A study on adenosine deaminase activity in pleural effusion of tuberculous and non-tuberculous origin

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Abstract

Introduction: Accumulation of fluid in the pleural space that exceeds the physiological amount is defined as pleural effusion. Pleural effusion can be either transudative or exudative. The exudative pleural effusion is predominantly observed in tuberculosis. Adenosine deaminase (ADA) level remains high in disorders where cellular immunity is stimulated. Tuberculosis is one such disease.

Materials and Methods: The cases were classified as two groups: 30 patients of tubercular pleural effusion were enrolled in Group I and 30 patients of non-tubercular pleural effusion were Group II. In non-tubercular pleural effusion, 6 cases were malignant effusions, 12 cases were parapneumonic effusions and 12 cases were transudative effusions, which include 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4 cases of hepatic cirrhosis. The following biochemical parameters in pleural fluid were estimated. They are Glucose, Protein and Adenosine deaminase.

Results: In this study mean pleural fluid glucose level was significantly higher in transudative when compared to exudative pleural effusions. In exudative pleural effusions, mean pleural fluid protein level was increased significantly as compared to transudative pleural effusions. The activity of ADA in tubercular effusion was significantly higher when compared to non-tubercular pleural effusions of different etiology such as malignant parapneumonic and transudative.

Conclusion: The assessment of ADA act as better marker for identifying tuberculosis than the traditional markers.

Keywords: Pleural effusion, Exudative, Transudative, Tuberculosis, Adenosine deaminase.
30 patients of tubercular pleural effusion and 30 patients of non-tubercular pleural effusion patients were considered as Group I and Group II. The study and objectives were explained to them and their consent in written form was taken.

**Sample Collection:** On confirmation of pleural effusion by X-ray and clinical examination, pleural fluid was aspirated from the posterior axillary line. 5ml of 2% lignocaine was infiltrated and Pleural fluid was aspirated from the site.

The following biochemical parameters in pleural fluid were estimated by different methods. They are Glucose by Glucose oxidase Peroxidase method, Protein by Biuret method and Adenosine deaminase by Giusti and Galanti method of enzymatic analysis.

**Statistical Analysis**

Data was expressed as Mean and Standard deviation (mean ±SD). Statistical significance between control and patient groups, the Z test was performed using SPSS software 16.0. The statistical significance was determined at 5% (p < 0.05) level.

**Results and Discussion**

Depending on the patient history, clinical examination, Chest X-ray and other investigations, 60 cases of pleural effusions were diagnosed and categorized into two groups. Group I (n= 30) tuberculous pleural effusion and Group II (n= 30) non-tubercular pleural effusion patients, which included 6 cases of malignant effusions, 12 cases of parapneumonic effusions and 12 cases of transudative effusions. The transudative effusions included 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4 cases of hepatic cirrhosis. (Table 1).

**Glucose levels in Pleural Fluid:** In comparison to pleural glucose values in tubercular with non tubercular of different etiology, it was observed that pleural fluid glucose in transudative effusions of congestive cardiac failure, nephrotic syndrome and hepatic cirrhosis (100.58 mg/dl ±6.24) had statistically significant higher values (p<0.001) than the exudative effusions of tuberculous, malignant and parapneumonic origin (65.12 mg/dl ±11.04). (Table 2 and Table 4). Parapneumonic effusions and tubercular effusions had low pleural fluid glucose because of utilization of glucose by the bacteria for the growth. Whereas in malignant pleural effusions, the growing malignant mass consumes ever increasing amounts of glucose at the expense of the host’s energy reserve. Most cancer cells have enzyme systems for both oxidative metabolism and anerobic glyogenic pathway utilizing majority of glucose produced by gluconeogenesis liberating lactic acid that often must be converted in to glucose before being utilized. In cancer there is an increase in energy expenditure and an inability to adjust the metabolic rate consequent to malnutrition. Growth continues throughout the entire day in malignant cells rather than follows the diurnal pattern of the metabolic activity of normal tissues. All these causes are responsible for low glucose level in malignant pleural effusions when compared to transudates.

