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Original Research Article

Quantitative Estimation of Adenosine Deaminase (ADA) Effects upon Cerebrospinal Fluid (CSF) Among Children with Tuberculous Meningitis in Tripura; North East India

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Conflict of interest: Nil

Abstract:

Background: Tuberculous meningitis is an endemic and prevalent disease among all reasons of meningitis with an incidence of 7 to 21% especially in developing countries. It has been stated that the currently available methods for diagnosis of TBM (tuberculous meningitis) are accompanied with very much low sensitivity and specificity. In the present scenario, Adenosine Deaminase (ADA) estimation can be significantly useful with less time duration, affordable and cost effective condition.

Aims and Objectives: To project the diagnostic significance of Adenosine Deaminase (ADA) activity in cerebrospinal fluid (CSF) in children suffering from tuberculous meningitis (TBM).

Materials and Methods: In this study, 200 children who have admitted in the wards of Paediatric department of Tripura Medical College, Agartala, Tripura, from October 2018 to November 2020 with positive features of meningitis prospected and included in this study after obtaining proper informed consent.

Results: Among the 200 patients, 112 were males while 88 were females. 34% of the study group consists of the cases of tuberculous meningitis while 22% and 23% were of bacterial and viral meningitis respectively. 21% patients obtained from the study group are free from any type of meningitis. The cut-off value of 10 U/L of CSF ADA considered in our study with 100% sensitivity and 97.06% specificity.

Conclusions: The suspected cases of meningitis among children are often associated with elevated level of ADA in CSF which may be a positive marker for the diagnosis of TBM so that the initial treatment can be started which will provide improved output.

Keywords: Adenosine Deaminase (ADA), cerebrospinal fluid (CSF), tuberculous meningitis (TBM), acid fast bacilli (AFB).

Introduction

Meningitis is referred as inflammation of the meninges consists of Arachnoid, Pia and Dura mater that covers the brain and spinal cord. The infections due to bacteria, viruses and mycobacterium are the most common cause of meningitis. On the other hand, the non-infectious causes include drugs, chemical compounds, malignancy and inflammatory conditions as CNS sarcoidosis, Bechet's syndrome, SLE etc. Meningitis can also be categorized as per temporal profile such as acute, sub-acute and chronic types. [1]

Meningitis presently considered as a medical emergency as it may be life threatening due to the inflammation itself proximal to the spinal cord and brain. If not diagnosed earlier, it can produce fatal long lasting neurological consequences like epilepsy, cognitive deficits, deafness and hydrocephalus. [2] CSF analysis and lumber puncture help to diagnose or exclude meningitis usually. Treatment includes early administration of antibiotics, and based upon accurate situations anti tuberculous and antiviral agents according to the suspected organism. To prevent complications from overactive inflammation, we use steroid as an adjunctive agent. [3]

According to Gupta BK et al (2010), tuberculous meningitis (TBM) is an endemic disease among other causes in developing countries which has an incidence of 7 to 21%. It is characterized with delayed onset of symptoms and produces irreversible neurological complications that lead to death if there is delay for the diagnosis and consecutive effective treatment. But presently the common methods for diagnosis of TBM comprises of very low sensitivity and specificity. Though light

microscopy of the CSF smear for the detection of acid fast bacilli (AFB) is very specific and rapid method, but the detection rate is only 30-40% as per Steingart K R et al (2006). Though sensitivity of Lowenstein-Jensen (L-J) medium's mycobacterium culture is higher than simple microscopy; but it requires several weeks of incubation. There are various types of genotypic assays those have been produced based upon nucleic acid amplification as per Abe C et al. However, high costs of the tests cause negative effects in their use particularly in developing countries. [4]

