

DIAGNOSTIC SIGNIFICANCE OF PLEURAL FLUID ADENOSINE DEAMINASE ACTIVITY IN TUBERCULOUS PLEURISY

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Abstract

Diagnosis of tuberculous pleural effusion (TPE) is difficult because of its non-specific clinical presentation and insufficient efficiency of conventional diagnostic methods. The study was carried out to evaluate the utility of adenosine deaminase (ADA) activity in pleural fluid for the diagnosis of TPE. ADA activity was measured in pleural fluid of 103 pleural effusion patients by colorimetric method using a commercial ADA assay kit. The diagnosis of TPE was made from pleural fluid examinations (including cytology, biochemistry, and bacteriology) and pleural biopsy. Patient with negative result of this methods were diagnosed by response of empirical treatment. Out of 130 cases, 62 (61.1%) had TPE and the remaining 41 (39.8%) had pleural effusion due to non tuberculous diseases. There was statistically significant difference ( $p < 0.001$ ) between the mean of pleural fluid ADA levels ( $70.82 \pm 22.54$  U/L) in TPE group and ( $30.07 \pm 22.93$  U/L) in non-TPE group. Of 62 TPE cases, microscopy for AFB and culture for *M. tuberculosis* in pleural fluid revealed positivity in 9.6% and 22.5% cases respectively, and biopsy of pleura showed typical epithelioid granuloma in only 43.5% cases. The cut-off value of ADA for diagnosing TPE was 40 U/L using a ROC curve, with a sensitivity of 94% and specificity of 88%. Positive and negative predictive value of ADA assay were 92% and 90% respectively. The overall test accuracy was 90%. Pleural fluid ADA assay is therefore a simple, rapid, highly sensitive and specific adjunct test for diagnosis of TPE.

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**Keywords:** Pleural fluid, adenosine deaminase, tuberculous pleural effusion

Introduction

Bangladesh ranks sixth in the world of tuberculosis (TB) disease burden with estimated 300,000 new cases and 70,000 death per year.<sup>1</sup> Pleural tuberculosis is a common manifestation of extrapulmonary TB and with or without pulmonary TB, is present in around 4% of all TB cases.<sup>2</sup> If undetected, it may resolve spontaneously, but untreated pleural TB is a progressive disease with high recurrence rate. Diagnosis of pleural TB is difficult because of non-specific clinical presentation and insufficient efficiency of traditional diagnostic methods due to paucity of bacteria in pleural cavity.<sup>3,4</sup> Pleural biopsy has been the gold standard in diagnosis but is invasive and often requires several attempts to locate the infectious loci.<sup>4</sup>

Pleural TB occurs as a result of a TB antigen entering the pleural space, usually through the rupture of a subpleural focus, followed by a local, delayed hypersensitivity reaction mediated by CD<sub>4</sub><sup>+</sup> T lymphocytes and macrophages. The activated macrophage enter the pleural cavity, producing adenosine deaminase (ADA) during its proliferation process.<sup>5</sup> ADA is an enzyme essential in the purine salvage pathway of DNA metabolism. The rise in ADA in tuberculous effusions has been studied elsewhere and it is higher than in other exudative pleural fluid.<sup>6,7</sup>

Present study tried to find out the significance of Adenosine deaminase activity in pleural fluid for the diagnosis of tuberculous pleural effusion.

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## Materials and Methods

### Patients

This cross sectional type of comparative study conducted in 103 pleural effusion patients admitted to in-patient departments of Bangabandhu Shiekh Mujib Medical University (BSMMU) and National Institute of Disease of Chest and Hospital (NIDCH) during the period of January 2008 to December 2008. All patients underwent thoracentesis and parietal pleural biopsy with Abram's needle by trained physicians. Patients with empyema thoracis, haemothorax and patients on anti-tuberculous treatment were excluded from the study.

### Laboratory methods

Aspirated fluid of all patients was subjected to cytological, biochemical and microbiological examination and ADA activity testing. Fifty milliliter of collected pleural fluid was centrifuged for 15 minutes at 3000 rpm and the supernatant was stored at 2-8 °C until ADA for a maximum of 24 hours. Haemolysed samples would give spuriously high ADA level and such specimens were excluded. ADA activity was determined by colorimetric method of Guisti and Galanti<sup>8</sup> using adenosine deaminase assay kit (Diazyme laboratories, USA). ADA levels were calculated and expressed in unit per liter (U/L). Centrifuged deposit was used for detecting AFB and bacteria by Ziehl-Neelsen and Gram's stains. Deposit was cultured in Lowenstein-Jensen and routine laboratory media for *Mycobacterium* and other bacteria respectively. Samples of sputum from patients who had productive cough were also subjected to microscopy and culture for AFB. A separate sample of fluid was collected for biochemical analysis (protein, sugar and LDH), total and differential cell count and cytological examination for malignant cells.

