Original article:

Diagnostic significance of adenosine deaminase in cerebrospinal fluid and blood of tubercular meningitis patients

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Abstract

Introduction: Tubercular meningitis (TBM) remains a major global health problem and is the most dangerous form of extra-pulmonary tuberculosis. Therefore early, simple and inexpensive diagnostic test has always been a matter of concern especially in a developing country like India. Therefore we aimed in our study to find out the diagnostic significance of cerebrospinal fluid (CSF) and blood adenosine deaminase (ADA) in TBM patients with an objective to yield valuable information about its relative sensitivity and specificity in CSF and blood separately.

Materials and Methods: A total of 130 participants of both sexes, between 1 to 70 years of age were recruited in this study, out which 80 were diagnosed cases of TBM and 50 were controls. Quantitative estimation of ADA was done in CSF as well as serum samples of the participants by the method of Galanti and Giusti. Results were analysed statistically to arrive at the final conclusion.

Observation & Results: CSF as well as serum ADA was found to be statistically significantly higher in cases of TBM as compared to controls. CSF ADA value was 13.95±3.91 IU/L in cases and 6.05±2.37 IU/L in controls (p<0.001) whereas serum ADA value was 71.96±16.33 IU/L in cases and 25.85±10.49 IU/L in controls (p<0.001). Further, at the cut off value of 9 IU/L for CSF ADA, the sensitivity was 91.25% in tubercular meningitis patients.

Conclusion: Our study gave supportive evidence that CSF ADA is a sensitive, rapid and economical test for the early diagnosis of TBM while the levels of ADA in blood cannot be used as a diagnostic tool for TBM.

Key words: ADA, tubercular meningitis, CSF

Introduction

Tubercular meningitis (TBM) remains a major global health problem especially in a developing country like India and it is the most dangerous form of extra-pulmonary tuberculosis. Tubercular meningitis is a progressive, severe disease which may lead to death if untreated. It is not only associated with the high mortality, but also associated with the occurrence of neurological deficit in the survivors.1 Therefore early, simple and inexpensive diagnostic test is not only inevitable but also fundamental for its prompt treatment to avert the dreaded complications of meningitis.2

For the diagnosis of TBM, clinicians rely mostly on the clinical findings and the confirmation is done by actual demonstration of the acid fast bacilli. For this, the microscopic examination, PCR or culture has the mainstay. The search of a simple biochemical test becomes necessary especially in a country like India where the incidence and prevalence of tuberculosis is high.
Adenosine deaminase (ADA) is found in all mammalian tissues and the chief physiological function of ADA is related to lymphocytic proliferation and differentiation. It is secreted by T-lymphocytes and macrophages during infections. It acts as a marker of cellular immunity and chronic inflammatory conditions like tuberculosis. Therefore we planned our study to evaluate the diagnostic significance of ADA in cerebrospinal fluid (CSF) as well as blood of the patients of tubercular meningitis and the relevance of these tests in the clinical practice.

Our study was aimed to find out the diagnostic significance of adenosine deaminase (ADA) in cerebrospinal fluid (CSF) as well as blood and to elucidate if there is any association between them in TBM patients with an objective to yield valuable information about the relative sensitivity and specificity of this parameter in CSF and blood separately.

**Materials and methods**

A total of 130 participants of both sexes, between 1 to 70 years of age were recruited in this study. Out of which, 80 were the patients of tubercular meningitis. These formed our cases group. The diagnosis was done on the basis of clinical findings, which included fever, headache, nausea and vomiting, karnig’s sign, neck rigidity, altered sensorium or focal neurological deficit and seizure. A past history of tuberculosis or any exposure to the open case of tuberculosis or discontinuation of any anti-tubercular treatment (ATT), positive radiological findings of chest and brain were also used in the diagnosis of TBM.

CSF samples were taken from the patients, for the microbiological examination, culture, cytochemistry as well as biochemical analysis. CSF proteins, glucose as well as ADA were thus estimated as part of biochemical investigations.

