Role of Adenosine Deaminase in Tubercular Pleural Effusion as a Diagnostic Index

Gindodia Pallavi G.¹, Dabhi Ajay S², M.Vadivelan³*

¹ Department of Medicine Global Hospitals, Hyderabad
² Department of Medicine Govt. Medical College, Vadodara
³ Department of Medicine JIPMER, Pondicherry

INTRODUCTION
Pleural effusion is an extra-pulmonary manifestation of tuberculosis (TB). TB remains one of the major causes of pleural effusion in India followed by suppuration and malignancy. Tubercular pleural effusion accounts for 4.6% of the total number of cases of extra-pulmonary TB¹. The definitive diagnosis of tubercular pleural effusion requires demonstration of Acid-Fast Bacilli (AFB) in Ziehl-Neelsen stained smear of pleural fluid or demonstration of characteristic granuloma in pleural biopsy.² ADA is a biological marker used in the diagnosis of tubercular pleural effusion. ADA was first identified by Conway and Cooke in 1939 in human erythrocyte³. It is present in the cytoplasmic fraction of T lymphocyte. An increased ADA activity is found where cell-mediated immunity is stimulated⁴. ADA may constitute a safe, cheap, useful and rapid diagnostic tool in the diagnosis of tubercular pleural effusion with a high degree of sensitivity and specificity.⁵

Aims & Objectives:
1. To study the level of Adenosine Deaminase (ADA) in pleural fluid and its correlation with various etiologies.
2. To evaluate the utility of ADA as a parameter in cases of tubercular pleural effusion.
3. To know the sensitivity and specificity of ADA in the diagnosis of tubercular pleural effusion.

*Corresponding Author
Dr. M. Vadivelan,
No.E-2, JIPMER Quarters, JIPMER Campus,
Dhanvantari Nagar P.O.,
Pondicherry-605006
Email:- mevadivelan@hotmail.com

MATERIALS AND METHODS
This prospective observational study was carried at a tertiary care Teaching Institute over a period of 2 years from June 2007 to May 2009. The study included 50 outdoor and indoor patients of exudative pleural effusion attending A.C.P.M.Medical College Hospital, Dhule, Maharashtra. The study was conducted after approval from the Institute Ethics Committee.

Inclusion criteria:
1. Patients above the age of 18 years.
2. Patients having radiologically confirmed pleural effusion in which diagnostic tapping of pleural fluid is possible.
3. Immunocompetent patients.

Exclusion criteria:
1. Patients suffering from enteric fever, viral hepatitis and renal disease.
2. Patients with past history of tuberculosis or history of anti-tubercular treatment in the past.

Laboratory Investigations:
1. Hemogram with Erythrocyte Sedimentation Rate (ESR)
2. Urine examination-Routine and microscopy
3. Blood urea and serum creatinine
4. Liver Function Tests (LFT)
5. Sputum examination-Gram’s staining and Acid-Fast Bacilli (by Ziehl-Neelsen stain)
6. Chest X-ray (PA view)
7. Pleural fluid analysis:
   a. Physical examination-Colour & appearance of pleural fluid
   b. Biochemical examination-Protein, glucose & Lactate Dehydrogenase (LDH)

ADA is a biological marker used in the diagnosis of pleural effusion. ADA was first identified by Conway and Cooke in 1939 in human erythrocyte. It is present in the cytoplasmic fraction of T lymphocyte. An increased ADA activity is found where cell-mediated immunity is stimulated. ADA may constitute a safe, cheap, useful and rapid diagnostic tool in the diagnosis of tubercular pleural effusion with a high degree of sensitivity and specificity.

Keywords: Pleural fluid Adenosine Deaminase (ADA), tubercular pleural effusion, extra-pulmonary tuberculosis
was carried out as per standard techniques of Galanti and Giusti.

c. Microscopic examination-Total cell count & differential cell count tubercular pleural effusion was diagnosed in patients with laboratory and radiologic investigations suggestive of TB and it was confirmed by- (1). Pleural fluid positive for AFB (2). Positive Mantoux test

RESULTS
Out of 50 cases of exudative pleural effusion included in this study, 34 were of tubercular etiology.

