

Research Article

Adenosine Deaminase levels in Cerebrospinal fluid among Tubercular Meningitis patients

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Abstract: Tuberculous Meningitis (TBM) is an endemic disease in developing countries, more so in low socio-economic status. CSF ADA (Cerebrospinal fluid Adenosine Deaminase) is thought to be released by T lymphocyte during cell mediated immune response to tuberculous infection. Adenosine deaminase (ADA) has been of interest for many years in TB diagnosis. The Present study has undertaken to know the levels of ADA in TBM and also comparison of ADA levels in TBM and other type of meningitis. This is a prospective study conducted in Siddhartha Medical College for one year. Based on CSF analysis meningitis cases were diagnosed and grouped in to various types. These CSF samples ADA levels are estimated. Statistical analysis has done with chi-square test. Children were most commonly affected in tubercular meningitis. The Mean CSF ADA levels are more in Tubercular Meningitis (28.7 ± 9.2) followed by Pyogenic meningitis (17.8 ± 7.7), Aseptic Meningitis (7.4 ± 8.4) and Fungal Meningitis (5.7 ± 5.1). CSF ADA levels cut-off value at 10 IU/L shown that sensitivity 96% and specificity 93.5% for the diagnosis of Tubercular Meningitis. CSF ADA levels estimation is a simple, rapid inexpensive, fairly sensitive and specific method. It also helps in early diagnosis and helps in differentiating tubercular meningitis and non tubercular meningitis.

Keywords: Tubercular Meningitis, Adenosine Deaminase, Cerebrospinal fluid, Non tubercular meningitis.

INTRODUCTION

Tuberculous Meningitis (TBM) is an endemic disease in developing countries [1], more so in low socio-economic status. Five lakh patients of tuberculosis die every year in India [2], 8.3% of which is childhood tuberculosis. Multidrug resistance in tuberculosis and acquired immuno-deficiency syndrome (AIDS) further worsen the outcome of this disease [3]. Incidence of TBM in developing countries is 7-12%. Delay in diagnosis and so in the start of effective treatment results in poor prognosis and sequelae in up to 25% of cases [4].

The newer methods for diagnosing tubercular disease is based on pheno and genotypic methods. For the detection of acid fast bacilli (AFB) in a smear, light microscopy is a common, rapid and specific method and is used the world over with a detection rate of 30-40% [5]. Sensitivity of culture on Lowenstein-Jensen (L-J) medium is higher than microscopy but it needs several weeks of incubation. A number of genotypic assays based on nucleic acid amplification have been designed including GenProbe amplified Mycobacterium tuberculosis direct test, Roche Amplicor MTB test, Cobas Amplicor test, Abbott LCx test, and the BD-

Probe Tec (Strand displacement amplification) test [6-10]. However, high cost involved in these tests prevents them to be widely used especially in developing countries.

There is an urgent need to improve early diagnosis. There are major hurdles in establishing a proven diagnosis in TBM. Central in diagnosis is the lumbar puncture. The procedure is invasive and particularly in patients in coma or with signs of raised intracranial pressure, it may not be safe. In many settings it may be impossible to perform brain imaging where TBM is common. If CT or MRI of the brain can be performed prior to lumbar puncture, it is still not clear how to make a reliable prediction who is at highest risk of coning or other complications. In many cases, TB treatment may have been started before lumbar puncture is performed, on clinical grounds; this affects the sensitivity of diagnostic tests. For nucleic acid amplification tests, contamination of samples may influence specificity.

Adenosine deaminase (ADA) has been of interest for many years in TB diagnosis. ADA was first isolated from calf mucosa in the early 1940 [11]. ADA

is ubiquitously present in the body, but especially in lymphoid tissue, levels are particularly high in active T lymphocytes, hence it is associated with disorders that induce T-cell mediated immune responses. It has been shown to be of value in the distinction of tubercular pleural effusions [12].

The use in differentiating between TBM and other forms of meningitis is attractive, because it is a relatively inexpensive and easy test, especially important in low income settings. Numerous studies have been published regarding the usefulness in TBM. A recent meta-analysis concluded that the mean sensitivity and specificity of ADA assays were 79 and 91%, respectively [13]. However, publication bias may have resulted in overestimation of diagnostic accuracy. Generally, ADA assays may be useful in confirming TBM, but raised levels may also be seen in other CNS disorders (Sarcoidosis, Meningeal lymphoma, Subarachnoid haemorrhage, Neurobrucellosis), rendering it too nonspecific [14-16]. It is not a useful test in HIV-positive patients [16]. CSF ADA is thought to be released by T lymphocyte during cell mediated immune response to tuberculous infection.

