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Original Article

Biochemistry Section

Diagnostic Importance of Cerebrospinal Fluid Adenosine Deaminase Levels in Tuberculous Meningitis

HARIKA KOLLI[1], SIVA PRABODH VUDDANDI[2], SAMATHA PAVULURI[3], KRISHNA SAI[4]

ABSTRACT

Introduction: Estimation of Cerebrospinal Fluid (CSF) Adenosine Deaminase (ADA) is used for early diagnosis of Tuberculous Meningitis (TBM) and has got importance in differentiating TBM from bacterial and viral types of meningitis. Hence, estimation of ADA in CSF can be carried out in all clinically suspected meningitis patients, especially in developing countries like India with high prevalence of Tuberculosis (TB).

Aim: To evaluate CSF ADA as a diagnostic test for TBM.

Materials and Methods: The prospective study was done on 80 cases admitted with meningitis in Government General Hospital Kakinada, Andhra Pradesh, India, in a period of eight months during August 2018 to March 2019. In all the subjects the concentrations of CSF ADA, Glucose, Total proteins, Calcium, and Phosphorus were assayed.

Results: The results shows that the mean values of CSF ADA in TBM were significantly increased (15.13±12.2) when compared with other types of meningitis due to stimulation of T-cells by *Mycobacterium* antigens. Mean values of CSF glucose were significantly decreased in bacterial and TBM. Mean values of CSF total proteins were increased in all three types.

Conclusion: Therefore, this study suggests the need for routine measurement of CSF ADA in the diagnosis of TBM and thus helps in early detection of TBM which warrants timely intervention leading to lowered morbidity and mortality.

Keywords: Bacterial meningitis, T-cells, Viral meningitis

INTRODUCTION

Meningitis is an infection within the subarachnoid space, TBM is a disease in developing countries especially with low socio-economic status [1]. It is an endemic disease. World Health Organisation (WHO) [2] estimates 10.4 million new TB cases each year and at least 100,000 individuals develop TBM annually, but this figure may be much higher. Untreated, TBM is uniformly fatal. ADA is an enzyme in the purine salvage pathway that catalyses the conversion of adenosine and deoxyadenosine to inosine and deoxyionosine respectively with the release of ammonia. It plays an important role in differentiating lymphoid cells and is present in abundance in active T-lymphocytes whose concentration is inversely proportional to the degree of differentiation, ADA is released by T-cells. ADA release occurs during Cell Mediated Immune response (CMI) to the tubercle bacilli. ADA is the marker of cell mediated immunity particularly as a marker of T lymphocyte activation. TBM is one of the most severe manifestations of extrapulmonary TB. In central nervous system infections TB commonly affects meninges, causing TBM. Usually confirmative diagnosis is not possible with meninges as bacteriological examination of finding Acid Fast Bacilli (AFB) is difficult. CSF culture is time consuming procedure and results obtained are disappointing due to low bacterial density. Even when it is not fatal, the sequelae are distressing and disabling [2]. Delay in diagnosis and so in the start of effective treatment results in poor prognosis and sequelae in up to 25% of cases [3]. Available methods of diagnosis of TBM were evaluated and all of them were found to have low sensitivity and specificity [4,5]. Hence, the diagnostic test which helps in differentiating various types of meningitis is chosen i.e., estimation of ADA enzyme activity in CSF.

The TBM can be diagnosed by detecting AFB and culture of *Mycobacterium tuberculosis* in CSF. AFB is seen on direct smear of CSF sediment in only 20% of the cases [6]. It takes 4-8 weeks for the AFB to grown in culture. Hence, there is a need for a simple, rapid, accurate, and specific test to confirm the diagnosis of TBM. ADA enzyme catalyses purines. ADA catalyses hydrolytic deamination of adenosine to inosine and ammonia. ADA is released by T lymphocytes and macrophages during infections. Therefore, ADA estimation has shown promising results in the diagnosis of tuberculous pleural, peritoneal, pericardial effusions, and TBM [7]. ADA levels have also been considered by several researchers to differentiate tubercular disease from non tubercular. ADA is a simple, time consumable cost-effective test performed in the laboratory which is used to differentiate TBM from the other types. Estimation of CSF ADA is for early diagnosis of TBM and has got importance in differentiating TBM from bacterial and viral types of meningitis. Numerous previous studies had demonstrated that CSF ADA estimation is useful in the diagnosis of TBM and can differentiate TBM from normal subjects or from other neurological disorder [8,9]. Hence, estimation of ADA in CSF can be carried out in all clinically suspected meningitis patients, especially in developing countries like India with high prevalence of TB. Therefore, this study suggests the need for routine measurement of CSF ADA in the diagnosis of meningitis and thus helps in early detection of TBM which warrants timely intervention which can lead to lower morbidity and mortality and also to understand CSF ADA activity in different types of meningitis and reinforce its diagnostic value in TBM.

