

Original Research Article

Analysis of cerebrospinal fluid adenosine deaminase level in tuberculous meningitis and validation of sensitivity and specificity

Shibeesh A. P., Kadeeja Beevi B.*, Balakrishnan Valliyot, Sarin S. M., Sarosh Kumar K. K.

Department of General Medicine, Academy of Medical Sciences, Pariyaram, (PO), Kerala, India

Received: 15 December 2017

Accepted: 25 December 2017

*Correspondence:

Dr. Kadeeja Beevi B.,

E-mail: dr.kadeeja.shaji@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Tuberculous meningitis is an important cause of morbidity and mortality in developing countries especially in India. The mortality associated with tuberculous meningitis is very high if not detected early and meticulous treatment is not given. CSF analysis and imaging are the most commonly used tools for diagnosis of meningitis. But these are often inadequate in making a definitive diagnosis. CSF Adenosine Deaminase estimation (ADA) is useful in differentiation of tuberculous meningitis from non-tuberculous meningitis. Though few studies have proved efficacy of Adenosine Deaminase level for the diagnosis, studies to assess the sensitivity and specificity of ADA levels were limited. This study was conducted to assess its usefulness and to validate the sensitivity and specificity of ADA level in tuberculous meningitis (TBM).

Methods: This was a prospective study conducted at Academy of Medical Sciences, Pariyaram for a period of 18 months from December 2013 to June 2015, Adenosine deaminase level was studied in the cerebrospinal fluid of 50 patients who got admitted with symptoms and signs of meningitis in the medical wards and intensive care units who fulfilled the inclusion criteria.

Results: In this study 50 patients were diagnosed clinically and with CSF analysis as meningitis. The mean cerebrospinal fluid adenosine deaminase activity was 23.08 ± 17.5 in Tuberculous meningitis 3.8 ± 1.92 U/l in Bacterial meningitis and 4.8 ± 2.3 U/l in Viral meningitis. The adenosine deaminase activity in Tuberculous meningitis cases were significantly higher than non-tuberculous meningitis. The sensitivity and specificity of this test for diagnosis of tuberculous meningitis was 90% and 100% respectively with ADA value of more than 10U/L.

Conclusions: This study found out that estimation of CSF Adenosine level is a very useful test for the diagnosis of tuberculous meningitis. The sensitivity and specificity attained in this study were comparable to other studies. This study also found out that ADA estimation is very useful in distinguishing tuberculous and viral meningitis.

Keywords: Adenosine deaminase, Cerebrospinal fluid, Tuberculous meningitis

INTRODUCTION

The global increase in the incidence of tuberculosis in both immunocompetent as well as immunocompromised individuals is a major health issue. The prevalence of tuberculosis is 40% in India and that of tuberculous meningitis varies from 0.5% to 13%. If not detected early and treated meticulously the fatality due to Tuberculous meningitis is very high. The post infective

sequelae of tuberculous meningitis are very distressing and disabling. Factors that have contributed to this increase are the acquired immuno-deficiency syndrome (AIDS), multi-drug-resistant tuberculosis (MDR-TB), advanced age, alcoholism, drug abuse, poverty, malnutrition, transmigration, and immuno-suppressive drugs.^{1,2} Usually meningitis is diagnosed by clinical trial of fever headache and neck rigidity and with typical CSF examination findings. At time it is very difficult to

