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ADENOSINEDEAMINASE ACTIVITY IN CEREBROSPINAL FLUID FOR DIAGNOSIS OF TUBERCULOSIS MENINGITIS

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ABSTRACT

Background:Tuberculous meningitis remains a serious clinical problem. Missed diagnosis and delayed treatment result in significant morbidity and mortality. Aims and Objectives: The study was aimed to estimate the cerebrospinal fluid adenosine deaminase levels in clinically suspected cases of meningitis and to evaluate the usefulness of CSF-ADA as a diagnostic test in tuberculosis meningitis.Methods: Adenosine deaminase activity (ADA) was studied in cerebrospinal fluid of 30 cases of tuberculous meningitis, 10 cases of pyogenic meningitis. 14 cases of aseptic meningitis and 15 controls (patients without any neurological disorders who were given spinal anesthesia).Results: The mean cerebrospinal fluid adenosine deaminase activity was 14.1±1.96; 4.92±1.27; 3.66±1.03 and 1.69±0.44 U/l in tuberculous meningitis, pyogenic meningitis, aseptic meningitis and control respectively. The adenosine deaminase activity in tuberculous meningitis cases was significantly higher. The sensitivity and specificity of this test for diagnosis of tuberculous meningitis was 100% and 97.44% respectively with ADA value of more than 10 U/L. Conclusion: Adenosine deaminase activity in CSF is a rapid, relatively inexpensive and easy procedure, can be of great value in the early diagnosis of tuberculous meningitis, help in earlier institution of appropriate treatment and thereby prevent mortality and complications.

KEY WORDS: Cerebrospinal fluid, Adenosine deaminase, Tuberculous meningitis.

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INTRODUCTION

Tuberculosis is a burning problem of the world. The global increase in the incidence of tuberculosis in both immunocompetent as well as immunocompromised individuals is a health issue of universal concern. Factors that have contributed to this increase are the acquired immuno-deficiency syndrome (AIDS) and the problem of multi-drug-resistant tuberculosis (MDR-TB)[1]. Advanced age, alcoholism, drug abuse, poverty, malnutrition, transmigration, lymphoma and immunosuppressive medication also contribute to increased susceptibility[2]. CNS tuberculosis, particularly tuberculous meningitis (TBM) remains a serious clinical problem. Missed diagnosis and delayed treatment result in significant mortality and morbidity. The use of polymerase chain reaction (PCR) to detect *mycobacterium tuberculosis* specific DNA may be of potential value. However, the facilities to perform the test are not available in all laboratories and many patients are not affordable because of its high cost. Adenosine deaminase (ADA) is an enzyme of purine catabolism, catalyzing hydrolytic deamination of adenosine to inosine. Detection of high level of ADA has shown promising results in the diagnosis of tuberculous pleural, peritoneal and pericardial effusions[3,4]. The present study is undertaken to assess the usefulness of adenosine deaminase assay for the diagnosis of tuberculous meningitis.

METHODOLOGY

A total of fifty four cases clinically suspected of meningitis, admitted in Basaveshwar Teaching & General Hospital, Gulbarga attached to M.R. Medical College, Gulbarga during the period between April 2004 to February 2006 and also in Government General hospital Gulbarga attached to M.R. Medical College, Gulbarga from April 2004 to January 2005 were selected. All the participants in the present study gave informed consent and the study was approved by the institutional ethics committee.

The cases were selected based on the following criteria

1. Age more than 12 years
2. Only fresh cases were taken, old partially treated cases were excluded.
3. Patient should not have received any specific therapy outside the hospital.
4. Some cases whose clinical picture is similar to meningitis like subarachnoid hemorrhage, cerebral malaria were excluded after relevant investigations.

Of the selected fifty-four cases, thirty cases were of tuberculous meningitis and ten cases of pyogenic meningitis and fourteen cases of aseptic meningitis. Fifteen patients without any neurological disorders who needed spinal anesthesia were included as controls. All the cases were thoroughly

examined clinically by performed proforma and investigated as follows

Ahuja et al[5] used a set of criteria using clinical features, CSF examination, CT findings and evidence of extra neural tuberculosis to divide suspected tuberculous meningitis patients into highly probable, probable and possible tuberculous meningitis groups, and tested its validation using bacterial isolation, polymerase chain reaction (PCR) test for tuberculosis, response to treatment and autopsy and found them reliable in making early diagnosis of tuberculous meningitis. In the present study, these valid set of criteria are used to categorize tuberculous meningitis patients.

