

Study of cerebrospinal fluid adenosine deaminase activity for differential diagnosis of tuberculous and non-tuberculous meningitis



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ABSTRACT

Background: Diagnosing a case of tuberculous meningitis (TBM) has always been challenging. Numerous previous studies have demonstrated that cerebrospinal fluid-Adenosine deaminase (CSF-ADA) estimation is useful in the diagnosis of TBM and can differentiate TBM from other types of meningitis. **Aims and Objectives:** The current study aims to look for a simple, rapid, cost-effective, and specific test to differentiate tubercular etiology from other causes of meningitis. **Materials and Methods:** A total of 80 patients admitted to the hospital with signs and symptoms of meningitis were selected and divided into two groups: Group 1 (Cases): 40 patients of TBM, Group 2 (Control): 40 patients of pyogenic and viral meningitis. The CSF ADA was estimated by enzymatic method, CSF Protein by turbidimetric method, and CSF Glucose by Glucose oxidase (GOD) and Peroxidase (POD) method. **Results:** CSF ADA levels (mean \pm SD) in the TBM and non-TBM groups were 10.874 + 7.72 IU/L and 2.44 + 1.757 IU/L, respectively (highly significant, $P < 0.001$). With the CSF ADA cut off as > 6 IU/L, the sensitivity of the test was 95%, and the specificity of 92.5%. On comparison of the values of CSF ADA in the two groups, the t value is -6.75 , and the difference in these two values was found to be highly significant ($P < 0.01$). **Conclusion:** ADA estimation in CSF is a simple, inexpensive, rapid, and specific method for making a diagnosis of tuberculous etiology in TBM. As a screening test, the determination of ADA activity in CSF can help the physician diagnose TBM early and reduce irreversible brain damage and neurologic sequelae by early administration of anti-tuberculous medications.

Key words: Cerebrospinal fluid; Tuberculosis; Meningeal meningitis; Viral; Meningitis; Bacterial

INTRODUCTION

A total of 1.6 million people died from tuberculosis (TB) in 2021 (including 187,000 people with HIV). Worldwide, TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV and AIDS).¹ Tuberculous meningitis (TBM) is the most serious complication of TB. Southeast Asia and Africa account for about 70% of TBM cases and 48% of deaths of adults due to TBM in 2019.² Incidence of TBM in developing countries like India is 7–12%.³ In the case of TBM, cerebrospinal fluid (CSF) is straw in color, containing more lymphocytes than neutrophils,

raised protein, and sugar less than the corresponding blood sugar. However, the similar chemical findings may be present in other conditions like bacterial or pyogenic meningitis.⁴ Smears of the CSF are diagnostic in only 5–20% of cases while culture has a sensitivity of 18–83%.⁵ Diagnosing TBM comes with several additional challenges, which are most acute in low- and middle-income countries. First, diagnosis can only be made using CSF, requiring a lumbar puncture. Secondly, once a sample is obtained, processing requires appropriate laboratory capacity. Finally, even with the best laboratory infrastructure, the currently available diagnostic tests (smear microscopy, molecular tests including Xpert

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MTB/RIF, or CSF culture) are only moderately sensitive and commonly yield false negative results.² Hence, it would be worthwhile to find simple, rapid, sensitive, and inexpensive diagnostic parameters of TBM. Of the several efforts made to develop reliable markers, adenosine deaminase (ADA) could be a promising parameter. ADA is an enzyme found in T-lymphocytes, an indicator of cell-mediated immunity. Elevated levels are found in various forms of TB making it a marker for the same.^{3,6} Numerous previous studies have demonstrated that CSF-ADA estimation is useful in the diagnosis of TBM and can differentiate TBM from other forms of meningitis. Sensitivities and specificities of ADA levels in CSF were found to be in the range of 44–100% and 71–100%, respectively.⁷ There is a lack of standardization in the ADA cut-off value of ADA for diagnosing TBM. The selected cut-off value, the control group, and the local prevalence of TB impact the sensitivity and specificity values for ADA.⁸ The present study aims to study CSF-ADA levels and to assess the diagnostic usefulness for discriminating between TBM and non-TBM. The unique aspect of this study is that CSF ADA has been evaluated as an efficient diagnostic test to differentiate TBM from non-TBM meningitis.

