Journal of Clinical Laboratory Analysis 15:236–238 (2001)

Significance of Cerebrospinal Fluid Adenosine Deaminase Isoenzymes in Tuberculous (TB) Meningitis

C.-M. Schutte,[1]* J.P.J. Ungerer,[2] H. du Plessis,[2] and C.H. van der Meyden[1]

[1]*Department of Neurology, University of Pretoria, Pretoria, Republic of South Africa*
[2]*Department of Chemical Pathology, University of Pretoria, Pretoria, Republic of South Africa*

Adenosine deaminase (ADA) exists as two isoenzymes, ADA_1 and ADA_2. It appears that the ADA_2 isoenzyme originates mainly from monocytes and macrophages. In tuberculous pleural effusions most of the ADA activity consists of ADA_2. The aim of this prospective study was to analyse ADA isoenzymes in the CSF of patients with meningitis to investigate whether the expected rise of the ADA_2 isoenzyme would occur in tuberculous meningitis. ADA isoenzyme analysis was performed on the CSF of 15 patients with tuberculous and 11 patients with bacterial meningitis by an automated kinetic enzyme coupled assay in the presence and absence of a specific ADA inhibitor. The ratio of ADA_2/ADA_{Total} was >0.8 in 14/15 patients with tuberculous meningitis. In bacterial meningitis the ratio was ≤0.8 in 10/11 patients. The ADA_2 isoenzyme is the major contributor to increased ADA activity in the CSF of patients with tuberculous meningitis, probably reflecting the monocyte–macrophage origin of the ADA. J. Clin. Lab. Anal. 15:236–238, 2001.

Key words: chronic meningitis; tuberculosis; diagnosis

Adenosine deaminase (ADA) is the catalysing enzyme for the deamination of adenosine (or deoxyadenosine) to inosine (or deoxyinosine) and ammonia. There are two isoenzymes of ADA namely, ADA_1 and ADA_2 (1), each encoded by different gene loci. While the ADA_1 isoenzymes can be found in all cells with highest activity in lymphocytes and monocytes, ADA_2 is mainly present in monocytes (2). It is well known that total ADA activity is increased in pleural fluid of patients with tuberculous (TB) effusions, as well as in cerebrospinal fluid (CSF) of patients with TB meningitis. However, high ADA activity is also often found in the CSF in bacterial meningitis, limiting the diagnostic utility of ADA determination in CSF (3).

It has been shown previously that ADA_1 and ADA_2 isoenzymes contribute independently to ADA increase in tuberculous pleural effusions. In TB effusions most of the measured ADA activity is due to the ADA_2 isoenzyme, probably reflecting monocyte–macrophage origin (4).

In this prospective study the composition of ADA enzymes in the CSF of patients with TB and bacterial meningitis was investigated to determine whether the trend found in tuberculous effusions was also present in CSF of patients with TB meningitis.

MATERIALS AND METHODS

Patients

The CSF of 15 consecutive patients with TB meningitis and 11 consecutive patients with bacterial meningitis presenting at the Pretoria Academic Hospital was investigated. The laboratory performing the ADA determination was unaware of the clinical diagnosis of the patients. Of the patients with TB meningitis, 7 were male and 8 female; the ages ranged from 15 to 45 years. All patients were Black. The time elapsed since beginning of symptoms (headache, neck pain, fever) and first ADA analysis at admission ranged from 5 days (one patient) to more than 2 weeks (5 patients).

Of the patients with bacterial meningitis, 9 were male and 2 were female; the ages ranged from 18 to 65 years, and all patients were Black. The time elapsed from beginning of symptoms to ADA analysis ranged from 2 days (6 patients) to 5 days (1 patient).

The activity of ADA and its isoenzymes was determined by an automated kinetic enzyme-coupled assay in the presence and absence of a specific ADA_1 inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (5). The Mann–Whitney *U*-test was used to determine whether the two groups of CSF differed significantly. Linear association tests (Pearson) were performed to establish whether correlations between ADA_1 and ADA_2 and lymphocyte/neutrophil counts in the CSF, respectively, were present.

