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The use of adenosine deaminase activity as a biochemical marker for the diagnosis of tuberculous meningitis

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Abstract

The diagnostic value of adenosine deaminase (ADA) activity was studied to evaluate its use in the differential diagnosis of tuberculous meningitis in the local setting. Cerebrospinal fluid (CSF) from 119 patients with meningitis and other conditions with central nervous system symptoms were collected and ADA activity determined by the colorimetric method of Guisti read at 628 nm. The CSF was also subjected to other laboratory examinations so as to provide the aetiological diagnosis. All 14 tuberculous meningitis patients had ADA activity greater than the cut off value of 9.0 IU/L. High ADA activity was also seen in 13 of 105 non-tuberculous cases giving a specificity of 87.6%. Even though the ADA activity determination is sensitive for tuberculosis, it was not specific enough to be used as a rapid diagnostic test. However when interpreted together with clinical signs and symptoms and other laboratory tests, it is a useful adjunctive rapid marker for tuberculosis.

Key words: Adenosine deaminase activity, tuberculosis, meningitis.

INTRODUCTION

Mycobacterium tuberculosis infection has been considered a minor and decreasing problem in industrialised countries while it has remained a severe problem in many developing countries. However, recently the incidence of tuberculosis has been increasing in the developed countries. This has been attributed to increases in the incidence of the acquired immunodeficiency syndrome (AIDS),' immigration, and the other primary risk factors for the disease such as poverty, malnutrition and overcrowded living conditions.[2] Currently, there are 20 million cases of tuberculosis (TB) worldwide with 8 million new cases each year. Three million deaths annually are directly attributable to tuberculosis.[3]

Tuberculous meningitis (TBM) is the most dangerous extrapulmonary diagnosis, accounting for 7-12% of TB cases in developing countries.[4] Although there are effective chemotherapeutic agents at present, the mortality and morbidity of TBM remains high, especially in patients receiving late treatment.[5]

Bacteriological methods of diagnosis are not satisfactory for early diagnosis because there are too few organisms in the cerebrospinal fluid (CSF) to be detected by direct smear. The culture of mycobacterium and its subsequent identification by biochemical tests generally require at least 3-6 weeks using solid media and 10-20 days using the radiometric Bactec System.[6,7]

Several rapid diagnostic methods have been described in recent years including immunological detection of tuberculosis infection by non-cultural methods, such as latex agglutination, radioimmuno-assay, and enzyme-linked immunosorbent assay, but these techniques lack sensitivity and/or specificity.[8,9,10] The use of DNA probes and gene amplification by polymerase chain reaction[11,12,13] in developing countries is of doubtful applicability since they require expensive equipment and specially trained staff.

Adenosine deaminase (ADA) is an enzyme which is widely distributed in mammalian tissue especially in lymphoid tissue. ADA activity can be a useful diagnostic indicator for patients with TBM since lymphocytosis is the main cellular response. ADA is released by lymphocytes and macrophages during the cellular immune response. Its main biological role is related to the proliferation and differentiation of lymphocytes. It catalyses the irreversible deamination of adenosine into inosine and ammonia. The specific activity of ADA is increased by 2-9 fold in transforming monocytes and can be detected as early as 3-4 hours after transformation, reaching a maximum after 16-24 hours.[14]

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ADA activity is high in T-lymphocytes and macrophages. The enzyme activity is inversely proportional to the degree of cell differentiation. The increase in levels of ADA is attributed to the mature stage of the T-cells. It has been reported that assay of ADA activity in CSF enables TBM to be distinguished from other meningitis.[15] This study was conducted to evaluate its use in the differential diagnosis of TBM and to obtain additional information about sensitivity, specificity, and suitability of the method in the local setting.

MATERIALS AND METHODS

Cerebrospinal fluid (CSF) samples were collected from 119 patients with meningitis and other conditions with central nervous system (CNS) symptoms from June 1992 to December 1993.

All the CSF samples are subjected to routine laboratory examination which include qualitative and quantitative cytology as well as glucose and protein concentration, gram stain, Ziehl-Neelsen stain, Indian ink stain and culture to provide an aetiological diagnosis.

