq 40$  for diagnosis of tubercular pleural effusion had Sensitivity=95.5%, Specificity=93.4%, Positive prediction value=94.4%, Negative prediction value=94.7% and Efficacy= 94.5%.

It was statistically significant ( $p$  value  $< 0.001$ ) [Table 4]. Similar observation was made in the study done by Bharat et al, Susmitha et al, by Mehta AA et al, Bergess LJ et al [9-12].

Bharat et al [9], consecutively selected 96 lymphocytic pleural fluid samples and divided them into two groups: tuberculous ( $n = 56$ ) and non-tuberculous ( $n = 40$ ). They found that the ADA with cut-off value of 40 for diagnosis of tubercular pleural effusion had a Sensitivity of 92%, Specificity of 90%, Positive prediction value of 92.8%, and Negative prediction value of 90%. In a study by Susmitha et al [10], ADA with cut-off value of  $\geq 40$  for diagnosis of tubercular pleural effusion had sensitivity of 97%, specificity of 93%, positive prediction value of 94% and negative prediction value of 97%. In a study by Mehta AA et al [11] in evaluation of 121 cases of exudative pleural effusion have found that ADA with cut-off value of  $\geq 40$  for diagnosis of tubercular pleural effusion had sensitivity of 85.7%, specificity of 80.8%, positive prediction value of 75% and negative prediction value of 89.5%. Bergess LJ et al [12], in retrospective study has evaluated 246 cases of exudative pleural effusion including 143 tubercular pleural effusion. They have found that, ADA with cut-off value of  $\geq 50$  for diagnosis of tubercular pleural effusion had a sensitivity of 91%, specificity of 81%, positive prediction value of 84% and negative prediction value of 89%.

The results of our study had sensitivity similar to above mentioned studies. This is partly because of the high prevalence of tubercular pleural effusion in this region, which increases the positive predictive value and efficiency of ADA concentration as a diagnostic marker. We made an attempt to evaluate and identify any correlation between pleural fluid ADA with sputum status, chest X-ray finding and pleural biopsy finding in tubercular pleural effusion patients.

In our study, pleural biopsy was done for 16 patients of tubercular pleural effusion. , on histopathological examination 11 were reported as tuberculosis and remaining 5 were inconclusive.

In our study, among 11 patients with pleural biopsy suggestive of tuberculosis, Pleural fluid ADA mean  $\pm$  Standard deviation was  $81.88 \pm 13.13$  and among 5 patients with pleural biopsy not suggestive of tuberculosis Pleural fluid ADA mean  $\pm$  Standard deviation was  $88.1 \pm 13.47$ . Pleural fluid ADA value of both group was comparable ( $p > 0.05$ ) (TABLE 7).

### Limitations

1. Number of patients studied is small. Small sample size may impede for detecting significant effects because of lack of power.

### Conclusions

1. ADA is a highly sensitive diagnostic marker of tubercular pleural effusion.

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