

Comparative analysis of cerebrospinal fluid adenosine deaminase activity in meningitis

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Summary

AIM: The purpose is to determine the cut-off value of adenosine deaminase (ADA) activity in cerebrospinal fluid (CSF) of patients with tuberculous and non-tuberculous meningitis, and to assess its value in differential diagnosis. **MATERIAL AND METHODS:** This study was conducted in 91 patients with meningitis in two university hospitals in Turkey. 24 patients had tuberculous meningitis (TBM), 25 purulent meningitis (PM), 25 aseptic meningitis (AM) and 17 neurobrucellosis (BM). ADA activity of CSF was quantified by colorimetry.

RESULTS: In our study, mean ADA values in CSF were 28.34 ± 14.83 IU/L in TB cases, 8.71 ± 5.83 IU/L in BM, 6.18 ± 2.54 IU/L in PM and 3.43 ± 3.48 U/L in AM cases. If we accept for CSF ADA an activity cut-off value of 12.5 IU/L for differential diagnosis of TBM and BM, its sensitivity was 92% and specificity was 88%. If we accept 12.35 IU/L for differential diagnosis of TBM and PM, its sensitivity was 92% and specificity was 100%. If we accept 6.45 IU/L for differential diagnosis of TBM and AM, its sensitivity was 100% and specificity was 92%. Additionally, we examined the cases after dividing them into two groups, viz. TB and non-TB. If we accept an ADA activity cut-off level of 11 IU/L for differential diagnosis of TB and non-TB by applying ROC analysis, its sensitivity was 92% and specificity was 90%.

CONCLUSION: The sensitivity and specificity for CSF ADA activity are markedly high in differential diagnosis of TB from non-TB. Hence CSF ADA activity may be used as a simple, cost-effective and reliable test for early differential diagnosis of TB.

Key words: ADA; CSF; non-tuberculous meningitis; tuberculous meningitis

Introduction

Tuberculous meningitis is the most severe form of tuberculosis, and its prevalence in the community is parallel to that of tuberculosis. Currently, despite treatment, mortality and sequel remain high [1]. Definitive diagnosis of tuberculous meningitis is established by observation of tuber-

culosis bacilli in Ehrlich-Ziehl-Neelsen (EZN) stains of cerebrospinal fluid (CSF) and/or isolation of bacteria in CSF culture. Unfortunately, the sensitivity of these methods is low and the conventional culture methods take a very long time, such as 4–6 weeks [2], to produce results. Mortality and sequel rates may be reduced considerably with early diagnosis and treatment. Therefore, studies are in progress to develop faster and more sensitive diagnostic methods [3]. Adenosine deaminase (ADA) is an enzyme involved in purine catabolism and plays a role in maturation of monocytes, macrophages and T lymphocytes. An increase in ADA levels is observed in tuberculosis as well as other bacterial infections in which the cellular immunity response is actively involved [3]. There are also studies reporting an increase in CSF ADA levels in tuberculous meningitis [4].

The aim of the present study was to determine the cut-off value by comparing CSF ADA activity among meningitis groups in two regions of Turkey where meningitis is prevalent, and to emphasise its importance in terms of differential diagnosis of tuberculous meningitis.

Material and method

The study was conducted in the Departments of Infectious Diseases and Clinical Microbiology of the Medical Faculties of Yuzuncu Yil University and Harran University. The study protocol was approved by the local ethics committee.

CSF was taken from a total of 91 patients treated and followed up with the diagnosis of meningitis who were included in the study between 2005–2010. CSF obtained by lumbar puncture were kept at -20 °C. CSF obtained from patients were divided into four groups, tuberculous meningitis (TBM), bacterial or purulent meningitis (PM), viral or aseptic meningitis (AM) and neurobrucellosis or brucella meningitis (BM). CSF adenosine deaminase activity was measured by colorimetry (Shimadzu UV 1240 spectrophotometer).

Diagnosis of TBM was based on the following characteristics; subacute or chronic outset and slow clinical progress, CSF WBC counts $>100/\text{mm}^3$ and mononuclear (MNL)

dominant (>60%), CSF protein >75 mg/dL (normal 15–45 mg/dL) and CSF glucose less than half of blood glucose obtained simultaneously, observation of tuberculosis bacilli in EZN staining of CSF, isolation of bacteria in CSF culture, presence of bacillary arachnoiditis and tuberculoma as observed radiologically by MRI, and response to antituberculous treatment.