Presently Adenosine Deaminase (ADA) measurement has been portrayed as extremely useful as it is cost effective, less time consuming and affordable. ADA represents itself as an enzyme in the salvage pathway abundantly present in the active T-lymphocytes. In course of cell mediated immune response, it is released by the T cells in the tubercle bacilli. ADA levels itself have been represented itself by many researchers for differentiation between tuberculous and non-tuberculous diseases. Moreover, its role for differentiation of tuberculous or pleural pathology, ascites and pericardial effusion from other reasons has been well proved. Various researches have been produced for its importance as a prompt, simple, fair and economic test in distinguishing tuberculous meningitis from other reasons of meningitis. So an attempt was performed to evaluate the CSF ADA level among the patients of suspected cases of meningitis and focus on its utility for differentiation of the several types of meningitis and prompt diagnosis of tuberculous meningitis itself. [5]

Aims & Objectives: To measure to efficacy of Adenosine Deaminase (ADA) effects upon Cerebrospinal Fluid in the pediatric subjects and its relation for the pathogenesis of tuberculous meningitis (TBM).

Material and Methods

This study is of cross-sectional type conducted in the Department of Biochemistry in collaboration with Department of Pediatrics, Tripura Medical College, Tripura, India from October 2018 to November 2020. The clinical signs and symptoms were considered for the diagnosis of meningitis.

Inclusion criteria: Children of age group of 6 months to 12 years with the positive features of meningitis were included in the study.

Exclusion criteria: Children of age less than 6 months and more than 12 years were excluded in this study.

Procedure: The different forms of meningitis patients were collected on the basis of clinical features and CSF cytochemistry. Tuberculous meningitis was confirmed if CSF culture was positive for M. tuberculosis or Ziehl-Neelson was positive for it. Particular disease was diagnosed in the low glucose and high protein content in the CSF with presence of lymphocytic pleocytosis along with negative fungal and bacterial cultures. Acute bacterial meningitis was confirmed in the patients with CSF with low glucose and high protein content, positive gram staining and bacterial culture along with presence of CSF neutrophilia. Viral meningitis was confirmed if there were negative mycobacterial, fungal and bacterial cultures with the presence of predominantly pleocytosis in the CSF.

All the details regarding history, clinical examination and laboratory investigation were obtained from the patients and details were kept in a predesigned Performa. Important clinical details were recorded regarding signs of meningeal irritation, duration of fever, cranial nerve palsies and focal neurological deficits. Laboratory investigations include CSF examinations with its appearance, biochemistry, cell counts, Gram staining, acid fast bacilli in India ink stain. Blood culture and sensitivity were also performed with Mantoux test and HIV test. The type of meningitis was diagnosed and treatment was started accordingly based on clinical and laboratory findings.

CSF ADA activity was determined at 37 degree Celsius as per method of Guisti and Galanti based upon Berthelot reaction, which is the formation of colored indophenol complex and ammonia is liberated from adenosine and then quantified spectrophotometrically. One unit of ADA is defined as the quantity of enzyme which is required to release 1 mmol of ammonia/min from adenosine as per standard assay conditions. Results were explained as units per liter per minute (U/L/min). Adenosine Deaminase (ADA) Activity Assay Colorimetric Kit (ab 204695) and Slectra Pro M fully auto analyzer was used for the determination of ADA activity in CSF.

Statistical Analysis: Statistical analysis was performed by using Sigma Stat statistical software in the study. Through the software results were obtained and plotted in the table or figures. All the findings were analyzed statistically as Mean and Standard Deviation.

Results

In our study it has been shown that the percentage of TBM is more prevalent among the age group of 5-10 years. The controls are almost equally distributed among all age groups, i.e, 6 months to 12 years (Table 1). Out of 68 cases, 34 were males while 34 were females, i.e, TBM was equally distributed in both sexes.

On the other hand, out of 132 controls, 78 were males, i.e, male sex was predominant. CSF ADA level was highest in patients with TBM (Table 2 and Figure 1). The mean value was 15.8647 U/L with a standard deviation of 2.8295. The mean value of ADA in control groups were 4.6678 U/L with SD of 1.7246 (Table 3 or Figure 2). Out of the 200 patients, 136 had ADA < 10U/L, out of this 132 belonged to control group, i.e , Non Tuberculous group and rest of had Tuberculous Meningitis. About 64 patients had CSF ADA ≥ 10 U/L and all of them belonged to TBM group (Table 4).

