

Comparative study of cerebrospinal fluid Adenosine deaminase activity in patients with meningitis

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ABSTRACT

Cerebrospinal fluid (CSF) adenosine deaminase (ADA) activity in tubercular meningitis (TBM) patients (n=20), non-tubercular meningitis (NTBM) patients (n=10) and non-tubercular non-meningitis (NTBNM) cases (n=15) were measured by the method based on Berthlots reaction. The mean CSF ADA activity in TBM (13.62 ± 8.45 IU/L) was found to be significantly higher as compared to NTBM (6.51 ± 2.41 IU/L, $p < 0.001$) and NTBNM (2.35 ± 1.16 IU/L, $p < 0.0001$) respectively. The sensitivity and specificity of CSF ADA activity was 85% and 88.0% respectively at cut-off value of 6.97 IU/L to diagnose tubercular meningitis. The specificity and sensitivity of CSF ADA for TBM was found to be 85.0% and 70.0% as compared to NTBM and 85.0% and 100.0% as compared to NTBNM. We propose that estimation of that ADA activity in CSF of TBM patients, using a cut off value 6.97 IU/L can diagnose differentially tubercular meningitis. Since, most developing countries have the dubious distinction of having higher prevalence and incidence of tuberculosis and lack of well equipped laboratory services for proper diagnosis of tubercular meningitis, measurement of CSF ADA activity can be a better and reliable approach for the rapid diagnosis and management of tubercular meningitis vis a vis other types of meningitis.

Keyword: Cerebrospinal fluid, Meningitis, Adenosine deaminase activity.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of mortality and morbidity in developing countries. The World Health Organization Reports puts to the record that globally, approximately 16 millions people are suffering from active TB with an estimated 8.5 million developing active TB each year, resulting in approximately 2 million deaths.¹ Recent data shows an estimated 47,315 cases of TB in Nepal with 21,245 new smear positive TB. There is no concrete data available on prevalence and incidence of tubercular meningitis in Nepal. This is the most dangerous form of extra pulmonary TB (PTB) occurring in 7.0-12.0% of TB patients in developing countries with high rate of mortality due to delay in diagnosis and proper treatment.² Due to complication in differentiating the various etiologies of meningitis, it is necessary to introduce simple, reliable and cost effective, method for rapid diagnosis of tubercular meningitis (TBM).

The isolation of *Mycobacterium tuberculosis* on culture of CSF takes a long time and cytology shows considerable overlap results for diagnosing different forms of meningitis.³ So there is a need of other modalities to support an etiological diagnosis of different types of meningitis.

Adenosine deaminase (ADA) is an enzyme that catalyses the deamination of adenosine forming inosine in the process.⁴ ADA activity increases in cell mediated immune response during T-cell differentiation and proliferation. Various studies have been conducted demonstrating CSF-ADA estimation as an enzymatic assay in diagnosis of TBM and can differentiate TBM from normal subjects or other infectious meningitis.⁵⁻⁷ In this particular paper we are evaluating the importance of assessing CSF ADA activity in the differential diagnosis of tubercular meningitis (TBM) as compared to other etiologies such as non-tubercular meningitis (NTBM) and non tubercular non meningitis (NTBNM) cases.

MATERIALS AND METHOD

This diagnostic study was conducted in the department of biochemistry in collaboration with the departments of medicine and microbiology, BP Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. The ADA activity was estimated in 45 cases which include 20 TBM patients, 10 NTBM and 15 NTBNM cases. The lumbar puncture (LP) was performed by trained medical officer in medicine ward of the same hospital. Informed consents were obtained from the patients and/or their guardians prior to performing the LP procedure. The TBM patients were confirmed by clear signs and symptoms with AFB and/or culture positivity for bacteria or good response to antituberculous drugs. In NTBM, the presence of other bacterial infection was confirmed by culture or staining smears positivity. The NTBNM cases included those who had no evidence of central nervous system (CNS) disorder or

bacterial/viral infections. This study was approved by the institute Ethical Review Committee of BPKIHS.