Based on clinical manifestations and laboratory results, the CSF samples were divided into various diagnostic categories. The tuberculous meningitis group included patients with positive CSF culture for M. ***tuberculosis.*** Since the sensitivity for culture is low, patients presenting with meningitis and tuberculosis at other sites as evidenced by positive chest X-ray, positive sputum culture, positive mantoux test, significant history of contact and exclusion of other infective causes supported by compatible CSF cytology and biochemistry were also included in this category.

CSF from patients with clinical manifestations of aseptic meningitis and previous history of viral infection together with normal CSF parameters were grouped into the viral meningitis category. The bacterial meningitis group included patients that were clinically suggestive of septic meningitis with either positive bacterial antigen in the CSF, culture positive or CSF findings suggestive of bacterial meningitis.

Cerebrospinal fluid samples which were culture positive for cryptococcus neoformans or contained either cryptococcal antigen or antibody or both, were grouped under the cryptococcal meningitis category. The benign intracranial hypertension group consisted of young adults, the majority being female, with increased intracranial pressure but negative CSF findings. CSF from patients with neurological conditions involving either the central or peripheral nervous systems were grouped in the neurological problem category. Those included in the miscellaneous group were extradural abscess, ketoacidosis, cardiovascular accident, septicaemia, brain tumour and chronic headache with hydrocephalus but negative CSF findings.

The ADA activity was determined on all CSF samples at 37°C by the colorimetric method of Guisti.[16] It was based on the indirect measurement of the formation of ammonium produced when ADA acts in an excess of adenosine. The absorbancy was measured at 628 nm and 3 replication of samples were measured. Levels of more than 9 IU/L was taken as positive.[17] ADA enzyme of a known activity of 10 IU/L as well as a CSF sample with a known activity were used as standard controls for intraassay accuracy in all the determinations. The release of ammonia was determined colorimetrically after development of an intensely blue indophenol with hypochlorite and phenol in alkaline solution.

RESULTS

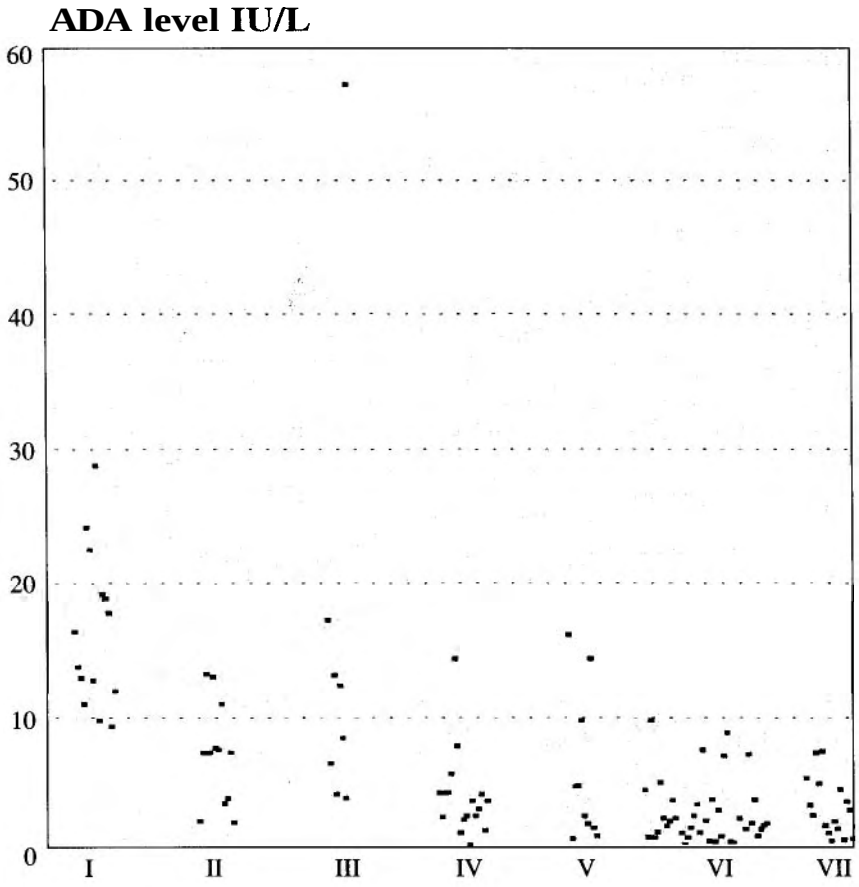
The 119 patients were categorised into 7 different groups based on the stated criteria. The mean value of ADA activity for the seven groups is shown in Table 1 and individual results are given in Fig. 1. In all the 14 patients with TBM and 13 out of 105 non-tuberculous cases the ADA activity was found to be higher than 9 IU/L giving a sensitivity of 1.O, a specificity 87.6 and a P value of <0.0001.

