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CEREBROSPINAL FLUID(CSF) ADENOSINE DEAMINASE(ADA) ACTIVITY IN PATIENTS WITH MENINGITIS

General Medicine

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ABSTRACT

Background:
Meningitis is inflammation of the meninges (pia, arachnoid and duramater) covering the brain and the spinal cord. The most common cause of meningitis is infections due to viruses, bacteria, mycobacteria, fungi and other microorganisms.
Objective:
To estimate the level of CSF Adenosine Deaminase level in different types of meningitis & To assess its usefulness in differentiating the various types (bacterial, viral and tuberculous) of meningitis.
Design & Methodology:
A prospective study conducted in 50 consecutive patients with signs and symptoms of meningitis in Meenakshi Medical College Hospital & research Institute, Kanchipuram. The different types of meningitis patients were separated on the basis of CSF cytochemistry and clinical features. Adenosine Deaminase(ADA) activity was determined in cerebrospinal fluid(CSF) by the method of Guisti and Galanti based on the Berthlot reaction that is the formation of coloured indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically.
Results & Conclusion:
The primary outcome studied was the CSF ADA level in various types of meningitis and comparison of the level of elevation in Tuberculous with other types of meningitis. Cases of tuberculous meningitis constituted 48% of the study group and bacterial and viral meningitis were 26% each. CSF ADA is a simple, time saving, inexpensive indirect test that helps in identifying the type of meningitis, differentiating tuberculous from nontuberculous etiology. Further among the nontuberculous group of meningitis, ADA values are lowest in viral meningitis and thus it can aid in distinguishing bacterial from viral etiology. CSF ADA cut-off of 10U/L considered in our study has 100% sensitivity and 92.85% specificity in differentiating tuberculous from nontuberculous etiology as early as possible, facilitating immediate management of these patients preventing morbidity and mortality due to delay in man agement.

KEYWORDS

Adenosine Deaminase; Cerebrospinal fluid; Meningitis; Tuberculosis

Introduction:
Meningitis is inflammation of the meninges (pia, arachnoid and duramater) covering the brain and the spinal cord[1]. The most common cause of meningitis is infections due to viruses, bacteria, mycobacteria, fungi and other microorganisms. The Non-infectious causes include malignancy, chemical compounds, drugs and inflammatory conditions like CNS Sarcoidosis, SLE, Behcet's syndrome, etc. Meningitis can also be classified according to the temporal profile as acute, subacute and chronic types.

Meningitis is a medical emergency as it can be life-threatening because of the inflammation's proximity to brain and spinal cord [1,2]. If not recognized earlier, it can lead to serious long term neurological sequelae like deafness, epilepsy, hydrocephalus and cognitive deficits [1,3].
Lumbar puncture and CSF analysis is used to diagnose or exclude meningitis. Management involves prompt administration of antibiotics, and in appropriate situations, antiviral and antituberculous agents according to the organism suspected. Steroids are used as an adjunctive agent to prevent the complications from overactive inflammation.

Tuberculous meningitis (TBM) is an endemic disease in developing countries with an incidence of 7-21%[4]. It presents with gradual onset of symptoms and results in irreversible neurological sequel and death if there is delay in diagnosis and start of effective treatment. But the available methods of diagnosis of TBM have too low sensitivity and specificity. Detection of acid fast bacilli (AFB) by light microscopy of the CSF smear is a rapid and specific method, but with a detection rate of only 30-40%[5]. Sensitivity of mycobacterial culture on Lowenstein-Jensen (L-J) medium is higher than microscopy but it needs several weeks of incubation. Recently number of genotypic assays especially molecular techniques such as GeneXpert MTB/RIF are developed which are helpful in the diagnosis of extra-pulmonary tuberculosis[6].

Also recently techniques such as Polymerase Chain Reaction(PCR) and Fluorimetry are useful in faster detection of Mycobacterium Tuberculosis[7,8]. However, high costs involved in these tests preclude their use especially in developing countries.

In these situations, Adenosine Deaminase (ADA) estimation has been profoundly useful. ADA is an enzyme in the purine salvage pathway which is found in abundance in active T-lymphocytes. It is released by T cells during cell mediated immune response to the tubercle bacilli. ADA levels have been considered by several researchers to differentiate tuberculous and nontuberculous diseases[9, 10, 11]. Its role in differentiating the tuberculous pleural, pericardial effusion and ascites from other causes has been well established. Several studies have also emphasized its importance as a simple, rapid, cost effective and fairly specific test in distinguishing TB meningitis from other causes of meningitis. Hence an attempt was made to estimate the CSF ADA level in patients with suspected meningitis and throw light on its use in differentiating the various types of meningitis.

Materials and Methods:
This is a prospective analytical study conducted in the patients admitted in medical wards of Meenakshi Medical College Hospital and Research Institute with clinical features suggestive of meningitis from January 2015 to December 2015.