MATERIALS AND METHODS

In this prospective study, a total of 80 patients with meningitis who have come with complaints of fever, headache, and seizures to medical Outpatient Department (OPD) and diagnosed clinically as meningitis and got admitted in Government General Hospital, Kakinada, Andhra Pradesh, India, during the period of eight months i.e., August 2018 to March 2019 were included in the study and they were divided into three groups- TBM, pyogenic meningitis and viral meningitis. Informed consent was obtained from the cases.

Convenience sampling was done for sample size. Out of the 80 cases, 57 were TBM, nine were bacterial and 14 were viral meningitis. In all the

cases Lumbar Puncture was done with strict aseptic conditions and CSF samples were collected and ADA estimation was carried out by PNP-XOD kit Method [10] using spectrophotometer. A cut-off reference value of >10 IU/L CSF ADA was considered to be positive as per the guidelines provided in the test kit literature. Total protein was measured by Biuret method [11], normal range is 15-45 mg/dL, Glucose by GOD-POD method [12], normal range is 45-75 mg/dL, Calcium by Arsenazo III method [13], normal range is 5.5-6 mg/dL, and Phosphorus by Molybdate UV Method [14] normal range is 1.5-2.1 mg/dL.

Inclusion criteria: Patients with age >18 years and <60 years, showing clinical features suggestive of meningitis were included in the study.

Exclusion criteria: Patients with sepsis, patients in whom lumbar puncture was contraindicated were excluded from the study.

Diagnostic Criteria of Meningitis Cases

The TBM is gradual in onset with weakness, prolonged low grade fever (more than two weeks), signs of meningial irritation, i.e., headache, vomiting, convulsion, neck rigidity, and Kernig's sign appear later. Confirmation demonstration of primary focus in lung on X-ray chest, CSF clear, colourless, cobweb formation when left for 12-24 hours, protein more than 60 mg% and sugar less than 2/3rd of corresponding blood sugar. CT scan of brain shows hydrocephalus, basal exudates, infarcts, tuberculomas.

Pyogenic meningitis (PM): Acute illness along with ear infection signs of meningeal irritation, i.e., headache, seizure, neck stiffness and Kerning's sign.

CSF: The CSF shows organism in gram's stained smear or culture is taken as diagnostic criteria. In the absence of organism, sugar less than half of corresponding blood sugar, and protein more than 60 mg% and response to intravenous (i.v.) antibiotics of 10-14 days.

Viral meningitis: Acute onset of fever, muscle ache, seizure and unconsciousness (if associated with encephalitis), clear CSF- raised protein and sugar more than 2/3rd of corresponding blood sugar value and absence of bacteria on gram's stain or culture.

Sample collection: The CSF samples were collected by standard lumbar puncture. Approximately, 3 mL of CSF sample was obtained and used for analysis of ADA, glucose, proteins, calcium and phosphorus.

STATISTICAL ANALYSIS

The data was analysed using descriptive statistics like mean and standard deviation. Correlation of ADA levels was observed among the three groups using Pearson correlation coefficient. The p-value was calculated according to student's paired t-test and p-value <0.05 is considered as significant.

RESULTS

In present study, there was high incidence of TBM in males as compared to females [Table/Fig-1]. In bacterial and viral meningitis there were no much significant differences. [Table/Fig-2] shows that maximum number of cases was in 31-50 years of age group and least in 51 and above years of age group in TBM.

Gender	Total cases	Bacterial	TBM	Viral
Male	64	7	48	9
Female	16	2	9	5
Total	80	9	57	14

[Table/Fig-1]: Shows sex distribution in patients with meningitis.