*Correspondence to: Dr. C.-M. Schutte, Department of Neurology, Private Bag X169, Pretoria 0001, Republic of South Africa.
E-mail: cschutte@medic.up.ac.za

Received 15 December 2000; Accepted 6 February 2001

RESULTS

The diagnosis of TB was made according to standard criteria including typical CSF findings, clinical findings, histology, and positive cultures of *Mycobacterium tuberculosis*. The criteria for the diagnosis of tuberculous meningitis were as follows: presence of *M. tuberculosis* on CSF examination; positive culture and/or PCR of *M. tuberculosis* from CSF; post mortem examination confirming tuberculosis; CSF findings of a predominantly lymphocytic pleocytosis with CSF-protein > 0.5 g/l and CSF–serum-glucose ratio of <50% together with typical clinical findings and/or presence of tuberculosis in other organs and response to anti-tuberculous treatment. The laboratory evaluation of the CSF findings is shown in Table 1. In 4 patients the diagnosis of TB was confirmed at post mortem; in 3 patients histologic evidence for TB was found on lymph node biopsies as previously described (6); in one patient CSF cultures were positive; and in 7 patients the diagnosis was made by typical clinical and CSF findings with clinical response to anti-tuberculous treatment.

In the patients with bacterial meningitis *Streptococcus pneumoniae* was cultured from the CSF in 8, while 2 patients had meningococcal meningitis, and one had streptococcal Group B infection. The laboratory evaluation of the CSF findings is shown in Table 2.

The results of the ADA activity studies are also shown in Tables 1 and 2. The ratio of ADA_2/ADA_{Total} was >0.8 in 14/15 patients with TB meningitis and ≤0.8 in 10/11 patients with bacterial meningitis. Statistical analysis shows a significant difference between the two groups ($P = 0.0001$). The one patient with TB meningitis with an ADA_2/ADA_{Total} ratio of <0.8 had marked hydrocephalus, and CSF was obtained from the ventricles when an external drain was placed. Two patients with bacterial meningitis had relatively high ratios of ADA_2/ADA_{Total} (0.88 and 0.79)—in both patients the CSF protein values were exceptionally high (17,200 and 17,700 mg/l, respectively), which possibly could have affected measurement of ADA. On statistical analysis no significant correlations, i.e., linear associations were present either between ADA_1 (or transformation of ADA_1) and CSF lymphocytes and neutrophils, respectively, or between ADA_2 (or transformations of ADA_2) and CSF lymphocytes and neutrophils, respectively. Biplots of ADA_1 and ADA_2 versus both neutrophils and lymphocytes also reflected no correlations.

TABLE 1. CSF findings in TB meningitis[a]

N	L	CSF Glu-S Glu	P	ADA_T	ADA_2	ADA_2/ADA_{Total}
270	42	0.9–6.2	3,400	3.5	3.2	0.91
55	128	0.7–5.5	2,960	7.5	6.1	0.81
77	108	0.8–5.7	1,850	10.5	9.9	0.94
12	470	1.5–5.2	2,300	10.5	9.7	0.92
0	244	6.4–1.6	3,000	16.5	14.3	0.87
0	385	1.0–3.43	2,190	22.2	20.6	0.93
24[b]	134	1.9–7.8	200	6.4	4.4	0.7
385	275	0.8–12.1	2,300	15.5	13.1	0.85
0	647	2.2–7.9	1,400	10.4	10.2	0.98
36	18	1.1–4.5	3,645	14.8	14.0	0.94
10	251	1.4–6.8	2,060	17.7	17.6	0.99
61	97	1.0–?	3,649	38.67	37.0	0.95
85	12	2.5–4.6	6,415	14.9	13.7	0.92
323	293	2.6–6.3	4,177	27.2	23.9	0.88
48	256	2.1–6.8	2,434	22.0	21.9	0.99

[a]N, neutrophils (/mm^3); L, lymphocytes (/mm^3); CSF Glu-S Glu, CSG glucose–serum glucose (mmol/l); P, protein (mg/l); ADA_{Total}, ADA total (U/l); ADA_2, ADA_2 isoenzyme (U/l).

[b]CSF is ventricular fluid collected when external drain was inserted; diagnosis of TB confirmed at postmortem.

