

Cerebrospinal Fluid Adenosine Deaminase Activity: A Valid Ancillary Test for Tubercular Meningitis

Chatterjee Gangadhar^{1,*}, Chandel Rittu², Choudhary Nikhil³, Abichandani Leela G.⁴

¹Assistant Professor, RCMC Govt. Medical College, Kolhapur, Maharashtra, ²Assistant Professor, Dr. Shankarrao Chavan Govt. Medical College, Nanded, Maharashtra, ³Junior Resident, ⁴Professor, Department of Biochemistry, Grant Govt. Medical College, Mumbai, Maharashtra

*Corresponding Author:

Email: ganga.chatterjee@gmail.com

Abstract

Background: Tuberculosis affects 1.80 million persons every year with 3.70 lakh annual deaths, ~ 10% of which are of meningeal involvement. Diagnosis of Tubercular meningitis is difficult with often under or over diagnosis. Delay in diagnosis and initiation of treatment results in poor prognosis and sequel in up to 25% of cases. Cerebrospinal fluid (CSF) Adenosine deaminase (ADA) is a simple, reliable, cost effective and rapid diagnostic test that can even be done in small clinical laboratory set up. We evaluated CSF-ADA as an ancillary test for tubercular meningitis (TBM).

Materials and Methods: Total 118 CSF samples were analyzed in this study under four different groups viz. TBM (n= 30), pyogenic meningitis (PM, n=24), aseptic meningitis (AM, n=20) and Controls (no meningeal involvement, n=44). Diagnosis of meningitis was done by clinicians on the basis of presence of signs of meningeal irritation and cytological and biochemical examination of CSF. CSF-ADA was estimated by method based on Berthelot reaction, which is the formation of a colored indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically. Kruskal-Wallis test with Dunnett's multiple comparison post-test was done to compare CSF-ADA activity in different groups. ROC curve analysis was done for CSF-ADA cutoff value.

Result: Mean CSF-ADA (U/L) value in TBM patients were significantly higher (24.37 ± 10.73) than in PM (14.28 ± 5.979), AM (10.32 ± 5.554) and Controls (6.520 ± 4.801), p -value < 0.0001 in TBM vs AM and TBM vs Controls. A cut-off value of 13.3 U/L gave a sensitivity of 91% and specificity of 90% when used to diagnose TBM.

Conclusion: CSF-ADA is not only simple, cost effective and rapid but also fairly sensitive and specific in diagnosis of TBM, especially in dilemma of differentiating the tuberculous etiology from non-infectious one. For these reasons CSF-ADA is a well performer as an ancillary test of TBM.

Keywords: TB (Tuberculosis), CSF (Cerebrospinal fluid), ADA (Adenosine Deaminase), TBM (Tubercular meningitis), PM (Pyogenic meningitis)

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-6377.2016.00028.9

Introduction

Tuberculosis is an epidemic in the developing countries, particularly India. India is the country with the highest burden of TB. The World Health Organization (WHO) statistics for 2014 give an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9 million⁽¹⁾. It is approximately 1/5th of global TB burden. Diagnosis and treatment of TB is becoming more difficult in recent times due to rapid emergence of multi drug resistance (MDR) and extremely drug resistant (XDR) TB cases. Tubercular meningitis (TBM) is one of the most feared complications of extra pulmonary tuberculosis. Among approximate 3.70 lakh annual death due to TB, ~ 10% is due to meningeal involvement⁽²⁾. Clinical features of

TBM are not much different from other meningitis. Neurological signs and symptoms are quite nonspecific in TBM and ranges from few or no clinical signs to stupor and coma, may be associated with paralysis⁽³⁾. Delay in diagnosis and initiation of effective treatment results in significant morbidity and mortality with sequel in up to ~ 25% of cases⁽⁴⁾. Different diagnostic modality for TBM is available like cerebrospinal fluid (CSF) culture, CSF PCR (polymerase chain reaction), CBNAAT (Cartridge based nucleic acid amplification technique). But these modalities are expensive and time consuming and not readily available in rural hospital setup⁽⁵⁾. So a diagnostic tool is required which is sensitive, inexpensive and able to provide early detection of TBM. Such test should increase the sensitivity of Ziehl- Neelsen staining and culture, but maintain the specificity. CSF-ADA (Adenosine Deaminase) is a marker which has been proposed in diagnostic aid in TBM⁽⁶⁾. Cerebrospinal fluid (CSF) Adenosine deaminase (ADA) is a simple, reliable, cost effective and rapid diagnostic test that can even be done in small clinical laboratory set up.