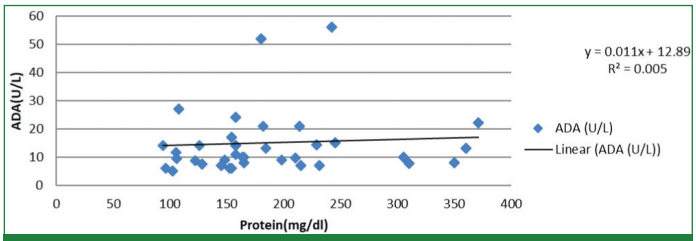
Age (years)	Bacterial	TBM	Viral
18-30	7	13	7
31-50	1	37	4
>51	1	7	3

[Table/Fig-2]: Shows age distribution in patients with meningitis.

The CSF ADA increased more significantly (<0.001) in TBM when compared to other types. CSF glucose was decreased more significantly (p<0.001) in bacterial type when compared with TB meningitis. CSF proteins was increased in all types (p<0.001) but more in TB Meningitis. CSF calcium and phosphorus were insignificant [Table/Fig-3]. In meningitis cases, significant elevation was observed in ADA and total protein levels whereas glucose, calcium levels were decreased than normal levels but the phosphorus levels were in the normal range only. CSF parameters were tested with one-way Analysis of Variance (ANOVA) between three groups of meningitis.

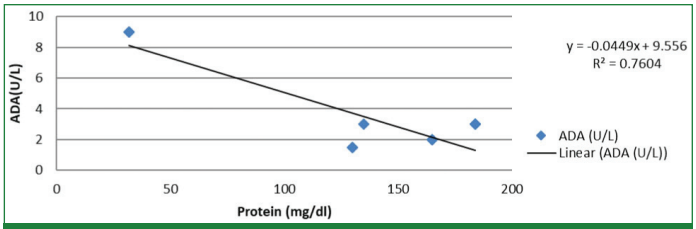
CSF parameters	TB meningitis (n=57)	Bacterial meningitis (n=9)	Viral meningitis (n=14)	Total meningitis cases (n=80)	p-value
ADA (U/L)	15.13±12.2	3.77±3.02	2.04±1.08	11.5±11.7	<0.001
Glucose (mg/dL)	42.72±12.58	36.13±2.70	66.85±14.98	46.4±15.4	<0.001
Protein (mg/dL)	198.8±77.13	128.56± 58.63	77.89± 21.21	168.9± 83.8	<0.001
Calcium (mg/dL)	4.6±0.54	4.64±0.53	4.74±0.49	4.6±0.5	0.667
Phosphorus (mg/dL)	1.70±0.28	1.74±0.32	1.69±0.24	1.7±0.3	0.905

[Table/Fig-3]: Shows mean and SD of CSF parameters in TB meningitis, bacterial meningitis and viral meningitis.

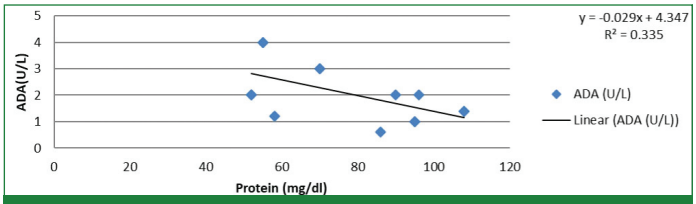


[Table/Fig-4]: Correlation of CSF ADA with total proteins in TB Meningitis.

Correlation of ADA levels was observed among the three groups using Pearson correlation coefficient. There was a slight positive correlation between CSF protein and ADA in TB Meningitis with r-value=0.08, whereas a significant negative correlation p-value=0.002 in bacterial group with r-value=-0.87 and significant negative correlation p-value=0.03 in viral group with r-value=-0.57 was seen [Table/Fig-4-6].



