Original Article

Study of Adenosine deaminase activity and IgM antibody level in the diagnosis of Tuberculosis: Hospital based cross-sectional study

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ABSTRACT

Background: Tuberculosis is one of the major causes of morbidity and mortality. Adenosine Deaminase (ADA) is an enzyme required for the conversion of adenosine to inosine through purine salvage pathway. The study was conducted to achieve following objectives: (1) To estimate Adenosine Deaminase (ADA) activity in pleural fluid and serum of tuberculous patients and determine its sensitivity and specificity, (2) To determine IgM antibody level in the serum of tuberculous patients and determine its sensitivity and specificity, (3) To determine pleural fluid to serum protein ratio in tuberculosis cases and controls.

Methodology: Hospital based comparative cross-sectional study was conducted. Subjects were grouped as pulmonary Tuberculosis (PTB) cases and non-PTB controls. Pleural fluid and serum of patients diagnosed with tuberculosis and controls were analyzed for ADA activity. Serum of both groups was tested for anti-TB IgM antibody. Results: In our study, 100 subjects (50 PTB cases and 50 non-PTB controls) were enrolled. Pleural fluid ADA activity was significantly higher in PTB cases (85.32±24.99 IU/L) than non-PTB controls (22.42±12.93 IU/L) (p<0.001). Serum ADA activity was also higher in PTB cases (32.40±7.07 IU/L) than controls (10.78±3.55 IU/L). The ADA assay had highest sensitivity (95.12%) and specificity (94.00%) at cut off of 40 IU/L in pleural fluid. For IgM antibody assay, a sensitivity and specificity of 63.10% and 82.0% respectively was obtained. Conclusion: The serum Adenosine Deaminase (ADA) is very useful marker of tuberculosis infection whereas serological test appears to be unreliable due to its low sensitivity.

1. Introduction

Tuberculosis is one of the major causes of morbidity and mortality, the global incidence of which is increasing by 0.4% per annum. TB is the second leading cause of death from an infectious disease worldwide (after HIV) which caused an estimated 1.8 million deaths in 2008.

Adenosine Deaminase (ADA) is an enzyme required for the conversion of adenosine to inosine through purine salvage pathway. The ADA activity is shown to be essential in the differentiation, proliferation and activation of lymphocytes and macrophages.

The host primarily regulates the tubercular infection through cell-mediated immune (CMI) response. CMI response causes macrophages to accumulate, become activated and destroy the bacilli when the latter are present at low levels. On contrary, the CMI response causes necrosis of tissues when bacillary antigens are present at high levels. Macrophage derived epitheloid giant cells and lymphocytes begin to accumulate at the site of bacilli and form granulomatous lesion. Dead and inactivated bacilli are rapidly destroyed within the granuloma with the release of large quantities of glycolipid and polysaccharide antigens in the associated lymph nodes and bloodstream which leads to humoral immune response. Also, delayed type hypersensitivity (DTH) and CMI response are induced as the viable mycobacteria release small quantities of glycoprotein antigens.

A number of mycobacterial antigens have been identified, such as 71, 65, 38, 23, 19, 16, 14 and 12-kDa proteins. The most extensively studied antigen is the 38-kDa protein which is an immunodominant lipoprotein antigen only specific for the M.
tuberculosis complex. IgG is considered to provide a strong evidence for the diagnosis of active tuberculosis both in children and in adults. IgM is the first antibody to be formed against any tubercular antigen and thus its measurement can be expected to provide diagnostic evidence for the recent tuberculosis.

In this study, we have measured the levels of ADA activity in pleural fluid and serum of PTB patients and non-PTB subjects and compared between the two groups. We have also measured protein concentration in pleural fluid and serum and attempt is made to correlate it with the tuberculosis infection. In order to observe the efficiency of antibody detection in the diagnosis of tuberculosis, we have measured the levels of IgM antibody in serum of the tuberculous patients and its sensitivity and specificity were determined.

