

Evaluation of Adenosine Deaminase Activity in Cerebrospinal Fluid: A Comparison Between Tuberculous and Non-Tuberculous Meningitis

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Abstract:

Background: Tuberculous central nervous system (CNS) infection is the most severe form of tuberculosis (TB). Tuberculous meningitis (TBM) is the commonest form of CNS TB caused by *Mycobacterium tuberculosis* bacilli and the diagnosis of TBM is often difficult. A cost-effective, reliable and rapid diagnostic test, which can be performed in any equipped laboratory, could be of help in the diagnosis of TBM. In the present study, we measured the adenosine deaminase (ADA) activity in cerebrospinal fluid (CSF) of TBM and non-TBM patients. **Objective:** To find out the diagnostic value of CSF ADA in the diagnosis of TBM by measuring the sensitivity and specificity of CSF ADA and comparing the CSF ADA level between TBM and non-TBM patients. **Method:** This cross-sectional observational study was done in the Neurology Department of BSMMU, Dhaka. ADA activity in the CSF of both TBM and non-TBM patients was estimated according to a method based on the DiazymeR commercial kit's manual using a semi-automated biochemistry analyzer. Comparison was done between the CSF ADA levels of TBM and non-TBM patients. The sample size was 80. The sampling method was purposive and included both sexes and all age groups. Diagnosis of TBM and non-TBM meningitis was based on appropriate diagnostic criteria. **Results:** The mean CSF ADA activity was found to be significantly higher in CSF of TBM patients, 12.9 ± 9.2 U/L (6.2-39.0 U/L), mean $\pm$ SD with range, than in the CSF of non-TBM meningitis patients, 5.1 ± 3.0 U/L (2.7-20.7 U/L); p value was <0.001 . A cutoff value of 9.3 U/L was used to differentiate TBM from non-TBM. The sensitivity and specificity of CSF ADA were 76.9% and 77.6% respectively, for the diagnosis of TBM when this cutoff value was used. **Conclusion:** This study demonstrated that the estimation of ADA level in CSF of TBM patients, using a cutoff value 9.3 U/L, may be useful for early diagnosis of TBM in our country, as this test can be performed in any equipped laboratory where more sophisticated methods are not available.

Key words: Adenosine Deaminase, Tubercular Meningitis, Non-Tubercular Meningitis, CSF Study

Introduction

Tuberculosis (TB) is the commonest chronic infectious disease worldwide after HIV. Nearly one-third of the global population (2.5 billion) is infected with *M. tuberculosis* and thus is at risk of developing this disease.¹ The WHO reports put to the record that globally approximately 16 million people are suffering from active TB, with an estimated 9 million developing active TB every year, resulting in 1.5 million deaths.¹ More than 90% of global TB cases and deaths occur in the developing world, where 88% of the cases are in the most economically productive age group (15-54 years).² TB is creating an immense adverse social and

economic burden in Bangladesh. Tuberculous CNS infection is the most severe form of TB, and it is endemic in developing countries and more so in low socio-economic status.³

Tuberculous meningitis (TBM) is the most common and severe form of CNS TB.⁴ About 7-12% patients who have TB, develop TBM in developing countries.⁵ The outcome of CNS TB is poor, despite treatment; the mortality, morbidity and sequelae remain high.⁶ Multi-drug resistant TB and AIDS further worsen the outcome.⁷ Delay in diagnosis and so in the start of effective treatment results in high mortality (20-69%) and sequelae in up to 50% of the cases.² So, early

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diagnosis and treatment are of paramount importance to prevent the irreversible neurologic sequelae and death. But the diagnosis of TBM continues to be a formidable clinical challenge, especially in the early course of the illness, even after the introduction of molecular tests.⁸ Additional challenges in implementing practical and useful methods to diagnose this disease remain largely unmet. The pathophysiology of this condition in which disproportionate inflammatory phenomena rather than the number of circulatory bacilli play a role, hinders AFB detection by staining and CSF culture.⁹ Sensitivity of AFB staining is 30-40% worldwide. Sensitivity of culture in BACTEC liquid media and Xpert MTB/RIF assay was 14.7% and 38.24% respectively.¹⁰ Moreover, culture in solid media takes 6 weeks for the result with nearly the same sensitivity.