Table 1: Types of patients included in study group

<table>
<thead>
<tr>
<th>Category of patients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubercular</td>
<td>34 (68)</td>
</tr>
<tr>
<td>Non-tubercular</td>
<td>16 (32)</td>
</tr>
<tr>
<td>1. Empyema</td>
<td>5</td>
</tr>
<tr>
<td>2. Malignancy</td>
<td>9</td>
</tr>
<tr>
<td>3. Dengue fever</td>
<td>1</td>
</tr>
<tr>
<td>4. Catheter induced effusion</td>
<td>1</td>
</tr>
</tbody>
</table>

Mean protein levels in tubercular pleural effusion were highest among all the study patients having an exudative pleural effusion.

Table 2: Protein level in pleural fluid

<table>
<thead>
<tr>
<th>Etiology of pleural effusion</th>
<th>TB</th>
<th>Empyema</th>
<th>Malignancy</th>
<th>Catheter induced</th>
<th>Dengue fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (gm %)</td>
<td>4.8</td>
<td>3.92</td>
<td>4.38</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean pleural fluid LDH level was high in patients with tubercular pleural effusion.

Table 3: Pleural fluid LDH level

<table>
<thead>
<tr>
<th>Etiology of pleural effusion</th>
<th>TB</th>
<th>Empyema</th>
<th>Malignancy</th>
<th>Catheter induced</th>
<th>Dengue fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (IUL)</td>
<td>477</td>
<td>234.2</td>
<td>411.3</td>
<td>278</td>
<td>370</td>
</tr>
</tbody>
</table>

The present study showed that 94% of cases of tubercular pleural effusion had predominance of lymphocytes in pleural fluid.

Table 4: Differential cell counts in pleural fluid

<table>
<thead>
<tr>
<th>Differential cell count</th>
<th>TB</th>
<th>Empyema</th>
<th>Malignancy</th>
<th>Catheter induced</th>
<th>Dengue fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorph predominant</td>
<td>2</td>
<td>6.25 %</td>
<td>80 %</td>
<td>1</td>
<td>11.11 %</td>
</tr>
<tr>
<td>Lymphocyte predominant</td>
<td>32</td>
<td>94.1 %</td>
<td>20 %</td>
<td>3</td>
<td>33.33 %</td>
</tr>
<tr>
<td>Malignant cells</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>55.55 %</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean pleural fluid ADA level in tubercular effusion was 59.88 IU/L while it was 35.62 IU/L in non-tubercular pleural effusion. Pleural fluid ADA was positive in 32 cases of tubercular effusion and 2 non-tubercular cases. It was negative in 14 non-tubercular effusions and in 2 cases of tubercular effusion. Sensitivity of pleural fluid ADA was calculated to be 94.12% and specificity as 87.5%, considering a cut-off value of pleural fluid ADA as 40 IU/L.

DISCUSSION
Tubercular pleural effusion is a diagnostic challenge due to its non-specific clinical presentation and the inadequacy in its diagnosis by the traditional diagnostic methods. The diagnosis of tubercular pleural effusion is generally made by the demonstration of M.tuberculosis in pleural fluid or pleural biopsy specimen or by the presence of granulomatous reaction in the pleura. Direct smear of pleural fluid for AFB is positive in less than 10% of the patients while culture of the fluid may yield the organism in about 25% of cases. The presence of granuloma in the pleura which is suggestive of tuberculosis is usually seen in 60% of the cases. Analysis of the fluid in tubercular pleural effusion is a useful diagnostic aid. Tubercular etiology is suggested by lymphocytic predominance in the effusion (Presence of lymphocytes > 50%). Estimation of pleural fluid ADA, an enzyme of purine catabolism has been shown to provide promising results in tubercular pleural effusion. Levels above 40 IU/L are highly suggestive of tubercular etiology. High ADA activity in tubercular pleural effusion is a reflection of the local activation of T lymphocytes. The gold standard for the diagnosis of tubercular pleural effusion is culture of M.tuberculosis from the fluid. However, it is time consuming. The advanced techniques in the diagnosis of tubercular pleural effusion are-