Diagnosis of BM, however, was based on the following criteria: acute or subacute onset, CSF WBC count >100/mm³ and MNL dominant (>60%), CSF protein >45 mg/dL, CSF glucose less than half of blood glucose obtained simultaneously, growth of pathogen in blood or CSF culture and/or blood standard tube agglutination test (STA) titre \geq 1/160 or positivity of CSF STA test at any titre and response to anti-brucellosis treatment.

Diagnosis of PM was made on the basis of the following features: acute onset and rapid clinical progress, CSF WBC count >1000/mm³ and polymorph nuclear (PNL) dominant (>75%), CSF protein >75 mg/dL and CSF glucose less than half of blood glucose obtained simultaneously, observation of bacteria in CSF gram staining or growth of pathogen in culture and response to antimicrobial treatment.

AM was diagnosed on the basis of the following: acute onset, insidious and slow clinical progress, CSF WBC count <1000 and MNL dominant (>60%), CSF protein close to normal (15–45 mg/dl), CSF glucose less than half of blood glucose obtained simultaneously, negative results in microbiological evaluation of CSF and serum in terms of purulent, tuberculous and brucella meningitis and full response to symptomatic treatment without antimicrobials.

Statistical analyses

In statistical analyses, descriptive statistics of mean, standard deviation, minimum and maximum values were used. In order to determine differences (if any) between groups in terms of ADA values, Kruskal-Wallis variance analysis was performed; Bonferroni adjusted Mann Whitney-U test was used to determine the group responsible for this difference. To establish cut-off values to differentiate TBM

and viral, TBM and purulent and TBM and brucella groups, ROC curve analysis was performed. In addition, ROC curve analysis was performed to determine the cut-off value for ADA in order to differentiate TBM and non-TBM groups. Statistical analysis was performed by SPSS (version 11.5) package program. Significance level was accepted as 5%.

Results

Out of 91 patients included in the study, there were 24 TBM, 25 PM, 25 AM and 17 BM cases. Age range of patients was 18–60 years; demographic characteristics are given in table 1. CSF findings and culture rates of meningitis types are shown in table 2. In our study, mean values for CSF ADA (for TBM, BM, PM and AM) are shown in table 3. CSF ADA levels of TBM cases were significantly high as compared to the other three meningitis groups separately ($p < 0.001$). The results are shown in table 3.

We also evaluated all cases in two groups: TBM and non-TBM patients. Following receiver-operating characteristic curves (ROC) analysis for ADA activity in TBM and non-TBM groups, and with a cut-off value of 11 IU/L for CSF ADA activity, we determined a sensitivity of 92% and a specificity of 90% for diagnosis of TBM. The area under the curve (AUC) is 0.965 (fig. 1). The distribution of CSF ADA activity in groups is shown in figure 2.

Discussion

TBM remains a major global health problem [5, 6]. The definitive criterion for the diagnosis of tuberculous meningitis is demonstration of *M. tuberculosis* in CSF, either by direct EZN stained smears or biological culture. However, the sensitivity of CSF EZN staining is 2–87%, and culture confirmation of *M. tuberculosis* in the CSF is only positive in 25–75% of cases [7–13].

Due to the high mortality and sequel rates in TBM meningitis, there is an urgent need for simple, rapid and sensit-

Table 1: Demographic characteristics of patients.

Meningitis	Men	Women	Total	Mean age \pm SD
TBM	10	14	24	34.20 \pm 21.32
PM	10	15	25	32.64 \pm 16.93
BM	9	8	17	40.10 \pm 23.12
AM	15	10	25	26.16 \pm 7.60
Total	44	47	91	32.26 \pm 17.42

Table 2: CSF findings of meningitis types.

CSF findings	TBM (%)	PM (%)	BM (%)	AM (%)
CSF culture (+)	25	36	23.5	0
CSF Gram (+)	0	20	0	0
CSF EZN (+)	20.8	0	0	0
Blood culture (+)	0	12	35.2	0
CSF Wright Test (+)	0	0	94.1	0
Blood Wright Test (+)	0	0	100	0
CSF protein (mg/dL)	200.70 \pm 126.62	272.76 \pm 35.08	147.64 \pm 131.10	39.20 \pm 15.6
CSF/blood glucose (mg/dL)	0.31 \pm 0.13	0.37 \pm 0.18	0.39 \pm 0.14	0.63 \pm 0.12
CSF leukocyte counts/mm ³	205.24 \pm 28.24	1359.26 \pm 292.38	222.05 \pm 23.55	86.20 \pm 51.30

CSF glucose / Blood glucose normal value: 0.50–0.66.
CSF protein normal value: 15–45 mg/dL

ive tests for early diagnosis. Some studies show that CSF-ADA is a more sensitive indicator than PCR [14].

