The clinical utility of Adenosine Deaminase (ADA) levels for the differentiation of tubercular and non-tubercular pleural effusion

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ABSTRACT

**Background:** Extra pulmonary TB accounts for 15% of all TB cases. Its incidence is much higher about 50% in HIV positive patients. Tuberculous pleurisy is present in around 4% of all TB cases. Tuberculosis is always the leading etiology of pleural effusions in the developing countries. **Aims and Objectives:** We have lot of investigations to diagnose pulmonary tuberculosis like sputum AFB, CBNAAT, LPA. These tests have some limitations in pleural fluid. So we are in need of a better test for diagnosis of tuberculous pleural effusion especially in high burden countries like India. **Methods:** In this study we have evaluated the usefulness of ADA level in pleural fluid for diagnosis of tuberculous pleural effusion. This study conducted from May 2017 to June 2018. **Conclusion:** This is a case control study done in a tertiary care center in Varanasi included 100 cases (Tuberculous pleural effusion) and 100 controls (Non Tuberculous pleural effusion).

Keywords: Tuberculosis, Adenosine Deaminase, Pleural effusion

Introduction

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis (MTB), mainly affecting the lungs. MTB infection leading to either a silent infection or turn into progressive disease. Burden of TB is a major health issue in developing countries. Two thirds of total TB cases were in eight countries. India (27%), China (9%), Indonesia (8%), Phillipines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%), South Africa (3%)¹. Pulmonary TB is the commonest form of TB, and a definite microbiological diagnosis is possible in most of the cases through sputum microscopy, culture and the use of molecular diagnostic tools like Cartridge Based Nucleic Acid Amplification Tests (CBNAAT) and Line Probe Assay (LPA).

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Pleural TB occurs as a result of a TB antigen entering the pleural space, usually through the rupture of a sub pleural focus, followed by a local, delayed hypersensitivity reaction mediated by CD4+ cells\textsuperscript{4}. This process may occur during primary or reactivation TB\textsuperscript{5}.

The presence of mycobacterial antigens in the pleural space elicits an intense immune response, initially by neutrophils and macrophages\textsuperscript{6,7}, followed by interferon (IFN) producing T-helper (Th) type 1 lymphocytes\textsuperscript{4,8}, resulting in a lymphocyte-predominant exudative effusion.

Diagnosis of tuberculosis in pleural effusion is challenging due to poor microbiologic confirmation rates from pleural fluid analysis\textsuperscript{15,16}. Even diagnostic tools like CBNAAT and interferon-gamma release assays (IGRA) have shown suboptimal diagnostic accuracy\textsuperscript{17,18}.

Conventional diagnostic tests for pleural TB include microscopic examination of the pleural fluid for acid-fast bacilli, mycobacterial culture of pleural fluid, sputum or pleural tissue, and histopathological examination of pleural tissue for Granulomatous inflammation. But these tests have recognised limitations for clinical use\textsuperscript{16}. As tubercle bacilli are mainly in the pleural wall and not within the fluid most of the times we could not find the microbiological evidence. So there is a need of simple, rapid and reliable diagnostic test to establish the etiology of pleural effusion.

Adenosine deaminase (ADA) is an enzyme which catalyses the deamination reaction of adenosine to inosine and plays an important role in the differentiation of lymphoid cells. Level of ADA increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. Adenosine deaminase (ADA), released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation. ADA1 is secreted by lymphocytes and monocytes, while ADA2 is secreted only by monocytes and is found in a higher concentration in TB pleuritis\textsuperscript{9,10}. The ADA2 isoenzyme released from monocytes and macrophages is the predominant contributor to total ADA activity\textsuperscript{11}. A high diagnostic accuracy of ADA activity measurement has been reported in several studies. Determination of ADA isoenzyme activity in pleural fluid may increase the accuracy of the test. However, because the additional yield is small, a study would require a very large sample size to demonstrate that isoenzymes have significantly higher specificity than total ADA activity. Usefulness of adenosine deaminase (ADA) estimation in pleural fluid may be
as a reliable chemical biomarker specially when there is suspicion of tuberculosis in endemic areas.

