

## A Study of Sensitivity and the Predictive value of Adenosine Deaminase enzyme for Diagnosis of Tuberculosis

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### Abstract :

**Introduction:** Pleural effusion can be caused by large varieties of etiologies; therefore it often presents with diagnostic dilemma. Tuberculosis is the most important cause of exudative pleural effusion in our country. It is often difficult to diagnose the tuberculous pleural effusion based on clinical, radiological, bacteriological, histological and laboratory findings. The estimation of Adenosine Deaminase (ADA) activity, which is inexpensive and easily available, can play a vital role for the diagnosis of tuberculosis as aetiology in the cases of pleural effusion.

**Materials & Methods :** A prospective study was carried out on the 50 patients of pleural effusion. The pleural fluid ADA level was done to check its sensitivity and the specificity for the diagnosis of tuberculous and non-tuberculous pleural effusion. **Observations :** Estimation of ADA level in pleural fluid for diagnosis of tuberculosis was interpreted with two arbitrary cut-off levels (35 U/L & 40 U/L). The sensitivity of ADA estimation in our study was 92.50% and 80% respectively for these two different cut-off levels, whereas the specificity was 80% and 100% respectively. **Conclusion :** ADA estimation in pleural fluid is a simple, rapid and inexpensive laboratory test. Though it cannot be used as a standard test for diagnosis of tuberculous pleural effusion, it can play a vital role as a corroborative investigation in diagnosis of tuberculosis.

**Key words:** Adenosine Deaminase (ADA) enzyme, Tuberculosis

### Abstract :

**Introduction:** Tuberculosis (TB) is a potentially fatal contagious disease caused by infection with Mycobacterium tuberculosis, which can affect almost any tissue or organ of the body, the most common site of the disease being the lungs. Tuberculosis remained a major cause of morbidity and mortality throughout the world; it has been reported as one of the most important public health problems of almost all regions by the World Health Organization (WHO). Approximately 3.8 million new cases of tuberculosis (all forms, pulmonary and extra-pulmonary) were reported annually by the WHO in the early 1990s; 90% of them from the developing countries. It is estimated that 8.8 million cases of tuberculosis occurred worldwide in 1995. <sup>(1)</sup> It is estimated that every year 50-100 million people are infected by TB bacilli and out of this, 10-20 million people develop overt disease and 3 million of them die. In other words, the bacilli cause more than 5,000 deaths everyday or about 1 death every 15 seconds. <sup>(2)</sup>

In India, tuberculosis is responsible for about 5,00,000 deaths every year. The annual rate of infection in India is about 1.6%. It is estimated that 1.5% of the Indian population above the age of 5 years is suffering from active pulmonary tuberculosis and one fourth of these i.e. about 0.4% population are sputum positive or infectious. <sup>(3)</sup>

Pleural effusion is an abnormal collection of fluid in pleural space. The large varieties of etiologies can cause pleural effusion; so, it often presents a diagnostic problem, even after extensive investigations. Tuberculosis is still the most important cause of exudative pleural effusion in our country. Its detection and differentiation from other causes constitutes a major problem. The diagnosis of tuberculous pleural effusion based on clinical, radiological, bacteriological, histological and many other laboratory findings have relatively low sensitivity and specificity.

Several new techniques have been introduced recently for the diagnosis of tuberculosis such as rapid & automated broth culture (BACTEC), gas chromatography and polymerase chain reaction (PCR); these techniques are effective but they are very costly and time consuming. These techniques also require sophisticated equipments and well-trained personnel. <sup>(4,5,6)</sup> In such scenario, a simple, rapid, reliable,

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inexpensive yet sensitive test is needed. Adenosine deaminase (ADA) estimation is one such test that might fulfill all the above requirements.

Adenosine deaminase is an enzyme, which is involved in catabolism of purine base. Its main physiological role is related to lymphocytic proliferation and differentiation. Adenosine deaminase activity is higher in T-cells with variation according to cellular differentiation. As a marker of cellular immunity, its activity is found to be elevated in those diseases in which there is a cell mediated immune response.<sup>(7)</sup> Recently, many researchers have emphasized the importance of the estimation of ADA activity in pleural fluid and serum as a diagnostic marker of tuberculous effusion.

The objective of the present study was to evaluate usefulness of estimation of ADA activity for the aetiological diagnosis of pleural effusion.

#### **Materials & Methods:**

The study was carried out at the department of TB and chest at tertiary health care center of Rajkot city of Gujarat, India over a period of 8 months.