Adenosine deaminase (ADA) activity assay: ADA activity was assayed on the same day of the collection of CSF samples. The ADA activity was measured by the spectrophotometric method described by Guisti and Galanti.⁸ Ammonia forms under treatment of adenosine to adenosine deaminase present in sample to give an intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution as determined by modification Berthelot's reaction. Sodium nitroprusside is used as the catalyst. The ammonia concentration is directly proportional to the absorbance of the indophenol measured at wavelength 620 nm. The reaction catalyzed by ADA was stopped at the end of one hour incubation by addition of phenol nitroprusside solution.

ADA activity was expressed as international unit (IU/L) by using the formula (Δ Absorbance of sample/ Δ Absorbance of standard) x50 IU/L

Adenosine was the product of SRL chemicals, India and all other chemicals used were of analytical grade from MERCK Chemical Company, India.

STATISTICAL ANALYSIS

Data were represented as Mean \pm SD and statistical analysis was done by using software SPSS version-13. The p-value <0.05 was established as statistically significant.

RESULTS

The total number of patients studied was 45. Of these, 20 were diagnosed of tubercular meningitis, 10 were non-tubercular meningitis and 15 cases of non-tubercular non-meningitis.

Table-1 depicts mean age and ADA activity (IU/L) among the study groups. There was no age variation among the patients groups studied. Mean serum ADA activity in TBM (13.69 ± 6.49 IU/L) was, significantly increased compared to the NTBM (6.51 ± 2.41 IU/L $p < 0.001$) and the NTBNM (2.35 ± 1.16 IU/L; $p < 0.0001$) groups.

Fig, 1 shows the comparative distribution of CSF ADA activity in different study groups represented in box-plot form. Distribution of CSF ADA activity was found to be increased in the order TBM>NTBM>NTBNM.

Table-2 shows sensitivity and specificity of CSF ADA activity. The sensitivity and specificity of CSF ADA activity was 85.0% and 88.0% respectively at cut-off value of 6.97 IU/L to diagnose TBM in cerebrospinal fluid (CSF). The specificity and sensitivity of CSF ADA for TBM was found to be 85.0% and 70.0% as compared to NTBM but it was found to be 85.0% and 100.0% as compared to NTBNM.

DISCUSSION

Most developing countries have the dubious distinction of having higher prevalence and incidence of tuberculosis and lack of well equipped laboratory services for proper diagnosis of tubercular meningitis. Early confirmatory diagnosis of TBM is difficult to establish because of its pleomorphic clinical presentation.⁹ Delayed diagnosis and treatment may be associated with many serious CNS complications.¹⁰ The most commonly used laboratory method for the definitive diagnosis of TBM is to demonstrate the presence of tubercle bacilli either by smear and/or culture. However, direct smear methods are often negative in CSF samples and culturing of MTB takes 4-6 weeks to show the growth.^{11,12} Although many sensitive tests involving molecular diagnostics have been available for rapid diagnosis of TB, yet, such a technology is not available in many developing countries. The estimation of ADA activity in body fluid therefore serves as a good and reliable tool in the diagnosis of TB pleural effusion and tubercular meningitis as well as their management specially when other clinical laboratory tests are negative within sensitive limit.¹³

Newer method such as those involving amplification of bacterial DNA by the PCR is incompletely assessed and not available for widespread use in most hospitals of the developing countries. The sensitivity of the PCR technique varies from 33.0% to 90.0% with a specificity of 88.0% to 100.0%.¹⁴ Various immunoassays such as antigen and/or Ab detection in CSF samples have been developed with variable sensitivities and specificities.^{5,8,9} ADA activity helps to differentiate TBM from non-TBM infection meningitis and non-neurological disorders.^{6,7,14} In present study, we have calculated an ADA cut-off value of 6.97 IU/L in CSF for the diagnosis of TBM infection. Using this cut-off value we have found sensitivity and specificity to be 85.0% and 88.0% for tubercular meningitis patients diagnosed on the basis of culture positivity. Baro *et al* proposed a cut-off value of 6.5 U/L/min and showed sensitivity of 83.3% and specificity of 88.3%.⁶ Gambhir *et al* reported a low sensitivity of 44.0% and specificity of 75.0% for ADA test with a cut off value 8 IU/L/min, which showed overlap between TBM and non-TBM patients, especially for infectious neurological disorders like pyogenic meningitis.¹⁵ Our result also suggests the importance of estimation of ADA activity in tubercular meningitis and in assessing the activation of T lymphocytes in pathogenesis of tubercular meningitis while differentiating the various

types of meningitis. The estimation of ADA activity in CSF therefore serves as relatively simple, inexpensive and reliable tool in the diagnosis of TBM and management specially when other clinical laboratory tests are negative within sensitive limit.

