Use of Adenosine Deaminase Activity in Pleural Fluid for the Diagnosis of Tubercular Pleural Effusion

ABSTRACT

Background: Adenosine deaminase (ADA) activity is a quick non-invasive method to diagnose tubercular pleural effusion (TPE) compared to other methods which are less diagnostic and time-consuming. A universal cut-off value for ADA in pleural fluid is needed since results vary widely from country to country and even within a given region.

The present study evaluated the effectiveness of the present cut-off point of (30 IU/L) in the diagnosis of TPE by determining the sensitivity and specificity and also evaluated the ability of the decreased cut-off point 25 IU/L to improve the sensitivity and specificity and thereby the diagnostic accuracy.

Materials & Methods: ADA was estimated in pleural fluid specimens of 104 patients who were admitted for pleural effusion by the colorimetric procedure of Galanti and Guisti.

Based on the cytological and radiological examination the patients were divided into Tubercular group and Non-tubercular group with 50 and 54 subjects respectively. The cut-off value for positive ADA result was 30 U/L according to clinical practice at this hospital.

Statistical analysis: A comparative correlation study of Adenosine deaminase levels in both groups was done based on Sensitivity/Specificity to diagnose the disease.

Results: The mean ADA level in Tubercular and in Non-tubercular group were 39.64 ± 15.6 IU/L and 13.17 ± 11.40 IU/L respectively with a p-value <0.001**, which is highly significant. The sensitivity, specificity was 74% and 90.74% respectively. Bringing down the cut-off value to 25 IU/L, the sensitivity improved from 74% to 84%, which is significant and the specificity decreased to 89% from 90% which is not significant.

Conclusion: The results of the present study it is suggested to bring down the cut-off value of Pleural Fluid ADA to 25 IU/L from 30 IU/L to improve the efficiency of this test in diagnosing Tubercular pleural effusion.

INTRODUCTION:

Tuberculosis is an endemic disease in several regions, particularly in developing countries. Its incidence also rises in developed countries. Pleural TB is one of the most common extra-pulmonary manifestations of the disease and may represent up to 10% of all cases. Tubercular pleural effusion (TPE) should be diagnosed at the earliest to avoid the occurrence of pulmonary or extra-pulmonary tuberculosis. Acute onset of fever, cough and pleuritic chest pain are the main presenting symptoms of the patients. The histological examinations of caseous lesions, microbiological methods such as acid-fast smears, and cultures take time for the confirmation of the diagnosis of tubercular pleural effusion and also have a low yield when done in pleural fluid. Acid fast bacilli (AFB) by the Ziehl-Neilsen on pleural fluid is positive in less than 5% of cases, and the culture on Lowenstein-Jensen medium takes more than four weeks and positive in less than 40% of cases. Therefore, a fast non-invasive test is required for the rapid diagnosis of TB.

Adenosine deaminase (ADA), which is produced from T-lymphocytes and also plays an important role in the maturation of the lymphocyte, is found to be a useful marker for the diagnosis of tubercular pleural effusion. The immune cellular response which occurs in the body against mycobacterium tuberculosis, suggests the possible role of ADA in the diagnosis of mycobacterium tuberculosis. Many studies support the fact that an increased ADA activity in body fluids also present in bacterial infections, rheumatologic diseases, and lymph proliferative disorders. The determination of adenosine deaminase activity in fluids including serum, CSF, pleural, peritoneal and pericardial is found to be useful in the diagnosis of tuberculosis. A universal reference cut-off value for ADA is not established yet in pleural fluid because of the varied results obtained among different countries and also in different regions of the same country. The present study was undertaken to evaluate the efficiency of present cut-off point for Adenosine deaminase activity (30 IU/L) for the diagnosis of tubercular pleural effusion by determining the sensitivity and specificity of the same and also to study whether the decreased cut off point of 25 IU/L essentially improves the sensitivity and specificity and thereby the diagnostic accuracy.

AIMS AND OBJECTIVE: Evaluation of the present cut-off point for Adenosine deaminase activity (30 IU/L) in the diagnosis of tubercular pleural effusion by determining the sensitivity and specificity of the same. Also evaluation of the ability of decreased cut off point to 25 IU/L to essentially improve the sensitivity and specificity and thereby the diagnostic accuracy.

MATERIALS & METHODS: 104 patients who were admitted for pleural effusion in a tertiary hospital in Bangalore were used as subjects for this study. Pleural tap was performed in these patients and the pleural fluid specimens were sent for the estimation ADA to the Biochemistry Laboratory of the hospital. The samples were analyzed immediately or within 48 hours after receiving the sample.

Patients both males and females aged between 20-60 years included in the study.

The patients were divided into two groups: The tuberculosis group included 50 cases confirmed by cytological examination of pleural fluid, radiological examination. The Non-tubercular group included 54 subjects with (1) malignant pleural effusion confirmed by cytological examination of pleural fluid/histological examination of pleural biopsy; (2) Pleural effusion of other known etiology such as congestive cardiac failure or para-pneumonic effusion. The cut-off value for positive ADA result was 30 U/L according to clinical practice at this hospital.

ADA analysis was done by the colorimetric procedure of Guisti and Galanti employing reagents optimized by Kaplan. All chemicals employed as stabilizing agents were of reagent grade.
A Comparative correlation study with 54 non-tuberculosis and 50 Tuberculosis patients was undertaken to study the Adenosine deaminase levels and its correlation based on Sensitivity/Specificity to diagnose the disease.

