CEREBROSPINAL FLUID ADENOSINE DEAMINASE ACTIVITY FOR THE DIAGNOSIS OF TUBERCULOUS MENINGITIS IN ADULTS

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Abstract. We studied adenosine deaminase (ADA) activity in cerebrospinal fluid (CSF) of 16 cases of tuberculous meningitis, 4 cases of cryptococcal meningitis, 5 cases of bacterial meningitis, 12 cases of eosinophilic meningitis, 26 cases of aseptic meningitis, 6 cases of carcinomatous meningitis and 108 cases with normal CSF. The mean CSF ADA values for the different groups were: 39.44 ± 41.46, 13.00 ± 7.43, 34.20 ± 40.81, 3.17 ± 4.82, 10.03 ± 9.23, 8.67 ± 13.60, and 2.58 ± 2.90 U/l, respectively. Comparing the ADA activity between patients with tuberculous meningitis and non-tuberculous meningitis, the receiver-operating characteristic (ROC) curve identified a CSF ADA level of 15.5 U/l as the best cut-off value to differentiate between the two, with a sensitivity of 75% and a specificity of 93%, with an area under the curve of 0.92. When tuberculous meningitis was compared with aseptic and carcinomatous meningitis, the ROC curve identified a CSF ADA level of 19.0 U/l as the best cut-off value for differentiation, with a sensitivity of 69% and a specificity of 94%, with an area under the curve of 0.83. The level of CSF ADA may be useful as a complementary tool in the early diagnosis of tuberculous meningitis.

INTRODUCTION

Tuberculous meningitis is a common infectious disease of the central nervous system in developing countries. Early diagnosis and treatment with chemotherapy and active management of the complications are of great importance to prevent the irreversible neurologic sequel and death. A definitive diagnosis of tuberculous meningitis depends on identifying *Mycobacterium tuberculosis* in the cerebrospinal fluid (CSF) by direct staining or culture. However, the diagnostic yield of CSF smears and cultures in our experience has been very low (Chotmongkol *et al*, 1996), and mycological cultures may take up to 8 weeks to yield results. Therefore, the diagnosis of tuberculous meningitis depends on the clinical manifestations of subacute to chronic meningitis with lymphocytic CSF and low CSF glucose levels. However, other forms of meningitis may mimic tuberculous meningitis. Certain patients with tuberculous meningitis may have CSF findings resembling aseptic meningitis. Several tests for the rapid diagnosis of tuberculous meningitis have been developed; all are based on examination of the CSF. These tests are considered indirect tests (usually measuring a product of the host response to this infection, such as adenosine deaminase, the radioactive bromide partition test and antibodies to the mycobacterial antigen) and direct tests [usually measuring a product of the infecting organism, such as 3-(2'-ketohexyl) indoline, detecting of tuberculostearic acid (a component of the cell wall of *M. tuberculosis*), mycobacterial antigens or fragments of

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mycobacterial DNA by polymerase chain reaction (Zuger and Lowy, 1991)]. These methods, except for adenosine deaminase, are too complicated or expensive for many laboratories.

Adenosine deaminase (ADA) is an enzyme involved in purine catabolism. It is considered as an indicator of cell-mediated immunity and is found mainly in T lymphocytes (Sullivan *et al*, 1977). Detection of CSF ADA activity in the diagnosis of tuberculous meningitis has been reported with good results (Ribera *et al*, 1987; Choi *et al*, 2002).

At Srinagarind Hospital, tuberculous meningitis accounts for 20% of community acquired-meningitis in adults. Previous reports of ADA levels in this infection have not been published in Thailand. The purpose of this prospective study was to define the diagnostic role of CSF ADA in tuberculous meningitis in adults.

MATERIALS AND METHODS

Study population

One hundred and seventy patients with symptomatic community acquired-meningitis in adults (age ≥15 years) admitted to Srinagarind Hospital, Khon Kaen were included in the study (from the equation $N = (Z\alpha/2)^2 P(1-P)/ e^2$, $\alpha = 5\%$, $Z_{0.025} = 1.96$, p = 90%, e = 0.1, prevalence of the disease = 20%). Informed consent was obtained before beginning the study. A detailed history and physical examination were taken and recorded in case-record format. Patients were excluded if they had a positive HIV antibody test or has previously received antituberculous drugs before admission.

CSF was sent for conventional diagnosis, including white blood cell count, protein and glucose concentrations, Gram stain, Ziehl-Neelsen stain, aerobic culture, cryptococcal antigen, and culture to identify *M. tuberculosis*. Other diagnostic examinations were performed as necessary. Two milliliters of CSF were sent for ADA assay. The laboratory technician was blinded to the diagnosis of each patient. The clinician was unaware of the ADA levels when the diagnosis was assigned.