The patients presented with pleural effusion, were educated about the study and written consents of the patients for participation in the study were obtained. All the patients were examined clinically and fully investigated. Routine haematological examination including haemoglobin estimation, total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate were done along with other investigations like X-ray of chest, serum proteins, blood urea, serum creatinine & fasting blood sugar. Sputum examination was done for detection of acid fast bacilli, pyogenic bacteria and for malignant cells.

Pleural fluid examination was carried out in all the patients to find its possible aetiology. Patients of tuberculous pleural effusion were diagnosed by clinical & radiological examination with supportive evidence of pleural fluid examination, sputum examination for acid fast bacilli, tuberculin test, histopathological examination for detection of tuberculous granuloma.

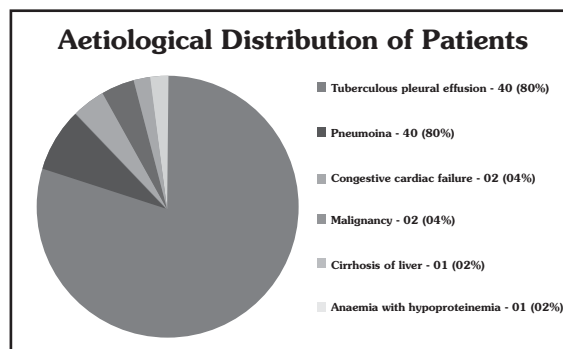
Patients of malignant pleural effusion were confirmed by sputum for malignant cells, pleural fluid cytology and fine needle aspiration cytology or biopsy examination. Patients of congestive cardiac failure were diagnosed by ECG, pleural fluid examination and echocardiography. Patients of synpneumonic pleural effusion were

confirmed by total WBC count, pleural fluid examination and Gram staining of pleural fluid and sputum examination as well as from the response to antibiotic treatment. Cirrhosis of liver was diagnosed by clinical and biochemical investigation, USG of abdomen, and ascitic fluid examination.

On the basis of above diagnostic criteria, 40 cases of tuberculous pleural effusion were selected by simple random sampling method (Group - A); & 10 cases of non-tuberculous pleural effusion were selected by simple random sampling method (Group - B). From each patient, from the tapped pleural fluid, adenosine deaminase enzyme (ADA) activity was measured by the spectrophotometric method.

#### **Observation:**

**Graph 1 : Distribution of patients according to aetiology**



In the present study, 40 patients were having tuberculous pleural effusion. Out of the 10 cases of non-tuberculous pleural effusion, 4 cases had pneumonia, 2 cases had congestive cardiac failure, 2 cases had malignancy, 1 case had cirrhosis of liver and 1 case had anaemia with hypoproteinemia. The maximum incidence of pleural effusion was found in the age group 31-40 years (32%) followed by the patients of the 41-50 years age group (22%). The male to female ratio was about 1.94:1.

In present study, 6% patients had a past history of tuberculosis and 10% patients had a family history of tuberculosis. They all had completed their treatment. 24 patients (60%) out of the 40 patients of tuberculous pleural effusion were smokers. The chest X ray of only 27.5% (11 out of the 40) patients of tuberculous pleural effusion showed evidence of pulmonary tuberculosis. While 47.5% (19 out of the 40) patients of tuberculous pleural effusion had positive tuberculin test (indurations more than or equal to 10 mm).

On pleural fluid examination, 76% (38 out of 50) patients had turbid pleural fluid, out of which 94% (36 out of 38) having evidence of tuberculous aetiology (vide supra). 12% (6 out of 50) patients had haemorrhagic pleural fluid, out of which 34% (2 out of 6) patients had evidence of malignancy. In both the selected patients of neoplastic aetiology, haemorrhagic pleural fluid was found; whereas all cases of transduative pleural effusion were having clear pleural fluid.