Adenosine deaminase, an enzyme of purine salvage pathway, catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine

respectively with release of ammonia. ADA plays an important role in proliferation and differentiation of T-lymphocytes. ADA is an important marker of cell mediated immunity. In tuberculosis, level of ADA increases due to stimulation of T-lymphocytes by mycobacterial antigens. Several authors have earlier reported high sensitivity and specificity of CSF-ADA in diagnosis of extra pulmonary tuberculosis like TBM⁽⁷⁾. In this study we have evaluated the performance of CSF-ADA as an ancillary test for diagnosis of TBM.

Materials and Methods

In this prospective study we have included 118 patients of the age range of 1 day to 70 years admitted to Grant Govt. Medical College & Sir JJ Group of hospitals, Mumbai during 2014-2015. Clinical and laboratory evidence of meningitis/ meningoencephalitis was taken as inclusion criteria. ADA enzyme activity was measured in CSF of four group of patients i.e. TBM (tubercular meningitis), PM (pyogenic meningitis), AM (aseptic meningitis) and controls. These four groups were characterized as given below:

TBM group (n= 30)

In this group TBM was defined by the presence of at least 1 of the following diagnostic criteria: (1) *M. tuberculosis* in CSF culture; (2) meningitis and presence of acid-fast bacilli on CSF smear; (3) meningitis associated with tuberculosis in another organ; or (4) clinical and/or laboratory evidence of TBM, with improvement after empirical treatment for tuberculosis⁽⁸⁾.

PM group (n= 24)

In this group CSF of patients showing organisms in Gram-stained smear or culture or presence of bacterial antigen on latex agglutination test was taken as diagnostic criteria. In the absence of organisms, cell count showing pleocytosis of 100/mm³ predominantly polymorphs, glucose level less than 50% of blood and

protein more than 60mg%. This was taken as inclusion criteria.

AM group (n= 20)

In this group CSF of patients showing absence of organisms in Gram-stained smear or culture and pleocytosis more than 10/mm³ predominantly polymorphs and glucose level more than 2/3rd of blood glucose level was taken as inclusion criteria.

Controls (n=44)

Patients without any neurological disorder who had been given spinal anaesthesia or were undergone cranial surgery for any other reasons (e.g. hydrocephalus) included as normal controls.

Specimen

CSF Obtained by Lumber Puncture with all aseptic precautions by clinician. Hemorrhagic CSF samples were excluded. All the CSF samples were further analyzed for cytology and biochemical parameters.

Method

Sensitive colorimetric method. CSF-ADA was estimated by method based on Berthelot reaction, which is the formation of a colored indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically.

Statistical Analysis

Data were tabulated in Microsoft[®] Excel spreadsheet and statistical analysis was carried out in Graph Pad[®] Prism v5.03 software. CSF-ADA values of different groups were expressed as mean±Standard Deviation (SD). Kruskal-Wallis test with Dunnett's multiple comparison post-test was done to compare CSF-ADA activity in different groups. ROC curve analysis was done for CSF-ADA cutoff value.

Observation

Table 1: Gender Distributions among different groups

Groups	Male	Female	Total	Ratio M:F
TBM	20	10	30	2:1
PM	12	12	24	1:1
AM	10	10	20	1:1
Control	25	19	44	1.32:1

It shows that males are more affected in TBM than females. In other types of meningitis no such gender predilection was observed.

Table 2: Age distributions among different groups

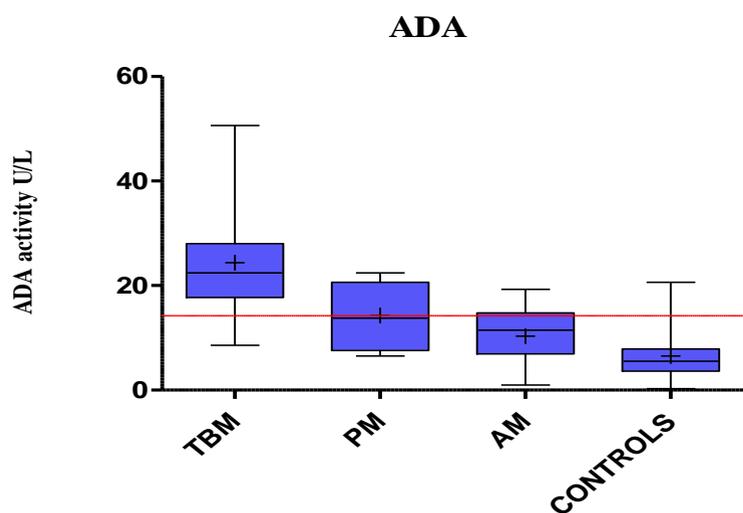
Age	TBM	PM	AM	Controls
0-6 months	0	13	3	6
7-12 months	2	4	2	2
1-10 years	4	2	2	4
10-18 years	2	2	3	4
18-50 years	16	1	8	20
>50 years	6	2	2	8

TBM was observed mainly in adult age group of 18-50 years in our study, whereas PM and AM were more common in early years of life.

