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CEREBROSPINAL FLUID ADENOSINE DEAMINASE AND LYSOZYME LEVELS IN THE DIAGNOSIS OF TUBERCULOUS MENINGITIS

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Summary. **Adenosine Deaminase Activity (ADA) and Lysozyme Activity (LYSA) were measured in the CSF of tuberculous meningitis (TBM) cases :26 bacteriologically positive TBM (Group 1), 61 bacteriologically negative TBM (Group 2), 10 non-tuberculous meningitis (Group 3) and 17 control subjects (Group 4). The mean ADA levels in different groups in that order were found to be 11.6., 4.5, 4.4 and 0.8 U/1 respectively. The mean LYSA levels in the same groups were 6.3, 2.1, 2.2 and 0.5 mcg/ml respectively. In bacteriologically positive TBM, the mean ADA and LYSA levels were significantly higher than the other three groups (P <0.0001). An ADA level of 4 U/I and LYSA level of 2 mcg/m1 were considered for the differential diagnosis of TBM. Based on this, the sensitivity and specificity of ADA and LYSA tests were 96%, 82% and 85%, 95% respectively. When both the criteria were considered, the sensitivity and specificity were 91% and 93% respectively. Combination of both test definitions could give additional support to the diagnosis in 49% of 61 clinically suspected but bacteriologically negative TBM cases. Correlation of ADA and LYSA levels in CSF was found to be statistically significant (r = 0.59; P < 0.01).**

Introduction

Confirmation of diagnosis of TBM is often difficult. Bacteriological confirmation of the disease is by demonstration of acid fast bacilli in CSF by microscopy and isolation of *M.tuberculosis* in culture. Since culture takes a long time and only less than 5% of TBM cases are positive by smear examination, there is an urgent need for simple, specific and rapid tests for the diagnosis of TBM.

Several indirect tests, such as lactate dehydrogenase level[1], lactate concentration[2], bromide partition test[3] and many direct tests such as detection of mycobacterial antigen[4,5] and presence of tuberculostearic acid[6] were suggested for the early diagnosis of TBM. None of these tests have been accepted as routine procedure in clinical microbiology laboratories[7]. T.M. Daniel[8] has reviewed the value of various direct and indirect tests for the rapid diagnosis of TBM.

ADA catalyses the deamination of adenosine into inosine and ammonia. It is secreted by T-lymphocytes and macrophages during infections. So, there has been interest in ADA as a marker of chronic inflammatory conditions such as tuberculous pleural effusions[9], tuberculous peritonitis[10] and TBM[7,11].

Lysozyme, secreted by the monocytic cell series, was reported by us[12] as useful for the differential diagnosis of meningitis. Hence, the objectives of the present study were to assess the usefulness of ADA test and to compare it with LYSA test in the diagnosis of TBM in children.

Material and Methods

Patients : The patients included in this study were children aged below 12 years, divided into 4 groups:

Group 1. (Confirmed TBM) : The CSF from all the 26 patients in this group was culture positive for *M.tubercu1osis*

Group 2. (Clinical TBM) : The 61 patients in this group were clinically diagnosed as suffering from TBM but their CSF was negative by smear and culture. The diagnosis in them was based on clinical features, history of contact, Mantoux test, biochemical findings of CSF and presence of

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evidence for tuberculosis elsewhere in the body.

Group 3. (Non-TBM) : The 10 patients in this group were clinically diagnosed as suffering from pyogenic meningitis. *S.pyogenes* was isolated from one and *H.influenzae* from the other patient and the CSF specimens from the remaining 8 patients were reported as culture sterile.

Group 4. (Control) : The CSF from patients with febrile fits(8), epilepsy (1), neuromuscular disorder (1), seizure disorder (1), cerebrovascular accident (3) and conditions such as hydrocele (2), anal fissure (l), where spinal anaesthesia was given constituted the control group. All the 17 CSF samples were biochemically normal and culture negative for AFB and non-AFB.

ADA assay : The ADA assay was performed as described by Giusti et al[13]. The CSF specimens were kept in the deep freezer (-20° C) for 7 to 10 days before the assay was done. The assay was set up on a routine basis and the details of the specimens were not known. On each day of the assay a pooled CSF sample was included as a control. The ADA content was expressed in U/I of CSF. Coefficient of variation was found to be 20% for a pooled CSF sample with a mean ADA level of 10.12 U/I in 9 consecutive assays.

Lysozyme assay : The lysozyme content was estimated by the turbidity measurement as described by Jain at al[14]. A pooled CSF sample was included to monitor the variation in the assay. The lysozyme content was expressed in mcg/ml of CSF. The coefficient of variation for the pooled CSF sample with a mean LYSA level of 1.43 mcg/ml was 15% in 7 consecutive assays.

AFB culture : All the CSF specimens were subjected to smear examination by fluorescence microscopy and processed for culture using multiple media as practised in this Centre[15].