distinguish between common type of meningitis like tuberculous, bacterial and viral meningitis. More accurate and rapid diagnostic tests are necessary to prevent the delay in specific treatment and prevention of sequelae. Acid Fast staining of CSF sediment is the most rapid method for detection of mycobacterium, but this method lacks sensitivity, seen only in 20% of the cases. Isolation of mycobacterium tuberculosis from CSF culture is time consuming (it requires 4-8 weeks) this method is insensitive if large amounts of CSF are not used. Detection of mycobacterium tuberculosis specific DNA by polymerase chain reaction is costly and not available in all laboratories. Studies show that CSF-ADA is a more sensitive indicator than PCR.³ Adenosine deaminase (ADA) is an enzyme required for the conversion of adenosine to inosine and found in many tissues particularly in active T lymphocytes. It is released by T cells during cell mediated immune response to *tubercle bacilli*. An increase in ADA levels is observed in tuberculosis as well as other bacterial infections in which the cellular immunity response is actively involved.⁴ Detection of high level of ADA has shown promising results in the diagnosis of tuberculous pleural, peritoneal and pericardial effusions. There are many studies reporting an increase in CSF ADA levels in tuberculous meningitis. However, no such studies have taken place in northern Kerala. This study was undertaken to assess the usefulness of adenosine deaminase assay for the diagnosis of tuberculous meningitis and validation of sensitivity and specificity of ADA.

METHODS

This was a prospective observational study conducted over a period of 18 months since December 2013 at Academy of Medical Sciences Pariyaram, a tertiary care centre situated in the north Kerala, India. Source of data - CSF samples were obtained from 50 patients with features of meningitis who were admitted in general medicine wards and intensive care unit. All those patients with an age more than 14 years with clinical features suggestive of meningitis, and those who gave consent for the study were included in this study. Exclusion criteria were those patient with acute infections at sites other than central nervous system., patients in whom lumbar puncture is contraindicated and all those patients on steroids.

Collection of data

Meningitis were classified based on typical clinical features and routine CSF analysis findings, other investigations and response to specific treatment. CSF Adenosine deaminase levels were estimated in all the cases. Triad of fever, headache and nuchal rigidity, altered mental status, nausea, vomiting and photophobia, seizures, signs of meningeal irritation were taken as the criteria for diagnosing meningitis. Meningitis were classified into 3 groups based on typical clinical features and CSF analysis findings, other investigations and

response to specific treatment. CSF Adenosine deaminase levels were estimated in all the cases.

Group 1: Tuberculous meningitis

Diagnosis of TBM was based on Insidious onset and slow progress, signs of meningeal irritation, associated tuberculosis of other organs., CSF showing Pleocytosis of >10 cells/ mm³ predominantly lymphocytes Proteins > 45 mg/dl. Sugar < 40 mg/dl or less than 40% of blood glucose concentration. Ziehl Neelson may be positive for AFB/Culture positivity for AFB, Neuroimaging: Meningeal enhancement, basal exudates and/or tuberculoma, and response to Anti tuberculous treatment

Group 2: Bacterial meningitis

Acute onset and rapid clinical progress with signs of meningeal irritation. CSF Findings includes Pleocytosis of >250 cells/ mm³ predominantly Neutrophils. Proteins > 45 mg/dl. Sugar < 40 mg/dl or less than 40% of blood glucose concentration. Gram stain positivity or Culture positivity Neuroimaging: Diffuse Meningeal enhancement, Abscess, Parameningeal focus response to Antibiotics.

Group 3: Viral meningitis

Acute onset and slow clinical progress with signs of meningeal irritation. CSF Findings includes Pleocytosis of >10 cells/ mm³ predominantly Lymphocytes Proteins > 45 mg/dl. Normal sugar. Neuroimaging: Diffuse Meningeal enhancement

Study design

A prospective clinical evaluation study is undertaken to study the predictive value of ADA in relation to various types of meningitis. Cerebrospinal fluid adenosine deaminase activity was estimated in all fifty cases spectrophotometrically by Guisti and Galanti method and comparison is made between the levels in tuberculous meningitis, pyogenic meningitis, viral meningitis. A CRP level estimation was conducted in all samples to assess the inflammation.

Reference Value of CSF ADA Normal<10 Positive >10 Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Binomial proportion test and Fisher Exact test has been used to find the significance of association of CRP and ADA levels with type of meningitis. Sensitivity, Specificity, PPV, NPV and Accuracy were calculated to know the diagnostic performance of CRP and ADA levels in relation to the types of meningitis. 90% confidence interval has been calculated in the present study.

