ADENOSINE DEAMINASE LEVELS IN CEREBROSPINAL FLUID AS A DIAGNOSTIC TEST FOR TUBERCULOUS MENINGITIS IN CHILDREN

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ABSTRACT

Adenosine deaminase activity (ADA) was estimated in cerebrospinal fluid (CSF) of 30 patients of tuberculous meningitis (TBM) and 10 patients each of partially treated pyomeningitis (PTM), aseptic meningitis (AM) and pyogenic meningitis (PM). Mean ADA levels in CSF of TBM patients were higher (18.22 U/L) as compared to 6.28 U/L, 3.43 U/L and 7.98 U/L in PTM, AM and PM respectively. This difference of ADA values in CSF between TBM and other types of meningitis was statistically significant ($p < 0.01$) different. Sensitivity and specificity of ADA levels in CSF of children to diagnose tuberculous meningitis was 66.6% and 90% respectively at 10 U/L cut off of ADA levels in CSF. ADA levels in CSF could also differentiate PTM, AM and PM from TBM with a specificity of 90%, 100% and 80% respectively.

INTRODUCTION

Tuberculous meningitis (TBM) is an endemic disease among socioeconomically disadvantaged communities in both developing and developed countries (1). In India, nearly 5 lakh people die due to tuberculosis each year (2). It represents 8.3 % of childhood tuberculosis (TB). The importance of this disease has increased since there is emergence of acquired immuno-deficiency syndrome (AIDS) and multidrug resistant TB (3). Tuberculous meningitis (TBM), the most dangerous form of extra pulmonary TB occurs in 7-12 % of TB patients in developing countries. Delay in diagnosis and institution of proper treatment is directly related to poor outcome and sequelae, which may be seen in 20-25 % of cases (4). A number of methods to diagnose TBM have been evaluated (5). However, all these methods have some drawbacks like the isolation of Mycobacterium tuberculosis on culture of CSF takes a long time. The cytology and biochemistry of CSF which is the main stay of diagnosis of different types of meningitis shows considerable overlap and one has to look for other modalities to support an etiological diagnosis. Levels of the enzyme adenosine deaminase, EC 3.5.4.4 (ADA) in cerebrospinal fluid (CSF) are also known to differentiate patients of TBM from aseptic meningitis and controls but most

of these studies are in adults (7, 8). Only one study conducted in children (6) has reported no significant difference in the mean ADA values in CSF of TBM and pyogenic meningitis. CSF ADA is thought to be released by T lymphocytes during cell mediated immune (CMI) response to tuberculous infection. None of the studies conducted so far had tried to correlate CSF ADA levels with stages of TB and the clinical parameters of CMI response like Mantoux positivity and BCG vaccination status.

In view of these observations ADA activity was estimated in CSF of children with TBM, PTM, AM & PM to assess its diagnostic value for TBM and to correlate with stages of TB and clinical parameters of CMI response to tuberculous infection.

MATERIALS AND METHODS

Activity of ADA was determined in 60 children of age range 6 months to 11 years admitted to department of pediatrics at the Postgraduate Institute of Medical Education and Research, Chandigarh, India during 1997-98. Clinical and laboratory evidence of meningitis/ meningo-encephalitis was taken as inclusion criteria. Enzyme activity was measured in CSF of four groups of patients i.e., TBM, PTM, AM and PM. These four groups were characterised as given below.

Pyogenic meningitis (PM) group

In this group, CSF of patients showing organisms in gram stained smear or culture or presence of bacterial antigen on latex agglutination was taken

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as diagnostic criteria. In the absence of organisms CSF showed pleocytosis of more than 100 cells/mm³ predominantly polymorphs, sugar less than half of blood sugar and protein more than 60 mg %. This was taken as inclusion criteria.

Partially treated pyomeningitis (PTM) group

This group consisted of patients in whom their CSF showed presence of organisms on gram stained smear or culture or presence of bacterial antigen. In the absence of organism CSF showed pleocytosis of more than 100 cells /mm³, sugar less than half of blood sugar, protein more than 60 mg % and who had received I.V. antibiotics for pyogenic meningitis for more than 48 hours before coming to hospital.