CSF samples were stored at 4°C from the time of collection until analyzed, within 1 week of collection. We determined the ADA levels by following the colorimetric method of Giusti (1974). Results were expressed as U/l.

Diagnostic classification

Tuberculous meningitis was confirmed if the CSF culture yielded *M. tuberculosis* or a positive Ziehl-Neelsen stain. Probable disease was diagnosed in the presence of a lymphocytic pleocytosis in the CSF with a high protein content and a low glucose content (<50% of matched plasma glucose), negative bacterial and fungal cultures, and a negative latex agglutination test for bacterial and cryptococcal antigens. Cryptococcal meningitis was diagnosed if either the Indian ink stain, CSF fungal culture or CSF cryptococcal antigen were positive. Acute bacterial meningitis was diagnosed in patients with CSF neutrophilia, a high protein content, a low glucose content and a positive Gram stain and bacterial culture. Diagnosis of eosinophilic meningitis was based on findings of ≥10% eosinophils in the CSF. Aseptic meningitis was diagnosed if there was a predominately lymphocytic pleocytosis in the CSF with a normal or mildly raised protein content, a normal glucose content, negative serology and bacterial, fungal and mycobacterial cultures. Carcinomatous meningitis was diagnosed if cytological examination was positive for malignant cells. A normal CSF result was reported when there were <5 leukocytes/mm^3 with a normal protein level, normal glucose level, negative culture and negative serology.

Ethics

The Ethics Committee of the Faculty of Medicine, Khon Kaen University, approved this research.

Statistical analysis

Means and standard deviations were given for continuous data. Following standard definitions, a 2x2 table of each level was used to calculate the sensitivity and specificity of ADA. The best cut-off value was selected using a receiver-operating characteristic (ROC) curve.

RESULTS

Study population

From June 2003 through December 2005, 214 patients were enrolled in the study. However, 37 patients were removed from the study because 32 patients had positive tests for HIV antibodies and 5 patients had previously received antituberculous drugs prior to admission. Therefore, 177 patients were studied.

Outcome

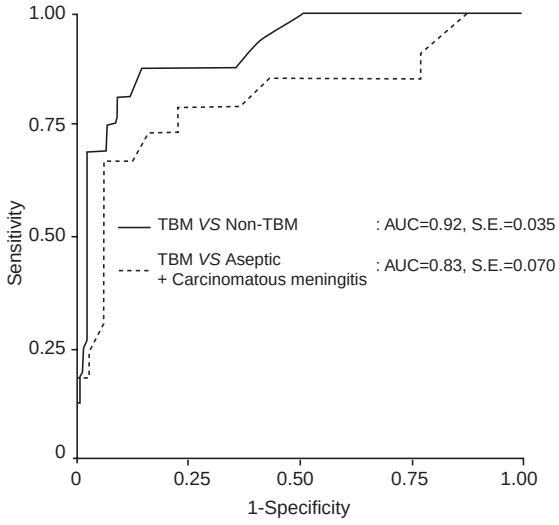
The 177 cases were comprised of: tuberculous meningitis in 16 (CSF culture positive for *M. tuberculosis* in 4 cases), cryptococcal meningitis in 4, bacterial meningitis in 5, eosinophilic meningitis in 12, aseptic meningitis in 26, carcinomatous meningitis in 6 and 108 normal cases. The values for CSF ADA and CSF total lymphocytes in the various groups are shown in Tables 1 and 2, respectively.

Table 1
CSF ADA levels in different groups.

Patient groups	ADA activity (U/l) Mean ± SD	Range
Tuberculous meningitis (n=16)	39.44 ± 41.46	3.0 - 139.0
Culture positive (n=4)	8.25 ± 6.02	3.0 - 16.0
Probable disease (n=12)	49.83 ± 43.16	14.0 - 139.0
Cryptococcal meningitis (n=4)	13.00 ± 7.43	2.0 - 18.0
Bacterial meningitis (n=5)	34.20 ± 40.81	1.0 - 102.0
Eosinophilic meningitis (n=12)	3.17 ± 4.82	0.0 - 18.0
Aseptic meningitis (n=26)	10.03 ± 9.23	0.0 - 48.0
Carcinomatous meningitis (n=6)	8.67 ± 13.60	0.0 - 36.0
Normal (n=108)	2.58 ± 2.90	0.0 - 14.0

Table 2
CSF total lymphocytes in different groups.