**Methods:**

The study was conducted in B.P Koirala Institute of Health Sciences, Dharan, Nepal. The study populations were the patients visiting the Institute during the tenure of my study from March 2011 to March 2012. This was a hospital based comparative cross-sectional study among patients admitted in the Medicine wards or visiting the Medicine OPD of B.P Koirala Institute of Health Sciences (BPKIHS) of eastern Nepal. It included patients with final diagnosis of tuberculosis as cases and patients without PTB as controls.

Fifty patients with final diagnosis of pulmonary tuberculosis (cases) and fifty with non-tuberculosis respiratory disease (controls) were selected by convenient sampling method.

Sample collection and storage

About 5 ml pleural fluid was collected through thoracentesis by a trained physician and 2 ml blood were also collected from the same patient. Ethical permission was obtained from Institutional Ethics Committee. Informed consent was taken from all the participants prior to the sample collection. Pleural fluid and blood were centrifuged at 2,500 rpm for 10 minutes and after separation they were kept at -20°C until analysis.

**ADA activity estimation**

Pleural fluid and serum ADA activity were measured at 37°C by simple sensitive spectrophotometric methods of Giusti and Galanti based on modified Berthelot's reaction.

**Measurement of IgM antibody**

Antibodies against tubercular antigen (recombinant 38kDa protein) were measured by Enzyme Linked Immunosorbent Assay (ELISA) using the commercial kit (Pathozyme Myco M) purchased from Omega Diagnostics, Scotland, UK according to the manufacturer’s instruction. Absorbance readings were taken in HumaReaderTM. From the absorbance values of all the samples, antibody index (AI) was calculated. AI was defined as:

\[
\text{Antibody index (AI) = } \frac{\text{OD of sample}}{\text{average OD of low positive control serum}} \times 2
\]

According to the manufacturer, the interpretation of antibody index (AI) is made as follows:

Negative result: a negative result should have an AI less than 0.8.

Suspected positive result: A low or suspected positive result should have an AI in the equivocal zone (greater than 0.8 but less than 1.2).

Positive result: A positive result should have an AI greater than 1.2.

**Measurement of protein in serum and pleural fluid**

Protein concentration was measured by principle based on Biuret test using kits purchased from Agappe Diagnostics, India.

**Data analysis**

Data analysis was performed using the statistical package for social sciences version 11.5 (SPSS-11.5). Data are presented as mean ± SD. Mann-Whitney U test was applied for comparison between cases and controls. Receiver Operating Characteristic (ROC) Curve was plotted to compare the sensitivity and specificity of the ADA assay and IgM antibody assay. Result was considered significant if p-value was less than 0.05 (95% confidence level).

**RESULTS**

In this study, one hundred (n=100) individuals were enrolled (from age 10 to 88 years). The cases included patients with confirmed diagnosis of Pulmonary Tuberculosis (PTB) based on microbiological and clinical investigations. The controls included patients with other diseases except PTB such as malignancy, emphysema or other infections.

Table 1 shows the comparison of pleural fluid ADA activity between the PTB cases and controls. The increase in pleural fluid ADA activity (85.32±24.99) was observed in PTB patients as compared to controls (22.42±12.93).

<table>
<thead>
<tr>
<th>Group</th>
<th>ADA Activity (Mean±SD) (IU/L)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTB</td>
<td>85.32±24.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>22.42±12.93</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Comparison of pleural fluid ADA activity between PTB and control subjects
The table 2 shows comparison of serum ADA activity between PTB cases and controls which shows higher activity in tuberculosis cases than in controls.

Table 2: Comparison of serum ADA activity between Pulmonary TB and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>ADA Activity (Mean±SD) (IU/L)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTB</td>
<td>32.40±7.07</td>
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</tr>
<tr>
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<td>10.78±3.55</td>
<td></td>
</tr>
</tbody>
</table>

The pleural fluid and serum protein concentration were determined and a ratio of pleural fluid to serum protein was calculated from these values (represented in table 3).

