

## DIAGNOSTIC SIGNIFICANCE OF ADENOSINE DEAMINASE IN PLEURAL TUBERCULOSIS

Rukhshan Khurshid, Najla Shore, \*Mahjabeen Saleem, \*Mammona Naz, \*Nazia Zameer

Department of Biochemistry and Physiology, Fatima Jinnah Medical College, Lahore and \*Institute of Biochemistry and Biotechnology, University of Punjab, Lahore.

**Background:** Tuberculosis (TB) is a major cause of pleural effusion, which in TB usually has lymphocytic and exudative characteristics. The analysis adenosine deaminase (ADA) activity is a very useful diagnostic approach to achieve a more rapid and precise diagnosis in the cases of pulmonary TB. **Methods:** Fifty male and fifty female patients presenting with tuberculous pleural effusion were included in the study. The patients were taken from the medical ward of Sir Ganga Ram Hospital. Activity of adenosine deaminase (ADA) was estimated by the technique of Sodium dodecyl sulphate electrophoresis (SDS-EF) using 10% polyacrylamide gel. **Results:** There is an increased level of enzyme ADA in pleural fluid of tuberculous patients as compared to normal subjects. **Conclusion:** Estimation of ADA activity may provide the basis for the rapid and efficient diagnosis of pleural TB in different clinical settings. However study should be carried out on large number of patients to reach a better conclusion.

**Key Words:** Adenosine deaminase, Pleural TB and Electrophoresis.

### INTRODUCTION

TB is no longer the scourge it once was, but it remains an important cause of morbidity and mortality worldwide. Recent estimates are that 8-10 million new tuberculosis (TB) cases occur each year in the world. 2-3 million die. In developing countries, TB is one of the most common opportunistic infections in people who are seropositive for HIV-1.<sup>1</sup> Fueled by increasing poverty, homelessness, immigration, drug abuse, declining prevention programs, and the HIV epidemic, its incidence in the developing and developed countries has increased dramatically. Strict distinction between "adult" and "childhood" patterns of TB should be avoided.<sup>2</sup>

Pleural effusion develops because of excessive filtration or defective absorption of accumulated fluid. Effusion may be a primary manifestation or a secondary complication of many disorders. Tuberculosis (TB) is a major cause of pleural effusion, which in TB usually has lymphocytic and exudative characteristics.<sup>3</sup> Exudates are due to pleural inflammation (pleurisy), with an increased permeability of the pleural surface to proteinaceous fluid. Inflammation or injury increases pleural membrane permeability to proteins and various types of cells and leads to the formation of exudative effusion. In general, exudates generally have protein concentration higher than 3 g/dL or a specific gravity of 1.020 on a refractometer.<sup>4,5</sup> Lymphatic obstruction may also contribute to accumulation of pleural fluid.<sup>6</sup>

Diagnosis can be made in a majority of patients from the clinical features, pleural fluid examination (including cytology, biochemistry and bacteriology), and pleural biopsy. Adenosine

deaminase estimation in pleural fluid is occasionally useful.<sup>3</sup>

Adenosine deaminase (ADA) is an endogenous tissue enzyme which is released into the serum in patients with many different types of malignancies and infections, including viral hepatitis, infectious mononucleosis, typhoid fever, and tuberculosis. In pleural fluid, elevated ADA levels are very commonly associated with tuberculosis. In CSF, ADA is elevated in cases of tuberculous meningitis.<sup>7,8</sup> It is reported that It is a pleural fluid marker for tuberculosis. The analysis of PCR and ADA activity, however, is a very useful diagnostic approach to achieve a more rapid and precise diagnosis in the cases of pTB.<sup>9</sup> It is found that Pleural fluid ADA levels in TB effusions were significantly higher than the non-TB effusions<sup>10</sup>. The molecular forms of ADA were studied<sup>11</sup> that in pleural effusions using the technique of SDS-PAGE. Analysis of the Adenosine deaminase in pleural fluid pinpoints many pulmonary and systemic diseases are known to cause pleural effusions.

Present study tried to find out the diagnostic significance of Adenosine deaminase in pleural tuberculosis using the technique of gel electrophoresis.

### MATERIAL AND METHOD

We studied ADA level in pleural fluid of fifty male and fifty female presenting with tuberculous pleural effusion to Sir Ganga Ram Hospital. Determination of adenosine deaminase (ADA) activity was carried out by using the technique of Sodium dodecyl sulphate electrophoresis (SDS-EF) using 10% polyacrylamide gel. Comparisons between groups were done using student's t-test.