Table 1: Distribution of age between cases and controls in the study

Age in Years	Cases	Cases	Controls	Controls
	Numbers	Percentage	Numbers	Percentage
<1	2	3%	10	8%
1-5	36	53%	50	38%
5-10	22	32%	48	36%
10-12	8	12%	24	18%
Total	68	100%	132	100%
Range	0.5-12		0.5-12	
Mean	4.854		5.5888	
SD	3.1309		3.4712	

Table 2: Mean CSF ADA with Standard Deviation between cases and controls in our study

	Numbers	Mean CSF ADA (in U/L)	Standard Deviation (in U/L)
Cases	68	15.8647	2.8295
Control	132	4.6678	1.7246
Total	200		

Table 3: Distribution of cases (TBM) and controls in term of CSF ADA cut off value-10 U/L in this study

CSF ADA Level (U/L)	Cases	Controls
<10	4	132
≥10	64	-
Total	68	132

Table 4: Diagnostic Performance of ADA in CSF with 10 U/L cut off value in relation to the types of Meningitis

Disease	Test (CSF ADA)		Total
	≥10	<10	
TBM	64(a)	4(c)	(a+b) 68
Non TBM	0(b)	132 (d)	(b+d) 132
Total	(a+b) 64	136 (c+d)	200 (a+b+c+d)