Table 3: Estimated CSF-ADA levels in different study groups

Groups	Mean (U/L)	±SD (U/L)
TBM	24.37	10.73
PM	14.28	5.98
AM	10.32	5.55
Controls	6.52	4.80

CSF-ADA was significantly higher in TBM group in comparison to all other groups. Among 30 cases of TBM in no case ADA level was less than 7.5U/L. In one case ADA value as high as 90.40U/L was observed.

**Fig. 1** Box-plot showing estimated CSF-ADA values in different study groups (Mean ± SD)**Table 4: Summary of 1-way ANOVA test**

Dunn's Multiple Comparison Test	Significant ($p < 0.05$)	Summary
TBM vs PM	No	ns
TBM vs AM	Yes	***
TBM vs CONTROLS	Yes	***
PM vs AM	No	ns
PM vs CONTROLS	Yes	***
AM vs CONTROLS	No	ns

(ns= not significant; ***= highly significant, $p < 0.001$)

On the basis of these results we carried out ROC analysis to find a cut-off value for CSF-ADA. We noticed that at a cut-off value of 13.31U/L it had a sensitivity of 91% and specificity of 98% to diagnose TBM. The ROC plot has area under curve (AUC) of 0.96 which is considered highly acceptable

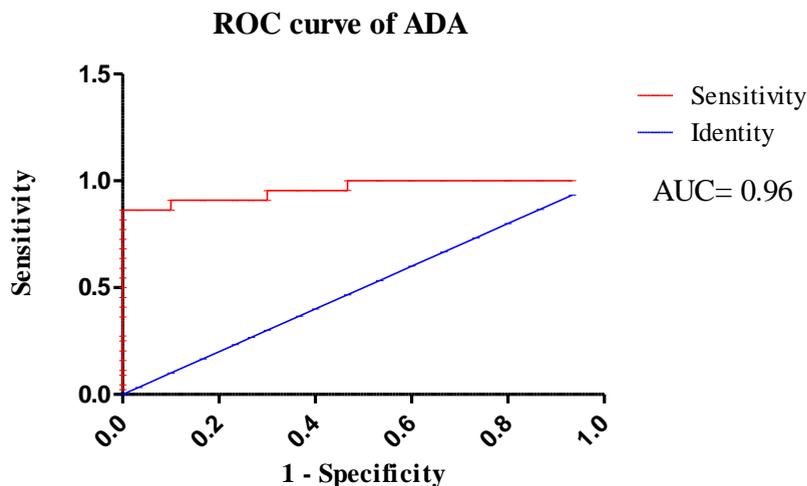


Fig. 2: ROC curve of ADA

Discussion

Diagnosis of Tubercular Meningitis is quite difficult with AFB staining which is less sensitive. It is difficult to 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⁽⁹⁾. CSF-ADA estimation was reported to be useful in diagnosing TBM and can differentiate TBM from normal subject or meningitis of different etiology. Others researchers have also observed the usefulness of CSF-ADA activity in the diagnosis of TBM⁽¹⁰⁾. 30 patients of TBM were included in this study to evaluate diagnostic ability of ADA in comparison to 24 PM, 20 AM and 44 normal control subjects. The peak incidence of TBM in this study was found in adult age group, more precisely between 21-40 years of age. Similar kind of observation was reported by Ramkrishna et.al⁽¹¹⁾.

In our study we found that CSF-ADA level in TBM is significantly higher than other groups. Equivalent results were reported by Mishra et al. and Gupta et.al^(12,13).

CSF ADA values of PM are intermediate to the TBM and AM, controls. Identical findings were published by Sarkar et al⁽¹⁴⁾.

Most relevant finding in our study is that ADA has performed appreciably well with a 97% specificity at 7.4U/L cut off value with moderate sensitivity. At 13.31U/L cut off value, we obtained 91% sensitivity and 98% specificity. Our main finding, the utility of ADA for diagnosis of TBM has been a matter of debate for many years; and meta-analysis by Tuon et. al and others have reported results similar to us: a specificity of 96% and a sensitivity of 59% for ADA values higher than 8U/l.⁽³⁾⁽¹⁵⁾.

Accuracy of test results vary according to the demographic area, incidence and prevalence of disease

among population, mixed patient characteristics, cut-off point, and laboratory specifications.⁽¹⁵⁾ In endemic areas of acute bacterial meningitis, ADA can give false-positive results, and most studies assessing clinical predictors for TBM have been conducted in such populations^(16,17). It may be the underlying reason why group of experts have developed the definition of TBM, have not included this test⁽¹⁸⁾.