The mean enzyme value was almost equal in TBM and bacterial meningitis being 16.33 ± 5.66 IU/L and 15.42 ± 13.93 IU/L respectively. The mean value for bacterial meningitis was high due to one extreme value of 57.1 IU/L observed in a case of fatal fulminant pneumococcal meningitis. Three other cases which had high ADA activity were, a case of H. *influenzae* meningitis in an eight-months-old boy and two other cases with high cell counts in the cerebrospinal fluid.

ADA activity was also high in 3 cases of cryptococcal meningitis. Cryptococcal antigen was detected in 2 of the 3 patients and cryptococcal antibody in the remaining one. One patient who was diagnosed as having Gullian Baire Syndrome and was given immunoglobulin had high ADA activity in the CSF. Three out of 10 patients with benign intracranial hypertension had ADA activity of more than 9

TABLE 1: ADA activity (IU/L) in the CSF of different groups of patients.

Group	Diagnosis	No. of patients	ADA (mean)IU/L
I	Tuberculous meningitis	14	16.33 ± 5.66
II	Cryptococcal meningitis	12	7.26 ± 3.80
III	Bacterial meningitis	8	15.42 ± 3.93
IV	Viral meningitis	17	4.16 ± 3.12
V	Benign intracranial hypertension	10	5.90 ± 5.60
VI	Neurological problem	41	3.04 ± 2.10
VII	Miscellaneous	17	3.13 ± 9.80



I Tuberculous meningitis
II Cryptococcal meningitis
III Bacterial meningitis
IV Viral meningitis
V Benign intracranial hypertension
VI Neurological problem
VII Miscellaneous

FIG. 1: Level of ADA activity in CSF from different diagnostic groups of patients.

IU/L. One of them had repeated cardiovascular accident, one was TPHA positive and one had no other coincidental finding and the reading was at the marginal 9.8 IU/L level. One patient with neurofibromatosis and gliomawas found to have high ADA activity in the CSF.

DISCUSSION

From this study it is apparent that the level of CSF ADA activity in tuberculous meningitis is high, but high levels are also observed in other conditions particularly bacterial meningitis. The activity is usually higher in CSF which contain numerous cells. These findings are in agreement with Donald *et al*[18] who observed a high ADA activity in CSF obtained at the initial diagnostic lumbar puncture from bacterial meningitis cases. Together with Hankiewies and Lesniak[19] he also noted a correlation between the level of adenosine diaminase activity and the level of CSF protein. Donald *et al* managed to illustrate in 2 of his patients the parallel movement of CSF ADA activity with CSF protein value.

Previous reports have shown the usefulness of determining ADA activity in the diagnosis of tuberculous pleural effusion, peritonitis and pericarditis with a sensitivity of 1 and a specificity of >0.95.[20,21] Ribera *et al*[17] reported a sensitivity of 1 and specificity of 0.99 for ADA activity for the diagnosis of tuberculous meningitis. They also found elevated ADA activity in the CSF of patients with bacterial meningitis which had high cell counts. Their findings were supported by Piras and Gakis.[22] Malan *et al*[23] reported a similar finding as ours although they claimed that the level of this enzyme in CSF is useful in distinguishing TBM from bacterial meningitis. On the basis of our results the ADA test is not specific enough to be used as a rapid diagnostic test. However when interpreted together with clinical signs and symptoms and other laboratory tests, it can be a useful adjunctive rapid marker for the diagnosis of TBM.