1. Polymerase Chain Reaction (PCR)- It is the laboratory method of amplification of the Deoxyribonucleic acid (DNA) in a specimen. This can be used on raw, uncultured specimens and is capable of detecting a single mycobacterium in a biological sample.
2. Ligase chain reaction- It is a recently developed target amplification system used for the detection of special mycobacteria. In research laboratories, it has been demonstrated to have a very high sensitivity and specificity and can also be performed rapidly.
3. Cultures- There are 3 types of conventional culture media- a) Lowenstein Jensen medium b) Agar based medium c) Liquid based medium

TK medium is a newer culture technique that uses multi-colour dye indicators that can identify M.tuberculosis rapidly. It can also be used in drug susceptibility testing. Adenosine Deaminase (ADA) is an enzyme present in the cytoplasmic fraction of the cell and a certain amount is located in the nucleus. ADA activity is 10 times higher in lymphocytes than erythrocytes and particularly in T lymphocytes with variation according to cellular distribution. Human ADA consists of 3 iso-enzymes-ADA1, ADA1 + CP & ADA2.
Role of Adenosine Deaminase in Tubercular Pleural Effusion

1. ADA$_1$-It is a monomeric protein with a molecular mass of approximately 35 kDa. It is most abundant in spleen, lymphocytes and monocytes.
2. ADA$_1$ + CP-It is composed of 2 ADA$_1$ connected via a combining protein. (CP) It has a molecular mass of approximately 280 kDa. It is present in the liver, lung, muscle, pancreas and kidney.
3. ADA$_2$-This could be detected only in monocytes. It suggests that ADA$_2$ is an enzyme unique to monocyte/macrophage cell lineage. Since ADA$_2$ reflects monocyte/macrophage origin, it is responsible for ADA activity in TB.

Quantitative estimation of ADA- ADA activity is measured by spectrophotometric method described by Giusti and Galanti$^{13}$. The substrate in this method is adenosine and the ammonia liberated is determined by Berthelot reaction.

Other studies on ADA levels in pleural fluid- ADA levels in pleural fluid were significantly increased in clinically diagnosed as well as in biopsy confirmed cases of tubercular pleural effusion in a study done by Maldhure et al$^{14}$. Valdes et al in their study concluded that high total ADA activity in tubercular pleural effusion is mainly due to an increase in ADA$_2$ iso-enzyme. Ratio of ADA$_2$ and ADA activity helps in differentiating tubercular pleural effusion from empyema, but it is not useful in differentiating tubercular and malignant pleural effusion$^{15}$. Mathur et al in their study reviewed ADA as an excellent marker for the diagnosis of tubercular pleural effusion. They however, concluded that determination of patterns of iso-enzymes of ADA does not enhance the overall diagnostic value of ADA activity in pleural effusion$^{16}$. Limitation of the study- Pleural biopsy was not performed in this study. Hence, the sensitivity or specificity of pleural fluid ADA in comparison to pleural biopsy cannot be known from the present study.

CONCLUSION
Pleural fluid ADA estimation is a simple, rapid, cheap and easily available laboratory investigation for the diagnosis of tubercular pleural effusion. Though it cannot be used as a general screening test or as a fool-proof standard test for the diagnosis of tubercular pleural effusion, it has a valuable role as an adjuvant investigation with other diagnostic investigations of tuberculosis in countries with a high prevalence of tubercular pleural effusion. Pleural fluid ADA estimation serves as an additive and supportive evidence for the diagnosis of tuberculosis.

REFERENCES