**Protein Levels in Pleural Fluid:** In comparing with the protein levels in tubercular and non tubercular pleural effusions, the pleural fluid protein in transudative effusions (2.05 g/dl ±0.36) showed statistically significant lower values (p<0.001) than the exudative effusion (tuberculous, malignant and parapneumonic) (5.27 g/dl ±0.61) and it is in agreement with the Light’s criteria (Table 3 and Table 4). The pleural fluid protein level helps in classifying transudates and exudates but it does not provide diagnostic information regarding the etiology of pleural effusion. In our present study, the pleural fluid protein in transudative effusions had statistically significant lower values than the exudative (tuberculous, malignant and parapneumonic) effusion It is in agreement with Light’s criteria to differentiate transudative and exudative effusions. The pleural fluid protein level helps in classifying transudates and exudates but it does not provide diagnostic information regarding the etiology of pleural effusion.16

**ADA Activity in Tubercular and Non Tubercular Pleural Effusion:** The ADA activity in tubercular and non-tubercular pleural effusion was compared and it was observed that pleural fluid ADA in tubercular effusions (85.76 U/L ±15.62) had statistically higher values (p<0.001) than the non-tubercular effusions of malignant, parapneumonic and transudative origin (23.3 U/L ±7.55). (Table 5 and Table 6). Tuberculosis is one of the dreadful disease and the severity of the disease can be judged by the fact that it affects all ages, irrespective of the sex. No other disease has so much socio economic health significance as tuberculosis in India and other tropical countries. In tuberculosis, the presence of antigens of mycobacteria in the pleural space causes delayed hypersensitivity which results in pleural effusion.17 In turn these antigens can enter the pleural space due to rupture of a sub pleural focus.18 The delayed hypersensitivity will cause the differentiation of lymphocytes and releases the lymphokines that activate the macrophages to increase mycobactericidal effect.19 The biochemical markers like adenosine deaminase, γ-interferon, and lysosome got utmost clinical importance in the early diagnosis of pleural tuberculosis.20 ADA is found in majority of cells, however its role mostly concerned to the proliferation and differentiation of T-lymphocytes. The enzyme ADA predominantly a T lymphocyte enzyme and its plasma activity is high in disease where cellular immunity is stimulated such as tuberculosis. In tubercular pleural effusion, the isoform ADA2 is predominant that accounts for 88% and ADA1 accounts for 70% of total activity of ADA.21 Monocytes or macrophages infected by mycobacteria are thought to be main source of ADA2 in pleural fluid. ADA2

International Journal of Clinical Biochemistry and Research, October-December, 2018/3(4):574-577 575
produced by macrophages in response to pathogen invasion leads to activation of immune cells at the site of inflammation. The non tuberculous lymphocytic pleural effusion includes malignant effusion, parapneumonic effusions, miscellaneous exudative effusions and transudative effusions. A high level of ADA is observed in parapneumonic effusions and emphysemas. The ADA level in non tuberculous does not exceed the 40 IU/L, which is considered as a cut-off for tuberculous effusions.

Table 1: Distribution of cases

| Groups | Type of pleural effusion | Number of cases | Percentage |
|--------|--------------------------|-----------------|------------|
| I      | Tuberculous              | 30              | 50%        |
| II     | Non tuberculous          | 30              | 50%        |

Group II cases were subdivided into three subgroups.

| Subgroups | Type of pleural effusion | Number of cases | Percentage |
|-----------|--------------------------|-----------------|------------|
| II-A      | Malignant                | 06              | 10%        |
| II-B      | Parapneumonic            | 12              | 20%        |
| II-C      | Transudative             | 12              | 20%        |

The above table shows distribution of 12 transudative cases which include 4 cases of congestive cardiac failure, 4 cases of nephrotic syndrome and 4 cases of hepatic cirrhosis.