The criteria are as follows:

A) Clinical features

- Fever and headache more than 14 days (mandatory)
- Vomiting, alteration of sensorium, focal deficits (optional).

B) CSF showing

- Pleocytosis with more than 20 cells
- More than 60% lymphocytes
- Sugar less than 60% of corresponding blood sugar
- Negative Indian ink preparation studies
- Malignant cytology (when relevant)

C) CT showing one or more of

- Exudates in basal cistern
- Hydrocephalus
- Infarcts
- Gyral enhancement

D) Active extraneural tuberculosis as evidenced by appropriate

- Radiological tests
- Microbiological tests or
- Ceseation necrosis on histopathological examination.

Tuberculous meningitis subgroups
Based on the above criteria the following classification is made

1. Highly probable ..... A + all of B, C, D
2. Probable..... A + any 2 of B, C, D
3. Possible..... A + any 1 of B, C, D

Diagnosis of Pyogenic Meningitis

It is done by Gram's staining and culture of CSF.

CSF ADA Activity

CSF-ADA activity was estimated in all fifty-four cases spectrophotometrically by Guisti and Galanti method and comparison is made between the levels in tuberculous meningitis, pyogenic meningitis, aseptic meningitis and controls. ADA is reported to be stable in serum for 3 days at 2-8ºC and in biological fluids for 2 days at 2-8ºC, as after this, ammonia may be released in the samples even without any microbial contamination.

Reference Values

CSF	Normal	< 10 U/L
	Positive	> 10 U/L

It is recommended that each laboratory establish its own normal range representing its patient population.

RESULTS

A total of 30 patients were studied (02 highly probable, 08 probable & 20 possible cases) based on the classification of tuberculosis meningitis patients using Ahuja clinical criteria as shown in Table-1. Among the 30 patients 17 were males 47.05% & 13 were females 43.33% peak incidence is seen in the age group of 21-30 years as depicted in Table-2. The mean CSF ADA levels were significantly higher in TB meningitis group (n=30) 14.1 U/L, when compared with Pyogenic meningitis group (n=10) 4.92 U/L, Aseptic meningitis group (n=14) 3.66 U/L and control group (n=15) 1.69 U/L as shown in Table-3. As per Table-4 there is no statistical difference in the CSF-ADA levels in culture positive and culture negative tuberculosis meningitis cases. The CSF sugar values were less than 40 mg% in 26 cases, and more than 40mg% in 4 cases of TB meningitis group. There is no correlation between CSF sugar and CSF ADA levels as shown in Table-5. The CSF protein levels in TB meningitis group showed direct correlation with CSF ADA levels as depicted in Table-6, similarly direct correlation was found between CSF lymphocyte count and CSF ADA levels in TB meningitis group as shown in Table-7. Taking 10 U/L as the cut-off value of CSF ADA

activity and comparing with CSF ADA levels in controls, a sensitivity of 100% specificity of 97.44% with a positive predictive value of 100% is obtained for this test.

Table-1
Classification of tuberculous meningitis patients using Ahuja Clinical Criteria

TBM sub-groups	No. of patients	Clinical criteria	Supportive CSF routine microscopy	CT positive cases	Evidence of extra neural tuberculosis
Highly probable (A+ all of B,C,D)	2	2	2	2	2
Probable (A+ any 2 of B,C,D)	8	8	8	6	2
Possible (A+ any 1 of B,C,D)	20	20	14	2	4

Table-2
Age wise distribution of Cases

Age in years	Male		Female		Total	
	No.	Percent	No.	Percent	No.	Percent
≤ 10	--	--	--	--	--	--
11 – 20	5	29.41	4	30.76	9	30.00
21 – 30	8	47.05	5	38.46	13	43.33
31 – 40	4	23.52	3	23.07	7	23.33
41 – 50	--	--	1	7.69	1	3.33
>50	--	--	--	--	--	--
Total	**17**	**100.00**	**13**	**100.00**	**30**	**100.00**