[Table/Fig-5]: Correlation of CSF ADA with total proteins in bacterial meningitis.
Five points instead of 9 cases is due to same values obtained for different cases; 2-3 points club at the same point



[Table/Fig-6]: Correlation of CSF ADA with total proteins in viral meningitis.
R^2 mentioned in the graph is coefficient of determination. r is Karl Pearson correlation coefficient

DISCUSSION

The present research differentiates tubercular disease from non tubercular. In present study, mean values of CSF ADA were significantly increased when compared with other types of meningitis, which may

be due to stimulation of T-cells by *Mycobacterium* antigens [15]. The ADA activity increases during mitogenic and antigenic response of T lymphocytes. T lymphocyte blastogenesis can be inhibited by ADA inhibitors. A deficiency of ADA is associated with severe defect in cell mediated immunity as well as humoral immune deficiency, predisposing the patients to opportunistic infection. ADA is released by T lymphocytes during CMI response, particularly during T-cell activation [16].

The ADA is now recognised as a marker of CMI response as well as an index for differentiation of TB and non TB infection ADA has a major role in proliferation and differentiation of T lymphocytes. It also acts in maturation of monocytes and transforming them to macrophages. ADA is an indicator of active cellular immunity significantly. In TB cell mediated immunity plays important role. Because of the stimulation of T-cells by mycobacterial antigens, ADA levels increases [17]. The CSF ADA elevation in TBM patients may be damaged blood brain barrier permitting ADA to enter into CSF blood or adjacent cerebral tissue or as a result of lymphocytic proliferation indicating local immune response [18].

Mean of ADA in was 15.13 U/L, Baheti R et al., [19] observed elevated mean values of CSF ADA 27.3 which correlates with the present study whereas study by Ramakrishna MR et al., showed that mean ADA levels in CSF of TBM patients were higher than in other types which correlate the present study [20]. The mean CSF ADA activity was higher in TBM patients than non TBM patients in Chotmongkol V et al., study, which correlates with the present study [21]. Mean values of CSF glucose were significantly decreased in bacterial type due to glycolysis by the invading bacteria.

Alavi MS et al., showed that CSF glucose was significantly decreased in all types of meningitis but the decrease was more in bacterial type when compared to other types of meningitis which correlates the present study [22]. Mean values of total proteins were significantly increased in tuberculous and bacterial meningitis [23]. Due to increased local synthesis of gamma globulins and passage through blood brain barrier, protein levels increases in TB Meningitis when compared with bacterial type [24].

Shekhar R, et al., showed that CSF protein was increased more significantly in TBM when compared to pyogenic type which correlates with the present study [25]. Mean values of CSF calcium and phosphorus study were insignificant in all the three groups [26]. CSF proteins showed positive correlation with ADA in TBM. Mishra OP et al., found that ADA level had positive correlation with CSF protein concentration which correlates with the present study [27], whereas in bacterial and viral CSF proteins showed significant negative correlation with ADA. In present study, authors found a significant rise of CSF ADA levels in TBM when compared with other types like bacterial and viral meningitis. Reddy KC et al., showed a significant rise of CSF ADA levels in TB Meningitis when compared with other types like bacterial and viral meningitis which correlates with the present study [28]. Agarwal AK et al., showed a significant rise of CSF ADA levels in TBM when compared with other types like bacterial and viral meningitis which correlates with the present study [29].

Limitation(s)

The major limitation of present study was small sample size; hence further studies are required to validate the role of CSF ADA in TBM.

CONCLUSION(S)

In present study, authors found a significant rise of CSF ADA levels in TBM when compared with other types like bacterial and viral meningitis. Hence, it is prudent to estimate CSF ADA levels for the diagnosis of TBM which helps in early detection which warrants timely intervention leading to lowered morbidity and mortality. Though demonstration of AFB in CSF, CSF cytochemistry, CSF culture are the gold standard for diagnosis of TBM, the CS ADA estimation is a cost effective and reliable means to establish a diagnosis of TBM and to differentiate it from non TB meningitis.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, NRI Medical College, Guntur, Andhra Pradesh, India.
2. Professor, Department of Biochemistry, NRI Medical College, Guntur, Andhra Pradesh, India.
3. Lecturer, Department of Biochemistry, NRI Medical College, Guntur, Andhra Pradesh, India.
4. Assistant Professor, Department of Biochemistry, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Harika Kolli,
NRI Medical College, Chinakakani, Guntur-522503, Andhra Pradesh, India.
E-mail: harikakolli23@gmail.com

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