Cerebrospinal Fluid Adenosine Deaminase Activity for the Diagnosis of Tuberculous Meningitis in Children

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Summary
Adenosine deaminase (ADA) activity was measured in the cerebrospinal fluid (CSF) of 27 subjects suffering from tuberculous meningitis (TBM), 19 from bacterial meningitis, 10 from encephalitis, and 10 control subjects. The mean CSF ADA level was significantly raised (P<0.001) in TBM patients as compared to other study groups. A cut-off CSF ADA level of >5 IU/l was considered for the diagnosis of TBM, and the test had sensitivity and specificity of 89 and 92 per cent, respectively. Overall, it was found to be a better test in comparison to any other single test for the diagnosis of TBM. Confirmed TBM patients had significantly higher CSF ADA activity when compared with clinical TBM (P<0.01) and the levels did not differ significantly among different stages of disease. The ADA level in TBM cases had significant correlation with CSF cell count (P<0.01), lymphocyte percentage (P<0.02) and protein concentration (P<0.02). Thus, the CSF ADA activity assay was found to be a simple, useful and rapid diagnostic test for the early recognition of TBM in children.

Introduction
Tuberculous meningitis (TBM) still remains an important cause of morbidity and mortality in children. Often it poses a diagnostic problem to the clinicians and the prognosis of disease is closely related to the stage at which the treatment is started. The definitive diagnosis of TBM depends upon the detection of acid fast bacilli in the CSF and culture of Mycobacterium tuberculosis, which takes about 6–8 weeks. The characteristic CSF cytological and biochemical changes are also variable and may even be absent.

Adenosine deaminase (ADA) is an enzyme which is required for lymphocyte proliferation and differentiation, and the principal biological activity is detected in T-lymphocytes. Raised levels of enzyme have been found in tuberculous pleural, peritoneal and pericardial fluids and cerebrospinal fluid (CSF) of patients with TBM. However, its level for the diagnosis of TBM in children was found to be less reliable in some studies in comparison to adults. The present study was conducted with the following objectives:

1. to evaluate the usefulness of CSF ADA activity for the diagnosis of TBM in children;
2. to find out the enzymatic activity in confirmed, clinical TBM at different stages of the disease;
3. to observe the relationship, if any, between CSF ADA activity with other CSF parameters.

Patients and Methods
A total of 66 children, aged 2 months to 12 years, were selected from those admitted to or attending the Out-Patients Department of Children Hospital, Banaras Hindu University, Varanasi, India, and were divided into four groups for the study.

Group-I (TBM)
1. Confirmed TBM. This group included patients with positive CSF culture for Mycobacterium tuberculosis or CSF profile as in meningitis along with culture/biopsy evidence from any other site.
2. Clinical TBM. This group included patients in whom CSF was negative by smear and culture, but had compatible history, CSF biochemical abnormalities, positive Mantoux/BCG test, lesion in chest X-ray, CT scan, and response to anti-tubercular therapy.

The patients of TBM were also divided into three stages for CSA ADA analysis.

Group-II (bacterial and partially treated bacterial meningitis)
This group included patients with clinical history,
positive CSF culture, and/or Gram’s stain, CSF showing polymorphonuclear leukocytosis, low sugar, high protein with or without positive blood culture and response to antibiotics.

**Group III (encephalitis)**
This group included patients with clinical history, normal CSF, or pleocytosis and or elevated protein with spontaneous resolution.

**Group IV (controls)**
This group included patients with seizures having normal CSF biochemistry, negative on culture, negative Gram’s staining, with no prior antibiotic therapy and uneventful subsequent course.

**ADA assay**
The CSF ADA assay was performed as described by Giusti. The CSF samples were kept in a deep freezer and assay was done within a week. Optical density was measured spectrophotometrically (using UV-1201 Shimadzu Spectrophotometer) at 265 nm in an assay mixture (final volume 2 ml) containing 0.025 mM adenosine, 10 mM Tris HCl (pH 7.4), 0.15 M NaCl, 1.25 per cent Glycerol and 0.1 ml CSF. One unit of activity represents the deamination of one micromole of adenosine per min at 37°C temperature and expressed as IU/l.