As mortality and sequelae rates may be reduced considerably with early diagnosis and treatment, many studies are in progress to develop faster, affordable, easily performed, more sensitive and more specific diagnostic methods. Adenosine Deaminase (ADA) is an enzyme with principal biological activity in the T lymphocyte. ADA is now being recognized as a marker of cell-mediated immunity, particularly as a marker of T lymphocyte activation.¹¹ Various studies have been conducted demonstrating CSF ADA estimation as an enzymatic assay in the diagnosis of TBM and can differentiate TBM from normal subjects or non-TBM.

Methods

Forty TBM and 40 non-TBM (20 bacterial and 20 viral meningitis) patients admitted in the Neurology, Pediatric Neurology and Internal Medicine Departments of BSMMU and DMCH have been taken as study subjects as per diagnostic criteria. Informed written consent was obtained as per the Institutional Review Board (IRB) before taking the cases. TBM cases were identified using the following diagnostic criteria⁷;

- Mycobacterium tuberculosis isolated from CSF by AFB stain or Gene X-pert or culture
- OR
- Clinical meningitis with negative Gram and Indian ink stains, plus sterile bacterial and fungal cultures of CSF, plus one or more of the following:
 - I. CSF: Cell count:50-500/cumm³, protein:100-200mg/dl, Glucose <40mg/dl.
 - II. Cranial CT/MRI consistent with TBM (Obstructive or non-obstructive hydrocephalus, tuberculoma in brain parenchyma or in meninges, brain oedema, basal meningeal Enhancement)
 - III. Chest X-ray consistent with active pulmonary tuberculosis

IV. Good response to anti-TB chemotherapy within 1 month.

In TBM cases, CSF was analyzed for ADA, biochemical, and cytological studies; Gram, AFB, and Indian ink staining; culture for bacteria, fungus, AFB; and Gene Xpert for MTB/RIF. The cases were evaluated in detail. Anti-TB medication and steroids were initiated in all TBM cases, and the response was monitored. Non-TBM cases included meningitis patients in whom TB was not suspected, and CSF was analyzed for bacterial or viral meningitis based on established diagnostic criteria.⁷

Patients with hypoxic encephalopathy, head injury, sub-arachnoid hemorrhage, infective foci anywhere in the body other than meninges; septicemia, cerebral malaria, and enteric fever or patients with meningitis but also having chronic liver disease, sarcoidosis, Rheumatoid Arthritis, or other malignancies were excluded from the study.

CSF samples were collected in four sterile tubes and distributed for different assays: 2 ml to Biochemistry (biochemical tests), 2 ml to Clinical Pathology (cytology), 6 ml to Microbiology (cultures, Gram/AFB/Indian ink staining, and ADA assay), and 2 ml to ICDDR'B (GeneXpert MTB/RIF). For ADA estimation, 2 ml of CSF was analyzed in the Microbiology Department of BSMMU using a semiautomated biochemistry analyzer following the Diazyme® kit manual.

The mean ADA levels in CSF of TBM and non-TBM cases were compared. Sensitivity, specificity, and the cutoff value of CSF ADA for diagnosing TBM were also determined using the ROC curve. For statistical analysis, SPSS software was utilized. Chi-square tests were used for qualitative variables, and unpaired t-tests and ANOVA were used for quantitative variables, with a p-value <0.05 considered statistically significant.

Results

Eighty meningitis patients were enrolled in the study, of which 50% (40) had TBM and the remaining 50% (40) had non-TBM. Among the non-TBM patients, 50% (20) had viral meningitis, and 50% (20) had bacterial meningitis. The mean

Table-1
Demographics and CSF ADA values between TBM and non-TBM patients

Variables	TBM (n=40) n (%)	non-TBM (n=40) n (%)	p value
Age (years) Mean ± SD	37.3 ± 17.0	39.5 ± 16.4	0.421 ^t
Male n (%)	21 (52.5)	28 (70.0)	0.108 ^c
CSF ADA (U/L) Mean ± SD	12.9 ± 9.2	5.1 ± 3.0	<0.001 ^t
CSF ADA (U/L) (Range)	6.2- 39.0	2.7- 20.7	

t: Unpaired t-test was done to measure the level of significance; c: Chi-square test was done to measure the level of significance

age in the TBM group was 37.3 years and in the non-TBM group was 39.5 years. Out of 80 male patients were predominant than female, 61.2 % (49/80) were male (Table 01). The mean ADA level in TBM group was 12.9 ± 9.2 U/L (6.2- 39.0 U/L). The mean ADA level in overall non-TBM group was 5.1 ± 3.0 U/L (2.7- 20.7 U/L).