Table 3: Comparison of ratio of pleural fluid to serum protein concentration between Pulmonary TB and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Ratio of pleural fluid to serum protein concentration (Mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary TB (n=50)</td>
<td>0.62±0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control (n=50)</td>
<td>0.25±0.50</td>
<td></td>
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</table>

The value of pleural fluid to serum protein ratio was higher in PTB cases than in controls.

Serum levels of anti-TB IgM antibody were determined by ELISA. From the absorbance of the test samples, antibody index (AI) was calculated as described in methods section. As represented in table 4, antibody index (AI) was higher in the PTB cases than in non-PTB controls.

Table 4: Comparison of antibody index (AI) between Pulmonary TB and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Antibody Index(AI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary TB (n=50)</td>
<td>1.20±0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control (n=50)</td>
<td>0.59±0.42</td>
<td></td>
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</tbody>
</table>

ROC curve was plotted to determine the sensitivity and specificity of ADA and IgM antibody assay. On ROC curve analysis for ADA assay, the best sensitivity and specificity were 95.12% and 94.00% respectively taking cut off value of 44 IU/L in pleural fluid. From the comparative analysis of ROC curve of the ADA and Antibody Assay, ADA assay was found to have more reliability for the diagnosis of TB than antibody test. The ROC curve for ADA assay and IgM assay is shown in the figure 1.

Discussion

The enzyme ADA is predominantly found in T lymphocytes and its raised activity in plasma is associated with infections such as tuberculosis in which cell-mediated immunity is stimulated. It can be inferred that the higher ADA activity in pleural fluid than in serum of PTB patients could be due to the local activation of T lymphocytes and synthesis of the enzyme in the pleural cavity. In non-tuberculous pulmonary diseases, ADA activity is relatively low. T lymphocytes are primarily involved in immune response against tuberculosis and once infection progresses, increase cell turnover takes place. Therefore, massive cell turnover may be the reason behind increase in protein concentration in pleural fluid in tuberculosis. Earlier studies have also shown increased levels of pleural fluid and serum ADA in a number of diseases where CMI is stimulated like typhoid, tuberculosis, and cancers.

The results of present study depicted in table 1 and 2 are in agreement to the previous study showing significantly increased ADA activity in PTB patients as compared to controls. The elevated level of ADA activity in pleural fluid and serum shows the involvement of either CMI or macrophage activation in pathogenesis of tuberculosis.

The determination of ratio of pleural fluid to serum protein concentration is helpful in classifying exudative or transudative pleural effusion. When the ratio is more than 0.5, it indicates the exudative pleural effusion. Tubercular infection in the lungs is usually accompanied by exudative pleural effusion, and thus this ratio can be helpful in assessment of tubercular pleural effusions.
The antibody index was significantly higher in PTB patients than controls indicating that IgM antibody assay may be used for screening tuberculosis infection. However, the sensitivity is low (63.10%) as determined from the ROC curve. Similar results were reported in 2008 by researchers using A60 as tubercular antigen.

Some studies point out that serological tests have high specificity and a much lower sensitivity in the adult population. The phase of the disease and the presence of mycobacteria in sputum are also the two important factors in which the test sensitivity depends on. Reasons for the low sensitivity of antibody test in the present study could be due to the chronic phase of the tuberculosis infection in which antibody level had been subsided.

The 38-kDa antigen is the most frequently studied serological antigen and it is a core component of many commercial TB serological test kits. The present study utilized the test kits containing recombinant 38-kDa antigen. Previous studies have reported lower sensitivities with the recombinant version of the 38-kDa antigen, with sensitivities of the ELISA technique ranging 16–36% for smear-negative patients and 36–67% for smear-positive patients. Therefore, serological tests cannot be taken as a reliable way of TB diagnosis.

WHO has been trying to develop new reliable tests for the rapid and accurate diagnosis of tuberculosis. The endorsement of new rapid test technique "Xpert MTB/RIF" in 2011 and negative policy on the use of commercial sero-diagnostics were one of the few steps taken by WHO.

Discussion

ADA activity assay has high sensitivity and specificity for TB diagnosis. However, serological tests for tuberculosis screening and diagnosis seem to be unreliable due to probability of more false negative results.

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