**Adenosine Deaminase Activity :**

**Table 1 : Serum Adenosine Deaminase (ADA) level in pleural fluid**

Nature of pleural effusion	No. of patients	Serum ADA U/L	
		Range	Mean ± S.D.
Tuberculous	40	29.0-81.0	50.25 ± 10.50
Malignant	02	17.0-36.0	26.50 ± 9.30
Synpneumonic	02	16.0-36.0	26.00 ± 9.30
Transduative	06	11.0-32.0	19.30 ± 5.76

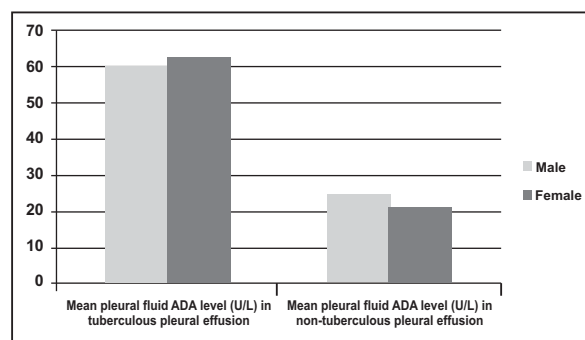
Table 1 shows that, the mean serum ADA levels were found to be remarkably high in tuberculous pleural effusion (mean 50.25 ± 10.50 SD) as compared to other types of pleural effusion.

**Table 2 : Statistical comparison of pleural fluid ADA levels in tuberculous and non-tuberculous pleural effusion**

Type of pleural effusion	No. of patients	Pleural fluid ADA (Mean ± S.D.)	SE(d)	't'	P
Tuberculous	40	50.25 ± 10.50	3.09	8.45	<0.001
Non-tuberculous	10	24.13 ± 8.25			

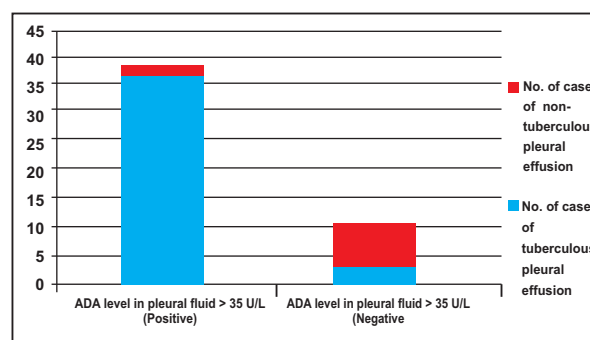
As shown in table 2, the Standard error of the difference [SE (d)] is 3.09; whereas the 't' value and the P value are 8.45 and <0.001 respectively. It clearly suggests that the observed difference of mean pleural fluid ADA levels between the tuberculous and non-tuberculous group is not by a mere chance but statistically it is highly significant.

**Graph 2: Sex distribution of mean pleural fluid ADA levels**



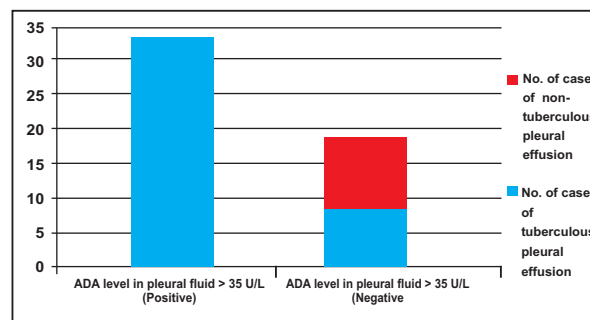
A minor difference was observed in the mean pleural fluid ADA levels between the male and the female patients of similar nature of pleural effusion, which is statistically insignificant.

**Graph 3: Nature of case distribution at cut-off ADA level of 35 U/L in pleural fluid**



Considering 35 U/L of ADA in pleural fluid (graph 3) as an arbitrary cut-off level, the test was positive for 37 patients of tuberculous pleural effusion and 2 patients of non-tuberculous pleural effusion whereas the test was negative for 3 patients of tuberculous pleural effusion and 8 patients of non-tuberculous pleural effusion. Thus, the specificity of the test comes out to be 80%; whereas the sensitivity of the test is 92.5%.

**Graph 4: Nature of case distribution at cut-off ADA level of 40 U/L in pleural fluid**



Considering 40 U/L of ADA in pleural fluid (graph 4) as an arbitrary cut-off level, the test was positive for 32 patients of tuberculous pleural effusion and no patients of non-tuberculous pleural effusion whereas the test was negative for 8 patients of tuberculous pleural effusion and all 10 patients of non-tuberculous pleural effusion. Thus, the specificity of the test becomes 100%, however the sensitivity of the test decreases to 80%.

Considering 40 U/L of ADA in pleural fluid (graph 4) as an arbitrary cut-off level, the test was positive for 32 patients of tuberculous pleural effusion and no patients of non-tuberculous pleural effusion whereas the test was negative for 8 patients of tuberculous pleural effusion and all 10 patients of non-tuberculous pleural effusion. Thus, the specificity of the test becomes 100%, however the sensitivity of the test decreases to 80%.