The diagnosis of meningitis was made on the basis of clinical symptoms and signs like headache, fever, nausea, vomiting, neck rigidity, presence of Kernig's and/or Brudzinski's sign, altered sensorium, any focal neurological deficit, cranial nerve palsies, seizures and/or signs of cerebral dysfunction ranging from confusion, delirium, declining level of sensorium from lethargy to coma.

50 Consecutive in-patients who had meningitis were prospectively enrolled and included in the study after informed consent.
Inclusion criteria:

Age > 12 years of age.
Patients with features of meningitis.

Exclusion criteria:
Age <12 years of age
Patients in whom lumbar puncture was contraindicated like:
- Those with coagulopathy(Partial Thromboplastin Time > 50, international normalised ratio> 1.4, platelet count <1,00,000/ mm^3)
- Those with local skin reactions and known spinal cord tumours.

Diagnostic classification of the subjects:
The different types of meningitis patients were separated on the basis of CSF cytochemistry and clinical features.

Tuberculous meningitis was confirmed if CSF culture yielded M. tuberculosis or a positive Ziehl - Neelson stain. Probable disease was diagnosed in the presence of a lymphocytic pleocytosis in the CSF with high protein content and low glucose content, negative bacterial and fungal cultures.

Acute bacterial meningitis was diagnosed in patients with CSF neutrophilia, high protein content, low glucose content and a positive gram stain and bacterial culture.

Viral meningitis was diagnosed if there was predominantly lymphocytic pleocytosis in the CSF with a normal or mildly raised protein content, a normal glucose content and negative bacterial, fungal and mycobacterial cultures.

Clinical and Laboratory Data:
History, clinical examination and laboratory investigation were carried out in all patients and details recorded in a predesigned proforma. Important clinical details regarding duration of fever, signs of meningeal irritation, focal neurological deficits and cranial nerve palsies were elicited. Laboratory examination included CSF analysis (appearance, cell counts, biochemistry, Gram, AFB & India ink stain), blood counts, blood culture & sensitivity, Mantoux test, HIV test. Based on clinical and laboratory data patients type of meningitis was confirmed and treatment started accordingly.

Collection of CSF sample:
This was done by lumbar puncture. Colour and cobweb formation was noted. Total and differential cell count was estimated. Biochemical analysis of protein, sugar, chloride and globulin was done. Gram's, India ink, and acid fast bacilli (AFB) staining performed to identify the causative organism. About 2 ml of CSF was used to find out the ADA level.

ADA activity measurement:
ADA activity in CSF was determined at 37°C according to the method of Guisti and Galanti based on the Berthlot reaction that is the formation of coloured indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically[12,13]. One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay conditions. Results were expressed as units per litre per minute (U/L/min).

Assessment of outcomes: The primary outcome studied was the CSF ADA level in various types of meningitis and comparison of the level of elevation in Tuberculous with other types of meningitis.

Statistical Analysis:
Descriptive statistical analysis was carried out in the study. The CSF ADA mean values were calculated for the different meningitis category patients. One-way Anova & Chi square test were used to determine statistical significance. Sensitivity, specificity, PPV, and NPV were calculated to know the diagnostic performance of ADA levels in relation to type of meningitis.

Observation and Results:
Tuberculous meningitis occurred more in the age group of 21 – 40 years. Bacterial meningitis was seen mainly in patients < 20 years of age. Viral meningitis was seen in all age groups. Out of 50 cases, 33 were males. All three types of meningitis (tuberculous, bacterial and viral meningitis) were more common in males. Of the 50 cases, 24 had tuberculous meningitis. Viral and bacterial meningitis constituted 13 cases each. The percentages of tuberculous, bacterial and viral meningitis were 48%, 26% and 26% respectively(**Table - 1**). CSF ADA level was highest in tuberculous meningitis, the mean value being 24.5 U/L. The mean value of ADA in bacterial meningitis was 4.54 U/L and viral meningitis patients had lowest mean ADA value of 2.65 U/L**(Table - 2).** Of the 50 patients, 28 had ADA <10 U/L. Out of this, 26 cases belonged to the viral and bacterial meningitis groups and the rest 2 had tuberculous meningitis. About 22 patients had CSF ADA >10 U/L and all of them belonged to tuberculous meningitis group**(Table - 3)**. **(Table – 4).**