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REFERENCES

1. Collins FM. Tuberculosis: The return of an old enemy. Crit Rev Microbiol 1993; 9(1): 1-16.
2. Diagnostic standards and classification of tuberculosis. Joint statement of the American Thoracic Society and The Centers for Disease Control. Am Rev Resp Dis 1990; 142: 725-35.
3. Kochi A. The global tuberculosis situation and the new control strategy of the World Health Organization. Tubercle 1991; 72: 1.
4. Tandon PN. Tuberculous meningitis. In: Vinken PJ, Brynn GW, eds: Handbook of Clinical Neurology, Vol 33. Amsterdam: North Holland Publishing Co., 1978: 195-262.
5. Molavi A, Le Frock JL. Tuberculous meningitis. Med Clin North Am 1985; 69: 315-31.
6. Kirihara JM, Hillier SL, Coyle MB. Improved detection times for Mycobacterium avium complex and ***Mycobacterium tuberculosis*** with the BACTEC radio-metric system. J Clin Microbiol 1985; 22: 841-45.
7. Morgan MA, Horstmeier CD, De Young DR, Roberts GD. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear negative specimens. J Clin Microbiol 1983; 18: 384-8.
8. Watt G, Zaraspe G, Bautista S, Llaughlin LW. Rapid diagnosis of TBM using enzyme linked immunosorbent assay to detect mycobacterial antigen and antibody in CSF. J Inf Dis 1988; 158: 681-6.
9. Kadival GV, Mazarelo TB, Chaparas SD. Sensitivity and specificity of ELISA in the detection of antigen in tuberculous meningitis CSF. J Clin Microbiol 1986; 23: 901-4.
10. Krambovitis E, McIllmurray MB, Lock PE, Hendrikse W, Holzel H. Rapid diagnosis of tuberculosis meningitis by latex particle agglutination. Lancet 1984; 2: 1229-31.
11. Shankar P, Manjunath N, Mohan KK, ***et al.*** Rapid diagnosis of TBM by polymerase chain reaction. Lancet 1991; 337: 5-7.
12. Gonzales RC, Hana BA. Evaluation of Gene-Probe DNA hybridization system for the identification of M. ***tuberculosis*** and ***M. avium-intracellulare.*** Diag Micro Infect Dis 1987; 8: 69-77.
13. Hermans DWM, Schuitema ARI, Van Soolingam D, ***et al.*** Specific detection of ***M. tuberculosis*** complex strains by polymerase chain reaction. J Clin Microbiol 1990; 28: 1204-13.
14. Fischer D, Van Der Weysen MB, Snyderman R, Kelly WN. The role of adenosine deaminase in human monocyte maturation. J Clin Invest 1976; 58: 399-407.
15. Segura RM, Paseual C, Ocana I, Martinez-Vazquez JM, Ribera E, Ruiz I, Pelegri MD. Adenosine deaminase in body fluids : A useful diagnostic tool in tuberculosis. Clin Biochem 1988; 22: 141-8.
16. Giusti G. Adenosine deaminase. In: Berg meyer HU, ed: Methods of enzymology analysis. Vol 2. New York : Academic Press, 1974 : 1092-9.
17. Ribera E, Martinez-Vazquez JM, Inma O, Segura RM, Carlos P. Activity of ADA in cerebrospinal fluid for the diagnosis and follow-up of tuberculous meningitis in Adults. J Inf Dis 1987; 155: 603-7.
18. Donald PR, Malam C, Schoeman JF. Adenosine deaminose activity as a diagnostic aid in tuberculous meningitis. J Inf Dis 1987; 156: 1040-1.
19. Hankiewicz J, Lesniak, M. Adenosine deaminase

in cerebrospinal fluid. Enzymologia 1972; 43: 385-95.

20. Ocana I, Martinez-Vazquez JM, Segura RM, Femandez De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids. Test for diagnosis of tuberculous pleural effusion. Chest 1993; 84: 51-3.
21. Ocana I, Martinez-Vazquez JM, Ribera E, Segura RM, Pascual C. Adenosine deaminase activity in the diagnosis of lymphocytic pleural effusions of tuberculous, neoplastic and lymphomatous origin. Tubercle 1986; 67: 141-5.
22. Piras MA, Gakis C. Cerebrospinal fluid adenosine deaminase ativity in tuberculous meningitis. Enzyme 1973; 14: 311-7.
23. Malam C, Donald PR, Golden M, Taljaard JJF. Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. J Trop Med Hyg 1984; 87: 33-40.