**Discussion :**

In the present study, the maximum cases of tuberculous pleural effusion were of the age group 31-40 years; Nagraja et al.<sup>(8)</sup> had also found the maximum incidence of tuberculous pleural effusion in the same age group, whereas Gediya<sup>(9)</sup> had found the maximum incidence of tuberculous pleural effusion in the age group of 21-30 years. In the case of tuberculous pleural effusion, the M:F ratio is 2.08:1 in our study, which is comparable to the M:F ratio of 1.73:1, found in the study done by Nagraja et al.<sup>(8)</sup> The reason for this male preponderance can be explained by the higher incidence of smoking in male patients as compared to female patients.

In the present study, 28% cases of tuberculous pleural effusion showed evidence of tuberculosis in the X-ray of

chest, which is comparable to 31% in the study done by Gediya.<sup>(9)</sup> Thus, X-ray of chest alone is a poor mode of investigation for the aetiological diagnosis of pleural effusion. In the present study, 48% cases of tuberculous pleural effusion had positive tuberculin test, which is comparable to 53% in the study done by Nagraja et al.<sup>(8)</sup> Thus; the tuberculin test has low sensitivity for the aetiological diagnosis of pleural effusion. In the present study, 90% cases of tuberculous pleural effusion had turbid pleural fluid, which is comparable to 93.75% in the study done by Gediya.<sup>(9)</sup>

In the present study, the mean ADA level in tuberculous pleural effusion was 50.25 U/L; which is comparable to 64.67 U/L in the study done by Rajendra Prasad et al.,<sup>(10)</sup> 58.60 U/L in the study done by Y. Aoki et al.,<sup>(11)</sup> and 54.70 U/L in the study done by E. Perez-Rodriguez et al.<sup>(12)</sup> All these studies show high level of ADA in pleural fluid in tuberculous patients. In the present study, the mean ADA level in non-tuberculous pleural effusion was 24.13 U/L; whereas the mean ADA level in non-tuberculous pleural effusion was 6.99 U/L in the study done by Rajendra Prasad et al.,<sup>(10)</sup> 25.90 U/L in the study done by Y. Aoki et al.,<sup>(11)</sup> and 18.30 U/L in the study done by E. Perez-Rodriguez et al.<sup>(12)</sup> All these studies show low level of ADA in pleural fluid in non-tuberculous patients.

Considering 35 U/L of ADA in pleural fluid as an arbitrary cut-off level, the specificity of the test comes out to be 80% and the sensitivity of the test is 92.5%. Whereas considering 40 U/L of ADA in pleural fluid as an arbitrary cut-off level, the specificity of the test is 100% and the sensitivity of the test is to 80%. These percentages of the test specificity and the test sensitivity correlate well with other studies as shows in table 3.

**Table 3 : The Specificity and the Sensitivity of ADA Level Test in Various Studies**

<b>Details of the study</b>	<b>Cut-off Value of ADA level in pleural fluid (U/L)</b>	<b>Specificity</b>	<b>Sensitivity</b>
Rajendraprasad et al. <sup>(10)</sup>	30	100 %	100 %
M. V. Nagraja et al. <sup>(8)</sup>	33	90 %	80 %
Y. Aoki et al. <sup>(11)</sup>	45	75 %	100 %
E. Perez-Rodriguez et al. <sup>(12)</sup>	40	92.5 %	88.8 %

**Conclusion:**

From the present study, we can conclude that, the X-ray of chest and tuberculin test has low sensitivity for the aetiological diagnosis of tuberculous pleural effusion. The mean pleural fluid ADA levels in tuberculous pleural effusion is significantly higher than that in non-tuberculous pleural effusion. There is no substantial difference in mean pleural fluid ADA levels among malignant pleural effusion, synpneumonic pleural effusion and transudative pleural effusion. The ADA level test for differential diagnosis of tuberculous and non-tuberculous pleural effusion has high specificity and sensitivity. Considering cut-off level of 35 U/L, the specificity of the test comes out to be 80% and the sensitivity of the test is 92.5%. Whereas considering 40 U/L of ADA in pleural fluid as an cut-off level, the specificity of the test is 100% and the sensitivity of the test is to 80%. Though it cannot be used for general screening or as a foolproof standard test for diagnosis of tuberculous pleural effusion, it can play a vital role as a corroborative investigation in diagnosis of tuberculosis.

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