Aseptic meningitis (AM) group

This group consisted of patients whose CSF showed absence of organisms on gram stain or culture and CSF pleocytosis with more than 10 cells/mm³, predominantly lymphocytes and sugar more than 2/3 of blood sugar value.

Tuberculous meningitis (TBM) group

In this group the patients had two or more of the following features on history; fever for > two weeks, contact with an adult with tuberculosis, positive Mantoux test. This group had CSF with absolute lymphocyte counts > 50 cells/mm³, protein more than 60 mg % and sugar less than 2/3 of blood sugar, chest X-ray showing skiagram suggestive of pulmonary TB, isolation of AFB from any site, CT scan showing evidence of chronic meningitis like hydrocephalus, basal exudates, infarcts, tuberculomas and histological evidence of tuberculosis.

Activity of ADA was assayed according to the method of Guisti (9). Two ml of CSF sample was collected in a sterile bottle at the time of admission. Patients in whom the lumbar puncture was traumatic were excluded from the study. The samples obtained were centrifuged at 2000 g for 10 min and the supernatant stored at -20°C until estimation. One unit of activity represented the deamination of one micromole of adenosine /min at 37°C and was expressed as U/L.

Statistical Analysis

The means $\pm$ standard error (SE) of ADA values was calculated for each group of subjects. Wilcoxon's rank sum test was used to determine statistical significance. Pearson's formula was used to calculate the coefficient of correlation (r-value). This study was approved by the Ethical Committee of Postgraduate Institute of Medical Education and

Research, Chandigarh, India.

RESULTS

Out of sixty cases, forty-three were males while seventeen females. The mean age of children was 4.15 years in TBM, 3.76 years in PTM, 0.86 years in PM and 6.06 years in AM. The average duration of illness in TBM, PTM, PM and AM was 40.3, 8.8, 5.7 and 5 days respectively. The PM group had the highest mean cell counts and TBM group had the highest mean protein values. There were Gram-negative bacilli in 4 cases of 10 patients of PM group and Gram-positive cocci in 3 cases out of 10 patients of PTM on Gram stained smears of CSF of these groups. Culture was positive in 2 of 10 (20 %) patients in PM group. One patient grew *E. coli* from blood as well as CSF. Other patients grew *H. influenzae* from CSF only.

The mean level of ADA in CSF was higher ($p < 0.01$) in TBM group of patients as compared to other groups (Table 1). Four patients in TBM group had much higher ADA levels ranging from 33.33-96.74 U/L while rest of patients (26/30) had ADA levels below 27 U/L.

Table 1. ADA levels in CSF of four groups

	ADA (U/L)			
	Mean	Median	(SE)	Range
TBM (n=30)	*18.22	12.25	(3.35)	1.0-96.7
PTM (n=10)	6.28	7.0	(0.91)	3.0-11.1
PM (n=10)	7.98	2.4	(3.56)	0.3-29.0
AM (n=10)	3.43	2.9	(0.86)	0.1-08.5
* $p < 0.01$ between TBM vs PTM or PM or AM TBM - Tubercular meningitis, PTM - Partially treated pyomeningitis, PM - Pyomeningitis, AM - Aseptic meningitis.				

In pyogenic meningitis group, two had higher ADA levels in CSF i.e. 29 U/L in each case while others (8/10) had values lower than 7.5 U/L. In AM group, one had ADA levels of 9.4 U/L and others (9/10) had below 8.5 U/L.

The sensitivity of ADA test in CSF at cut off of 10 U/L was 66.6 %. The specificity was highest while taking AM group as control (100 %) and was lowest while taking PM group as control (80 %). The overall specificity was 90 % (Table 2).

Table 2. Sensitivity and specificity of the test at 10 U/L CSF ADA levels for diagnosis of TBM

Groups	Sensitivity (%)	Specificity (%)
TBM VS (PTM+PM+AM)	66.6	90
TBM VS PTM	66.6	90
TBM VS PM	66.6	80
TBM VS AM	66.6	100
TBM - Tubercular meningitis, PTM - Partially treated pyomeningitis, PM - Pyomeningitis, AM - Aseptic meningitis.		