Of the TBM patients, majority had CSF protein level more than 80 mg/dL. Most of the viral meningitis patients had protein level less than 40 mg/dL. Among the bacterial meningitis patients, protein level was found to be between 40 – 80 mg/dL predominantly**(Table - 5)**. In TBM group, 14 patients had cell count less than 50 cells/µL and 10 patients had cell count more than 50 cells/µL. Most of the bacterial meningitis patients had cell count more than 100 cells/µL. All the viral meningitis patients had cell count less than 50 cells/µL**(Table - 6)**. In tuberculous and bacterial meningitis group, majority had low sugar (<40 mg/dL). All the viral meningitis patients had normal CSF sugar**(Table - 7)**. Of the 24 TBM patients, 14 had mantoux positivity and 10 were mantoux negative. Of the 14 mantoux positive TBM patients, 12 had ADA more than the cut off level i.e. more than 10 U/L(**Table - 8**). Of the 24 TBM patients, 7 had developed focal neurological deficits and cranial nerve palsies. Out of these patients one had Right hemiparesis, one had left monoparesis, and one had paraparesis. 2 patients had developed 3rd cranial nerve palsy and another 2 had developed 6th cranial nerve palsy. Of the 24 patients with TBM, 8 had positive findings in neuroimaging in the form of basal exudates (4.17%), meningeal enhancement (20.83%) and both meningeal enhancement and basal exudates together in 8.33% patients.

Discussion:
There is considerable urgency in establishing the correct diagnosis in patients with meningitis since specific therapy is most effective when instituted early in the course of illness. While waiting for cultures to confirm the diagnosis, irreversible brain damage can occur. Clinical features, CSF cytology, biochemistry and microbiology results are many a time inconclusive with regard to confirmation of diagnosis. So, in suspected meningitis patients, specific treatment for tuberculous or non-tuberculous (bacterial or viral) etiology is usually begun on the basis of presumptive clinical diagnosis.

Moreover, in the diagnostic evaluation of meningitis, sophisticated methods are necessary which are not routinely available in developing countries like ours. In this situation, CSF Adenosine deaminase (ADA) estimation which is simple, cost effective and rapid helps in differentiating the etiology of meningitis when other findings are ambiguous. Hence this simple test aids in prompt and appropriate treatment of this medical emergency and helps in saving many lives.

A total of 50 clinically suspected cases of meningitis admitted in our hospital, were studied. 50 cases comprised of tubercular meningitis-26, bacterial meningitis-13 and viral meningitis-13 cases. CSF ADA activity was estimated in these patients.

Adenosine deaminase level was clearly higher in patients with tuberculous meningitis in our study, consistent with various earlier studies [14, 15, 16, 17, 18]. By using 10 U/L as the cut off value, the test had sensitivity of 100% and specificity of 92.85% in identifying cases with tuberculous meningitis. The positive predictive value and the negative predictive value were 91.66% and 100% respectively. The higher level of ADA in tuberculous meningitis was statistically significant ($p<0.001$).

In comparison to our study, Gupta et al in their study established that CSF ADA level of 10 U/ L as a cut off value exhibited 94.73 % sensitivity and 90.47% specificity in differentiating tuberculous from non-tuberculous meningitis. Various other studies used different cut off level for ADA, as 3.3 U/L in the study by Rajendra Prasad et al with sensitivity of 100% and specificity of 97.87%, 6.5 U/L in the study done by Rajesh Baheti with sensitivity of 95.83 % and specificity of 92.85% and 15.5 U/L in the study by Verajit Chotmongkol in Thailand with sensitivity and specificity of 75% and 93% respectively. Parr-Ruiz et al(2015) demonstrated the optimal ADA cut off was 11.5 IU/L with 91 % sensitivity and 77.7 % specificity in low endemicity area[15].

The mean ADA level in CSF in cases with tuberculous meningitis was 24.5 U/L. It is considerably higher than that reported by other workers (11.7- 15.7 U/L). The range of CSF ADA level in TBM in our study was 6.0 U/L to 84.3 U/L.

Solari et al(2013) shows that ADA in CSF above 6 U/l in patients with a meningeal syndrome has a high positive likelihood ratio for TBM[16].

The mean ADA level in CSF in bacterial meningitis and viral meningitis was 4.54 U/L and 2.65 U/L respectively. This is in comparison to the mean ADA level of bacterial meningitis (3.80±1.92 IU/L) and viral meningitis (1.85±1.43 IU/L) in the study done in Sri Siddhartha Medical College, Tumkur.

Among the nontuberculous etiology, thus ADA level was helpful in differentiating between viral and bacterial meningitis.
Age and Sex distribution of meningitis:

Most of the patients belonged to the 21-40 years age category followed by 41-60 years age group. Majority of the tuberculous meningitis patients belonged to the 21-40 years age group. Bacterial meningitis patients were younger (<20 years). Viral meningitis occurred in all age groups.

There was a male predominance of patients in our study, male to female ratio of 1.9.

CSF Biochemistry Characteristics:
Higher CSF protein levels were found in tuberculous and bacterial meningitis patients and 5 patients with TBM had CSF protein more than 120mg/dL and one had protein level of 2600mg/dL.

