Evaluation of CSF ADA Level in diagnosis of Tubercular Meningitis

P. L Prasad[1], Sandhya Chauhan[2], Bhawana Khurana[3]

ABSTRACT

Introduction: Adenosine Deaminase Activity (ADA) was measured in 60 patients of TBM, 51 patients of Acute Bacterial Meningitis and 45 Controls and with the help of ROC (Receiver Operating Characteristic) curve, a cut-off point was identified to determine the best level for ADA activity which can differentiate Tuberculous Meningitis in early stages reliably from Acute Bacterial Meningitis. Study aimed to evaluate the significance of Cerebrospinal fluid Adenosine Deaminase level in diagnosis of tubercular meningitis and to find out cut off level with optimum specificity and sensitivity.

Material and methods: ADA activity in the CSF was calculated based on ammonia liberated from

Adenosine and quantified spectrophotometrically in children aged 6 months to 16 years who got admitted as cases of Suspected Meningitis in the Indoor Patient Department of Paediatrics of a Tertiary Level Hospital and Teaching Institute. Arithmetic mean values and standard deviation of ADA in each group were measured. A receiver operating characteristic curve was plotted to identify various cut-off points to determine the best level for ADA activity. The following measures of accuracy of the Diagnostic test e.g., sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), Positive Predictive Value (PPV) and Negative Predictive Value were computed.

Results: The mean and Median level of ADA in CSF was significantly higher in TBM group of patients ($p<0.05$) (10.00 and 11.00, SD±4.13, 95%CI 8.9333-11.0667) as compared to patients with Acute Bacterial Meningitis (6.15 and 6.00, SD±3.90, 95% CI 5.0598-7.2539) and Controls (5.733 and 7.00, SD±2.47, 95%CI 4.9908-6.4759). The area under curve (AUC) was at the CSF ADA cut off level of 10U/L, the sensitivity was 68.3%, specificity was 92.7%, PPV was 85.4%, NPV was 82.4% and the Positive Likelihood Ratio was 9.7.

Conclusion: Spectrophotometric analysis of ADA is a rapid diagnostic test and at value of 10U/L can help in diagnosing TBM and differentiating it from Acute Bacterial Meningitis in early stages.

Keywords: CSF, ADA, Tubercular Meningitis

INTRODUCTION

Central Nervous system infections are the commonest cause of CNS disease in children. These infections are potentially serious with a high rate of complications and risk of long term morbidity. Meningitis in children is a medical emergency and requires prompt initiation of management since the outcome is critically dependant on early treatment. TBM is the commonest type of meningitis in children between 9 months to 5 years of age in the developing countries and as many as 60% of cases of meningitis are tuberculous in origin.[1] Case fatality rates from Tubercular meningitis (TBM) in India is around 1.5 per 100,000 population.[2] In India, there is a dearth of epidemiologic data on the etiological profile of causative organisms of meningitis in children. The available studies suggest that despite the availability of adequate medications, the mortality and morbidity rate due to tubercular meningitis remains significantly high in India and other developing countries.[3] Whatever may be the etiological profile, due to low yield of organisms in CSF, the main challenge in Indian scenario is to differentiate between TBM and Acute Bacterial Meningitis (ABM) in early stages.

A reliable, cost-effective and rapid diagnostic test, feasible in any standard pathology laboratory is, therefore, a necessity. ADA is one such test which can be performed easily during CSF examination. A study was undertaken to evaluate the utility of CSF adenosine deaminase (ADA) level in diagnosing tubercular meningitis and differentiating it from other types of meningitis.

MATERIALS AND METHOD

The study has been carried out in a tertiary care teaching hospital at Bareilly, U.P for duration of 18 months. This cross sectional study included 111 patients who were admitted in the Indoor Department of Paediatrics with clinical features of fever, headache, vomiting and altered sensorium. All patients in the age group of 6 months to 16 years admitted in pediatric ward or PICU with complaints of fever, headache, vomiting and altered sensorium were enrolled in the study. Patients who received antibiotic treatment or anti tubercular treatment prior to admission and those with post traumatic meningitis, brain abscess, meningitis in post operative neurosurgical condition and partially treated pyogenic meningitis were excluded.

The patients were subjected to detailed history taking and physical examination. They were categorized as Suspected Meningitis (Group 1). Age and sex matched 45 children were taken as control (Group 2). Cerebrospinal Fluid (CSF) was examined for cytology, glucose, total protein concentration, Gram stain, Ziehl-Neelson stain, ADA level and Bacterial and tubercular bacilli Culture and sensitivity.