At higher cut off of ADA value in CSF (11 U/L), only 18/30 patients were positive for TBM decreasing the sensitivity to 60 % from 66.6 %. However, there was no change in the specificity (Table 3). At lower cut off of ADA value in CSF (9 U/L), sensitivity measured to 70 % but specificity decreased from 90 % to 86.6 %. So ADA levels of 10 U/L was taken as cut off value for diagnosis of TBM with overall sensitivity and specificity 66.6 % and 90 % respectively.

Mean ADA levels in CSF were lower (1.83 U/L) in case of pyogenic meningitis with low CSF cell counts as compared to TBM (12.53 U/L) with similar counts of cells (Table 4). In TBM group, levels of ADA in CSF were also correlated with cell counts, clinical & biochemical parameters but found no correlation except protein (Table 5). There was no significant variation in ADA levels of CSF of TBM patients with immunization (BCG +ve & BCG -ve), Mantoux status and TB stages (Table 6).

DISCUSSION

Routine CSF laboratory parameters may not be helpful to differentiate TBM from other meningitis like partially treated pyomeningitis and aseptic meningitis. Demonstration or isolation of acid-fast bacilli on CSF smear or culture is usually difficult (10). This aim keeps looking for simple and rapid tests that can help to diagnose TBM and differentiate TBM from other meningitis. Results of our study indicate that ADA levels in CSF are of great value in diagnosis of TBM and in differentiating TBM from other meningitis i.e. PM, PTM & AM.

Levels of ADA in CSF of adult patients of TBM have been evaluated in few earlier studies (6-8). Raised levels of ADA in CSF are not specific to meningitis and this should be kept in mind when interpreting the test. Raised levels, in other conditions particularly in certain intracranial tumors has also been noted (11).

We found a positive correlation of ADA levels in CSF with CSF proteins but no significant correlation of ADA levels in CSF and CSF pleocytosis in our patients. Whereas Malan *et al.* (6) found a positive correlation between CSF ADA levels, CSF proteins and CSF pleocytosis in patients of TBM. In their study they have also observed that in cases of

Table 3. Sensitivity and specificity of ADA levels in CSF of patients at different ADA levels cut off

CSF ADA (U/L) cut off	No of Patients				Sensitivity (%)	Specificity (%)
	TBM (n=30)	PM (n=10)	AM (n=10)	PTM (n=10)		
9	21	2	0	2	70	86.6
10	20	2	0	1	66.6	90
11	18	2	0	1	60	90
TBM - Tubercular meningitis, PTM - Partially treated pyomeningitis, PM - Pyomeningitis, AM - Aseptic meningitis.						

Table 4. Variation of ADA levels in CSF with CSF pleocytosis group

Groups		Mean ADA (U/L)				
TBM	0	11.5	12.31	27.53	16.5	19.00
PM	0	0	1.83	7.5	3.65	14.88
AM	2.31	6.75	4.6	0	0	0
PTM	0	0	9.45	6.32	3.0	0
<50		50-100	100-200	200-500	500-1000	>1000
CSF cell counts / mm ³						
TBM - Tubercular meningitis, PTM - Partially treated pyomeningitis, PM - Pyomeningitis, AM - Aseptic meningitis.						

Table 5. Relationship of ADA levels in CSF with other CSF parameters in TBM patients (n = 30)

	Mean	SE	Correlation Coefficient (r)	P-Values
ADA (U/L)	18.22	3.35	—	—
Age (years)	4.15	1.80	-0.1589	> 0.1 (NS)
Duration of illness (days)	40.3	12.37	0.1657	> 0.1 (NS)
Total cell Counts/mm ³	431.47	200.8	0.0358	> 0.1 (NS)
Protein (mg/dl)	266.87	122.02	0.8088	< 0.01 (S)
Sugar (mg/dl)	62.33	3.46	0.1347	> 0.1 (NS)

pyogenic meningitis with low cell counts, the mean ADA levels in CSF were lower than in case of TBM with a similar cell counts. They did not study comparison of ADA levels in CSF of TBM patients with PTM and correlation with TB stages and CMI response markers. ADA assay has not been compared with PCR on pediatrics samples neither in the literature nor by us.

The mean ADA levels in CSF of TBM cases of pediatric age groups have been reported to be ranging between 11.6-13.7 U/L in earlier study (6).