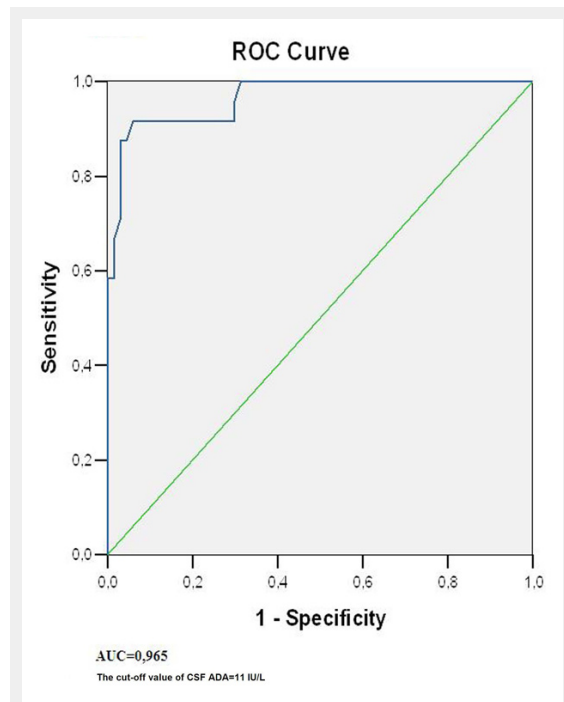


Figure 1
The receiver-operating characteristic (ROC) analysis curve for ADA in tuberculous meningitis and non-tuberculous meningitis.

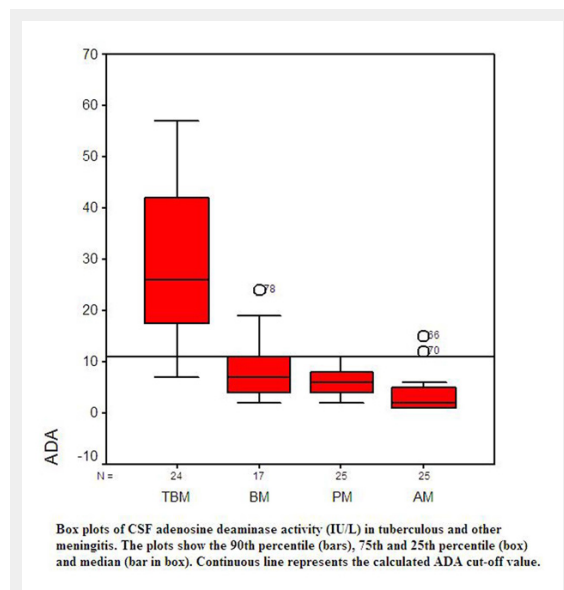


Figure 2
Box plots of CSF-ADA activity (IU/L) in tuberculous and non-tuberculous meningitis (BM, PM and AM).

For various aetiologies the diagnostic value of ADA levels in CSF in meningitis cases has been investigated in a number of studies, and ADA levels were found to be significantly high in tuberculous meningitis groups as compared to bacterial and viral meningitis groups [2, 3].

Choi et al. reported that the sensitivity of the ADA test for TBM compared with AM was 0.83 and the specificity was 0.95 when a cut-off value of 7 IU/L was used [4]. López-Cortés et al. reported that the sensitivity of the ADA test for TBM compared with AM was sensitivity of 48% and specificity of 100% when a cut-off ≥ 10 IU/L was used [15]. In our study, if the cut-off value of CSF ADA activity for TBM and AM differentiation was accepted as 6.45 IU/L, the sensitivity of the test for tuberculous meningitis was 100% and specificity was 92%. Choi et al. reported that the sensitivity of the ADA test for TBM compared with PM was 0.58 and the specificity was 0.89 when a cut-off value of 10 IU/L was used [4]. In our study, when the cut-off value for CSF ADA activity for differential diagnosis of TBM and PM was accepted as 12.35 IU/L, we found that the sensitivity of the test for the diagnosis of TBM was 92% and the specificity was 100%.