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Table-1: Age (yrs) and CSF ADA activity (IU/L) in tubercular meningitis non-tubercular meningitis and non-tubercular non-meningitis subjects (results are expressed in Mean \pm SD)

Variables	TBM(n=20)	NTBM(n=10)	NTBNM(n=15)
Age (yrs)	28.24 \pm 3.84	29.4 \pm 2.41	29.24 \pm 4.89
ADA activity (IU/L)	13.69 \pm 6.49 ^{a*} , ^{b**}	6.51 \pm 2.41 ^{c*}	2.35 \pm 1.16

*p<0.001, **p<0.0001, ^a TBM vs NTBM, ^bTBM vs NTBNM, ^c NTBM vs NTBNM

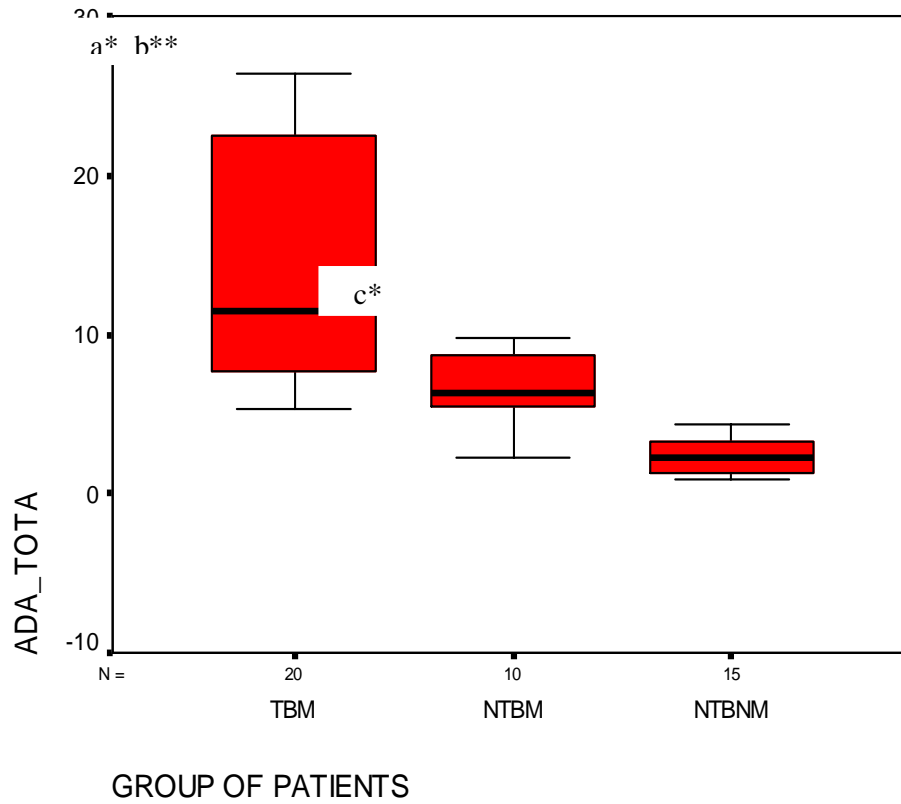


Fig. 1. Box plots chart for CSF ADA activity (IU/L) in tubercular meningitis (TBM), non-tubercular meningitis (NTBM) and non-tubercular non-meningitis (NTBNM) subjects. N= number of individuals in study subjects. * and ** represent $p < 0.001$ and $p < 0.0001$ respectively and superscript a, b and c represents TBM vs NTBM, TBM vs NTBNM, NTBM vs NTBNM respectively. Dashed line represents calculated cut-off value for CSF ADA activity (6.97 IU/L) for diagnosis of TBM

Table-2: Sensitivity and Specificity of overall tubercular meningitis, non-tubercular meningitis and non-tubercular non-meningitis study subjects.

Variables	TBM	NTBM	NTBNM
Sensitivity	85.0%	85.0%	85.0%
Specificity	88.0%	70.0%	100.0%