Table 6. ADA levels in TBM patients with TB stages, mantoux test and immunization status (n = 30)

	Number of Patients (%)	ADA (U/L) (Mean)	P value
Mantoux (>10 mm)	16 (53.33)	22.62	> 0.1
Mantoux (<10 mm)	14 (46.67)	13.20	
BCG -	11 (36.67)	17.37	> 0.1
BCG +	19 (63.33)	18.72	
Stage I	2 (6.67)	11.65	> 0.1
Stage II	10 (33.3)	22.10	> 0.1
Stage III	18 (60)	16.85	> 0.1

A relatively higher range of ADA values in CSF (15.7-21.3 U/L) has been observed in adult TBM patients (7,8). These results show that levels of ADA vary in different age groups and lower activity is observed in CSF of pediatric patients with TBM. This may be due to difference in immunological reactivity to tubercular antigen in children as compared to adults.

In the present study, mean ADA levels in CSF were highest in TBM patients as compared to PM, AM & PTM. Ribera et. al. have also demonstrated

similar findings in their study of adult TBM patients but they have not compared the ADA levels in CSF of TBM and PTM patients. None of the previous studies mentioned the ability of ADA levels in CSF to differentiate TBM from partially treated pyomeningitis. We observed that ADA in CSF could differentiate TBM from partially treated pyomeningitis with a specificity and sensitivity of 90 % and 66.6 % respectively.

In conclusion, ADA estimation in CSF is a simple, inexpensive, rapid and fairly specific method for aiding a clinician in making the diagnosis of tuberculous meningitis when confronted with a common dilemma of distinguishing it from partially treated pyomeningitis and other meningitis. For above reasons, ADA estimation in CSF of TBM patients should find a place in routine laboratory methodology.

REFERENCES

1. Mastroianni, C.M., Paolotti, F., Lichter, M.D., Agostino, C., Vullo, V. and Delia, S. (1997) Cerebrospinal fluid cytokines in patients with tuberculous meningitis. *Clin Immunopathology* 84, 171-176.
2. Tandon, P.N. (1999) Neurotuberculosis: Clinical aspects. In: *Neurology in Tropics*. Chopra, J.S. and Sawhney, I.M.S. (eds.) Churchill Livingstone Ltd. 358-389.
3. Thwaites, G., Chan, T.T.H., Mai, N.T.H. et al. (2000) Tuberculous meningitis. *J Neurol Neurosurg and Psychiatr* 68, 289-299.
4. Garcia-Monco, J.C. (1999) CNS Tuberculosis. In: *Neurologic Clinics*. Marra, C.M. (ed.). 17(4), 737-760.
5. Bothamley, G.H. (1995) Serological diagnosis of tuberculosis. *Eur. Respir. J.* 8, 676s-688s.
6. Malan, C., Donald, P.R., Golden, M. and Talard, J.J.F. (1984) Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. *J. Trop. Med. Hy.* 87, 33-40.
7. Piras, M.A. and Gakis, C. (1972-73) CFS adenosine deaminase activity in tuberculous meningitis. *Enzyme* 14, 311-317.
8. Ribera, E., Jose, M., Martinez-Vasquez, J.M., Inma, Ocana, Rosa, M, Segura, R.M. and Pascual, C. (1987) Activity of adenosine deaminase in cerebrospinal fluid for the diagnosis and follow-up of tuberculous meningitis in adults. *J. Inf. Dis.* 155, 603-607.
9. Giusti, G. (1974) Adenosine deaminase. In: *Methods of enzyme analysis*. Bergmeyer, H.U. (ed.). New York: Academic Press. 1092-1099.
10. Molavi, A. and Le Frock, J.L. (1985) Tuberculous Meningitis. *Med. Clinics. North Am.* 69, 315-331.
11. Martinez-Vasquez, J.M., Ocana, I. and Ribera, E. (1986) Adenosine deaminase activity in diagnosis of tuberculous peritonitis. *Gut.* 27, 1049-1053.
12. Coovadia, Y.M., Dawood, A., Ellis, H.E., Coonadia, H.M. and Daniel, T.M. (1986) Evaluation of adenosine deaminase activity and antibody to mycobacterium tuberculosis antigen 5 in cerebrospinal fluid and the radioactive bromide partition test for the early diagnosis of tuberculosis meningitis. *Arch. Dis. Child* 61, 428-435.