There are few studies on ADA activity in neurobrucellosis, but high ADA levels are nevertheless determined in CSF [16]. As far as we know from our research, only two studies have reported on ADA values in neurobrucellosis apart from case reports [15, 17]. One of these studies was conducted by López-Cortés et al., and they investigated ADA levels in only 5 neurobrucellosis cases among other meningitis groups. In two of these five cases ADA was found to be high [15]. Abduljabbar also investigated ADA levels in 5 neurobrucellosis cases and determined ADA values of 20 IU/L in cases with high lymphocyte levels in CSF. In this study, ADA was only investigated in neurobrucellosis cases and was not compared to other meningitis cases [17]. In our study, P value of CSF ADA activity in a series of neurobrucellosis was found to be significant both in comparison to AM and TBM ($P < 0.001$). Since laboratory and clinical symptoms of neurobrucellosis are similar to TBM, differential diagnosis is crucial. Furthermore, brucella Wright test may not always yield positive results. In some cases growth in culture may be delayed, and growth rate is usually low [6]. Therefore, in differential diagnosis of TBM and neurobrucellosis ADA can be used as a reliable, simple and rapid test. Moreover, brucellosis is prevalent in countries where tuberculosis is widespread [18].

A number of studies have been carried out on the differentiation of tuberculous meningitis from non-tuberculous meningitis through CSF ADA levels. The non-tuberculous meningitis group consists of pyogenic meningitis, aseptic meningitis and non-infectious disorders, plus, in certain cases, cryptococcal meningitis and neurobrucellosis. Dif-

Table 3: Descriptive statistics and results for ADA in four groups.

Meningitis	Mean	SD	% 95 CI		Minimum	Maximum	P
TBM	28.34 a	14.83	22.08	34.61	6.80	57.00	0.001
BM	8.71 b	5.835	5.71	11.71	2.10	23.50	
PM	6.18 bc	2.54	5.13	7.23	2.10	11.40	
AM	3.43 c	3.48	1.99	4.87	0.80	14.80	

Significant difference between a and other three groups. Significant difference between b and c. No significant difference between b and bc and between c and bc.

ferent values were reported for cut-off values of CSF ADA activity and for sensitivity and specificity rates.

Chotmongkol et al. reported a cut-off value of 15.5 IU/L with sensitivity of 75%, specificity of 93% and area under the curve in a receiver-operating characteristic (ROC) curve as 0.92 to differentiate tuberculous from non-tuberculous meningitis [19]. Moghtaderi et al. reported a cut-off value of 10.5 IU/L, sensitivity as 81% and specificity as 86% respectively. The area under the curve (AUC) is 0.870 [3]. Kashyap et al. reported a cut-off value of 11.4 IU/L for TBM patients and calculated sensitivity and specificity rates of 82% and 83% respectively [20]. Rana et al. reported the cut-off value as 10 IU/L, sensitivity as 66.6% and as specificity 90% respectively [21]. In a meta-analysis evaluating ten studies, the authors reported that cut-off values for CSF ADA in TBM were between 8.5–15.5, sensitivity was between 50–100% with a mean of 0.79 (95%CI 0.75–0.83) and specificity was between 60–100% with a mean of 0.91 (95%CI 0.89–0.93). Mean area under the curve (AUC) was reported as 0.915 [22].

In our study, when the cut-off value was accepted as 6.7 for TBM, sensitivity was determined as 100% and specificity as 69%. If the cut-off value was accepted as 10.5, sensitivity was 92% and specificity 88%. When the cut-off value is 11 IU/L, sensitivity is 92% and specificity is 90%. The area under the curve (AUC) was found to be 0.965 (fig. 1).

Moghtaderi et al. and Choi et al. reported that CSF ADA values over 15 IU/L are strong indicators for the diagnosis of tuberculous meningitis [3, 4]. In our study we determined that CSF ADA levels over 20 IU/L are strong indicators for TBC. Inclusion of neurobrucellosis cases in our series caused the upper limit to increase.

Different values for CSF ADA in various studies may be due to study method, gender, age and race factors. Besides this, the disease stage during which ADA is determined may also cause changes in ADA levels [23]. Multicentre studies in different populations are needed to determine the standard CSF ADA levels.

Currently, TBM and early diagnosis is a global issue and is becoming more and more crucial. All relevant studies share the view that ADA is a useful test in early differential diagnosis of TBM. On the other hand, there is no consensus on standardisation of the cut-off value [2, 4, 22].

In conclusion, when the cut-off value for CSF ADA was accepted as 11 IU/L to differentiate TBM from other meningitis cases, both sensitivity and specificity were determined at a markedly high level. CSF ADA activity is a simple, cost-effective and reliable biochemical test for differential diagnosis of TBM. Nevertheless, multicentre studies are needed for standardisation of the test.

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