**ORIGINAL ARTICLE**

# Adenosine Deaminase as a Diagnostic Aid to Ascertain Tuberculous Etiology of Pleural Effusions

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## ABSTRACT

**Aim:** To determine the sensitivity and specificity of pleural fluid ADA for the diagnosis of tuberculous pleural effusion taking the cut off value of ADA as ≥ 40 mg/dl.

**Methods:** It was a prospective, analytical study comprised of 220 patients having pleural effusion. Sputum smear examination and sputum culture for mycobacterium tuberculosis, pleural fluid examination for protein, glucose, cell count, malignant cells, Gram's stain, AFB, pleural fluid culture for mycobacterium tuberculosis and other relevant investigations were performed. ADA was measured in pleural fluid by colorimetric method of Guisti and Galanti.

**Results:** Tuberculosis was the final clinical diagnosis in 164 (74.5%) patients having pleural effusion out of which 161 (98.2%) patients had ADA level ≥ 40 IU/L and only 3 (1.8%) had ADA level < 40 IU/L. None of the patients of pleural effusion with other than tuberculous etiology except 3 patients of para-pneumonic effusions had ADA level ≥ 40 IU/L. The cut off value of 40 IU/L for pleural fluid ADA level was found to have 98.17% sensitivity and 94.64% specificity.

**Conclusion:** Pleural fluid adenosine deaminase level >40% is a sensitive and specific marker of tubercular etiology in cases of pleural effusion.

**Keyword:** Tuberculosis, pleural effusion, adenosine deaminase

## INTRODUCTION

Pulmonary tuberculosis is the most common etiology of pleural effusion in countries like Pakistan, where it accounts for over 50% of total cases of pleural effusions[1,2,3]. Diagnosis of pulmonary tuberculosis is confirmed mainly by sputum examination of acid fast bacilli (AFB), while the diagnosis of tuberculous pleural effusion requires investigations of pleural fluid biochemistry, cytology and even the pleural biopsy. The yield of AFB in pleural fluid and that of the pleural biopsy are very low whereas the culture of Mycobacterium is too much time consuming and again is negative in almost >80% cases. Enzyme-linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) are other sophisticated modalities for the confirmation of TB which are neither widely available in the country nor pocket-friendly for the most of the patients. Definitive diagnosis of tuberculous pleural effusion is often challenging in as many as half of the patients, pleura is the only site of infection[5]. Tuberculin test is non-specific and finding can be negative[6]. Thus, a treating physician needs a simple, rapid and reliable diagnostic test to establish the aetiology of pleural effusion.

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Pleural fluid adenosine deaminase (ADA) has been shown to be a useful biochemical marker of tubeculous pleural effusion and provides a reliable basis for a treatment decision, particularly in areas where the disease is as common as in Pakistan. Rahim et al. favor the application of ADA in pleural fluid as the diagnostic tool for tuberculous pleural effusion without performing an invasive procedure like the pleural biopsy[7]. However, high levels of ADA can also be found in patients with neutrophilic effusions such as parapneumonic effusions or empyemas[8]. Moreover, various authors have suggested different cutoff levels for the differentiation of tuberculous and non-tuberculous pleural effusions.

This prospective study was carried out to determine the usefulness of pleural fluid ADA in establishing the diagnosis of tuberculous pleural effusion taking the cut off value as ≥40 mg/dl favouring tuberculous etiology.

## MATERIALS AND METHODS

It was a prospective, analytical study comprised of 220 consecutive patients of pleural effusion, both male and female, above the age of 12 years who attended the Medical Units of various teaching hospitals including Services and Jinnah Hospital Lahore, Nishtar Hospital Multan and some private clinics from January 2011 and August 2013. Patients having history or evidence of past typhoid fever, acute viral hepatitis and active cirrhosis were

excluded. Detailed history was taken and thorough clinical examination was done in every patient and they were then subjected to a battery of investigations which included complete blood counts, urinalysis, chest x-ray PA and lateral view, sputum smear examination for AFB and sputum culture for mycobacterium tuberculosis, pleural fluid examination for protein, glucose, cell count, malignant cells, Gram's stain, AFB, pleural fluid culture for mycobacterium tuberculosis and other relevant investigation as per need of cases. ADA was measured in pleural fluid by colorimetric method of Guisti and Galanti[9].