Acute Bacterial Meningitis was diagnosed if along with

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clinical meningitis, all of the following were present: a) Duration of illness ≤6 days, b) CSF total leucocyte count >1000 cell/mm³, c) CSF neutrophils ≥75%, d) CSF sugar ≤40 mg/dl, e) response to antibiotic therapy in 72 hrs of admission. The patients were considered to be suffering with TBM if along with fever lasting for more than 2 weeks and CSF evaluation showing pleocytosis more than 20 cells with more than 60% lymphocytes, protein more than 100 mg/dl and sugar <60% of random blood sugar. In addition to the above mandatory criteria any two of the following: (a) evidence of extra neural tuberculosis, (b) positive Family history, (c) positive mantoux test >10mm, (d) abnormal CT findings two or more of the following (i) exudates in basal cisterns, or in sylvian fissure, (ii) hydrocephalus, (iii) infarct, and (iv) gyral enhancement.[3]

ADA activity in CSF was determined according to a method based on the Berthlot reaction, which is the formation of a colored indophenol complex from ammonia liberated from adenosine, and quantified spectrophotometrically.

STATISTICAL ANALYSIS

Medians, arithmetic mean values and standard deviation of ADA in CSF were measured in cases of TBM, ABM and in Controls. A receiver operating characteristic (ROC) curve was plotted to identify various cut-off points to determine the best level for ADA activity in the CSF of patients with TBM. Comparisons were labeled as statistically significant at p-value of <0.05.

The following measures of test accuracy for each study, e.g., sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), Positive Predictive Value (PPV) and Negative Predictive Value were computed. The analysis was based on the Receiver Operating Characteristic (ROC) curve.

RESULTS

Out of the 111 suspected cases of meningitis, 39 were in the age group of 6 months-5 years (20 males and 19 females) and 72 were in the age group ≥ 6 years (36 males and 36 females). 45 patients were enrolled in the Control Group (27 in the age group of 6 months-5 years, 18 in the age group ≥ 6 years with 37 males and 8 females).

Based on criteria decided for the study, 60 cases were diagnosed as Tubercular Meningitis and 51 cases were of Acute Bacterial Meningitis. The mean age of the children in TBM was 7.12±3.62 years, and in the ABM were 7.27±4.27 years and in the Controls group were 6.61±4.48 years.

The mean and Median level of ADA in CSF was significantly higher (p<0.05) in TBM patients as compared to patients with Acute Bacterial Meningitis and Controls Group. 41 patients in the TBM group had ADA levels >10u/l whereas

ADA Values	TBM (n=60)	ABM (n=51)	Control (n=45)
Mean	10.0000	6.1569	5.7333
Median	11.0000	6.0000	7.0000
95%CI	8.9333-11.0667	5.0598-7.2539	4.9908-6.4759
Ranges	1.00-23.00	1.00-16.00	1.00-9.00
S.D.	4.12927	3.90063	2.47166
P value		P<0.01	P<0.01

Table-1: Comparison of CSF ADA between TBM, ABM and Control

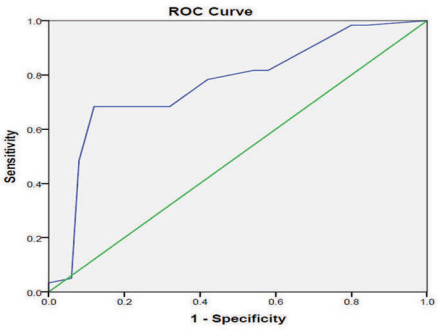

Figure-1: Sensitivity and specificity

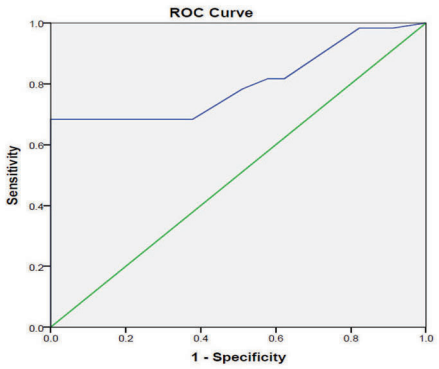

Figure-2: Sensitivity and specificity

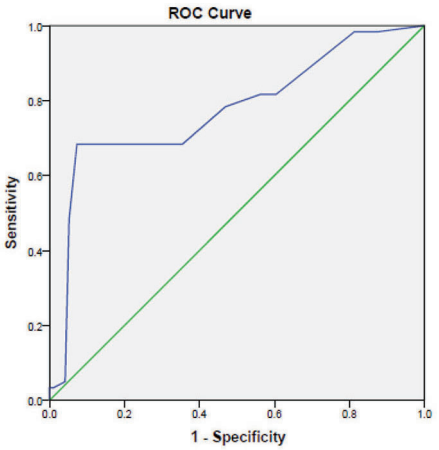

Figure-3: Sensitivity and specificity

Cut Off CSF ADA u/l	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR-positive
7.5	78.3%	53.1%	51.1%	79.7%	1.7
8.5	68.3%	88.5%	78.8%	81.7%	5.9
10	68.3%	92.7%	85.4%	82.4%	9.37

Table-2: Positive and Negative predictive values

19 had values below 10u/l. Seven patients suffering from ABM had ADA >10u/l and the rest had values below 10u/l. In the Controls Group, none of the patients had ADA values more than 10u/l.