The Present study has undertaken to know the levels of ADA in TBM and also comparison of ADA levels in TBM and other type of meningitis.

MATERIALS AND METHODS

This is a 1 year prospective study conducted in Siddhartha Medical College, Vijayawada. All the patients presenting with headache, fever, headache, nausea, vomiting, neck rigidity, signs of meningitis like Kernigs or Brudzinski's sign or microbiologically, clinically, radiologically suspected to be tuberculosis were included. Complete history and significant findings of general examination were noted. Routine investigations were followed. Informed consent has taken from all patients and ethical committee has approved.

Fresh CSF samples were collected under aseptic precautions in three parts. Those patients who are contraindicated for lumbar puncture are excluded. First part processed for diagnosing meningitis by noting pressure, cell type and count, glucose and protein levels. Second part of CSF for bacterial and fungal culture along with AFB staining, Negative staining for Cryptococcus. Third part of CSF for ADA estimation. Meningitis cases are separated into groups based on Cytochemistry.

1. Tubercular Meningitis diagnosed by slight elevation in pressure, Protein >40mg%, cell count

>50/ mm³ more of lymphocytes, Cob web appearance, Culture and AFB staining revealed M.tuberculosis.

2. Pyogenic Meningitis diagnosed by elevation of pressure, protein >40mg%, cell count >100/mm³ more of Polymorphoneutrophils, sugar \leq 50% of blood sugar, cloudy appearance, Culture and gram staining revealed the presence of bacteria.
3. Aseptic Meningitis diagnosed by normal or slight elevation in pressure, protein >40mg%, cell count >100/mm³ more of lymphocytes, Sugar is normal or slightly reduced.
4. Fungal Meningitis diagnosed by elevation in pressure, protein >40mg%, cell count >50/mm³, Sugar \leq 50% of blood glucose, Fungal culture and staining revealed Cryptococcus
5. ADA estimation has done with fresh samples by colorimetric method using spectrophotometric method at optimum wavelength of 628nm as described by Giusti [17].

RESULTS

Total number patients presented with meningitis were 128. Among 128 patients, most common type of Meningitis was Tubercular Meningitis. The Incidence of various types of meningitis has depicted in Table no: 1. Among 51 Tubercular Meningitis cases, 28 (54.9%) were females and 23 (45%) were males. There is a female preponderance in this study. Most commonly affected age group was 21-40 (37%) years followed by children about 1 - 10 years (28%).

The common clinical symptoms presented among Tubercular Meningitis patients were tabulated in Table No: 2. According to this most common clinical presentations are fever, nausea, vomiting, headache and signs of meningeal irritation like Kernig's sign, Brudzinski's sign, and Neck rigidity.

Levels of ADA in CerebroSpinal Fluid estimated among different types of Meningitis. ADA levels observation were depicted in Table No: 3 along with mean and standard deviation values. The Mean ADA levels are more common in tubercular meningitis followed by pyogenic meningitis.

CSF ADA levels comparing between Tubercular Meningitis and Non-Tubercular Meningitis with ADA cut off value 10 IU/L has tabulated in Table No: 4. On Comparing Tubercular and Non tubercular Meningitis of CSF ADA levels at cut-off value 10 IU/L by Chi-Square test it is statistically significant ($P < 0.05$).

Table No: 1 Incidence of various types of Meningitis.

Meningitis Types	No. of cases	Percentage (%)
Tubercular Meningitis (TM)	51	39.8
Pyogenic Meningitis (PM)	38	29.6
Aseptic Meningitis (AM)	27	21
Fungal Meningitis (FM)	12	9.3

Table 2: Percentage of Clinical Presentations in TBM

S.No.	Signs and Symptoms	No. of cases (n=51)	Percentage (%)
1	Fever	45	88.2
2.	Headache	37	72.5
3.	Nausea, Vomiting	42	82.3
4.	Altered sensorium	24	47
5.	Signs of Meningeal Irritation	39	76.4
6.	Focal Neurological deficit	17	33.3