Rest 50 subjects formed our control group. These were the patients who visited the hospital’s out-patient departments or admitted for other neurological problems not arising from any infective etiologies. CSF samples were collected for the biochemical investigations and ADA was estimated in them.

Blood samples were also collected in both the groups (cases as well as controls) for ADA estimation.

Quantitative estimation of ADA was done in the CSF as well as in the serum samples of the participants by the method of Galanti and Giusti based on Chaney and Marbach modification of Berthelot reaction.

Results were analysed statistically to arrive at the final conclusion.

**Results**

Tubercular meningitis is the most dangerous form of extra pulmonary tuberculosis which results from the haematogenous spread of primary or post primary pulmonary disease or from the rupture of subependymal tubercle into the subarachnoid space. Hence, it is important that the infection is diagnosed early and treated properly to minimize the morbidity and mortality. There are a number of tests for the diagnosis of tuberculosis. Recently, ADA has gained the attention of healthcare workers as a simple, rapid and inexpensive test for the diagnosis of tuberculosis.

We carried out the present study to evaluate the diagnostic significance of ADA levels both in CSF and serum of the tubercular meningitis patients.

Following were the findings of our study:

Tubercular meningitis can occur at any age but majority of our patients were in pediatric age group as depicted in the figure 1.
It was observed that the levels of adenosine deaminase in the CSF and serum showed remarkable variations in different age groups. Comparison of mean values of CSF-ADA and serum ADA in different age groups (Table 1 and Figure 2) in the present study revealed that the mean value of both CSF-ADA (16.8 IU/L) and serum ADA (77 IU/L) was elevated in 60-70 years age group.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value of CSF-ADA (IU/L)</td>
<td>11.00</td>
<td>12.26</td>
<td>9.29</td>
<td>11.57</td>
<td>13.07</td>
<td>14.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Mean value of Serum-ADA (IU/L)</td>
<td>46.3</td>
<td>51.3</td>
<td>49.3</td>
<td>59.21</td>
<td>62</td>
<td>67.1</td>
<td>77</td>
</tr>
</tbody>
</table>

Table 1: Differences of Mean Value of CSF-ADA and Serum ADA in different age groups of TBM cases.

Figure 2: Mean values of ADA in CSF and serum of the TBM patients.
Table 1 and figure 2 also indicate that there is a decreased mean value of both CSF-ADA (11 IU/L) and serum ADA (46.3 IU/L) in 0-10 years of age groups. Further on comparing the levels of CSF and serum ADA among cases and control groups, we found that the values were significantly higher in tubercular meningitis patients as compared to control subjects (p value <0.001) (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>CSF-ADA (IU/L)</th>
<th>Serum ADA (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>TBM</td>
<td>80</td>
<td>13.95</td>
<td>3.91</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>6.05</td>
<td>2.37</td>
</tr>
</tbody>
</table>

Table 2: Comparison of levels of CSF ADA and serum ADA in TBM patients and control groups

One of the important objectives of our study was to establish a cut off level for CSF ADA and serum ADA because different studies by different workers suggest that there is a lack of consensus over the cut off values of serum or CSF ADA levels owing to the lack of standardisation of the methods used for ADA estimation.

Thus, for this purpose different cut off levels were used and their sensitivities and specificities were compared (Table 3 and 4). CSF-ADA estimation with the cut off value of 9 IU/L (Table 3) in the present study had the maximum sensitivity and specificity i.e. 91.25% and 90% respectively.

Table 3: Sensitivity and specificity at different cut off points of CSF-ADA

However serum ADA estimation had a maximum specificity at the cut off level of 61 IU/L i.e. 95% but then there is a little compromise regarding its sensitivity at this level i.e. 84.81% (Table 4).

Table 4: Sensitivity and specificity at different cut off points of Serum ADA
Further there was a statistically significant correlation between CSF and serum ADA level in tubercular meningitis patients (correlation coefficient, r=0.314, p value <0.01).

**Discussion**

Tubercular meningitis is a severe, progressive disease which may lead to death if untreated. It is not only associated with high mortality, but also with the occurrence of neurological deficit in survivors. Hence, it is important that infections are diagnosed early and treated properly to minimize the morbidity and mortality.