Student’s t-test was used to analyse the data for statistical significance, and correlation and regression coefficients were also calculated among different parameters.

**Results**
The mean CSF ADA activity in different study groups is presented in Table 1; their individual levels are shown in Fig. 1. The mean ADA activity was significantly higher (P<0.001) in TBM patients in comparison to bacterial meningitis, encephalitis, and controls. In the TBM patients, 24 of 27 (89 per cent) cases had ADA levels of > 5 IU/l, whereas only three out of 39 (8 per cent) non-TBM group had values above this level and these cases belonged to partially treated bacterial meningitis. By taking a cut-off ADA level of > 5 IU/l for the diagnosis of TBM, the test had sensitivity and specificity of 89 and 92 per cent, respectively. The relative sensitivity, specificity, predictive values and accuracy of this CSF ADA level were compared with other routine tests performed for the diagnosis of TBM and is shown in Table 2. Overall, CSF ADA was found to be a better test for the diagnosis of TBM as compared to any other single test.

Out of 27 TBM patients, four were confirmed TBM and the rest 23 cases belonged to clinical TBM. The mean CSF ADA activity in the confirmed TBM (15.5 IU/l) was significantly raised (P<0.01) as compared to clinical TBM group (8.3 IU/l). The CSF ADA levels in relation to various stages of TBM are given in Table 3. The mean enzymatic activity rose from stage I to III TBM, but the difference was found to be statistically insignificant.

Table 4 shows the correlation of CSF ADA activity with other CSF parameters in TBM patients. It was observed that enzymatic activity had significant positive correlation with cell count (P<0.01), lymphocyte percentage (P<0.02) and protein con-


**TABLE 2**
The sensitivity, specificity, predictive values, and accuracy of various parameters for the diagnosis of tuberculous meningitis

<table>
<thead>
<tr>
<th>Screening parameter</th>
<th>Cut-off</th>
<th>Mantoux test positivity</th>
<th>CSF cell count (&lt;500/mm³)</th>
<th>CSF lymphocyte (&gt;70%)</th>
<th>CSF protein (≥100 mg/dl)</th>
<th>CSF sugar (&lt;40 mg/dl)</th>
<th>CSF AFB smear and culture positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>88.9</td>
<td>62.9</td>
<td>96.3</td>
<td>100</td>
<td>85</td>
<td>66.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.3</td>
<td>100</td>
<td>76.9</td>
<td>33.4</td>
<td>53.4</td>
<td>56.4</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>88.9</td>
<td>100</td>
<td>74.3</td>
<td>52.9</td>
<td>56.0</td>
<td>51.4</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>92.3</td>
<td>79.6</td>
<td>96.7</td>
<td>100</td>
<td>84</td>
<td>70.9</td>
<td>61.9</td>
</tr>
<tr>
<td>Accuracy</td>
<td>90.9</td>
<td>82.1</td>
<td>84.8</td>
<td>61.9</td>
<td>66.7</td>
<td>60.6</td>
<td>63.6</td>
</tr>
</tbody>
</table>

All values expressed as percentages.

**TABLE 3**
CSF ADA levels in relation to stages of TBM (n = 27)

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of cases</th>
<th>CSF ADA (IU/l)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2</td>
<td>6.7±1.0</td>
<td>6.0-7.5</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>11</td>
<td>7.4±4.4</td>
<td>4.0-17.0</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>14</td>
<td>11.3±5.9</td>
<td>5.5-22.5</td>
<td></td>
</tr>
</tbody>
</table>

P-value: Stage I v. II, NS; Stage I v. III, NS; Stage II v. III, NS.

The result of the present study showed that mean CSF ADA level in TBM patients (9.4 IU/l) was significantly raised (P<0.001) as compared to bacterial meningitis, encephalitis and controls; a finding similar to that of other studies.10-14 The mean CSF ADA levels in TBM cases of pediatric age groups have been reported to be ranging between 11.6-13.7 IU/l in previous studies.6-8 A relatively higher mean CSF ADA values (15.7-21.3 IU/l) have been observed in adult TBM patients.9,10,16 These results show that ADA secretion by T-lymphocytes in response to mycobacterial antigen vary and lower activity observed in CSF of pediatric TBM patients may be due to difference in immunological reactivity to tubercular antigen in children as compared to adults.