Aims and objectives

To look for a simple, rapid, cost-effective, and specific test to differentiate tubercular etiology from other causes of meningitis.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted in a tertiary care hospital in the Department of Biochemistry from January 2015 to December 2016. The study protocol was approved by the Institutional Ethics Committee (D-1214139–139). Prior informed consent was taken from patients who participated in the study. The sample size was calculated from OpenEpi software with 95% CI.⁹ A total of 80 patients of either sex with age more than 18 years were included in this study and divided into two groups: Tubercular (cases) and non-tubercular meningitis (pyogenic and viral meningitis) control. The patients who were admitted to the hospital with signs and symptoms of meningitis were allotted in case and control group based on simple randomization.

Inclusion criteria

1. Patients with meningitis who have clinical symptoms
2. Patients with clinical signs like headache, fever, nausea, vomiting, neck rigidity, presence of Kernig's and/or Brudzinski's sign, altered sensorium, seizures, and/or signs of cerebral dysfunction.

Exclusion criteria

1. Patients receiving treatment for TB
2. Patients with pseudo-meningitis or Meningism.

Sample collection

A 2 mL of CSF was collected and sent for microbiological, and cytological examination. The remaining sample was processed for ADA, protein, and sugar estimation in the central clinical laboratory. The CSF sample was subjected to CSF ADA estimation by enzymatic method, CSF Protein by turbidimetric method, and CSF Glucose by GOD-POD method in the biochemistry department. DATA ANALYSIS All the calculations were done using Microsoft Excel and statistical analysis was done using SPSS statistics software. The collected data were analyzed by applying the student's unpaired test. The ($P < 0.05$) was taken as statistically significant while the ($P < 0.001$) was taken as statistically highly significant. Sensitivity, specificity, and positive and negative predictive values were calculated. Based on the above-mentioned parameters, the receiver-operating characteristic (ROC) curve was plotted.

RESULTS

A total of 101 patients were selected for the study, out of which forty patients (50%) who completed the criteria were labeled as cases, while the remaining 40 (50%) patients were labeled as controls. These 40 patients were further classified as bacterial meningitis (25) and viral meningitis (15). The remaining patients were taken as non-conclusive.

Demographic features

Out of a total of 80 patients, 52 were male and 28 were female. The age distribution of different cases of meningitis is highlighted in Table 1. As per the age distribution, comparatively, a greater number of cases were found in the more than 60 years age as compared to other age groups.

CSF ADA analysis

Out of 102 patients studied for meningitis, 40 had fulfilled the criteria for tubercular meningitis. Among these 40 patients, 38 had CSF ADA level above the cut-off value and 2 had CSF ADA below the cut-off. Out of 40 patients who were classified as non-tubercular, 37 had CSF ADA below the cut-off and 3 had above the cut-off. The Cut-off has been taken as 6IU/L.

As mentioned in Table 2, CSF ADA activity in the case group ranged between 1.1 and 37.11 U/L with a median of 10.87 and mean SD of 7.72 ($ADA \geq 6$). The CSF ADA activity in the case group was significantly higher than the control group ADA activity which ranged between

Table 1: Sensitivity and specificity of CSF ADA in meningitis cases

Factors	ADA (percentage)
Sensitivity	95
Specificity	92.5
Positive predictive value	92.68
Negative predictive value	94.8
t-value	-6.756
P-value	<0.00001

t-value and P value was determined by unpaired t' test. CSF: Cerebrospinal fluid, ADA: Adenosine deaminase

Table 2: CSF ADA levels in both types of meningitis

IU/L	Mean	Range	Standard deviation
Cases	10.874	(1.1–37.1)	7.72
Controls	2.44	(0–5.1)	1.757

CSF: Cerebrospinal fluid, ADA: Adenosine deaminase

0 and 5.1 IU/L with a mean of 2.44, with mean SD as 1.757(ADA <6) as shown in Table 2.

With a cut-off of 6IU/L, the sensitivity and specificity of CSF ADA were found to be 95% and 92.5%, respectively. The positive predictive value is 92.68 and the negative predictive value is 94.8. On comparison of the values of CSF ADA in the two groups, the t value is -6.75, and the difference in these two values was found to be highly significant (P<0.01) (Table 1).