Table-3
CSF ADA levels in different groups

Group	No. of cases	CSF ADA Levels (U/L)		
		Mean	SD	Range
A-TB meningitis	30	14.1	1.96	10.2-17.2
B-Pyogenic meningitis	10	4.92	1.27	3-10.1
C-Aseptic meningitis	14	3.66	1.03	1.2-4.9
D-controls	15	1.69	0.44	1-2.5

A Vs B, t=13.84, df=38, p<0.001
A Vs C, t=18.71 df=42 p<0.001
A Vs D, t=24.02 df=43 p<0.001

Table-4
CSF ADA levels in TB Meningitis in relation to CSF culture

CSF culture	No. of cases	CSF ADA Levels (U/L)		
		Mean	SD	Range
Positive	4	14.04	1.96	10.2-17.2
Negative	26	14.45	2.20	11.8-16.5

t=0.38, df=28 p>0.05

Table-5
CSF ADA levels in TB meningitis in relation to CSF sugar

CSF sugar (mg%)	No. of cases	CSF ADA Levels (U/L)		
		Mean	SD	Range
Less than 40	26	14.19	1.97	10.2-17.2
More than 40	4	13.7	2.05	11.8-16.50
t=0.52, df=28 p>0.05				

Table-6
CSF ADA levels in TB meningitis in relation to CSF proteins

Protein (mg%)	No. of cases	CSF ADA Levels (U/L)		
		Mean	SD	Range
A: 0-100	8	11.76	0.99	10.2-13.5
B: 101-200	11	14.06	1.35	12.4-16.5
C: >200	11	15.82	0.99	14.8-17.2
A Vs B, t=5.22, df=17, p<0.001				
A Vs C,t=11.32 df=17 p<0.001				
B Vs C,t=4.14 df=20 p<0.001				

Table-7
CSF ADA levels in TB meningitis in relation to CSF lymphocyte count

Lymphocyte count (%)	No. of cases	CSF ADA Levels (U/L)		
		Mean	SD	Range
A: 0 – 50	3	10.8	0.65	10.2 – 11.5
B: 51 – 70	5	12.66	0.79	11.8 – 13.5
C: 71 – 90	12	14.72	1.32	11.8- 16.5
D: 91 – 100	10	15.05	1.91	12.4 – 17.2
A Vs B,t=7.24, df=7, p<0.001				
A Vs C,t=7.11 df=13 p<0.001				
B Vs D,t=5.83 df=11 p<0.001				

Table-8
Calculation of sensitivity, specificity and positive predictive value for CSF ADA in diagnosis of tuberculous meningitis

Cut off value	TBM group (n=30)	Non-TBM group (n=39)	Sensitivity	Specificity	Positive predictive value
≥ 10 U/L	30	1	100%	97.44%	96.77%

TBM 30 Cases
PYOM 10 cases
ASPM 14 cases
Controls 15 cases
Total 39 cases

DISCUSSION

Thirty patients of tuberculous meningitis are studied in detail and diagnostic ability of ADA is evaluated in comparison with ten cases of pyogenic meningitis, fourteen cases of aseptic meningitis and fifteen patients without any neurological disorders who needed spinal anesthesia. The peak incidence in the present study was found in young adults in the age group of 21-30 years (43%). It is similar to Virmani et al[6] who observed 35.5% in their study. According to the present study, the incidence in males was 56.66% and in females 43.33%. The incidence in both males and females is consistent with the study done by Gambhir et al[7].In the present study, history of fever is present in all the cases (100%). It is low-grade, more in the evening, associated with night sweats. In Khatua et al[8] study the incidence of fever was 87%, while it was 58.9% in the study carried out by Virmani et al[6]. In the present study, 6 cases i.e., 20% had the past history of tuberculosis, whereas in Ahuja et al[5] study, it was 10%. In the present study, there are two highly probable cases, eight probable cases and twenty possible cases of tuberculous meningitis. This is almost consistent with Gambhir et al[7] study, but there were no cases in highly probable group in that study. Mean CSF ADA level was significantly higher in TBM patients (14.1±1.96) as compared to pyogenic meningitis (4.92±1.27), aseptic meningitis (3.66±1.03) and controls (1.69±.44). In Gambhir et al[7] study, mean CSF ADA level in TBM patients was 9.61±4.10 U/L and was significantly elevated as compared to viral encephalitis and enteric encephalopathy cases. In the study of Prasad et al[9], the mean CSF ADA levels were 6.43±1.93, 1.89±0.91, 0.90±0.45 and 0.64±0.57 U/l in tuberculous meningitis, pyogenic meningitis, aseptic meningitis and controls respectively. Ribera E et al[10] found that the mean CSF ADA values in tuberculous meningitis (15.7±4.3 U/L) which was clearly higher than for the other patients (1.4±1.5 U/L).Malan C et al[11]studied 30 cases of tuberculous meningitis. CSF-ADA levels differentiated tuberculous meningitis cases from those with asceptic meningitis being higher than 4U/l in all and higher than 6 U/L in 90% of cases of tuberculous meningitis, but lower than 4U/l in normal controls.