Early confirmatory diagnosis of TBM is difficult to establish because of its pleomorphic clinical presentation. Delayed diagnosis and treatment may be associated with many serious CNS complications.

The most commonly used laboratory method for the definitive diagnosis of TBM is to demonstrate the presence of tubercle bacilli either by smear and/or culture. However, direct smear methods are often negative in CSF samples and culturing of Mycobacterium tuberculosis takes 4–6 weeks to show the growth. Newer methods such as those involving the amplification of bacterial DNA by the PCR and comparable systems are incompletely assessed and not available for the widespread use in the developing countries. The sensitivity of the PCR technique varies from 33% to 90% and the specificity from 88% to 100% which implies that in this technique, DNA is amplified several times which increases the sensitivity of this method tremendously but then the specificity is compromised several folds especially in the presence of even minute contamination. Moreover the sample volume has to be adequate for the success of this technique.

Various immunoassays such as antigen and antibody detection in CSF samples have been developed with variable sensitivities and specificities. But despite of the extensive work on TBM, only a few diagnostic tests are available. Currently, TBM and early diagnosis is a global issue and is becoming more and more crucial. All relevant studies share the view that ADA is a useful test in early diagnosis of TBM.

In our study also, it was observed that the CSF ADA levels were significantly high in tubercular meningitis patients as compared to the controls. So far very few studies have addressed to the importance of the evaluation of serum ADA levels and thus we evaluated the serum ADA levels also in the tubercular meningitis patients and compared with the control subjects. Interestingly, these levels were also found to be significantly higher in patients as compared to controls.

ADA is present in every tissue but its level is 10 times higher in lymphocytes than in erythrocytes and particularly in T-lymphocytes which always vary according to cellular differentiation. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes. ADA deficiency is associated with severe defects in the cell mediated and the humoral immune system predisposing the patient to opportunistic infections. In several diseases, where cellular immunity is stimulated, there is an increase in ADA levels in serum and other body fluids.

We also aimed to find out the association between CSF and serum ADA levels in tubercular meningitis and found a significant positive correlation between CSF and serum ADA levels in patients as compared to the controls. This was attributed to the fact that there is stimulation of T-cells by mycobacterial antigens in TB patients. Thus there is an increase in the ADA levels in the body fluids including serum.

Moreover, to evaluate the efficacy of CSF and serum ADA to be used as diagnostic tools we further established the cut off values for both
CSF and serum ADA by comparing their sensitivities and specificities at different levels. On comparing the sensitivities of CSF ADA and serum ADA, it was found that the sensitivity of CSF ADA was higher especially when a cut off value of 9 IU/L was chosen and the sensitivity of serum ADA was less as compared to CSF ADA. This gives us supportive evidence about the use of CSF ADA being a more reliable screening test as compared to serum ADA. Regarding the specificity of both CSF ADA and serum ADA, it was found that the specificity was 95% when higher cut off values were chosen in both the cases, but then the sensitivity is highly compromised. This may go in favour of these cut offs i.e. 11 IU/L for CSF ADA and 61 IU/L for serum ADA to become diagnostic markers.

Keeping in mind and comparing both the sensitivity and specificity of CSF ADA and serum ADA, we came to the conclusion that there is a strong evidence that CSF ADA can be used as an appropriate screening test for tubercular meningitis when the cut off value of 9 IU/L is used, owing to its high sensitivity and specificity.

As far as the status of serum ADA is concerned, it lags behind being an appropriate test for the purpose of screening or diagnosis of tubercular meningitis. This is attributed to its low sensitivity and specificity at different cut offs used in our study. Nevertheless serum ADA has 95% specificity at a cut off value of 61 IU/L. But at this cut off there is a compromise on its sensitivity. So it can be used as supportive or complementary test for the diagnosis of tubercular meningitis but not solely as a screening or diagnostic test.

However an extensive research over a wide population based study would be more effective in consolidating the establishment of the use of ADA in CSF and serum as the putative diagnostic markers for the tubercular meningitis.

References


