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RESEARCH ARTICLE

# ADENOSINE DEAMINASE AND ITS ISOENZYME AS A DIAGNOSTIC MARKER IN TUBERCULAR PLURAL EFFUSION

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**ABSTRACT**

Mortality and residual morbidity is quit high in tubercular meningitis particular in developing countries like India. Several investigations have been developed for early diagnosis but this sill poses a lot of diagnostic problem and financial burden. So, the present study was performed to search a quick and definite investigation for easy and rapid diagnosis of tubercular meningitis and pleural fluid ADA and its isoenzymes activity was selected to differentiate between tubercular and non-tubercular exudative pleural effusion. Among 87 exudative pleural effusion patients, 55 were tubercular, 16 were empyemic and 16 were malignant selected. Pleural fluid total ADA and ADA2 activity were measured and compared within the different types of exudative pleural effusion. The pleural fluid ADA activity had no significant different among exudative pleural effusion. But when ADA2 was selected it can efficiently differentiated tubercular form non-tubercular origin. ADA2 activity than total ADA in pleural fluid are elevated in suspected tubercular pleural effusion cases and it is a simple, rapid, inexpensive and has got definite diagnostic value. It is thus a useful biochemical marker for the early diagnosis of tubercular pleural effusion.

**Key Words**: Pleural effusion, Tuberculosis, Adenosine deaminase, Isoenzyme.

## INTRODUCTION

Pleural tuberculosis is the most common presentation of extrapulmonary tuberculosis and the most common cause of pleural effusion worldwide.[1,2] Conventional methods for the diagnosis of tubercular pleural effusion have proven inefficient. It is well known that because of the low number (or even absence) of *M. Tuberculosis* in pleural effusion and direct examination of pleural fluid and Ziehl-Neelsen staining requires bacillar concentrations of 10,000/ml and, therefore, has a low sensitivity (0 to 1%).[3,4] Although a culture is more sensitive (11 to 50%), [5,6] it requires 2 to 6 weeks to grow *Mycobacterium tuberculosis* and a minimum of 10 to 100 viable bacilli. The sensitivity of pleural biopsy specimens is reportedly higher whether by culture (39 to 79%) [4,5] or histologic evaluation (71 to 80%).[3,4] However, this procedure requires greater expertise, is more invasive, and is subject to sampling error. Radiological imaging techniques are also sometime given confusing diagnosis. Several relatively newer techniques have been developed to make the diagnosis including recognition of serum IgG antibody to selected Mycobacterium antigen by Enzyme Immunoassay (EIA) and identification of Mycobacterium DNA through gene amplification by Polymerase Chain Reaction (PCR), interferon gamma. Serum IgG towards tubercular antigen is poorly correlated with extrapulmonary tuberculosis and technique is costly, required sophisticated instrumentation. [7] PCR has relatively low sensitivity in body fluids (42-81%) [8-10] and is fairly expensive. Moreover, it gives positive result for mycobacterium in patients with history of treated tuberculosis. Non-viable organisms are detected and negative PCR makes the diagnosis very unlikely. The sensitivity of an elevated interferon level appears better (89-99%) [11-12] but relatively only few studies of its use have been reported and the assay is expensive and cannot be done in routine laboratory. So, the present study was performed to search a quick, simple, low cost investigation for easy and rapid diagnosis of tubercular pleural effusion and resolve the diagnostic dilemma. One such biochemical parameter is estimation of Adenosine deaminase (ADA) and its isoenzymes activity in pleural fluid.

Adenosine deaminase an enzyme required for purine degradation is widely distributed in human tissues [13]. ADA helps in proliferation and differentiation of lymphocytes especially T lymphocytes. ADA is a significant indicator of active cellular immunity.[14] There are 2 isoforms of ADA, ADA-1 and ADA-2. The ADA1 isoenzyme is found in all the cells with highest concentration in lymphocytes and monocytes, whereas ADA-2 is released by monocyte macrophages [15] when they are stimulated by the presence of live microorganisms in their interior. Hence, this study was designed and conducted to assess the role of pleural fluid ADA2 activity in the early laboratory diagnosis of tubercular pleural effusion.

## MATERIAL AND METHODS

### Study area

The present study was conducted in the department of Biochemistry with the collaboration of department of Medicine and Pathology of Burdwan Medical College, Burdwan, and West Bengal, India.

### Selection of subjects

In this hospital based cross-sectional study a total of 87 patient paediatric age group varying from 20 to 80 years old were selected as case by simple random sampling after informed consent had been received from parents or guardians of the subjects between February 2011 and May 2013. Out of these 55 were suffering from

 

tubercular pleural effusion and rest of the 32 patients were grouped as non-tubercular exudative pleural effusion, among which sixteen patients were due to empyema and malignant causes each.

In present study, the diagnosis of tubercular and non-tubercular pleural effusion was made by clinical examination as well as examining pleural fluid using (i) Cell count (ii) Histopathological examination (iii) Quantitative estimation of glucose and protein (iv) Histopathological examination (v) Gram's stain, Ziehl-Neelson (Z-N) stain (vi) cultured on Lowenstein-Jensen medium (vii) Radiological investigations.