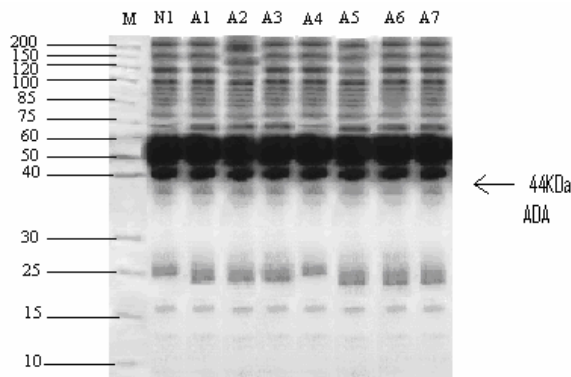
## RESULTS

The mean age of male and female was in the range of 42-46 years. Mean age of male was 45.72 years and of female was 43.74 years. Mean protein level was  $3.39 \pm 1.75$  gm/dl in male and female was  $3.0 \pm 1.86$  gm/dl with mean specific gravity of male was  $1.020 \pm 0.01$  and in female was  $1.020 \pm 0.01$ . By comparing the level of fluid protein with the standard level of normal subjects it is observed that although the level of fluid protein having a lower level in patients but this shows no significant difference (Table-1).

Level of Adenosine deaminase was determined by applying the sample of both patients and of normal subjects on polyacrylamide gel (a technique of gel electrophoresis). It is observed that a 44 KDa band with density 0.0372 was observed in male patient. On the other in normal control subject the same band having a density of 0.0159 was observed. This shows an increased level of enzyme ADA in patients as compared to normal subjects (Figure-1).

**Table-1: Level of fluid protein and specific gravity of male and female patients**

Parameters	Male (50)	Female (60)
Age (years)	$45.72 \pm 19.22$	$43.74 \pm 16.09$
Protein (gm/dl)	$3.39 \pm 0.24$	$3.02 \pm 0.26$
Sp. Gravity	$1.020 \pm 0.01$	$1.020 \pm 0.01$



**Figure-1: serum protein profile of patients and control subjects. N represent normal and A represent the patients. M represents markers.**

## DISCUSSION

The combination of PCR and ADA activity determination allowed the selective increase of sensitivity and specificity for probable and confirmed cases compared to individual methods. Positive and negative predictive values for these individual or combined methods were maintained over a wide range of prevalence of pleural TB in the patient population presenting with pleural effusions.<sup>12</sup>

According to our study the mean age of male patients was 45.72 year. While a study reported<sup>13</sup> that the mean age of male patient with pleural TB was 61.00 years.

Present study tried to find out the level of fluid proteins in a group of patients. It is observed that the level of fluid protein having a low level as compared to normal standard protein ( $>3$ gm/dl). However it is reported<sup>14</sup> that the pleural fluid, total protein concentration was between 5.1-5.5 g/dl.

Electrophoretic profile of pleural fluid of male patients and normal subjects was carried out to find out the level or density of ADA. It is observed that the density of 44 KDa ADA was 0.0372. On the other, in the fluid of normal subjects (fluid having a protein of 5-6 gm/dl) the 44 KDa ADA have a density of 0.0159. According to a study, adenosine deaminase exists in its smallest molecular form (ADA-S) of  $<42$  kDa in primate and rodent brain, intestine and liver, human erythrocytes, avian liver and in bovine spleen and intestine.<sup>15</sup> The enzyme is a monomeric protein of molecular weight 44000.<sup>16</sup> It is highlighted that pleural cells become activated and produce cytokines as a response to mycobacteria. Intra macrophage and direct cytotoxic elimination of mycobacteria, granuloma formation, and fibrosis are the main facets of this reaction. It has been shown that the most useful diagnostic test is the level of adenosine deaminase in the pleural fluid. Elevation of these compounds in lymphocytic pleural effusions is virtually diagnostic of tuberculous pleurisy.<sup>17</sup>

## CONCLUSION

Estimation of ADA activity may provide the basis for the rapid and efficient diagnosis of pleural TB in different clinical settings. However study should be carried out on large number of patients to reach a better conclusion.

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**Address for Correspondence:**

**Dr. Najla Shore**, Department of Physiology, Fatima Jinnah Medical College, Lahore. Tel: +92 300 4172859

**E-Mail:** Shorenajla@hotmail.com