ROC curve analysis for CSF ADA activity

Cut-off was determined based on the Receiver Operating Curve in which the cut-off was taken as 4, 6, 10, 14 IU/L. Based on the sensitivity and specificity of CSF ADA activity, ROC was plotted at different cut-off levels as mentioned above. On analysis of the ROC curve for cases and control group, the ideal CSF ADA cut-off value was 6 IU/L, which was associated with a sensitivity of 95% and a specificity of 92.5%. By increasing the CSF ADA cut-off value to >10 IU/L, specificity increased to 95% but sensitivity dropped to 42.5%. By decreasing the cut-off value to <4 IU/L, sensitivity increased to 97.5% and specificity was 70% as highlighted in Figure 1.

CSF protein and glucose analysis

The mean CSF protein level was 165.52 mg/dL in some cases which is higher than the normal range (15–45 mg/dL). The mean CSF protein in control patients is 138.25 mg/dL which is also higher than the normal range, but it is lower than the cases.

The mean glucose levels in TBM meningitis were 29.7 mg/dL which is lower than the normal range of CSF glucose (45–80 mg/dL) and the mean CSF glucose of the

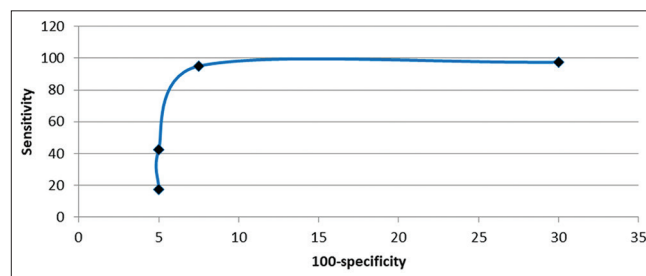


Figure 1: Receiver-operating characteristic curve for different cut-offs of cerebrospinal fluid adenosine deaminase levels

control group is 32.6, lower than the normal range but higher than cases.

White blood cell (WBC) count

The mean total leukocyte count was found to be higher in control than in cases (116.9 vs. 218.3). On the other hand, the average lymphocyte count was found to be higher in TB cases as compared to the control group (96.6 v/v 40.7) with statistical significance (P<0.05).

DISCUSSION

TBM remains a major global health problem. The increasing number of new cases, especially in patients with immunodeficiency disorders, clearly illustrates that the problem is not only in developing countries but also in developed countries.¹⁰ Definite diagnosis for TBM requires the detection of tubercle bacilli in CSF, and the gold standard laboratory test for the diagnosis of TBM is culture. Culture methods require up to 8 weeks but have variable sensitivity with a wide range. This delay makes this test impractical for the clinician to make a differential diagnosis of TBM, so there is an urgent need to improve the efficiency of diagnostic methods.¹¹ It is estimated that in more than 50% TBM patients, microbiological confirmation is not achieved.⁹ Until recently, the lack of accessible and timely tests has contributed to a delay in diagnosis and subsequent morbidity and mortality for many patients, particularly those in resource-limited settings making it challenging for clinicians.¹²

Demographic factors

In the present study, the incidence of TBM was slightly higher in the older age group (>60 years). However, the findings of the age parameter were not significant which concurs with a similar study.¹³ In this study, the male/female ratio was 1.35 in TBM with a male pre-ponderance, concurring with a similar study conducted by Gangadhar C et al.³

CSF ADA levels in both types of meningitis

Piras and Gakis have suggested increased CSF ADA activity as a diagnostic marker for TBM. Following their lead, many previous studies have suggested that CSF ADA activity

could be used as a method for differentiating TBM from non-TBM.¹³ In this study, CSF ADA activity was evaluated that reinforce a clinical diagnosis of TBM. It was found that the ROC curve identified a CSF ADA activity of 6 IU/L as the best cut-off value to differentially diagnose TBM from other types of meningitis.

