Cerebrospinal fluid Adenosine deaminase: its evaluation as a marker for diagnosing tuberculous meningitis in paediatric patients.

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Abstract: Five hundred thousand patients of tuberculosis die every year in India. Delay in diagnosis and in initiating treatment results in poor prognosis. This disease is affecting the children more and more. Meningeal tuberculosis has acquired an endemic shape with about 12% incidence. The aim of the present study is to evaluate Cerebrospinal fluid Adenosine deaminase: as a marker for diagnosing tuberculous meningitis

Eighty paediatric patients of age group 6-24 months having symptoms and signs of meningitis were divided into two groups: tuberculous and non-tuberculous, as per the accepted criteria. The CSF was drawn and ADA levels estimated in all patients.

Out of 38 tuberculous patients, 36 had CSF ADA at or above the cutoff value, while 02 had below. Out of 42 non-tuberculous patients, 04 patients had at or above while 38 were below the cutoff value. Results of our study indicate that ADA levels in CSF are not only of considerable value in the diagnosis of TBM; CSF ADA level 10 IU/L as a cutoff value exhibited 94.73% sensitivity; 90.47% specificity; 90.00% positive predictive value and 95.00% negative predictive value.

Cerebrospinal fluid Adenosine deaminase estimation is less expensive, rapid and fairly specific method for the diagnosis of tuberculous etiology in TBM; it may prove to be diagnostic marker for diagnosing tuberculous meningitis in paediatric patients.

KeyWords: Adenosine deaminase (ADA), Cell-mediated immunity (CMI), Cerebrospinal fluid (CSF), Tuberculous meningitis (TBM).
Adenosine deaminase levels (ADA) has been considered by several researchers to differentiate tubercular disease from non-tubercular [6,7,8,19,20 & 21]. ADA is released by T cells during cell mediated immune response (CMI) to the tubercle bacilli. Literature survey reveals that some reports are in favor while some are against the role of CSF ADA in the diagnosis of TBM; we planned the present study not only to estimate ADA levels in CSF but also to evaluate its role as a marker for diagnosing tuberculous meningitis.

II. Material And Methods

After obtaining the informed consent from the guardian of the patient, routine procedures of history taking, clinical examination and routine investigations were carried out. A total of 80 patients form paediatric population falling in the age group of six to twenty four months having symptoms and signs of meningitis, admitted in the Pediatrics ward from Jan 09 to November 12 at Subharti Medical College and associated ChhatrapatiShivajiHospitals, Meerut, U P, India were included in the present study. Presence of first or a minimum of two of the following accepted criteria was adopted to label a case as tuberculous:

1. Bacteriological proof of the presence of Mycobacterium tuberculosis; either positive direct smear or AFB culture in sputum and/or CSF.
2. Tissue biopsy showing caseating granulomas.
3. Radiological findings consistent with TB.
4. Clinical presentation consistent with TB.
5. Definite clinical and radiological improvement in two months after specific anti-tubercular treatment.
6. History of contact with current disease and positive reaction (>10 mm induration) to 5 tuberculin unit (TU) purified protein derivative (PPD).

Amongst a total of 80 patients, 56 children were male and 24 were female. Out of these, 38 patients having fulfilled the criteria were labeled as tuberculous, while the other 42 were labeled as non-tuberculous. Amongst tuberculous group (n=38) only four patients had evidence of presence of Mycobacterium (10.53%); two in CSF smear, one in sputum smear and one in CSF culture positive.

Lumbar puncture was undertaken in each case and atleast 2 ml of clear CSF was collected in a sterile tube without anticoagulant for total ADA quantification. The enzyme was stable for 24 hours at 25°C, for 7 days at 4°C and for 3 months at -20°C [9,10]. Hemorrhagic CSF was excluded from the study. This CSF was subjected to biochemical and microscopic examination. Total ADA activity was estimated in all these patients by the method reported by Guisti[9] by estimating the rate of hydrolysis of adenosine to ammonia and inosine. The ammonia so formed on reaction with Phenol and hypochloride gave a blue colored indophenol complex which was measured spectrophotometrically. The results were expressed as IU/L. One international unit of total ADA is defined as the amount of enzyme required to release 1μmol ammonia per minute from adenosine under standard assay conditions. The cutoff reference range of 10 IU/L for CSF ADA was taken as positive for TB, as required by the manufacturer of the chemicals used. The values of CSF-ADA in tuberculous (n=38) and non-tuberculous (n=42) are expressed in terms of Mean ± S D. Z-test (double sample) was applied to test the significant difference between the two groups for their ADA levels. Further, sensitivity, specificity, positive predictive value and negative predictive value were also calculated.

III. Observations And Results

CSF ADA at or above the cutoff value was observed in 36 patients in tubercular group (n=38), while 02 had ADA below cutoff value. In non-tubercular group (n=42), 04 patients were found to have CSF ADA at or above the cutoff value while 38 had values below the cutoff (Table 1).

In tuberculous group ADA activity in CSF ranged between 9.2 to 110 U/L with a median of 22, mean ± S D as 27.1684 ± 22.4563while in non-tuberculargroup ADA activity ranged between 2 to 10.5 U/L with a median of 6, mean ± S D as 6.0619 ± 2.5399.

On comparison of CSF ADA in the two groups, Z value was 6.0345 and p value was .000097, the difference was found to be highly significant (p<.001) (Table 1).The screening for TBM by estimating CSF ADA activity was evaluated according to the standard formulae. The sensitivity of the test was found to be 94.73%, specificity 90.47%, positive predictive value 90.00 % while negative predictive value was 95.00% (Table 2).