**Collection of samples**

Thoracocentesis was performed in all the patients to obtained pleural fluid under aseptic precautions where about 30 ml of pleural fluid was obtained. and the collected fluid was centrifuged for 10-15 min at 1500 rpm; supernatant was separated and stored at -20$^0$C for ADA and its isoenzyme activity analysis.

**Assay of total ADA and ADA isozyme activity**

The activities of the total ADA and the ADA isozymes were assayed using an automatic analyzer (TBA-80FR; Toshiba, Tokyo, Japan) with a commercial kit according to the instructions of the manufacturer (Toyobo, Osaka, Japan). The enzyme activity was determined by quantifying inosine liberated from the substrate, adenosine (10 m*M*). Inosine was converted by purine nucleoside phosphorylase into hypoxanthine, which was further converted by xanthine oxidase into uric acid and hydrogen peroxide. The hydrogen peroxide was then converted into the quinone dyes by peroxidase, 4-aminoantipyrine, and *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-m-toluidine, and the absorbance at 548/700 nm was measured. ADA2 activity was measured in the presence 0.35 m*M* erythro-9-(2-hydroxy-3- nonyl) adenine hydrochloride (EHNA; Toyobo) since ADA1 activity is blocked by EHNA at that concentration but ADA2 activity is unaffected. [16] Total ADA activity was measured in the absence of EHNA, and ADA1 activity was then calculated by subtracting the ADA2 activity from the total activity. For this study we used commercial calibrator material (DDR-1, Toshiba, Tokyo, Japan marketed by ERBA) and control material [DDC-1R (Fluid/CSF), Toshiba, Tokyo, Japan marketed by ERBA]. Intra assay CV% was 2.3 and inter assay CV% was 3.1.

**Statistical analysis**

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows.

**RESULT**

The age of the patients ranged from 20-75 years and their characteristics are shown in Table 1. Among eighty seven pleural effusion patients, 45 (51.72%) were male and 42 (48.28%) were female.

**Table 1**: Personal profile and clinical details of healthy persons and patients suffering from pleural effusion

| Groups | Male | Female | Age (years) |
|---|---|---|---|
| Empyemic pleural effusion (n = 16) | 7 | 9 | 20-55 |
| Tubercular pleural effusion (n = 55) | 28 | 27 | 21-59 |
| Malignant pleural effusion (n = 16) | 10 | 6 | 45-80 |

The mean value of pleural fluid total ADA activity in tubercular, empyemic and malignant pleural fluid were 23.44, 19.79 and 18.29 IU/L respectively. The ADA2 activity was 6.31, 15.38 and 6.28 in empyemic, tubercular and in malignant pleural effusion respectively as shown in the Table 2.

**Table 2:** ADA activity in CSF in control, tubercular, viral, pyogenic, cerebral malaria

| **Groups** | **Total ADA activity in pleural fluid (IU/L)** | **ADA2 activity in pleural fluid (IU/L)** |
|---|---|---|
| Empyemic pleural effusion (n = 16) | 19.79 ± 6.69 | 6.31 ± 1.37 |
| Tubercular pleural effusion (n = 55) | 23.44 ± 11.96 | 15.38 ± 2.86 |
| Malignant pleural effusion (n = 16) | 18.29 ± 3.92 | 6.28 ± 1.8 |

***n = number of subject; Values are mean ± SD***

In the Table 3 it was observed that when comparison of means of all exudative pleural effusions was analysed it was found that the CSF ADA activity among all type of pleural effusion under study had no significant different among them ($p > 0.05$)

 

**Table 3:** ANOVA with Bonferroni correction showing multiple comparisons between different types of exudative pleural effusions with significance of difference

| Dependent variable | Factors (I) | Factors (J) | Mean difference (I-J) | Significance at 95% CI |
|---|---|---|---|---|
| ADA activity (IU/L) in pleural fluid | 1 | 2 | 3.65 | 0.319 |
| | | 3 | 5.15 | 0.098 |
| | 2 | 1 | -3.65 | 0.319 |
| | | 3 | 1.5 | 0.376 |
| | 3 | 1 | -5.15 | 0.098 |
| | | 2 | -1.5 | 0.376 |

**p value significant ( p < 0.05) at 95% Confidence interval (CI); 1 = Tubercular pleural effusion, 2 = Empyema, 3 = Malignant pleural effusion.*

But when isoenzyme of ADA that is ADA2 was used as a parameter to compare among the all exdutative cause of pleural effusion it was found that CSF ADA2 activity in case of tubercular pleural effusion was significantly elevated than other types of exudative pleural effusion as shown in the Table 4.