ROC curve analysis for CSF ADA activity

In this study, mean CSF ADA activity was found to be significantly higher in TBM than in non-TBM meningitis. In comparison to the mean CSF ADA activity value of 10.87 ± 7.7 for TBM determined by this study, other studies had the following values: Cho et al. (12.7 ± 7.5 U/L: 1.1–32.5),¹³ Gupta BB et al. (18.1 IU/L \pm 19.176 IU/L),¹⁴ Mishra et al. (9.4 ± 5.4 , 4–22.5),¹⁵ Moghtaderi et al., (9.6 ± 4.1 IU/L, 6.2–54.6)⁶ Kashyap et al., (14.31 ± 3.87),¹⁶ Sun et al., (14.1 ± 5.4 : 2.8–44.1),¹⁰ Karsen et al., (28.34 ± 14.83 : 6.8–57).¹⁷ The wide range of CSF ADA activities in TBM (1.1–37.1 IU/L) as well as the widely variable mean values in this study were comparable to the above-mentioned studies. The suggested ideal cut-off values are variable from 1 IU/L to 20 IU/L, reaching no consensus on the ideal cut-off value for CSF ADA activity. In the present study, raising the cut-off value for CSF ADA activity from 4 U/L to 6 IU/L to 10 U/L to 14 U/L led to a rapid decline in sensitivity (from 97.5% to 95% to 42.5% to 17.5%, respectively), but specificity progressively increased (from 70% to 92.5% to 95%, respectively). The overall results of the present study are comparable to previous studies.^{10,13,18} It indicates that a cut-off level of 6 U/L gives good sensitivity and specificity.

CSF protein and glucose analysis

In the present study, CSF protein levels in TBM patients were significantly higher than in the non-tubercular group which supports the results of other previous studies. Moghtaderi et al., reported CSF protein value as 80 mg/dL in the TBM group and 72 mg/dL in the non-TBM group. P-value was not found to be significant between these two groups. Similar results have been found in the present study with CSF protein higher in the TBM group than in the non-TBM.^{6,17,19} Similarly, the glucose levels were found to be significantly lower than non-tubercular meningitis. In Moghtaderi et al., study, median CSF glucose was 34 mg/dL in tubercular meningitis patients whereas 43 mg/dL in the patients of non-tubercular meningitis, and the $P=0.03$ which is significant. Jasani et al., found that the mean CSF glucose of TBM-positive patients was 29.87 whereas for pyogenic it was 32.45 and for viral meningitis mean CSF glucose was 66.10 which has similar to the result of this study.^{6,17,19-21}

It has been suggested from previous studies that lymphocyte count increases in TBM patients since TBM mainly affects cell-mediated immunity.^{9,13,19,21} In the present

study, lymphocyte count was significantly increased in the TBM group as compared to the non-TBM group, thereby supporting the previous studies.

Limitations of the study

Limited sample size, restricted to single hospital.

CONCLUSION

Based on the findings of this study, it can be concluded that ADA estimation in CSF is not only simple, inexpensive, and rapid but also a specific method for making a diagnosis of tuberculous etiology in TBM, especially when there is a dilemma of differentiating the tuberculous etiology from non-tuberculous. As a screening test, the determination of ADA activity in CSF can help the physician diagnose TBM early and reduce irreversible brain damage and neurologic sequelae by early administration of anti-tuberculous medications.

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REFERENCES

1. World Health Organisation. Tuberculosis; 2023. Available from: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis> [Last accessed on 2023 Apr 21].
2. Dodd PJ, Osman M, Cresswell FV, Stadelman AM, Lan NH, Thuong NT, et al. The global burden of tuberculous meningitis in adults: A modelling study. *PLOS Glob Public Health*. 2021;1(12):e0000069. <https://doi.org/10.1371/journal.pgph.0000069>
3. Gangadhar C, Rittu C, Nikhil C and Leela AG. Cerebrospinal fluid adenosine deaminase activity: A valid ancillary test for tubercular meningitis. *Int J Clin Biochem Res*. 2016;3(2):145-149. <https://doi.org/10.5958/2394-6377.2016.00028.9>
4. Prasad MK, Kumar A, Nalini N, Kumar P, Mishra B, Lata D, et al. Diagnostic accuracy of cerebrospinal fluid (csf) adenosine deaminase (ADA) for tuberculous meningitis (TBM) in adults: A systematic review and meta-analysis. *Cureus*. 2023;15(6):e39896. <https://doi.org/10.7759/cureus.39896>
5. Murthy JM. Tuberculous meningitis: The challenges. *Neurol India*. 2010;58(5):716-722. <https://doi.org/10.4103/0028-3886.72178>
6. Moghtaderi A, Niazi A, Alavi-Naini R, Yaghoobi S and Narouie B. Comparative analysis of cerebrospinal fluid adenosine deaminase in tuberculous and non-tuberculous meningitis. *Clin Neurol Neurosurg*. 2010;112(6):459-462. <https://doi.org/10.1016/j.clineuro.2009.12.006>
7. Chander A and Shrestha CD. Cerebrospinal fluid adenosine deaminase levels as a diagnostic marker in tuberculous meningitis in adult Nepalese patients. *Asian Pac J Trop Dis*. 2013;3(1):16-19. [https://doi.org/10.1016/S2222-1808\(13\)60004-3](https://doi.org/10.1016/S2222-1808(13)60004-3)