Data was collected with the help of a pre-designed questionnaire. Informed consent was taken from all patients. The demographic and clinical information about every patient was entered in respective column of the questionnaire. The data was entered to and analysed with SPSS version 19.0. Pearson Chi-square and Fisher exact tests were used to calculate *p* value as the cut off value of ADA level in pleural fluid was used as the variable of interest. Any *p* value less than 0.05 was considered statistically significant.

## RESULTS

Mean±SD age of the patients was 28±9.34 (Range= 12-54) years. Males 133(60.45%) constituted majority of the patients with pleural effusion as compared to the females 87(39.55%) making up a male to female ratio 3:2. Larger proportion of the patients 153(69.54%) were rural dwellers as compared to lesser number of patients from urban population, 67(30.46%). Majority of the patients (65.45%) were belonging to poor socioeconomic backgrounds having median±IQR number of family members of 7±4 and most of them 156(70.91%) had single bedroom house.

Tuberculosis was the final clinical diagnosis in 164(74.5%) patients having pleural effusion followed by para-pneumonic effusions 25(11.4%) and malignancy i.e., lung cancer or disseminated malignancy 18(8.2%). Uncommon etiologies included liver cirrhosis 3(1.4%) and others as shown in Table 1.

Out of 164 patients of pleural effusion with tuberculous eitiology, 161(98.2%) patients had Adenosine deaminase level ≥ 40 IU/L and only 3(1.8%) had ADA level < 40 IU/L. None of the patients of pleural effusion with other etiologies except 3 patients of para-pneumonic effusions had ADA level ≥ 40 IU/L as shown in Table 2**.** Thus, the cut off value of 40 IU/L for pleural fluid ADA level was found to have a high sensitivity of 98.17 % (95% Confidence Interval = 94.74%–99.60%) and a fairly good specificity of 94.64% (95% CI=85.11%–98.82%) as shown in Table 3.

Table 1: Etiology of pleural effusion in study population (n=220)

| Etiology of Pleural effusion | Frequency | %age |
|---|---|---|
| Tuberculosis | 164 | 74.5 |
| Pneumonia | 25 | 11.4 |
| Malignancy | 18 | 8.2 |
| Congestive cardiac failure | 02 | 0.9 |
| Liver cirrhosis | 03 | 1.4 |
| Chronic kidney disease | 01 | 0.5 |
| Pericardial effusion | 02 | 0.9 |
| Constrictive pericarditis | 01 | 0.5 |
| Hypoalbuminemia | 01 | 0.5 |
| Rheumatoid Arthitis | 01 | 0.5 |
| Subphrenic abscess | 02 | 0.9 |

Table 2: Adenosine deaminase cut off value 40 IU/L

| Diagnosis | Pleural fluid Adenosine Deaminase level | | Total |
|---|---|---|---|
| | < 40 IU/L | ≥ 40 IU/L | |
| Tuberculosis | 3 | 161 | 164 |
| Pneumonia | 21 | 4 | 25 |
| Malignancy | 18 | 0 | 18 |
| Congestive cardiac failure | 2 | 0 | 2 |
| Liver cirrhosis | 3 | 0 | 3 |
| Chronic kidney disease | 1 | 0 | 1 |
| Pericardial effusion | 2 | 0 | 2 |
| Constrictive pericarditis | 1 | 0 | 1 |
| Hypoalbuminemia | 1 | 0 | 1 |
| Rheumatoid Arthitis | 1 | 0 | 1 |
| Subphrenic abscess | 2 | 0 | 2 |