Higher CSF cell counts were found mainly in patients with bacterial meningitis and 5 of them had cell count more than 200 cells/µL.

Lower CSF sugar levels were found in both tuberculous and bacterial meningitis patients.

Complications and associated characteristics of TBM:
Focal neurological deficits and cranial nerve palsies were found in 7 TBM patients. Mantoux positivity in tuberculous meningitis was found in 14 cases out of 24 cases. Extraneural TB was found in 7 TBM patients in the form of pulmonary TB. In TBM patients, positive CT/MRI findings were observed in 8 of the 24 patients.

2 patients in our study were HIV positive and they had developed tuberculous meningitis. HIV positivity did not affect the ADA level in these patients. GC Ghosh et al demonstrated a statistically significant difference in cerebrospinal fluid ADA levels between tuberculous meningitis and cryptococcal meningitis cases[17].

Highest CSF-ADA levels were seen in patients with evidence of hydrocephalus and no correlation was seen between development of infarcts, diffuse cerebral atrophy, and normal CT/MRI scans[18]. CSF-ADA levels correlated inversely with morbidity and mortality, and were found to be higher in patients with remnant neurologic deficit and in those who expired[18].

Conclusion:
CSF protein values were higher and sugar values lower in patients with tuberculous and bacterial meningitis. CSF cell counts were higher in patients with bacterial meningitis.

The conclusions that can be drawn from our study, regarding CSF Adenosine Deaminase (ADA) estimation are as follows:

- CSF ADA is a simple, time saving, inexpensive indirect test that helps in identifying the type of meningitis, differentiating tuberculous from nontuberculous etiology.
- Further among the nontuberculous group of meningitis, ADA values are lowest in viral meningitis and thus it can aid in distinguishing bacterial from viral etiology.
- CSF ADA estimation can be done at routine labs spectrophotometrically and assayed according to the method of Guisti.
- CSF ADA cut-off of 10U/L considered in our study has 100% sensitivity and 92.85% specificity in differentiating tuberculous from nontuberculous etiology as early as possible, facilitating immediate management of these patients preventing morbidity and mortality due to delay in management.

However, as the study was limited to a small population due to financial and laboratory constraints, analysis of a larger group would definitely give an insight into the further finer relationship between CSF ADA and clinical severity and outcome of meningitis patients.

Ethical approval:
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:
Informed consent was obtained from all individual participants included in the study.

Table - 1: Distribution of types of Meningitis

Type of Meningitis	No. of cases	% of cases
Bacterial	13	26
Tubercular	24	48
Viral	13	26
Total	50	100

Table – 2: Mean CSF ADA level in various types of Meningitis

Type of Meningitis	Mean CSF ADA Level (U/L)
Bacterial	4.54
Tubercular	24.5
Viral	2.65

Table – 3: Distribution of Meningitis with respect to CSF ADA cut off level-10 U/L

CSF ADA Level (U/L)	No. of cases	Bacterial	Tubercular	Viral
<10	28	13	2	13
>10	22	-	22	-
Total	50	13	24	13

Table – 4: Diagnostic Performance of CSF ADA (at 10 U/L cut off) in relation to the type of Meningitis

	Test (CSF ADA)		
Disease	>10	<10	Total
Tubercular	22 (a)	2 (c)	24 (a+c)
NonTubercular (Bacterial & Viral)	0 (b)	26 (d)	26 (b+d)
Total	22 (a+b)	28 (c+d)	50
P value < 0.001 (statistically significant) Sensitivity = 22/22 * 100 = 100% Specificity = 26/28 * 100 = 92.85% Positive Predictive Value = 22/24 * 100 = 91.66% Negative Predictive Value = 26/26 * 100 = 100%.			

Table – 5: CSF Protein level according to the type of Meningitis

CSF Protein (mg/dL)	No. of cases	Tubercular	Bacterial	Viral
<40	11	0	1	10
40 – 80	24	11	10	3
80 – 120	10	8	2	0
>120	5	5	0	0
Total	50	24	13	13

Table – 6: CSF cell count analysis according to the type of meningitis

CSF cell count (cells/µL)	No. of cases	Tubercular	Bacterial	Viral
<50	28	14	1	13
50 -100	6	6	0	0
100 – 200	10	3	7	0
>200	6	1	5	0
Total	50	24	13	13

Table – 7: CSF sugar level according to the type of Meningitis

CSF Sugar (mg/dl)	No. of cases	Tubercular	Bacterial	Viral
<40	29	17	12	0
>40	21	7	1	13
Total	50	24	13	13

Table – 8: Mantoux status with regard to CSF ADA level in Tuberculous Meningitis

Mantoux/ CSF ADA	<10 U/L	>10 U/L
Positive	2	12
Negative	0	10
Total	2	22

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