TABLE 2. CSF findings in bacterial meningitis[a]

N	L	CSF Glu-S Glu	P	ADA_{Total}	ADA_2	ADA_2/ADA_{Total}
672	250	0.1–8.9	4,298	10	5.2	0.52
684	24	2.1–7.1	4,991	6.7	2.2	0.33
138	0	0.1–13.5	17,200	9.8	8.6	0.88
1,311	30	0.3–16.4	17,700	9.4	7.5	0.79
600	75	1.6–8.3	1,310	56.4	25.4	0.45
5,894	507	1.3–5.1	5,720	4.6	2.1	0.45
2,200	110	0.1–17.2	5,870	3.8	1.5	0.39
2,536	208	1.9–5.5	2,370	27.5	7.3	0.26
6,313	12	0.1–6.3	3,910	5.1	3.9	0.76
12,100	22	0.2–9.5	5,810	6.3	2.5	0.39
4,620	110	1.3–4.8	2,120	15.8	9.9	0.63

[a]N, neutrophils (/mm^3); L, lymphocytes (/mm^3); CSF Glu-S Glu, CSG glucose–serum glucose (mmol/l); P, protein (mg/l); ADA_{Total}, ADA total (U/l); ADA_2, ADA_2 isoenzyme (U/l).

DISCUSSION

The ADA_2 isoenzyme was found to be the major contributor to total ADA activity in the CSF of patients with TB meningitis, with a median contribution of 90%. In bacterial meningitis, the median ADA_2 isoenzyme contribution was 51%.

The origin of ADA activity in the CSF of patients with TB meningitis is uncertain, but studies based on isoenzyme occurrence in body fluids suggest a monocyte–macrophage origin (7). In another recent study it was found that the ADA_1 isoenzyme was responsible for all the ADA activity in lymphocytes while ADA_2 was present only in monocytes (2). The increased ADA activity in CSF in TB meningitis therefore is probably due to monocyte–macrophage activation. In the bacterial meningitis group the ADA activity probably originates from neutrophils—the most abundant cell present in the CSF in bacterial meningitis—and lymphocytes. Total CSF ADA activity is often also increased in bacterial meningitis, decreasing the specificity of ADA determination in the diagnosis of TB. However, as this study shows, measurement of the ADA isoenzymes could help to distinguish between bacterial and TB infections in the CSF.

In conclusion, the ADA_2 isoenzyme is the major contributor to increased ADA activity in the CSF of patients with TB meningitis, probably reflecting monocyte–macrophage origin of the ADA. Thus, the same trend found in studies of

tuberculous pleural effusions where the ADA_2 isoenzyme is the major contributor to increased ADA activity is also found in cerebrospinal fluid of patients with TB meningitis.

ACKNOWLEDGMENTS

We thank Dr. Piet Becker for help with the statistical analysis of the data.

REFERENCES

1. Hirschhorn R, Ratech H. Iso-enzymes of adenosine deaminase. In: Rattazzi MC, Scandalios JG, Whitt GS, editors. Iso-enzymes: Current Topics in Biological and Medical Research, Vol 4. New York: Liss; 1980. p 131–157.
2. Ungerer JPJ, Oosthuizen HM, Bissbort SH, Vermaak WJH. Serum adenosine deaminase: iso-enzymes and diagnostic application. Clin Chem 1992;38:1322–1326.
3. Coovadia YM, Dawood A, Ellis ME, Coovadia HM, Daniel TM. Evaluation of adenosine deaminase activity and antibody to *M. tuberculosis* antigens in CSF and the radioactive bromide partition test for the early diagnosis of tuberculous meningitis. Arch Dis Child 1986;61:428–435.
4. Ungerer JPJ, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its iso-enzymes in tuberculous effusions. Chest 1994;106:33–37.
5. Oosthuizen HM, Ungerer JPJ, Bissbort SH. Kinetic determination of serum adenosine deaminase. Clin Chem 1993;39:2182–2185.
6. Schutte C-M, Van der Meyden CH, Labuschagne JH, Otto D. Lymph node biopsy as an aid in the diagnosis of intracranial tuberculosis. Tuber Lung Dis 1996;77:285–286.
7. Gakis C, Calia G, Naitana A, Ortu AR, Contu A. Serum and pleural adenosine deaminase activity. Chest 1991;99:1555–1556.