Although pleural fluid ADA may not be a perfect discriminator, its level is considerably elevated in patients with tuberculous pleural effusion. Therefore, presence of raised pleural fluid ADA may be considered a useful marker for diagnosis of tuberculous pleural effusion, especially in patients with exudative and lymphocytic pleural effusion in high TB burden settings. These patients can be started on anti-tuberculous therapy if no other investigation can provide a definite diagnosis\textsuperscript{13,14}. Similarly, low pleural fluid ADA may be useful in excluding TPE, especially in a patient with low pre-test probability \textsuperscript{12,13}. These patients usually require additional investigations to establish the etiology of pleural effusion.

Considering this a prospective hospital based study was designed to determine the pleural fluid ADA level and its interpretation in tubercular pleural effusion.

**Material and Methods**

This study conducted in Sir Sunderlal Hospital, BHU, Varanasi from May 2017 to June 2018. Patients more than 15 years of age who were undergone pleural aspiration for the first time included in this study.

Cases were defined by anyone of the following criteria:

1. TB PCR positive in pleural fluid.
2. Cough more than 2 weeks, fever with evening rise, night sweating, significant weight loss, loss of appetite. Any of these two symptoms with chest X-ray changes suggestive of tuberculosis.

Controls were defined by the pleural effusion which did not meet the criteria for cases.

The aspirated samples were collected under aseptic precautions and separated into 3 samples. One sample sent for ADA, one sample sent for routine microscopy, another one sent for Nested PCR. Total 100 cases and 100 controls were registered in study after detailed explanations of this study and written consent. Statistical analyses were done.
Observation & Discussion

In this study total 200 study subjects were consented and included, 100 cases and 100 controls. Mean age in the control group was 50.97 years, whereas in cases it was 41.7 years.

Table 1: Distribution of study subjects with respect to age groups

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>15-25</td>
<td>34</td>
<td>34.0</td>
</tr>
<tr>
<td>26-35</td>
<td>11</td>
<td>11.0</td>
</tr>
<tr>
<td>36-45</td>
<td>12</td>
<td>12.0</td>
</tr>
<tr>
<td>46-55</td>
<td>17</td>
<td>17.0</td>
</tr>
<tr>
<td>56-65</td>
<td>14</td>
<td>14.0</td>
</tr>
<tr>
<td>66-75</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>&gt;75</td>
<td>7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

In the control group 72 male and 28 female were enrolled, whereas in the cases 71 male and 29 females were enrolled. There is no statistical difference in sex between cases and controls.

Table 2: Sex wise distribution of cases and controls

<table>
<thead>
<tr>
<th>Sex</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Pleural fluid characteristics (mean values)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar (mg/dl)</td>
<td>112.4±64.7</td>
<td>84.4±53.6</td>
</tr>
<tr>
<td>Protein (gm/dl)</td>
<td>3.59±1.77</td>
<td>4.92±1.35</td>
</tr>
<tr>
<td>Total Cell (/cu mm)</td>
<td>4826±23142</td>
<td>26593±87379</td>
</tr>
<tr>
<td>Neutrophil Count</td>
<td>3457±21862</td>
<td>21589±77140</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>1157±1522</td>
<td>4422±10039</td>
</tr>
<tr>
<td>ADA (IU/L)</td>
<td>28.8±11.48</td>
<td>106.16±92.55</td>
</tr>
</tbody>
</table>

The sugar values in cases were reduced than controls (112 in controls vs. 84 in cases). Protein levels in cases were higher than controls (3.59 in controls vs. 4.92 in cases). ADA level is significantly higher in cases than controls (28 in controls vs. 106 in cases, p<0.001).
Most of the cases (94%) were having >50 IU/L ADA value in pleural fluid. Most of the controls (97%) were having <50 IU/L ADA value in pleural fluid which is statistically significant.

If we are considering the cut off value for ADA is 50, then the sensitivity of ADA for diagnosis is 97% & specificity is 94%, Positive Predictive value is 94%, negative Predictive value is 97%, Diagnostic accuracy is 95%.

## Conclusion

We can use pleural fluid ADA level for differentiating tubercular pleural effusion from non tubercular pleural effusion. At ADA 50 IU/L cut off value in pleural fluid has acceptable sensitivity and specificity & diagnostic accuracy. In majority of the cases pleural fluid routine microscopy is the initial investigation of choice to identify the etiology. We can use ADA also during initial tapping to diagnose or rule out tuberculosis. And it can be done as a day care procedure.

## References