In the present study, a cut-off CSF ADA level of > 5 IU/l was considered for the diagnosis of TBM with sensitivity and specificity of 89 and 92 per cent, respectively. Overall, it was found to be a better diagnostic test in comparison to any other single test like CSF biochemistry, Mantoux test and CSF smear

**TABLE 4**
Relationships of CSF ADA with other CSF parameters in TBM patients (n = 27)

<table>
<thead>
<tr>
<th></th>
<th>ADA (IU/l)</th>
<th>Cell count (per/mm³)</th>
<th>Lymphocyte (%)</th>
<th>Protein (mg/dl)</th>
<th>Sugar (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>9.4</td>
<td>228.6</td>
<td>91.6</td>
<td>210.7</td>
<td>37.9</td>
</tr>
<tr>
<td>SD</td>
<td>5.4</td>
<td>133.6</td>
<td>6.7</td>
<td>144.5</td>
<td>18.3</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.5776</td>
<td>0.4503</td>
<td>0.4380</td>
<td>0.2336</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.01</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SE of r</td>
<td>0.163</td>
<td>0.178</td>
<td>0.179</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>b value</td>
<td>0.023</td>
<td>0.363</td>
<td>0.016</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>a value</td>
<td>4.10</td>
<td>-23.83</td>
<td>6.03</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

Discussion
The raised ADA activity under antigenic stimulation shows its importance in rapid proliferation of cells in order to prevent the accumulation of toxic metabolites and thus reflects good cell mediated immunity.13

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and culture positivity for *Mycobacterium tuberculosis* (Table 2) Ribera et al.\(^5\) have also demonstrated similar findings in their study of adult TBM patients.

Previously conflicting results have been shown regarding the diagnostic value of CSF ADA activity in children as compared to adult TBM patients. Many studies have demonstrated a high diagnostic value with sensitivity of 96–100 per cent in adults.\(^{9,10,14,15}\) However, Malan et al.\(^6\) and Coovadia et al.\(^8\) found overlapping CSF ADA levels between TBM and bacterial meningitis pediatric patients, and the authors concluded that it is of lesser help in diagnosis of childhood TBM. In our study, only three cases had false positive levels in non-TBM group and they belonged to partially treated bacterial meningitis. Moreover, our observation seems to confirm the usefulness of CSF ADA activity for the diagnosis of TBM in children.

A significantly higher CSF ADA activity has been observed in confirmed TBM as compared to clinical TBM cases (*P* < 0.01); a finding in accordance to the observation of Selvakumar et al.\(^7\) In contrast, no significant difference between the two groups has been reported by other workers.\(^8,15\) The higher enzymatic level in confirmed TBM may be due to persistent antigenemia leading to more pronounced immunological response. This is further supported by the observation of Ribera et al.\(^9\) who showed an increase in CSF ADA level during the first 10 days of anti-tuberculous therapy and the authors pointed out that increase in enzymatic activity may be because of greater stimulation of T-cells by the release of antigen due to bactericidal effect of chemotherapy.

A comparison of CSF ADA level is relation to different stages of TBM showed that enzymatic activity did not differ significantly between various stages of disease indicating that raised level was detectable even in stage I TBM, although this group had only two cases. Thus, it can also help in early diagnosis of the disease.

The mean CSF ADA activity in TBM patients correlated well with CSF total cell count, lymphocyte percentage and protein concentrations. This was in agreement with data of Malan et al.\(^6\) and Prasad et al.\(^15\) However, CSF sugar level did not produce any significant difference in CSF ADA levels in these patients. The increase in CSF ADA activity with increasing CSF cell count or lymphocyte percentage in these patients thus indicate local immune response due to lymphocyte proliferation in response to antigen. Alternatively, the raised enzymatic level may be because of seepage across the damaged blood brain barrier permitting ADA to enter in the CSF from blood or adjacent cerebral tissue.\(^6\)

Thus, it is evident that CSF ADA activity determination is a useful test for the early diagnosis of TBM. Since it is simple, relatively inexpensive and takes less time to perform, it can be included as rapid diagnostic test for TBM in children.

### References