Effusions were classified as transudates or exudates using Light's criteria<sup>9</sup> and this required a blood sample to be collected on the day of thoracentesis in order to measure total protein and LDH. Three pieces of pleural tissue were taken for histology.

### Diagnostic criteria

The diagnosis of tuberculous pleural effusion was made if any of the following was satisfied: (a) presence of AFB in pleural fluid by microscopy or culture; (b) presence of granuloma in pleural biopsy tissue; (c)

AFB positive sputum with no alternate explanation for exudative pleural effusion; and (d) clinical features were compatible with TB of exudative effusion and there was clear response to anti-TB drugs.<sup>10,11</sup>

Malignant pleural effusion was diagnosed when malignant tissue was shown by pleural tissue or cytopathology. Some of the malignant cases were diagnosed by fiber optic bronchoscopy. Effusion was considered parapneumonic when there was an acute febrile illness associated with pneumonia and complete response to antibiotic treatment. Rest of the non-tuberculosis patients (nephrotic syndrome, congestive cardiac failure and rheumatoid arthritis) were diagnosed by standard clinical procedure.

### Statistical analysis

Data were compiled and analyzed by using SPSS version 15.0. Cut-off value of ADA level for the diagnosis of tuberculous pleural effusion was established by using receiver operating characteristic (ROC) in the same version of SPSS. Comparison of two groups was done by *chi-square test*.

## Results

Out of 103 pleural effusion cases, 62 (60.1%) were diagnosed as tuberculous pleural effusion (TPE) and 41 (39.1%) were non-TPE cases. The non-TPE cases group included 30 (29.1%) cases with malignant pleural effusion, 8(7.7%) patients with parapneumonic effusion and 3 cases of effusion due to nephrotic syndrome, cardiac failure and rheumatoid arthritis each. Among the 62 of TPE, 49 (79%) were male and 13 (21%) were female with a male female ratio 3.8:1. The mean age of all tuberculous cases was  $35.85 \pm 14.59$  years.

Through analysis of a ROC curve, the optimal cutoff value was determined to be 40 U/L (Figure-1). The area under the curve was 0.928 and standard error was 0.032(95% CI: 0.865-0.990). Based on this cutoff value ADA sensitivity and specificity were 94% and 88% respectively. Positive predictive value was 92% and negative predictive value was 90%. The overall test accuracy was 90%.

The ADA levels obtained in pleural fluids of the study groups were shown in Figure-2. The horizontal line shows the cutoff level. Mean ADA in TPE group was  $70.82 \pm 22.54$  U/L versus  $30.07 \pm 22.93$  U/L in non-TPE group ( $P < 0.001$ ). Five of 41 non-TPE cases had

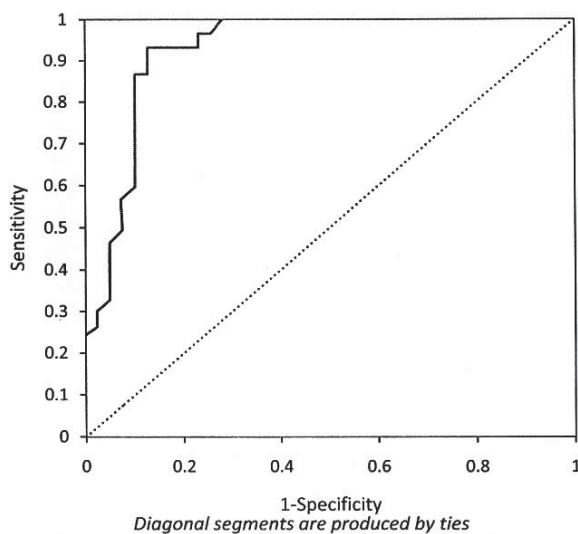
**Table-1:** Correlation of ADA activity with bacteriological examination in pleural fluid and histological evaluation of pleural biopsies among TPE cases (N=62).