In recent years newer diagnostic modalities, particularly molecular techniques like GeneXpert MTB/RIF, have been developed and they are contributing appreciably to the diagnosis of extrapulmonary forms of tuberculosis including TBM^(19,20). However rolling out these tests require considerable time, resources and technical expertise. Therefore the diagnostic tests which are already available, implemented, cheap and easy to perform should be encouraged. In this context, our study shows that CSF-ADA is a valid ancillary test in diagnosis of TBM.

References

1. World Health Organisation. TB Statistics for India – National & state statistics TB statistics for India. 2015.
2. Sharma S, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res.* 2004;4(1):316–53.
3. Tuon FF, Higashino HR, Lopes MIBF, Litvoc MN, Atomiya AN, Antonangelo L, et al. Adenosine deaminase and tuberculous meningitis--a systematic review with meta-analysis. *Scand J Infect Dis.* 2010;42(3):198–207.
4. Nethala SV, Anke G. Adenosine Deaminase levels in Cerebrospinal fluid among Tubercular Meningitis patients. 2015;3(11):914–8.
5. Jasani JH, Bhuvu K V, Vachhani AB, Patel VR, Sankalp M. Value of Adenosine Deaminase Level for the Differential diagnosis various meningitis . *Int J Biol Med Res.* 2012;3(1):1644–7.
6. Arjun Khanna, Virendra Atam, M. L. Patel, Rajesh Verma AG. Evaluation of cerebrospinal fluid adenosine. *Ann Niger Med.* 2010;4(2):51–4.

7. Lakkana Boonyagars SK. Use of Adenosine Deaminase for the Diagnosis of Tuberculosis: A Review. *J INFECT DIS ANTIMICROB AGENTS*. 2010;27(2):111–8.
8. Schutte CM, Ungerer JPI, Du Plessis H, Van Der Meyden CH. Significance of cerebrospinal fluid adenosine deaminase isoenzymes in tuberculous (TB) meningitis. *J Clin Lab Anal*. 2001;15(5):236–8.
9. V.P.Amudha, B.Cinthujah GS. Diagnostic Utility of CSF Adenosine Deaminase and C - Reactive Protein Estimation In Meningitis in Adults. *INDIAN J Appl Res*. 2014;4(2):8–9.
10. Verajit Chotmongkol, Yaovalak Teerajetgul CY. CEREBROSPINAL FLUID ADENOSINE DEAMINASE ACTIVITY FOR THE DIAGNOSIS OF TUBERCULOUS MENINGITIS IN ADULTS. *SOUTHEAST ASIAN J TROP MED PUBLIC Heal Stat*. 2006;37(5):948–52.
11. RAMAKRISHNA M R, TRUPTI R R, SRINIVASA RAO K, SRINIVAS T HB. ADENOSINEDEAMINASE ACTIVITY IN CEREBROSPINAL FLUID. *Int J Pharma Bio Sci*. 2013;4(1):344–51.
12. Gupta BK, Bharat A, Debapriya B, Baruah H. Adenosine Deaminase Levels in CSF of Tuberculous Meningitis Patients. *J Clin Med Res*. 2010;2(5):220–4.
13. Rana S V, Singhal R K, Kumar L. Adenosine deaminase levels in cerebrospinal fluid as a diagnostic test for tuberculous meningitis in children. *Indian J Clin Biochem*. 2004;19(2):5–9.
14. Dn S, Mi H, Akm S, Fa Q. Presentation of Tuberculous Meningitis Patients: Study of 30 Cases. 2013;25(01):25–8.
15. Solari L, Soto A, Agapito JC, Acurio V, Vargas D, Battaglioli T, et al. The validity of cerebrospinal fluid parameters for the diagnosis of tuberculous meningitis. *Int J Infect Dis*. 2013;17(12).
16. Thwaites GE, Chau TTH, Stepniewska K, Phu NH, Chuong L V, Sinh DX, et al. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. October. 2002. p. 1287–92.
17. Chacko F, Modi M, Lal V, Prabhakar S, Rana S V., Arora SK. Diagnostic efficacy of adenosine deaminase levels in cerebrospinal fluid in patients of Tubercular meningitis: A comparison with PCR for *Mycobacterium tuberculosis*. *Annals of Neurosciences*. 2010. p. 126–30.
18. Marais S, Thwaites G, Schoeman JF, Toeroek ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infectious Diseases*. 2010. p. 803–12.
19. Kösters K, Nau R, Bossink A, Greiffendorf I, Jentsch M, Ernst M, et al. Rapid Diagnosis of CNS Tuberculosis by a T-Cell Interferon- γ Release Assay on Cerebrospinal Fluid Mononuclear Cells. *Infection*. 2008. p. 596–600.
20. Jain RS, Handa R, Nagpal K, Prakash S. Clinicoradiological improvement of intracranial tubercular abscess with medical management alone. *BMJ Case Reports*. 2014.