Statistical analysis: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance., Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (inter group analysis)

Diagnostic statistics viz. Sensitivity, Specificity, PPV, NPV and Accuracy, PLR, NLR, DOR have been computed to find the correlation of ADA for diagnosis with tuberculosis patients. Student t test (Two tailed, independent), Sensitivity and Specificity using standard statistical software package.\(^{(44,45,46)}\)

RESULTS:

Table 1: Age distribution of patients studied

Table 2: Gender distribution of patients studied.

Table 3&Fig1: Levels of ADA IU/L in two groups of patients; 1. Tubercular group and 2. Non-
tubercular group.

Table 4&Fig2: Mean of ADA in two groups of patients

Table 5: Components of diagnostic accuracy with a cut off value of 30 IU/L

Table 6 Components of diagnostic accuracy with a cut off value of 25 IU/L

DISCUSSION: ADA is required for converting Adenosine to Inosine in the Purine salvage pathway\(^{(16,21)}\). Its role in immune response was first identified by Giblett in severe combined immunodeficiency disease (Absence of ADA) in 1972. Increased levels were found in diseases such as Typhoid, Lymphoma, Leukaemia, and Tuberculosis which stimulates T-Lymphocyte response.

Table 1: Age distribution of patients studied

<table>
<thead>
<tr>
<th>Age in years</th>
<th>NON-Tuberculous group</th>
<th>Tuberculous group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>20-30</td>
<td>6</td>
<td>11.1</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>7.4</td>
</tr>
<tr>
<td>41-50</td>
<td>11</td>
<td>20.4</td>
</tr>
<tr>
<td>51-60</td>
<td>33</td>
<td>61.1</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>100.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>50.8±11.19</td>
<td>35.3±12.92</td>
</tr>
</tbody>
</table>

Table 2: Gender distribution of patients studied

<table>
<thead>
<tr>
<th>Gender</th>
<th>NON-Tuberculous group</th>
<th>Tuberculous group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Male</td>
<td>43</td>
<td>79.6</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>20.4</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>100.0</td>
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</table>

Table 3: Levels of ADA IU/L in two groups of patients

<table>
<thead>
<tr>
<th>Component of diagnostic accuracy</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90.74%</td>
</tr>
<tr>
<td>Specificity</td>
<td>88.10%</td>
</tr>
<tr>
<td>PPV</td>
<td>79.03%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>84.6%</td>
</tr>
<tr>
<td>PLR</td>
<td>7.4</td>
</tr>
<tr>
<td>NLR</td>
<td>0.22</td>
</tr>
<tr>
<td>Diagnostic odds ratio</td>
<td>31.04</td>
</tr>
</tbody>
</table>

Table 4: Mean of ADA in two groups of patients

<table>
<thead>
<tr>
<th>Component of diagnostic accuracy</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>82%</td>
</tr>
<tr>
<td>Specificity</td>
<td>89%</td>
</tr>
<tr>
<td>NPV</td>
<td>88.10%</td>
</tr>
<tr>
<td>PPV</td>
<td>84%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>85.5%</td>
</tr>
<tr>
<td>PLR</td>
<td>7.45</td>
</tr>
<tr>
<td>NLR</td>
<td>0.20</td>
</tr>
<tr>
<td>Diagnostic odds ratio</td>
<td>36.44</td>
</tr>
</tbody>
</table>
The present study evaluated the usefulness of ADA with a cut-off value of 30 IU/L to diagnose Tubercular pleural effusion cases efficiently. This study showed the significance of ADA in diagnosis of TPE with sensitivity and specificity of 90-100% and specificity of 89-100% utilizing GALANTI AND GIUSTI method (16). Gupta, D.K (47) found in 53 cases of pleural effusion 36 were of tubercular etiology, concluded the mean ADA level in tubercular cases as 50.75 IU/L while in cases of Non tubercular group comprising other causes as 14.47 IU/L and 28.65 IU/L respectively. His study showed the diagnostic value of ADA in diagnosis of TPE with sensitivity and specificity of 100% respectively for diagnosing tuberculosis. Burgess L.J (48) showed ADA activity in tubercular effusion was higher at a level of 50 IU/L with the sensitivity and specificity being 90% and 89% respectively. The most important finding of our study was the high negative predictive value for a cut off of 30 IU/L this allowed us to exclude TB in our community where there is a relatively high prevalence of TB pleuritis. Positive likelihood ratio of 7.4 showed moderate increase in the likelihood of disease and negative likelihood ratio of 0.22 to 0.20, increase in diagnostic odds ratio from 31.66 to 36.44 and decrease in negative likelihood ratio from 7.4 to 7.45 and decrease in negative likelihood ratio from 90% to 89% and from 89% to 88% which is not significant. But the specificity and negative predictive value of the study decreased from 90% to 89% and from 89% to 88% which is not significant. There is also significant increase in positive likelihood ratio from 7.4 to 7.45 and decrease in negative likelihood ratio from 0.22 to 0.20, increase in diagnostic odds ratio from 31.66 to 36.44 and increase in accuracy from 84.6% to 85.3% shows that decreasing the cut off value to 25 IU/L increases the diagnostic accuracy of the test. So from the results of the present study it is suggested to bring down the cut off value of Pleural Fluid ADA to 25 IU/L from 30IU/L to improve the efficiency of this test in diagnosing Tubercular pleural effusion.

### References

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works and what does not?


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