ADA activity in CSF was weighted to emphasize different cut off points. Sensitivity was 80% at a cut off value of 7u/l and decreased to 68.3% at the cut off value of >10u/l. However, in this range, the specificity increased from 53% to 92.7%. At the cut off value of >10u/l, the positive predictive value was 85.4% and the Positive Likelihood ratio (LR) was 9.7 (fig) and a Negative Likelihood ratio of 0.34 (table-1).

The Optimum cut off for the diagnostic index by which to classify a patient as having TBM was, therefore, found to be >10u/l. But at this cut off, 19 out of 60 TBM patients were missed (table-2).

The area under the curve (AUC) was 0.766 (95%CI:0.67-0.86) when ADA values of TBM are compared to that in Acute Bacterial Meningitis (figure-1).

When ADA activity in CSF is used to differentiate TBM from Controls, the AUC is 0.801 (95%CI:0.72-0.89) (figure-2). On comparing the ADA activity in CSF of the probable TBM Vs non-TBM patients, the AUC is 0.775 (95% CI 0.694-0.856) (figure-3).

This ROC curve plot (fig 1) is showing the sensitivity and specificity at various cut off levels. At an ADA cut off of 3.5U/L, the sensitivity is highest of 98.3% but the specificity is hardly 18.7%. According to this plot, the best cut off value of ADA in CSF to differentiate TBM from non-TBM patients is 10.0U/L with a sensitivity of 68.3% and specificity of 92.7%.

On comparing the CSF ADA results, no difference was found in different age groups and similarly no difference was found in male Vs female children.

DISCUSSION

Meningitis is a severe, life-threatening infection and requires immediate initiation of treatment to reduce mortality and morbidity. Outcome of the disease depends on the appropriate treatment of the suspected or known causative organism. The condition becomes complicated in our country due to endemicity of tuberculosis. The biggest challenge at the time of the presentation of the patient is to differentiate TBM in its early stages from ABM so that timely antitubercular treatment can decrease the mortality, morbidity and long term sequelae associated with TBM.

Early clinical diagnosis of any form of meningitis is challenging because of non-specific symptoms and low number of organisms in cerebrospinal fluid (CSF). Moreover, the diagnosis of TBM in early stages can be difficult and may be based only on clinical and preliminary cerebrospinal fluid (CSF) findings without definitive microbiologic confirmation. Given the relatively low sensitivity of acid-fast smear (20%-40%) and inherent delay in culture along with its low sensitivity (40-80%), numerous studies have been conducted in search of cheap, rapid, sensitive and specific methods of diagnosis for TBM.[4,5]

Adenosine Deaminase (ADA) is an enzyme which is involved in purine catabolism and plays a role in the catabolism of monocytes, macrophages and T lymphocytes. An increase in ADA is observed in tuberculosis as well as other infections in which the cellular activity response is actively involved. Its measurement is simple, inexpensive and rapid. The diagnostic value of ADA levels in CSF in meningitis cases has been investigated in studies, and ADA levels were found to be significantly high in TBM groups as compared to other bacterial meningitis and viral meningitis. Thus it can be used as a supportive diagnostic finding for TBM along with the routine CSF analysis.[6]

Studies conducted on the diagnostic accuracy of ADA in CSF for finding the different cut offs and sensitivity and specificity of this test in diagnosing TBM have largely focused on either adult or a mixed population. There are few studies that have considered only children as the study population, so this study was designed to find out the accuracy of ADA estimation in differentiating TBM in early stages from acute bacterial meningitis and other meningitis in children.

The present study included children between the age groups 6 months to 16 years. Rana et al included children in the age group 6 months-11 years in their study. Few other studies have included children varying from 2 months to 12 years.[7,8,9,10]

The mean ADA levels recorded in the present study were 10U/L in TBM which was significantly higher ($p<0.01$) than that of ABM (6.16U/L) and controls (5.7U/L). The mean CSF ADA values in TBM ranged from 10.0U/L to 23.0U/l in the different studies and were significantly higher than the CSF ADA values in other CNS infections.

We observed CSF ADA levels more than 10U/L in 7 out of 51 cases of ABM whereas none had levels above 8U/L in the controls group. CSF ADA level was considerably higher in all cases of TBM in previous studies[7,8,9,10] as compared to the present study where out of 60 cases of TBM, 19 had ADA levels below the cut off of 10U/L.[10] had ADA value of 4U/L, 2 had ADA value of 6U/L, 6 had ADA value of 7U/L, and there was 1 patient who had ADA value of only 1U/L).