ADA in pleural fluid	Peural fluid positive for		Pleural biopsy granuloma positive(n)
	AFB by microscopy n	<i>M.tuberculosis</i> by culture n	
ADA Positive n = 58	6	12	27
ADA Negative n=4	0	2	0
Total N=62	6(9.62%)	14(22.50%)	27(43.54%)

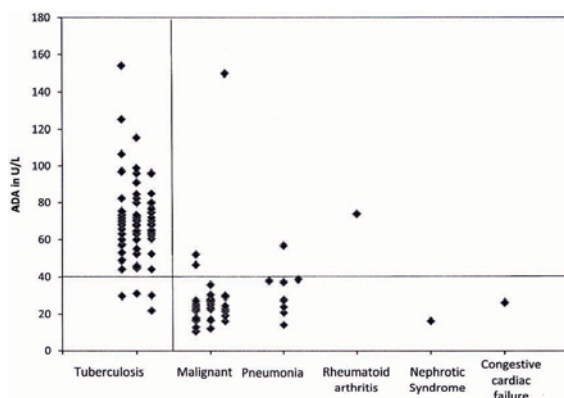
ADA levels above diagnostic cutoff and ADA level of 4 TPE cases remained less than 40 U/L. Among 4 ADA negative TPE cases, 2 were culture positive for *M.tuberculosis* and 2 cases were diagnosed by response to anti TB treatment. Pleural biopsy for granuloma, pleural fluid microscopy for AFB, and culture for *M.tuberculosis* revealed positivity in 43.54%, 9.62% & 22.5% of TPE cases respectively (Table-I).

## Discussion

The diagnosis of TPE continues to be a challenge in clinical practice. In the present study, the commonest cause of pleural effusion were found to be tuberculosis (60.2%) followed by malignancy (29.1%) and pneumonia (7.7%). These findings were similar to those



**Fig-1.** ROC plot of pleural fluid ADA activity. Dotted diagonal line indicates the line of no discrimination



**Fig-2.** Adenosine deaminase levels in pleural fluids of the studied groups

observed in other studies.<sup>12,13</sup> Only a few cases of pleural effusion from congestive cardiac failure, rheumatoid arthritis and nephrotic syndrome were noted in the study. The mean age group was  $35.85 \pm 14.59$  for TPE patients which is similar to that in previous studies.<sup>14,15</sup> Seventy nine percent of TPE cases were male and 21% were females with a male female ratio of 3.8:1. Similar male predominance was also observed in other studies.<sup>16,17</sup>

TPE represent an immunological reaction to relatively few AFB in pleural space. Hence direct examination of pleural fluid by Ziehl-Neelsen (Z-N) staining has low sensitivity (0-10%).<sup>14,18,19</sup> In the present study, Z-N staining of pleural fluid revealed positivity in 9.6% cases and pleural fluid culture yielded *M.tuberculosis* in 22.5% cases. Culture requires a minimum of 10-100 viable bacilli and, therefore, is more sensitive than Z-N staining.<sup>20</sup> Majority of series showed diagnostic yields of <30%.<sup>18,21,22</sup> Presence of granulomatous inflammation is frequently used as a diagnostic criteria for TPE,<sup>23,18</sup> and biopsy of parietal pleura showed typical epithelioid granuloma in 50% to 84% of TPE cases.<sup>23,24,25</sup> In this study, pleural biopsy showed diagnostic granuloma in only 43.5% of TPE cases. The low positivity might be due to non repetition of pleural biopsy in this series. Moreover, biopsy requires greater expertise and subject to sampling error to locate the infection foci.<sup>4</sup>

Many studies have confirmed the utility of ADA for diagnosis of tuberculous pleural effusion.<sup>12,13,26</sup> With a most suitable cut-off of level of 40 U/L in pleural fluid estimated from ADA ROC analysis, this study found high diagnostic sensitivity (94%) and specificity

(88%). Chen *et al.*<sup>27</sup> reported the sensitivity and specificity for ADA as 79-100% and 80.5-96% respectively using 30-50 U/L cut-off level while summarizing the results of eight previous studies. This study revealed high levels of ADA activity in pleural fluids of tuberculous patients as compared to non tuberculous pleural effusion cases ( $p < 0.001$ ). Five patients of non-TPE group had ADA more than 40 U/L including 3 with malignancy, one with pneumonia and one rheumatoid arthritis patient. An ADA value of 150 U/L was detected in patient with lymphoma. This increased activity is probably due to malignant proliferation of undifferentiated T lymphocyte. It is thought that false positive diagnosis of TPE by ADA can be significantly reduced if ADA measurement is limited to lymphocytic pleural fluid because in most studies false positive findings were reported from parapneumonic effusion and empyema, which were usually neutrophil predominant.<sup>28</sup> Of the 62 patients with TPE, 4 showed pleural ADA activities below the threshold value. Of 4 patients with false negative, 2 were co-existent with malignancy and one had millitary tuberculosis. This could explain why immunodeficiencies render the ADA test falsely negative.

Estimation of ADA activity may provide the basis for rapid and efficient diagnosis of TPE with a high sensitivity (94%) and specificity (88%) when cut-off set at 40 U/L. Its low cost, ease of performance, short assay time, and high diagnostic efficiency render it a very useful adjunct test for the diagnosis of TPE.

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