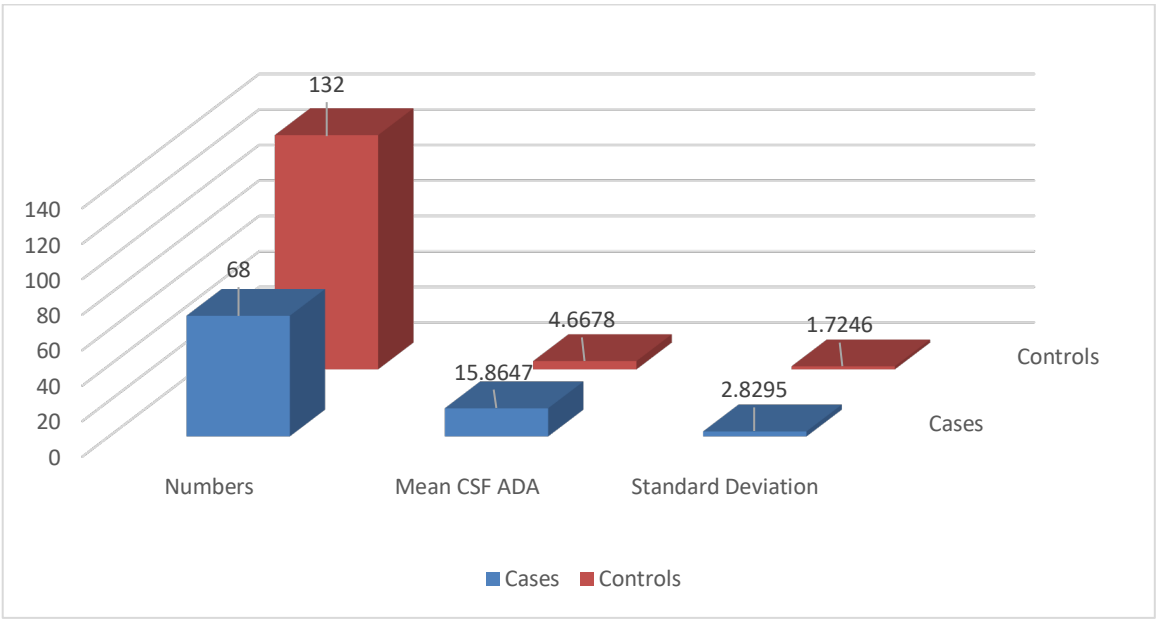

Figure 1: Mean CSF ADA among Cases and Controls

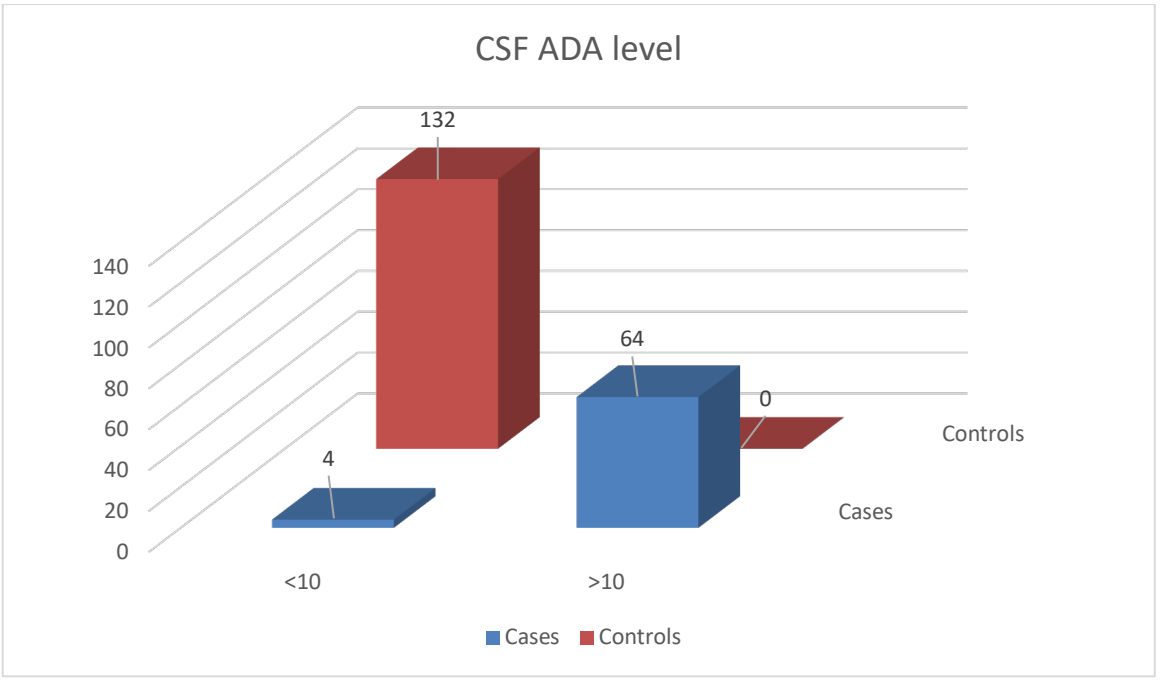

Figure 2: Distribution of Cases (TBM) and Controls with respect of CSF ADA cut off value of 10 U/L

Discussion

In this study, Tuberculous Meningitis (TBM) group consists of 68 cases with 34 males and 34 females, where the control group included 132 patients with 78 males and 54 females. The distribution of age (6 months to 12 years) is similar to both case and control group. In the present study, ADA activity in CSF was estimated.

Since particular and accurate therapy is most efficient when instituted early during the course of illness, there is considerable urgency for establishment of accurate and prior diagnosis among patients with meningitis.

The diagnosis itself of TBM is considerably tough task across the world. Delayed treatments associated with slow diagnosis are common features, so there is emergence for improving the diagnostic tests. Though detection of Acid fast bacilli (AFB) in CSF is considered to be the best diagnostic test for TBM yet it consists of the detection rate of only 15 to 20%. It is very much time consuming (4-8 weeks) to receive positive culture results in regular LJ (Lowenstein-Jensen) medium and positive in 50% of cases only. So culture is considered as the gold standard to obtain the diagnosis of TBM.

Conventional techniques such as radiometric BACTEC and Roche SepticChek as a biphasic culture increase the speed of recovery from mycobacterium tuberculosis within 7 to 10 days. So it is very much time consuming to wait for those methods or culture reports to confirm the specific diagnosis. Delayed treatment may also be associated with irreversible brain damage etc. Many procedures have been conducted for the diagnosis of TBM but the best diagnostic test still considered being controversial except solo detection of mycobacterium. CSF cytology, microbiology, biochemistry and clinical features are remained inconclusive as per confirmation of diagnosis. So in case of suspected meningitis patients, accurate treatment for both tuberculous and non-tuberculous (viral or bacterial) etiology is usually started with the association of presumptive clinical diagnosis.