Significant values

- + Suggestive significance 0.05<p<0.10
- * Moderately significant 0.01<p 0.05
- ** Strongly significant p 0.01.

All the statistical calculations were done by using software namely Statistical Package for Social Science (SPSS) 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0.

RESULTS

Among the 50 patients studied 27 were males and 23 were females. About two third of cases reported were of tubercular meningitis 60%, followed by viral 22% and bacterial meningitis. 18%. Tuberculous meningitis occurred more in the middle age group of 30-60 years, viral meningitis was more in < 40 age groups, whereas bacterial meningitis was seen in all age groups. Tuberculous meningitis was more common in males 70.4% compared to females 47.8%. The incidence of bacterial and viral meningitis was more in females 21.7% & 30.4% compared to males 18% and 22% more than 94% of patients of meningitis had fever, headache and vomiting. About 60 % of them had associated seizure as the predominant symptom. Focal deficits were seen in five cases, four patients had lateral rectus palsy and one had associated left hemiparesis, all of them had tuberculous meningitis. About 32% of the patients had associated type 2 diabetes mellitus and majority of them were having tuberculous meningitis, one patient with tuberculous meningitis had disseminated tuberculosis.

Two patients with Bacterial meningitis had Chronic suppurative otitis media, ADA activity was found to be the highest in tubercular meningitis with mean value of 23.08 ±17.5. Patients with bacterial meningitis had mean ADA of 3.8 ±1.92.and in viral meningitis it was 4.8 ± 2.3 (Figure 1).

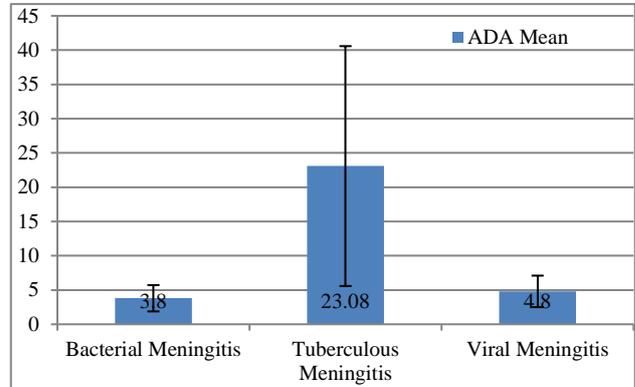


Figure 1: Levels of ADA in the different types of meningitis.

Table 1: p value of ADA.

Type of meningitis	Number	ADA no %	ADA-p value
Tuberculous meningitis	30	27	0.001**
Bacterial meningitis	9	0	-
Viral meningitis	11	0	-
Total	50	27(54%)	

Table 2: Diagnostic performance of ADA in relation to the type of meningitis.

Type of meningitis tuberculous	Number	Sensitivity	Specificity	PPV	NPV	Accuracy
Meningitis	30	90	100	100	88	94
Bacterial meningitis	9	0	34.2	0	60	28
Viral meningitis	11	0	34.2	0	52	24

At CSF ADA levels of 10 IU/L, the sensitivity and specificity of ADA with respect to tubercular meningitis was 90% and 100% respectively with an accuracy of 94%. This value was statistically significant with p value <0.001 (Table 1 and 2). There was a total of 3 deaths, 2 of tubercular meningitis and one of bacterial meningitis.

DISCUSSION

Infections involving the CNS, particularly meningitis and encephalitis are likely to arouse tremendous anxiety in both the patients and physician. Perhaps this to be expected considering the high mortality rates associated with these infections and the neurologic sequelae that may persist in those who recover. The initiation of treatment in suspected cases of meningitis can often be

delayed due to the lack of confidence in the presently available laboratory tests. Reliable, cost effective, rapid diagnostic tests which can be performed in any standard pathology laboratory could be of help in the differentiation of various types of meningitis in adults. In this regard, CSF Adenosine deaminase activity can be used as a rapid test in the differential diagnosis of tuberculous meningitis from non-tuberculous meningitis. There have been various studies on the use of CSF ADA in tuberculous meningitis.