Table-2

Sensitivity and specificity at different cutoff values of ADA for the diagnosis of TBM by gene Xpert MTB/RIF assay using ROC curve (n=80; 9.3 is the best cutoff value)

ADA level	Sensitivity	Specificity
8.8	76.9	73.1
9.0	76.9	76.1
9.3	76.9	77.6
9.9	69.2	79.1
10.8	69.2	80.6

Table 02 showed that an ADA level of 9.3 U/L is the best cutoff value for diagnosing TBM compared to the gene Xpert MTB/RIF assay, based on the ROC curve. At ADA levels below 9.3 U/L, sensitivity remains the same but specificity decreases. At ADA levels above 9.3 U/L, sensitivity decreases while specificity increases.

Table-3

CSF ADA positivity (>9.3 U/L) and negativity (<9.3 U/L) in TBM and non-TBM groups using a cutoff value of 9.3 U/L (n=80)

CSF ADA (U/L)	TBM (n=40) n (%)	Non-TBM (n=40) n (%)	p value
>9.3	19 (47.5)	6 (15.0)	0.002
≤9.3	21 (52.5)	34 (85.0)	

Chi-square test was done to measure the level of significance

ADA (>9.3 U/L) was positive in 19/40 TBM and 6/40 non-TBM cases, negative in the remainder (Table 03). ADA activity demonstrated a sensitivity of 76.9% (10/13) and specificity of 77.6% (52/67) for diagnosing TBM, with overall positivity in 31.25% (25/80) of cases (Table 04). The positive predictive value was 40%, the negative predictive value was 94.5% and the overall accuracy of the test for detecting TBM was 77.5 %. The ROC curve demonstrated the diagnostic performance of ADA for TBM, with an AUC of 0.806 (95% CI: 0.689–0.923; SE: 0.060; p <0.001), indicating moderate accuracy (Figure 01).

Discussion

TBM frequently poses a diagnostic problem even after extensive investigations. In this study, the mean ADA of CSF between TBM and non-TBM were compared. The best ADA cut off value (9.3 U/L) was found for the diagnosis of TBM

Table-4

Performance of ADA activity in comparison to Gene Xpert MTB/RIF assay for the diagnosis of TBM

ADA category	Gene Xpert MTB/RIF		Total n (%)
	Positive n (%)	Negative n (%)	
>9.3 (U/L)	10 (76.9) (True Positive)	15 (22.4) (False Positive)	25 (31.2)
≤9.3 (U/L)	3 (23.1) (False Negative)	52 (77.6) (True Negative)	55 (68.7)
Total	13 (100.0)	67 (100.0)	80 (100.0)

in comparison to Gene Xpert MTB/RIF assay according to the ROC curve. The sensitivity and specificity of ADA for the diagnosis of TBM were also evaluated. In the present study, the biochemical parameters of CSF were similar to the standard results of various meningitides. Identification of AFB in the CSF through both smear and culture methods remains one of the most important and widely available means to diagnose CNS TB. Despite its importance among the diagnostic methods used for TB, traditional staining and culture remain relatively insensitive, most likely due to the typical paucity of AFB in a clinical case of CNS TB. CSF AFB staining sensitivity was <20%.¹²

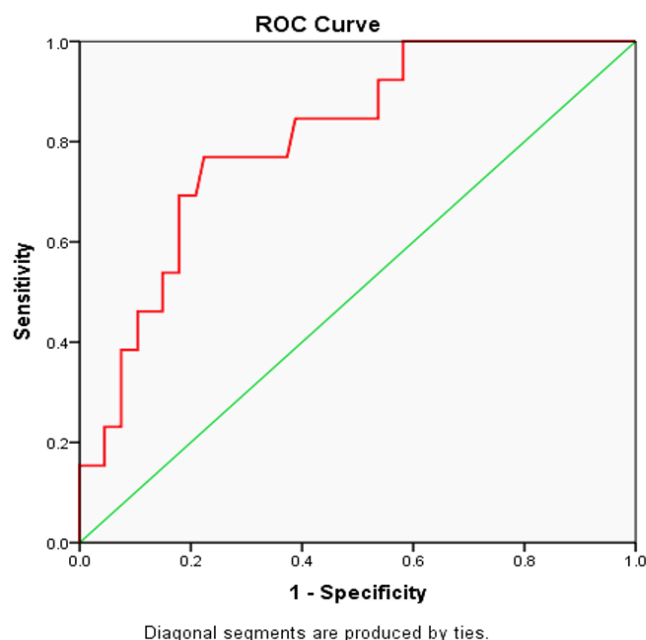


Figure 1: ROC curve for the detection accuracy of CSF ADA in the diagnosis of TBM