Table 3: CSF ADA levels in various Meningitis types

S.No.	Meningitis Types	Range of ADA IU/L	Mean
1.	Tubercular Meningitis (TM)	6.8-38.7	28.7±9.2
2.	Pyogenic Meningitis (PM)	3.9-22.9	17.8±7.7
3.	Aseptic Meningitis (AM)	2.7-12.1	7.4±8.4
4.	Fungal Meningitis (FM)	3.1-10.8	5.7±5.1

Table No: 4 CSF ADA levels with cut-off value 10 IU/L among different types of Meningitis

Meningitis types	Sensitivity (%)	Specificity (%)	PPV	NPV
TM vs NTM	96	93.5	90.7	97.2
TM vs PM	96	92.1	94.2	94.5
TM vs AM	96	96.2	98	92.8
TM vs FM	96	91.6	98	84.6

TM=Tubercular Meningitis; NTM=NonTubercular Meningitis, PM=Pyogenic Meningitis; AM=Aseptic Meningitis; FM=Fungal Meningitis; PPV=Positive Predictive Value; NPV=Negative Predictive Value.

DISCUSSION

Diagnosing Tubercular Meningitis is quiet difficult with AFB staining which is less sensitive. We cannot differentiate accurately tubercular meningitis from other types of infectious Meningitis with routine CSF laboratory parameters. Accurate diagnosis of Tubercular Meningitis is needed for early treatment.

CSF-ADA estimation was reported to be useful in diagnosing TBM and can differentiate TBM from normal subject or patients with other neurological disorders [18]. CSF-ADA estimation is a useful method to differentiate TBM from aseptic meningitis [19]. Others researchers have also observed the usefulness of CSF-ADA activity in the diagnosis of TBM [20, 21].

Among 51 Tubercular Meningitis samples, most commonly affected age group was 21-40 (37%) years followed by children about 1-10 years (28%). Children (1-10 years) have higher mean ADA levels in CSF than other age groups in the present study. Malan C *et al* [22] reported mean ADA levels in CSF of TBM

cases of Peadiatric age groups ranging between 11.6-13.7 IU/L. A relatively higher range of ADA values in CSF (15.7-21.3IU/L) has been observed in adult TBM patients [23, 24]. The results shown different in different age groups may be due to immunological difference.

The Mean CSF ADA levels are more in Tubercular Meningitis (28.7±9.2) followed by pyogenic meningitis (17.8±7.7), Aseptic Meningitis (7.4±8.4) and Fungal Meningitis (5.7±5.1). Ribera *et al* have also demonstrated similar finding but his study was in TBM patients of adult age group [24]. The Mean CSF ADA values observed by Chotmongkol Vet *al* [25] for the different groups were: Tubercular Meningitis - 39.44±41.46, Cryptococcal Meningitis-13±7.43, Bacterial Meningitis-34.20±40.81, Eosinophilic Meningitis-3.17±4.82, Aseptic Meningitis-10.03±9.23, Carcinomatous Meningitis-8.67±13.60. Other studies like Rajesh baheti *et al.*; [26], Satya vati rana *et al.*; [27], Gupta *et al.*; [28] also reported that higher ADA

levels of CSF in Tubercular Meningitis compared to other meningitis etiology.

In the present study with CSF ADA levels cut-off value at 10 IU/L shown that sensitivity 96% and specificity 93.5% for the diagnosis of Tubercular Meningitis. Sensitivity was 96% to all Meningitis with same cut-off value; specificity was variable among different groups, more specificity for Aseptic Meningitis (96.2%) to differentiate tubercular and other types of meningitis. Kashyap *et al.*; [29] take cut off value of 11.39 U/L and have obtained sensitivity of 82% and specificity as 83% in TBM cases. Rana *et al* [30] take 10 U/L as cutoff value for diagnosis of TBM and found sensitivity 66.6% and specificity 90%. CSF ADA level of 15.5 U/L as the best cut off value to differentiate between the two, with a sensitivity of 75% and a specificity of 93%, with an area under the curve of 0.92 [25]. The sensitivity and specificity was 73.9% and 92.6% respectively when a cut-off value of ADA of 10U/L was used, with an accuracy of 84% [31].

In Conclusion, estimation of CSF ADA levels to diagnose Tubercular Meningitis from other types of Meningitis. It is a simple, inexpensive, fairly sensitive and specific test. Estimating ADA levels helps in early diagnosis and treatment and which in turn helps in reducing the spread of disease.

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