8. Tuon FF, Higashino HR, Lopes MI, 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.
<https://doi.org/10.3109/00365540903428158>
9. Seid G, Alemu A, Dagne B and Gamtesa DF. Microbiological diagnosis and mortality of tuberculosis meningitis: Systematic review and meta-analysis. *PLoS One.* 2023;18(2):e0279203.
<https://doi.org/10.1371/journal.pone.0279203>
10. Sun Q, Sha W, Xiao HP, Tian Q and Zhu H. Evaluation of cerebrospinal fluid adenosine deaminase activity for the differential diagnosis of tuberculous and nontuberculous meningitis. *Am J Med Sci.* 2012;344(2):116-121.
<https://doi.org/10.1097/MAJ.0b013e318238fee3>
11. Iqbal I, Farhana A, Zahoor D and Bashir H. Detection of tuberculous meningitis by various microbiological modalities at a tertiary care hospital in North India. *Indian J Microbiol Res.* 2020;7(3):273-280.
<https://doi.org/10.18231/j.ijmr.2020.049>
12. Foppiano Palacios C and Saleeb PG. Challenges in the diagnosis of tuberculous meningitis. *J Clin Tuberc Other Mycobact Dis.* 2020;20:100164.
<https://doi.org/10.1016/j.jctube.2020.100164>
13. Cho BH, Kim BC, Yoon GJ, Choi SM, Chang J, Lee SH, et al. Adenosine deaminase activity in cerebrospinal fluid and serum for the diagnosis of tuberculous meningitis. *Clin Neurol Neurosurg.* 2013;115(9):1831-1836.
<https://doi.org/10.1016/j.clineuro.2013.05.017>
14. Ghosh GC, Sharma B and Gupta BB. CSF ADA Determination in early diagnosis of tuberculous meningitis in HIV-infected patients. *Scientifica (Cairo).* 2016;2016:5820823.
<https://doi.org/10.1155/2016/5820823>
15. Mishra OP, Loiwal V, Ali Z, Nath G and Chandra L. Cerebrospinal fluid adenosine deaminase activity for the diagnosis of tuberculous meningitis in children. *J Trop Pediatr.* 1996;42(3):129-132.
<https://doi.org/10.1093/tropej/42.3.129>
16. Kashyap RS, Kainthla RP, Mudaliar AV, Purohit HJ, Taori GM and Dagainwala HF. Cerebrospinal fluid adenosine deaminase activity: A complimentary tool in the early diagnosis of tuberculous meningitis. *Cerebrospinal Fluid Res.* 2006;3:5.
<https://doi.org/10.1186/1743-8454-3-5>
17. Karsen H, Koruk ST, Karahocagil MK, Calisir C and Baran FC. Comparative analysis of cerebrospinal fluid adenosine deaminase activity in meningitis. *Swiss Med Wkly.* 2011;141:w13214.
<https://doi.org/10.4414/sm.w.2011.13214>
18. 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(5):424-430.
<https://doi.org/10.1016/j.clineuro.2010.02.012>
19. Cao D, Wang T, Wang Y and Han J. Analysis of cases with cerebrospinal fluid characteristics similar to tuberculous meningitis. *Biomed Res Int.* 2022;2022:9692804.
<https://doi.org/10.1155/2022/9692804>
20. Jasani JH, Bhuva KV, Vachhani AB, Patel VR, Sancheti SM, Patel D, et al. Value of adenosine deaminase level for the differential diagnosis various meningitis. *Int J Biol Med Res.* 2012;3(1):1644-1647.
21. Gu J, Xiao H, Wu F, Ge Y, Ma J and Sun W. Prognostic factors of tuberculous meningitis: A single-center study. *Int J Clin Exp Med.* 2015;8(3):4487-4493.

Authors Contribution:


SA- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **SV-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **AA-** Design of study, statistical analysis and interpretation, review manuscript.

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