And for the diagnostic evaluation of meningitis, accurate and unbiased methods are required which are not usually present in centers in the developing countries. In this condition, estimation of CSF ADA (Adenosine Deaminase) is cost effective, rapid and simple, which helps in distinguishing the causes of meningitis when other findings are non-specific. So this simple test provides the appropriate and prompt diagnosis and quick treatment for the tuberculous meningitis that saves many lives.

In our study, it is proved that Adenosine Deaminase level was definitely higher among the patients with tuberculous meningitis. The findings are similar as compared to various earlier studies, like Satya Vati Rana et al (2004), Sharad Jain et al (2005), Amulya C Belagavi et al (2008) and Anil Chandra et al (2012) respectively. [6] By considering 10 U/L as the cut-off value, the sensitivity and specificity are of 100% and 97.06% respectively in diagnosed cases of tuberculous meningitis. The positive predictive value was 94.12%. While the negative predictive value was 100%. The positive likelihood ratio was 34 and the

negative likelihood ratio was only 00. The increased level of ADA in tuberculous meningitis was considered to be statistically significant with p value<0.001.

In this case-control study, the case group of TBM consists of mean value of ADA in CSF as 15.864 U/L accompanied with standard deviation of 2.8295 U/L. This study is relatively close to the studies conducted by other researchers (11.7-15.7 U/L); eg:- Anil Chandra et al (2010), Dr. Aniruddha Debnath et al (2015) etc. 1.7 U/L to 23.3 U/L was the range of CSF ADA level of TBM in this study. 4.6678 U/L was the mean ADA level in CSF in control group with standard deviation of 1.7246. This depicts the increased level of CSF ADA in TBM. It was also concluded that 64 cases out of 68 cases had ADA levels of >10U/L in CSF. [7]

The ADA level in CSF is increased in our study significantly (p<0.001). This study is similar with the study conducted by Amulya C Belagavi et al (2008) with 14.14±7.44 U/L as the mean value of ADA of CSF in TBM group. The study conducted by Gupta et al (2010) is also similar to this study which shows that CSF ADA level of 10 U/L as a cut-off value produces 94.73% sensitivity and 90.47% specificity differentiation of tuberculous from non-tuberculous meningitis. Several other studies projected different cut off level of ADA as 3.3 U/L by the study conducted by Rajendra Prasad et al (1991) with sensitivity of 100% and specificity of 97.87% respectively. Similarly the study conducted by Rajesh Baheti et al used the cut-off level for ADA as 6.5 U/L with sensitivity of 95.83% with specificity of 92.85% respectively. [8]

CSF ADA level has been increased in tuberculous meningitis while C-reactive protein or CRP level in CSF is increased in pyogenic meningitis. On the other hand, viral meningitis shows the low level of ADA and CRP in CSF. So the differentiation among bacterial, viral and tuberculous meningitis can be done by measuring CSF level of ADA and CRP both. [9] So it can be said that the estimation of increased ADA level in CSF in suspected cases of meningitis among children will be a pathfinder for the diagnosis of TBM so that treatment can be started as early as possible to provide improved outcome. [10]

Conclusion

The conclusion which can be reflected in our study is that ADA level of CSF is an inexpensive, simple and time saving indirect test which helps for the identification of the types of meningitis and differentiating the tuberculous and non-tuberculous origin of it.

On the other hand, among the non-tuberculous groups of meningitis, values of CSF ADA are lowest in viral meningitis which may be useful for distinguishing viral and bacterial etiology. The estimation of CSF ADA levels can be done in routine laboratories through spectrophotometer and assaying can be provided according to method of Guisti. The cut-off value of CSF ADA of 10 U/L considered being 100% sensitive and 97.06% specific in our study which differentiates tuberculous from non-tuberculous origin of meningitis as soon as possible, providing early management of these patients that prevents increased morbidity and mortality because of delay in management.

However the main limitation of our study is that it consists of small population, so further research and analysis of a comparatively larger group would surely provide an insight into the further intense relationship of adenosine deaminase activity in CSF along with clinical features and output of patients infected with tuberculous meningitis.

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