IV. Discussion

Demonstration of AFB in direct smear, culture, cytochemistry, and CT scan are the various means to confirm the etiology of TB. The visualization of AFB in direct smear or in cultures of CSF is present in very low percentage of patients and in majority of patients it is negative and so the diagnosis is usually difficult [11]. In the present study we found the evidence of AFB only in 10.53% of the tubercular cases. Newer methods such
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The ADA had been considered as a marker of cell-mediated immunity and its activity has been observed in various infections including TBM. Both humoral and cell-mediated immunity play an important role in TBM infection. It has been suggested that ADA activity in CSF may help to differentiate TBM from non-TB infectious meningitis. Not only this, CSF - ADA has been reported to be useful in differentiating TBM from normal subjects and patients with other neurological disorders. CSF – ADA estimation is also a useful method to differentiate TBM from aseptic meningitis. Other researchers have also observed the usefulness of CSF-ADA activity in the diagnosis of TBM.

We have observed a statistically highly significant difference in the CSF - ADA levels of meningitis due to tuberculosis and non-tuberculous etiology (P < .001) (Table 1), which indicates that ADA levels in CSF are of considerable value in diagnosis of TBM and in differentiating this disease from other etiologies. At 10 IU/L cut-off value of CSF ADA our study exhibited sensitivity of 94.73%, specificity of 90.47%, positive predictive value 90.00% and overall accuracy 95.00% (Table 2) for the diagnosis of tuberculous meningitis.

The levels of ADA in CSF of adult patients of TBM have been evaluated in earlier studies. Elevated levels of ADA in CSF are not specific to meningeal inflammatory disease but it can be a test for confirming its etiology with good predictive value. Raised ADA levels have also been noted in other conditions particularly in certain intracranial tumors. In earlier study the mean ADA levels in CSF of TBM cases of pediatric age groups has been reported to be ranging from 11.6-13.7. The level of 15.7-21.3 has been observed in adult TBM patients. These results show that levels of ADA vary in different age groups in TBM. This might be due to difference in immunological reactivity to tubercular antigen in children as compared to adults.

Literature reports that when the cutoff value 11.39 IU/L was taken; the researcher obtained sensitivity of 82% and specificity as 83% in TBM cases. Rana et al. have taken 10 IU/L as cutoff value for diagnosis of TBM and found sensitivity as 66.6% and specificity 90% Baheti et al. found that CSF ADA may differentiate tuberculous from non-tuberculous meningitis even at a cut-off level of 6.5 IU/L. Gupta et al. observed that ADA levels in nontuberculous disease rarely exceeded the cut-off value; set for tuberculous disease. They have further observed that ADA estimation is not only a fairly sensitive and specific test (more than 90%), helpful in differentiating tubercular from non-tubercular etiology; both in pulmonary and extra-pulmonary disease but is also simple, inexpensive and rapid. For these reasons this test may help in early diagnosis, improve the prognosis and reduce spread of disease and sequelae.

In the present study, median ADA levels in CSF were significantly higher in TBM patients as compared to those with other etiologies. Ribera et al. have also demonstrated similar finding but his study was in TBM patients of adult age group.

Although Tuon et al. in their meta-analysis concluded that, ADA cannot distinguish between bacterial meningitis and TBM, but these observations are based on studies having heterogeneity, publication bias and nonstandardized method for ADA estimation and so further studies are needed to obtain reliable observations.

Gupta et al. have further found the sensitivity of this test to be 94.73%; specificity 90.47%, positive predictive value is 90.00% and negative predictive value 95.00% and they concluded that ADA estimation in CSF is not only simple, inexpensive and rapid but also fairly specific method for making a diagnosis of tuberculous etiology in children with meningitis.

The authors are of the opinion that estimation of CSF- ADA is less expensive, easy to perform, provides instant results and the present study exhibited a high specificity and sensitivity with fairly good negative and positive predictive values; therefore estimation of ADA in CSF may be established as a marker for diagnosis of tuberculous etiology in paediatric patients of meningitis.

V. Conclusion

Cerebrospinal fluid Adenosine deaminase estimation is less expensive, rapid and fairly specific method for the diagnosis of tuberculous etiology in TBM; especially when there is a dilemma of differentiating the tuberculous etiology from non-tuberculous and for this reason ADA analysis may find a place as a marker for diagnosing tuberculous meningitis. CSF- ADA is not only helpful in the diagnosis of TBM but can also be useful to differentiate it from other causes with fairly good accuracy; and which is simple, quick to perform and cost effective test.
Table 1: Distribution of the cases according to accepted criteria and CSF ADA levels:

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No of cases</th>
<th>ADA levels in U/L</th>
<th>No of cases</th>
<th>Mean ± SD</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous</td>
<td>38</td>
<td>ADA ≥ 10</td>
<td>36</td>
<td>27.1684 ± 22.4563</td>
<td>6.0345</td>
<td>.000097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADA &lt; 10</td>
<td>02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-tuberculous</td>
<td>42</td>
<td>ADA ≥ 10</td>
<td>04</td>
<td>6.0619 ± 2.5399</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADA &lt; 10</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td></td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (p<.001) shows highly significant difference between tuberculous and non-tuberculous groups.

Table 2: Distribution of the cases according to true and false positivity and negativity with statistical accuracy:

<table>
<thead>
<tr>
<th>Cell entries</th>
<th>Number of cases</th>
<th>Statistical indices of diagnostic accuracy</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>36</td>
<td>Sensitivity</td>
<td>94.73%</td>
</tr>
<tr>
<td>False Negative</td>
<td>02</td>
<td>Specificity</td>
<td>90.47%</td>
</tr>
<tr>
<td>True Negative</td>
<td>38</td>
<td>Positive predictive value</td>
<td>90.00%</td>
</tr>
<tr>
<td>False Positive</td>
<td>04</td>
<td>Negative predictive value</td>
<td>95.00%</td>
</tr>
</tbody>
</table>

Reference