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.

Introduction

Accumulation of fluid in the pleural space that exceeds the physiological amount is defined as pleural effusion. Effusion develops when there is an alteration in hydrostatic and osmotic forces that affects the formation of pleural fluid, when lymphatic drainage is impaired, or when mesothelial or capillary endothelial permeability is increased.¹

Pleural effusion can be either transudative or exudative.² Transudative pleural effusions are usually bilateral due to systemic conditions results in elevated capillary hydrostatic pressure or declined pleural oncotic pressure. The exudates are more often unilateral, associated with localized disorders that increase vascular permeability or interfere with lymphatic resorption.³⁴ The transudative pleural effusions are due to congestive cardiac failure, nephrotic syndrome and left ventricular failure. The transudative pleural effusion occurs because the increased amount of fluid in the lung interstitial space exists in part across the visceral pleura. This overwhelms the capacity of the lymphatics in the parietal pleura to remove the fluid.⁵ The exudative pleural effusions are due to tuberculosis, malignancy and pneumonia. Parapneumonic effusions are associated with bacterial pneumonia, lung abscess or bronchiectasis.⁶

Tuberculosis is the prime cause of exudative pleural effusion in our country and this is due to tuberculosis protein hypersensitivity in the pleural space. The exudate pleural fluid predominantly contains small lymphocytes.⁷ Adenosine deaminase catalyzes the conversion of adenosine to inosine.⁸ Its main biological activity is detected in T-lymphocytes and its level remains high in disorders where cellular immunity is stimulated. Tuberculosis is one such disease.⁹,¹⁰

Materials and Methods

The study was undertaken at Maharaja’s Institute Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, during the period December 2016 to March 2018. Patients attended at MIMS OPD of Chest and TB department and admitted in the Medical / Surgical wards, during the study period were chosen for the study. The study subject comprised of 60 cases of pleural effusions of different etiology in the age group between 25 to 65 years of both the sexes. The diagnosis is based on detailed history, clinical examination and chest X-ray. The Institutional Ethical Committee (Ref. No. MIMS/IEC/Lr. No. 027/2016) approval was also taken.

Inclusion Criteria: Patients with tuberculous pleural effusion, malignant effusion, parapneumonic effusion, congestive cardiac failure, nephrotic syndrome and hepatic cirrhosis were included.

Exclusion Criteria: Patients with HIV, Viral hepatitis and age greater than 65 years were excluded

The cases were classified as two groups.
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 (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.

**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. In turn these antigens can enter the pleural space due to rupture of a sub pleural focus. The delayed hypersensitivity will cause the differentiation of lymphocytes and releases the lymphokines that activate the macrophages to increase mycobactericidal effect. The biochemical markers like adenosine deaminase, γ-interferon, and lysosome got utmost clinical importance in the early diagnosis of pleural tuberculosis. 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. Monocytes or macrophages infected by mycobacterium are thought to be main source of ADA₂ in pleural fluid.
produced by macrophages in response to pathogen invasion leads to activation of immune cells at the site of inflammation.22 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.23

Table 1: Distribution of cases

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of pleural effusion</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculous</td>
<td>30</td>
<td>50%</td>
</tr>
<tr>
<td>II</td>
<td>Non tuberculous</td>
<td>30</td>
<td>50%</td>
</tr>
<tr>
<td>Group II cases were subdivided into three subgroups.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Malignant</td>
<td>06</td>
<td>10%</td>
</tr>
<tr>
<td>II-B</td>
<td>Parapneumonic</td>
<td>12</td>
<td>20%</td>
</tr>
<tr>
<td>II-C</td>
<td>Transudative</td>
<td>12</td>
<td>20%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of pleural effusion</th>
<th>Pleural fluid Glucose range (mg/dL)</th>
<th>Mean ± SD (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculous</td>
<td>50-90</td>
<td>66.9±10.58</td>
</tr>
<tr>
<td>Group II cases were Non tuberculous subdivided into three subgroups.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Malignant</td>
<td>65-87</td>
<td>76.17±8.11</td>
</tr>
<tr>
<td>II-B</td>
<td>Parapneumonic</td>
<td>49-61</td>
<td>55.17±3.67</td>
</tr>
<tr>
<td>II-C</td>
<td>Transudative</td>
<td>91-110</td>
<td>100.58±6.24</td>
</tr>
</tbody>
</table>

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

Table 3: Protein levels in pleural fluid

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of pleural effusion</th>
<th>Pleural fluid Protein range (g/dL)</th>
<th>Mean ± SD (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculous</td>
<td>4.1-6.6</td>
<td>5.36±0.73</td>
</tr>
<tr>
<td>Group II cases were Non tuberculous subdivided into three subgroups.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Malignant</td>
<td>4.9-5.4</td>
<td>5.01±0.26</td>
</tr>
<tr>
<td>II-B</td>
<td>Parapneumonic</td>
<td>4.7-5.7</td>
<td>5.17±0.33</td>
</tr>
<tr>
<td>II-C</td>
<td>Transudative</td>
<td>1.5-2.6</td>
<td>2.05±0.36</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Transudates Mean ± SD</th>
<th>Exudates Mean ± SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural fluid Glucose (mg/dl)</td>
<td>100.58±6.24</td>
<td>65.12±11.04</td>
<td>14.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pleural Fluid Protein (g/dl)</td>
<td>2.05±0.36</td>
<td>5.27±0.61</td>
<td>23.58</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of pleural effusion</th>
<th>Pleural fluid ADA range (U/L)</th>
<th>Mean ± SD (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tuberculous</td>
<td>54-117</td>
<td>85.77±15.6</td>
</tr>
<tr>
<td>Group II cases were Non tuberculous subdivided into three subgroups.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>Malignant</td>
<td>24-36</td>
<td>31.83±5.11</td>
</tr>
<tr>
<td>II-B</td>
<td>Parapneumonic</td>
<td>12-33</td>
<td>25.08±5.92</td>
</tr>
<tr>
<td>II-C</td>
<td>Transudative</td>
<td>11-25</td>
<td>17.25±4.13</td>
</tr>
</tbody>
</table>

The above table shows the adenosine deaminase levels of different pleural effusion groups.
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.

References

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