Table 2: Glucose levels in pleural fluid

| Groups | Type of pleural effusion | Pleural fluid Glucose range (mg/dL) | Mean ± SD (mg/dL) |
|--------|--------------------------|------------------------------------|------------------|
| I      | Tuberculous              | 50-90                              | 66.9±10.58       |
| II     | Non tuberculous          |                                    |                  |
| II-A   | Malignant                | 65-87                              | 76.17±8.11       |
| II-B   | Parapneumonic            | 49-61                              | 55.17±5.67       |
| II-C   | Transudative             | 91-110                             | 100.58±6.24      |

Group II cases were Non tuberculous subdivided into three subgroups.

The above table shows the glucose levels of pleural fluids in different groups of pleural effusion.

Table 3: Protein levels in pleural fluid

| Groups | Type of pleural effusion | Pleural fluid Protein range (g/dL) | Mean ± SD (g/dL) |
|--------|--------------------------|-----------------------------------|-----------------|
| I      | Tuberculous              | 4.1-6.6                           | 5.36±0.73       |
| II     | Non tuberculous          |                                    |                  |
| II-A   | Malignant                | 4.9-5.4                           | 5.01±0.26       |
| II-B   | Parapneumonic            | 4.7-5.7                           | 5.17±0.33       |
| II-C   | Transudative             | 1.5-2.6                           | 2.05±0.36       |

Group II cases were Non tuberculous subdivided into three subgroups.

The above table shows the protein levels of pleural fluids in different groups of pleural effusion.

Table 4: Pleural fluid glucose and protein levels in transudative and exudative pleural effusion

| Biochemical Parameters | Transudates Mean ± SD | Exudates Mean ± SD | Z value | p value |
|------------------------|-----------------------|--------------------|---------|--------|
| Pleural fluid Glucose (mg/dL) | 100.58±6.24          | 65.12±11.04        | 14.73   | <0.001 |
| Pleural Fluid Protein (g/dL) | 2.05±0.36            | 5.27±0.61          | 23.58   | <0.001 |

The above table shows the glucose and protein levels of pleural fluids in different groups of pleural effusion.

Table 5: Adenosine deaminase (ADA) in pleural fluid

| Groups | Type of pleural effusion | Pleural fluid ADA range (U/L) | Mean ± SD (U/L) |
|--------|--------------------------|-------------------------------|-----------------|
| I      | Tuberculous              | 54-117                        | 85.77±15.6     |
| II     | Non tuberculous          |                               |                 |
| II-A   | Malignant                | 24-36                         | 31.83±5.11     |
| II-B   | Parapneumonic            | 12-33                         | 25.08±5.92     |
| II-C   | Transudative             | 11-25                         | 17.25±4.13     |

Group II cases were Non tuberculous subdivided into three subgroups.

The above table shows the adenosine deaminase levels of different pleural effusion groups.
Table 6: Adenosine deaminase activity in tubercular and non tubercular pleural effusion

| Type of pleural effusion | ADA range (U/L) | Mean ± SD (U/L) |
|--------------------------|-----------------|-----------------|
| Tubercular               | 54-117          | 85.77±15.6      |
| Non tubercular           | 11-37           | 23.3±7.55       |

Z value: 19.7 p value<0.001

The above table shows the adenosine deaminase levels in the pleural effusion of tubercular and non tubercular patients.

**Conclusion**

In comparison to non tubercular pleural effusion, the tubercular pleural effusion showed a marked and significant elevation of pleural fluid ADA. The association of pleural effusion tuberculosis and the rise of ADA can be considered as early and sensitive marker for the early diagnosis and to prevent further disease severity.

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**How to cite this article:** Basha S. J, Raju D.S.S.K. Kumar M. A. A study on adenosine deaminase activity inpleural effusion of tuberculous and non tuberculous origin. Int J Clin Biochem Res. 2018;5(4):574-577.