According to recent studies, both CSF volume and duration of the microscopic evaluation are independently associated with bacteriological confirmation of CNS TB, suggesting that a minimum of 6 ml of CSF fluid should be examined

microscopically for a period of 30 minutes. A recent study showed the sensitivity of AFB culture in BACTEC was also poor, only 14.7%.¹⁰ However, in the present study, all cases of CSF Z-N staining for AFB and AFB culture in Lowenstein-Jensen media were negative. Lack of optimum expertise, short time observation and lack of logistics at different levels of the laboratory, from sample processing to reporting, may be the cause. Repeated CSF study increases the sensitivity of culture and AFB staining.⁷ We could not do repeated LP due to some limitations. The sensitivity of the Xpert MTB/RIF assay was 38.2% in a recent study, which was nearly similar to our study.¹⁰

In this study, Xpert MTB/RIF assay-positive TBM cases were 13 (32.5%). In this study, the mean ADA in TBM group was 12.9 ± 9.2 U/L (6.2- 39.0 U/L) and the mean ADA in the non-TBM group was 5.1 ± 3.0 U/L (2.7- 20.7 U/L). Mean ADA in bacterial meningitis was 5.7 U/L and in viral meningitis was 3.8 U/L. Out of 80 cases of meningitis, ADA was positive (>9.3 U/L) in 25 cases (31.2 %) and negative (<9.3 U/L) in 55 cases (68.7 %). Out of 40 TBM cases, ADA was positive in 19 cases (47.5%) and was negative in 21 cases (52.5 %). On the other hand, out of 40 non-TBM cases, ADA was positive only in 6 cases (15%) and negative in 34 cases (85%). In bacterial meningitis cases, ADA was positive in 4 cases (20%) and negative in 16 cases (80%). In viral cases, ADA was positive in only 2 cases (10%) and negative in 18 cases (90%). For the diagnosis of TBM, the sensitivity of CSF ADA was 76.9 % and the specificity was 77.6 % when cut-off value of ADA 9.3 U/L was used. Mean ADA in TBM and non-TBM, sensitivity and specificity of ADA at a cut-off value of 10 U/L (near 9.3 U/L) for the diagnosis of TBM in different studies were nearly similar to the present study.

Mean ADA in TBM: 13.5 U/L, mean ADA in non-TBM: bacterial-7.6 U/L, viral-6.7 U/L, sensitivity of ADA was 83.3 % and specificity was 87.5 % at ADA cut off value 10 U/L.5 Mean ADA in TBM:14.3 U/L, mean ADA in non-TBM: 9.2 U/L.¹³ Gupto et al in 2010 also found 10 U/L as the best cutoff value of CSF ADA for the diagnosis of TBM, which was almost similar to our best cut-off value (9.3 U/L).¹⁴ In different studies, along with our study, we observed that the lower the cut-off value, the higher the sensitivity of ADA for the diagnosis of TBM and vice versa. But, the higher the cutoff value, the higher the specificity of CSF ADA for the diagnosis of TBM.

By the present study and several previous studies, it was apparent that the level of CSF ADA activity in TBM was significantly higher in comparison to the overall non-TBM group. So, on the basis of the present result, we can say, CSF ADA may be a useful adjunctive rapid marker for the diagnosis of TBM when it is interpreted with clinical signs, symptoms and other laboratory tests. The estimation of ADA activity in CSF is relatively simple, less expensive and may be a reliable tool for the diagnosis and management of TBM

in early stage, especially when other clinical laboratory tests are inconclusive. Our study along with several previous studies, reemphasized that CSF ADA level measurement may be used as a good, rapid, reliable laboratory test for the diagnosis of TBM at least in low socioeconomic conditions where the incidence and prevalence of TB is high.^{13,15,16}

Conclusion

CSF ADA levels were significantly higher in TBM patients (12.9 ± 9.2 U/L) than in non-TBM cases (5.1 ± 3.0 U/L; $P < 0.001$). At a cutoff of ~ 9.3 U/L, ADA showed 76.9% sensitivity, 77.6% specificity, 40% PPV, 94.5% NPV, and 77.5% accuracy. CSF ADA estimation is a simple, inexpensive, and rapid test that provides fair diagnostic accuracy for TBM, particularly when differentiation from non-TBM meningitis is challenging. ADA values around 10 U/L may favor a diagnosis of TBM.

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Conflicts of Interest

The authors declare that they have no conflicts of interest related to this work.

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