Adenosine deaminase is an enzyme of purine catabolism leading to hydrolytic deamination of inosine and ammonia, which is abundantly found in active T lymphocytes ADA has shown promising results in diagnosis of tuberculous pleural, peritoneal and

pericardial effusion and tubercular meningitis. CSF ADA activities are raised in tuberculous meningitis and their use has been suggested to help differentiate tuberculous meningitis from viral and bacterial meningitis.⁴

Sang-Ho Choi et al studied ADA activity in CSF of 182 patients with meningitis.⁵ The mean ADA level in the tuberculous meningitis group was 12.7 ± 7.5 U/l and it was significantly higher than the other groups (3.10 ± 2.9 U/l; $p < 0.001$). The sensitivity and specificity was 0.83 and 0.95 respectively when a cut-off value of 7U/l was used.

Pettersson et al reports sensitivity of 1.0 and specificity of 0.99 when a cut-off value of 20 U/l was used, but in that study, there were only 3 enrolled tuberculous meningitis patients.⁶

Chotmongkol V et al identified a CSF ADA level of 15.5U/l as the best cut-off value to differentiate tuberculous meningitis and non-tuberculous meningitis, with a sensitivity of 75% and specificity of 93%.⁷ When tuberculous meningitis was compared with aseptic and carcinomatous meningitis, a CSF ADA level of 19.0 U/l was the best cut-off value for differentiation, with a sensitivity of 69% and a specificity of 94%.

In the present study, a total of 30 patients were diagnosed as tubercular meningitis based on the clinical features and CSF analysis and response to anti tuberculous treatment. The mean ADA activity was 23.08 ± 17.5 U/l in the tuberculous meningitis group; 3.8 ± 1.92 in the bacterial meningitis group; 4.8 ± 2.3 in the viral meningitis group. Comparing the ADA activity in the 3 groups, the difference was found to be statistically significant ($p < 0.001$) in the tuberculous meningitis group compared to the other groups. The sensitivity and specificity were 90% and 100% respectively when a cut-off value of ADA of 10U/l was used.

Malan C et al showed that in both bacterial and TBM groups, the mean ADA level in the CSF was significantly higher than in aseptic meningitis ($p < 0.001$), but a significant difference was not shown between bacterial meningitis and TBM groups.⁸

In our study, we found that the mean value of CSF ADA was 3.80 ± 1.92 in pyogenic meningitis. Some studies have reported a lower efficacy of this test and show an overlap between tuberculous meningitis and bacterial meningitis, so we used the higher cut-off value of 10 U/l in order to increase the sensitivity of ADA and overcome this lacuna.

The value of ADA in the differential diagnosis of bacterial meningitis and fungal meningitis is controversial and there has been no cut-off value of CSF ADA activity. Martinez et al reported that CSF ADA activities were not significantly different between the group with tuberculous meningitis and the group with

cryptococcal meningitis in AIDS patients.⁹ According to a study by Lopez, CSF ADA levels were raised in cases of neurobrucellosis and cryptococcal meningitis.¹⁰ It has been postulated that the selective increase of ADA depends on the degree of stimulation of T lymphocytes rather than the total numbers. Ena et al reported CSF ADA elevation in tuberculous meningitis patients with significant T cell depleted AIDS.¹¹

Gambhir IS et al found that the mean CSF ADA levels in TBM patients was 9.61 ± 4.10 U/l and was significantly elevated as compared to viral encephalitis and enteric encephalopathy cases; but the difference was insignificant in comparison to pyogenic meningitis and cerebral malaria.¹² It has been suggested by Piras and Gakis that ADA levels in CSF may help differentiate TBM from viral meningitis and further that TBM and bacterial meningitis differ clearly from one another as regards the relationship of ADA to the number of cells.¹³