Table 3: Confidence interval

| Indicator | Value | 95% Confidence Interval |
|---|---|---|
| Sensitivity | 97.58% | 93.90% to 99.32% |
| Specificity | 94.55% | 84.86% to 98.80% |
| Positive predictive value | 98.17% | 94.74% to 99.60% |
| Negative predictive value | 92.86% | 82.69 to 97.98% |

## DISCUSSION

While enlisting the causes of pleural effusion, it is common practice to classify these causes according to whether the effusion is transudative or exudative – based on the protein or lactate dehydrogenase (LDH) content of the fluid. This classification requires chemical examination of pleural fluid which is routinely performed in all cases. The frequency of various causes of transudative effusion e.g. left ventricular failure, cirrhotic liver disease,

hypoalbuminemia etc and those of the exudative effusion e.g. malignancy, parapneumonic effusions and tuberculosis greatly vary in different countries and regions of the world. In South East Asia including Pakistan, the most common cause of pleural effusion is tuberculosis which contributes more than half of the cases[2,3].

The problem with diagnosing tuberculous effusion lies in the fact that various laboratory techniques like demonstration of acid fast bacilli or culture which are possible means to confirm the diagnosis of tuberculosis in patients having pleural effusion are usually difficult and in most cases negative[10]. Modern techniques such as those involving the amplification of bacterial DNA by polymerase chain reaction and comparable systems, are not available for pervasive use in the developing countries like Pakistan. Routine laboratory findings may not be helpful to differentiate tuberculous etiology of pleural effusion from other causes.

Adenosine deaminase (ADA) is an enzyme in the purine salvage pathway that catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine with the release of ammonia. It plays important role in differentiating lymphoid cells. Raised ADA concentrations are found in active T lymphocytes and these concentrations are inversely proportional to the degree of differentiation[11]. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes. Likewise, a deficiency of adenosine deaminase is associated with severe defects in the cell-mediated and humoral arms of the immune system, predisposing the patient to opportunistic infections. With this background, ADA has been considered as a marker of T-cell activity and cell mediated immune response. That's why raised level of ADA can help us in differentiating between the diseases against which cellular limb of immune response is the predominant mechanism of immune reaction from those diseases in which humoral response (antibody-mediated immune response) plays vital role. As, cell-mediated response is the predominant form of immune reaction in tuberculous infection, while both cell-mediated and humoral responses are elicited by most non-tuberculous infections in human body, ADA activity can fairly differentiate tuberculous etiology from the rest of the causes of pleural effusion.

Piras et al. were first to report high ADA in tubercular pleural effusion [12]. Subsequently several workers explored its efficacy in the diagnosis of tuberculosis[13] and determined that pleural fluid ADA level less than 40 U/L virtually excludes the diagnosis of tuberculosis[14]. In other terms, an ADA level equal to or more than 40 U/L means the etiology of pleural effusion is nothing but tuberculosis. Meta-analysis of a large number of studies conducted between 1966 and 1999 concluded that the test performance was reasonably good[15] in establishing tuberculous etiology in patients with pleural effusion.

In current study, it was found that cause of pleural effusion was tuberculosis in 74.5% cases. Pneumonia (11.4%) and disseminated malignancy (8.2%) were second and third commonest causes of pleural effusion whereas in only a few cases the effusion developed due to other etiologies as shown in Table 1. A recent study by Rehan et al. (2013) from Pakistan found that three commonest causes of pleural effusion were tuberculosis, pneumonia and malignancy which contributed 53.33%, 17.33% and 12% cases of exudative effusions respectively[16].

Authors have found pleural fluid ADA as a sensitive and specific diagnostic adjunct to ascertain tuberculous etiology in cases of pleural effusion, a remark which is in agreement with the literature cited above. Current study has not taken into account the affordability and availability of this test. A more detailed effort to cross-validate the sensitivity and specificity of this test alongwith its socioeconomic and logistic implications will be valuable input in the long fight of an old yet mammoth human slayer.

## CONCLUSION

Pleural fluid adenosine deaminase level >40% is a sensitive and specific marker of tubercular etiology in cases of pleural effusion.

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