Mishra OP et al[12] found that mean CSF-ADA level was significantly raised in TBM as compared to partially treated bacterial meningitis patients. The CSF ADA values in pyogenic meningitis cases are intermediate to the respective values of Gambhir et al[7] and Prasad et al[9]. In the aseptic meningitis group, the values of ADA are higher as compared with the respective values in Prasad et al[9], but not in the range of tuberculous meningitis group, as well as ADA levels in control groups of the present study were higher when compared to the values of Prasad et al[9]. The ADA values in tuberculous meningitis group in the present study when compared with the ADA levels in other groups such as pyogenic meningitis, aseptic meningitis and controls, the p-value was <0.001 and is statistically significant. There was no statistical difference in the CSF-ADA levels in culture positive and culture negative tuberculous meningitis cases. Similar observation was made by Prasad et al[9]. The CSF sugar levels also did not produce any significant difference. Similar observation was made by Prasad et al[9]. CSF protein levels were raised in all the cases of TBM. When the protein levels were compared with CSF-ADA levels, there was significant correlation between CSF ADA levels and CSF protein levels. These findings are consistent with the findings of Prasad et al[9] study and Gambhir et al[7] study. CSF lymphocyte counts raised in all the cases. There is a definitive correlation between the CSF ADA values and lymphocyte count. This is simple to explain as the adenosine enzyme is required for T-lymphocyte proliferation and differentiation. The findings of the present study are consistent with Prasad et al[9] and Gambhir et al[7] studies. In the present study by using cut-off value of 10 U/L, the test has sensitivity of 100% and a specificity of 97.44%. This is

consistent with Prasad et al[9] who observed a sensitivity of 100% and specificity of 97.5% using 3.3 IU/L as cut-off values. According to Gambhir et al[7] using 8 IU/L as cut-off value for diagnosis of TBM a sensitivity of 44% and specificity of 75% was observed. According to Ribera E et al[10], the sensitivity of the test for diagnosing tuberculous meningitis was 1 and specificity 0.99. Mishra OP et al[12] found a sensitivity of 62.5% and specificity of 88.9%. The test can be of great aid in reaching a presumptive diagnosis when spinal puncture fails to show a reduced glucose content or other characteristic clues in the CSF. The adenosine deaminase test is a simple and low-cost procedure that may be included as a routine laboratory method, especially in those countries with a high prevalence of tuberculosis. It is therefore, evident that determining ADA level in the CSF is a simple and very useful test for the early diagnosis of tuberculous meningitis.

CONCLUSION

This study was undertaken to evaluate the diagnostic utility of CSF-ADA activity in tuberculous meningitis. Thirty cases of tuberculous meningitis were studied. The mean CSF ADA value in tuberculous meningitis was statistically significant ($p<0.001$) when compared to the CSF ADA values in pyogenic meningitis, aseptic meningitis and controls. CSF ADA levels correlated with CSF lymphocyte count and protein levels but not with sugar levels. Taking the cutoff value of 10 U/L, the CSF ADA estimation in tuberculous meningitis has a sensitivity of 100% and specificity of 97.44% in the present study. Though the number of the controls, pyogenic meningitis and aseptic meningitis cases in this study is small, adenosine deaminase activity in CSF is a rapid, relatively inexpensive and easy procedure, can be of great value in the early diagnosis of tuberculous meningitis, help in earlier institution of appropriate treatment and thereby prevent mortality and complications.

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