CONCLUSION

In this study out of the total 50 patients, 30 patients were diagnosed clinically and with CSF analysis as tuberculous meningitis, a comparative ADA activity among three common meningitis was analysed and it was found out that estimation of CSF ADA is useful. The CSF ADA level has a sensitivity of 90% and specificity of 100% when a cut of value of ADA 10iu/L was used. As in previous studies, it is apparent from the results of our study that the level of ADA in CSF was considerably elevated in tuberculous meningitis compared with viral meningitis. This conclusion has proved to be extremely beneficial in the treatment of viral meningitis also where patients have been started inadvertently on prolonged courses of ant tuberculous medication with the misdiagnosis of tubercular meningitis.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee of Academy of Medical Sciences, Pariyaram

REFERENCES

1. Bernaerts A, Vanhoenacker FM, Parizel PM. Tuberculosis of the central nervous system: Overview of neuroradiological findings. *EurRadiol.* 2003;13:1876-90.
2. Radhakrishnan K, Kishore A, Mathuranath PS. Neurological tuberculosis. In: Sharma SKed. *Tuberculosis*, 1st Ed., New Delhi; Jaypee Brothers. 2001:209-28.
3. Rana SV, Chacko F, Lal V, Arora SK, Parbhakar S, Sharma SK, et al. To compare CSF adenosine deaminase levels and CSF-PCR for tuberculous meningitis. *Clin Neurol Neurosurg.* 2010;112:424-30.

4. Moghtaderi A, Niazi A, Alavi-Naini R, Yaghoobi S, Narouie B. Comparative analysis of cerebrospinal fluid adenosine deaminase in tuberculous and non-tuberculous meningitis. *ClinNeurol Neurosurg.* 2010;112:459-62.
5. Choi SH, Kim YS, Bae IG, Chung JW, Lee MS, Kang JM, et al. The possible role of cerebrospinal fluid adenosine deaminase activity in the diagnosis of tuberculous meningitis in adults. *Clin Neurol Neurosurg.* 2002;104:10-5.
6. Pettersson T, Klockars M, Weber TH. Diagnostic value of cerebrospinal fluid adenosine deaminase determination. *Scand J Infect Dis.* 1991;23:97-100.
7. Chotmongkol V, Teerajetgul GY, Yodwut C. Cerebrospinal fluid adenosine deaminase activity for the diagnosis of tuberculous meningitis in adults. *Southeast Asian J Trop Med Public Health.* 2006;37(5):948-52.
8. Malan C, Donald PR, Golden M, Talijsaard JJF. Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. *J Trop Med Hyg.* 1984;87:33-40.
9. Ribera E, Martinez-Vazquez JM, Ocana I, Segura RM, Pascual C. Activity of adenosine deaminase in cerebrospinal fluid for the differential diagnosis and follow-up of tuberculous meningitis in adults. *J Infect Dis.* 1987;155:603-7.
10. Cortes LLF, Cruz-Ruiz M, Gomez MJ, Hernandez JD, Mejias JE, Pahon J, et al. Adenosine deaminase activity in the cerebrospinal fluid of patients of aseptic meningitis: utility in the diagnosis of tuberculous meningitis or neurobrucellosis. *Clin Infect Dis.* 1995;20(3):525-30.
11. Ena J, Crespo MJ, Vallis V, De Salamanca RE. Adenosine deaminase activity in cerebrospinal fluid: a useful test for meningeal tuberculosis, even in patients with AIDS. *J Infect Dis.* 1988;158:896.
12. Gambhir IS, Mehta M, Singh DS, Khanna HD. Evaluation of CSF-Adenosine deaminase activity in tubercular meningitis. *JAPI.* 1999;47(1):192-4.
13. Piras MA, Gakis C. Cerebrospinal fluid adenosine deaminase activity in tuberculous meningitis. *Enzyme.* 1973;14:311-7.

Cite this article as: Shibeesh AP, Kadeeja BB, Valliyot B, Sarin SM, Sarosh Kumar KK. Analysis of cerebrospinal fluid adenosine deaminase level in tuberculous meningitis and validation of sensitivity and specificity. *Int J Res Med Sci* 2018;6:438-42.