Patient groups	Total lymphocytes (per mm^3) Mean ± SD	Range
Tuberculous meningitis (n=16)	290.19 ± 479.45	10.0 - 1,568.0
Culture positive (n=4)	200.25 ± 239.32	24.0 - 536.0
Probable disease (n=12)	320.17 ± 542.14	10.0 - 1,568.0
Cryptococcal meningitis (n=4)	240.50 ± 210.69	80.0 - 547.0
Bacterial meningitis (n=5)	1,604.60 ± 3,471.49	3.0 - 7,814.0
Eosinophilic meningitis (n=12)	421.25 ± 417.23	1.0 - 1,218.0
Aseptic meningitis (n=26)	178.00 ± 230.71	8.0 - 765.0
Carcinomatous meningitis (n=6)	317.40 ± 476.15	9.0 - 1,150.0
Normal (n=108)	0.46 ± 1.34	0.0 - 5.0



TBM = Tuberculous meningitis; AUC = Area under the curve; S.E. = Standard error.

Fig 1–Receiver-operating characteristic curves for CSF ADA.

Comparing the ADA activity between the tuberculous meningitis cases (n=16) and non-tuberculous meningitis cases (n=161), the ROC curve identified a CSF ADA level of 15.5 U/l as the best cut-off value to differentiate between the two, with a sensitivity of 75% and a specificity of 93%, with an area under the curve of 0.92. When tuberculous meningitis was compared with aseptic and carcinomatous meningitis (N=32), the ROC curve identified a CSF ADA level of 19.0 U/l as the best cut-off value to differentiate, with a sensitivity of 69% and a specificity of 94%, with an area under the curve of 0.83 (Fig 1).

DISCUSSION

In the present study, we calculated a CSF ADA cut-off value of 15.5 U/l to differentiate between tuberculous meningitis and non-tuberculous meningitis. Generally, routine CSF laboratory parameters may be helpful in the diagnosis of bacterial, cryptococcal and eosinophilic meningitis. In clinical practice there are diagnostic difficulties in differentiating tuberculous meningitis from other lymphocytic CSF, especially aseptic meningitis. We calculated a CSF ADA cut-off value of 19.0 U/l to differentiate between tuberculous meningitis and aseptic meningitis and carcinomatous meningitis, in which lymphocytes predominate in the CSF.

In the present study, certain patients with tuberculous meningitis had CSF ADA values more than 100 U/l, which have never been reported in previous studies (Ribera *et al*, 1987; Pettersson *et al*, 1991; Prasad *et al*, 1991; Choi *et al*, 2002). This may be due to racial differences. We also found the mean CSF ADA levels and CSF total lymphocyte counts in culture-positive tuberculous meningitis patients were lower than in those with probable disease. This may be reflect host defensive mechanisms.

In conclusion, ADA estimation in the CSF is simple, inexpensive and a rapid method to help the physician make an early diagnosis of tuberculous meningitis. This test should be done as a routine laboratory test on the CSF in areas with similar incidences of tuberculous meningitis exist.

REFERENCES

Choi SH, Kim YS, Bae IG, *et al.* The possible role of cerebrospinal fluid adenosine deaminase activity in the diagnosis of tuberculous meningitis in adults. *Clin Neurol Neurosurg* 2002; 104: 10-5.

Chotmongkol V, Jitpimolmard S, Thavornpitak Y. Corticosteroid in tuberculous meningitis. *J Med Assoc Thai* 1996; 79: 83-90.

Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. Vol 2. New York: Academic Press, 1974: 1092-9.

Pettersson T, Klockars M, Weber TH, Somer H. Diagnostic value of cerebrospinal fluid adenosine deaminase determination. *Scand J Infect Dis* 1991; 23: 97-100.

Prasad R, Kumar A, Khanna BK, *et al.* Adenosine

deaminase activity in cerebro-spinal fluid for diagnosis of tuberculous meningitis. *Ind J Tub* 1991; 38: 99-102.

Ribera E, Martinez-Vazquez JM, Ocana I, Segura RM, Pascual C. Activity of adenosine deaminase in cerebrospinal fluid for the diagnosis and follow-up of tuberculous meningitis in adults. *J Infect Dis* 1987; 155: 603-7.

Sullivan JL, Osborne WR, Wedgewood RJ. Adenosine deaminase activity in lymphocytes. *Br J Haematol* 1977; 37: 157-8.

Zuger A, Lowy FD.Tuberculosis of the central nervous system. In: Scheld WM, Whitley RJ, Durack DT, eds. Infections of the central nervous system. New York: Raven Press, 1991: 425-56.