In the study by Rana et al[11], the sensitivity of ADA test in CSF for diagnosing TBM at cut off of 10 U/L was 66.6%. The specificity was highest while taking Aseptic Meningitis as control (100%) and was lowest while taking Pyogenic meningitis group as control (80%). The overall specificity was 90%. The study by Shinde et al[9] calculated the sensitivity and specificity of CSF ADA test at a cut of level of 10U/L(sensitivity 90%,specificity 97.14%,accuracy 95%) as well as 15U/L (accuracy 94% sensitivity 86.11%, specificity 98.43%).Study by Bindu et al[8] calculated a sensitivity of 90.62% and a specificity of 95.65% at a cut off of 10U/L.

In the present study, we tried to find out the cut off value with maximum specificity and sensitivity. A Receiver Operating Characteristic curve was plotted to identify various cut off points to determine the best level for ADA activity in the CSF of patients with TBM. At a cut off value of 7U/L of CSF ADA level, sensitivity was found to be 80% and specificity decreased to 68.3%. Moreover, at the cut off value of 10U/L, specificity increased from 53% to 92.7%. At this cut off value

of 10U/L, the study recorded the highest positive predictive value at 85.4%, the Positive Likelihood ratio (LR+) at 9.36 and the negative likelihood ratio(LR-) was 0.34. As diagnostic tests with negative likelihood ratio less than 0.2 can help in ruling-in a disease, this means that a positive ADA value cannot definitely label a patient as that of TBM but a negative ADA value can definitely label a patient as not having TBM. The AUC was 0.76. The results in the present study also suggest that on lowering the cut-off value of ADA, the sensitivity of the test increases, although the specificity of the test declines severely. At a cut off of 3.5U/L, only one patient diagnosed as TBM would be missed. In a study by Ekermans et al, at a cut off of 2.0U/L, 13 out of 92 cases of TBM were still missed although both the LR+&LR- were quite significant.[16] Thus, both the resent study as well as other stdies suggest a reasonable performance of CSF ADA, missing out of cases is still a concern.

Various other studies have evaluated the diagnostic significance of CSF ADA activity at different cut off points. Moghtaderi et al[12] in 2009 conducted a study on a mixed population of adults and children. They also plotted CSF ADA at different cutoff value of 9.5, 10.5, 11.5 U/l and 12.5 U/L. They reported a decrease in sensitivity from 86% at cut off value of 9.5U/L to 76% at the cut off value of 12.5U/L. In the same range, they reported an increase in specificity from 71% to 91%, and that of PPV from 75% to 89%. The highest accuracy (83%) was noted at the cut off value of 10.5U/L which is quite similar to the present study. Choi et al reported that the sensitivity of the ADA test for TBM compared with ABM was 0.83 and the specificity was 0.95 when a cut-off value of 7 IU/L was used and was 0.58 and the specificity was 0.89 when a cut-off value of 10 IU/L was used.[13] López-Cortés et al reported that the sensitivity of the ADA test for TBM compared with ABM of 48% and specificity of 100% at a cut-off of 10 IU/L.[14] In study by Karsen et al[6], when the cutoff value for CSF ADA activity for differential diagnosis of TBM and ABM was accepted as 12.35 IU/L, the sensitivity of the test for the diagnosis of TBM was 92% and the specificity was 100% whereas when the cut-off value of CSF ADA activity for TBM and ABM differentiation was accepted as *6.45 IU/L,* the sensitivity of the test for TBM was100% and specificity was 92%.

The results of present study suggest the sensitivity and specificity of ADA varies widely at different cut off value of CSF ADA, There is no consensus for accepted cut off value for CSF ADA level.

A study by Solari et al[15], which evaluated the utility of individual changes in protein, glucose, chloride, and ADA levels and the presence of lymphocytic pleocytosis in CSF as key elements for guiding decision-making in the first hours after admission, when appropriate initiation of anti-tuberculous treatment can prevent disability and mortality, reported ADA as the parameter that in isolation performed the best, followed by protein level (area under the ROC curve 82.1% and 75.5%, respectively). Protein level was the most sensitive test, while ADA was the most specific one. The latter attained a positive likelihood ratio of 10.7.

The present study did not evaluate the correlation of ADA with CSF pleocytosis, sugar or protein, and moreover, the diagnosis of Probable TBM was made considering clinical history and physical examination to be the gold standard. Out of 60 probable cases, 41 had ADA levels more than the appropriate cut off value as found by the ROC curve. That means the ADA values can be used as a bedside rapid complementary tool to assist clinical history and physical examination in correctly diagnosing TBM.

Conclusion - The sensitivity of the test in diagnosing TBM could have been further increased when combined with the levels of CSF pleocytosis, protein and sugar levels.

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