**Table 4:** ANOVA with Bonferroni correction showing multiple comparisons between different types of exudative pleural effusion with significance of difference

| Dependent variable | Factors (I) | Factors (J) | Mean difference (I-J) | Significance at 95% CI |
|---|---|---|---|---|
| ADA2 activity (IU/L) in pleural fluid | 1 | 2 | 9.07 | <0.001* |
| | | 3 | 9.1 | <0.001* |
| | 2 | 1 | -9.07 | <0.001* |
| | | 3 | 0.03 | 0.945 |
| | 3 | 1 | -9.1 | <0.001* |
| | | 2 | -0.03 | 0.945 |

** p value significant ( p < 0.05) at 95% Confidence interval (CI); 1 = Tubercular pleural effusion, 2 = Empyema, 3 = Malignant pleural effusion.*

The usefulness of CSF ADA2 level as a biomarker for diagnosis of tubercular pleural effusion was evaluated using Receiver Operative Characteristic **(**ROC) curve analysis and the optimal cut-off value was determined to be 19.6 IU/L (Figure 1). The area under the curve (AUC) for suspected tubercular pleural effusion group was 0.933 and standard error (SE) was 0.032 (95% confidence interval (CI) =86.1% - 99.2%, P <0.001). Based on the cut-off value of 19.6 IU/L, the pleural fluid ADA2 sensitivity and specificity were 82.45% and 97.82% respectively. Positive predictive value was 96.18% and negative predictive value was 84.7%. The accuracy of the test in suspected tubercular pleural effusion cases was 89.1% (Table 5).

**Table 5:** Validity of CSF ADA2 as a diagnostic test in suspected cases of tubercular pleural effusion

| Study Group | Sensitivity % (CI) | Specificity % (CI) | PPV % (CI) | NPV % (CI) | Accuracy % |
|---|---|---|---|---|---|
| Tubercular pleural effusion | 82.45 | 97.82 | 96.18 | 84.7 | 89.1 |

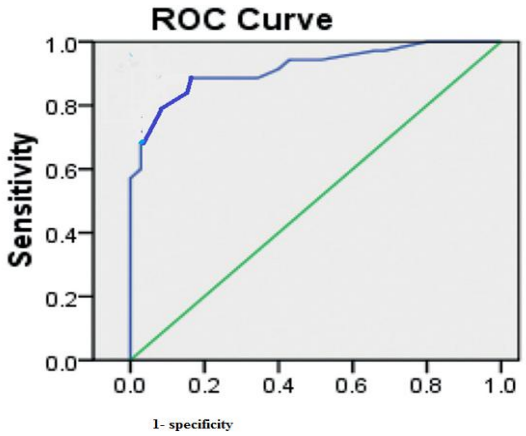


**Figure 1:** Receiver Operative Characteristic **(**ROC) curve of CSF ADA2 activity. Diagonal line indicates the line of no discrimination. Area Under the ROC Curve (AUC) curve is 0.933

 

## DISCUSSION

Exudative lymphocytic pleural effusions are commonly encountered in clinical practice but they often constitute difficult diagnostic problems. The three most common causes are tubercular, malignant and empyemic.[17] For tuberculosis, the limitation of diagnostic tests are few positive staining and culture from pleural fluid, and time consuming for identification.[18,19] So the present study was conducted to choose a diagnostic parameter that will help to diagnose properly and as early as possible to minimize the morbidity and mortality. ADA measurement in pleural fluid is the simple and inexpensive test that was used as parameter where cellular immunity is stimulated as in tuberculosis [20,21] and it was found that there was no significant difference of pleural fluid ADA activity in all types of exudative pleural effusion and thus the yield of total ADA may be low in setting to differentiate tubercular from other types of exudative pleural effusion as one old study. [22] The possible explanation may be from ADA value in most assays detected total ADA which includes ADA-1 and ADA-2. Thus, fluid with high cell counts can have high total ADA and may be undifferentiated from tubercular pleural effusion. [23]But determination of the individual ADA2 isoenzyme in the present study could help in distinguishing between the increase activity in CSF especially between tubercular and non-tubercular causes as ADA 2 isoenzyme was found to be primarily responsible for total ADA activity in tubercular origin, which mostly reflects monocyte-macrophage activity by live phagocytosed microorganisms. [24]

ADA2 has a good diagnostic potential to differentiate tubercular from other forms of exudative pleural effusion. In the present study CSF ADA2 activity 19.8 IU/L as a cut-off value exhibited a sensitivity of 81.65%, specificity of 97.42% for the diagnosis of tubercular pleural effusion. In addition to this, the positive predictive value of test is 96.85% with overall accuracy being 88.2%. Thus a cut-off CSF ADA2 level of 19.6 IU/L may differentiate tubercular from non-tubercular pleural effusion in this study area.

## CONCLUSION

We concluded that the ADA2 activity instead of total ADA in tubercular pleural effusion is much higher than the other aetiology. Using 19.6 IU/L as the cut off, it is possible to avoid other expensive, lengthy, non-reliable investigation to diagnose tubercular pleural effusion. Thus, activity of CSF ADA2 estimation seems to have the potential for being a single test for the diagnosis of pleural effusion of tubercular origin tubercular in developing countries where prevalence of tuberculosis is quite high.

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