Non-AFB culture : The CSF specimens from the control and Non-TBM groups were examined for Non-AFB by the conventional procedures.

Statistical method : Univariate statistical analysis was carried out for the groups, separately. The comparisons of the means of various groups of meningitis were made using Kruskal-Wallis non-parametric one-way ANOVA test. The association between ADA and LYSA levels was determined using Pearson correlation coefficient and their significance using t-test. Unless otherwise stated, a statistical significance indicates P value less than 0.05.

Results

The frequency distribution, mean, standard deviation and range of ADA values in different groups are given in Table 1. The mean level of ADA in confirmed TBM was 11.6 U/l and was significantly higher than the other three groups (P <0.000l). In the confirmed TBM group, 25 (96%) of 26 had ADA values more than 4 U/l. All the 17 in the control group and 7 of 10 in Non-TBM group had values less than 4 U/l. So, an ADA level of 4 U/l and above was considered diagnostic of TBM. With this criterion, the sensitivity and specificity of the test were 96% and 82% respectively.

The frequency distribution, mean, standard deviation and range of LYSA levels in different groups are given in Table 2. The mean level of LYSA in confirmed TBM was 6.3 mcg/ml and was significantly higher than the other three groups (P < 0.0001). In confirmed TBM, 23 (88%) out of 26 had LYSA values higher than 2 mcg/ml. All the 17 in the control group and 9 out of 10 in the Non-TBM group had values less than 2 mcg/ml. So, a LYSA value of 2 mcg/ml and above was considered diagnostic of TBM. With this criterion, the sensitivity and specificity of the test were found to be 85% and 95% respectively.

The results of ADA and LYSA tests in bacteriologically positive and negative TBM groups are presented in Table 3. In bacteriologically positive TBM, 25 (96%) out of 26 were positive by ADA test whereas 23 (88%) were positive by LYSA test. By applying either of the two tests a probable diagnosis of TBM could be made in 49% of 61 cases without bacteriological confirmation.

The correlation coefficient between the ADA and LYSA levels in the confirmed TBM was found to be statistically significant (r = 0.59; P < 0.01)

Discussion

In the present study, ADA levels ranged from 2.5 to 25.5 U/l in confirmed TBM. In pediatric populations, Malan et al[16] and Coovadia et al[7] reported a range of 4.7 to 32.7 U/l and 7.1 to 32.5 U/l respectively. A range of 9.1 to 22.7 U/l was reported by Ribera et al" in adults. These results

Table 1. *Distribution of ADA levels.*

ADA (U/l)	Groups			
	Confirmed TBM	Clinical TBM	Non-TBM	Control
0-	0	25	3	17
2-	1	10	4	
4-	6	15		
8-	10	8	3	
12-	9	3		
Total	26	61	10	17
Mean	11.6	4.5	4.4	0.8
SD	6.2	4.3	4.0	0.5
Range	2.5-25.4	0.1-20	1.0-10.9	0.0-1.6

Table **2.** *Distribution of LYSA levels*

LYSA (mcg/ml)	Confirmed TBM	Clinical TBM	Non-TBM	Control
0-	3	38	5	15
l-	1	4	4	2
2-	2	6		
4-	11	9		
8-	9	4	1	
Total	26	61	10	17
Mean	6.3	2.1	2.2	0.5
SD	3.4	2.8	3.9	0.6
Range	0.0-10.8	0.0-10.5	0.3-13.1	0.0-1.6

TaBLE 3. *Results of ADA and LYSA tests in bacteriologitally positive and negative TBM*

Test status	Bact. neg.TBM		Bact. pos.TBM	
	Number	%	Number	%
Both positive	15	24.5	22	85
ADA positive LYSA negative	11	18.0	3	11
LYSA positive ADA negative	4	6.5	-	-
Both negative	31	51.0	1	4
Total	61	100	26	100

show that ADA secretion by T-lymphocytes in response to mycobacterial infection is similar in different ethnic groups.

In this investigation, an ADA value of 4 U/l and above was considered consistent with tuberculous pathology. Mann et all[7] used the same criterion for the differential diagnosis of meningitis. Malan et all[6] reported a value of 6 U/l as the cut off point for classification; Coovadia et al[7] had a cut-off point of 10 U/l for differentiation while in adults Ribera et al[11] considered a value of 9 U/l as the significant level. The variations in the diagnostic cut-off levels in these reports could be due to the type of controls investigated.

In this study, a LYSA level of 2 mcg/ml was considered as diagnostic while Matti Klockars et all[8] used 1.5 mcg/ml as the cut off point.

In this study, 3 of 10 Non-TBM patients had ADA values more than 4 U/l . similarly, Malan et all[6] reported that 13 (31%) of 42 patients with bacterial meningitis had values higher than 9 U/l. Coovadia et al[7] observed that 14 of 16 Non-TBM patients had ADA levels more than 10 U/I. It is, therefore, evident that the CSF from patients with pyogenic meningitis had also elevated ADA levels. This aspect should be taken into consideration while interpreting ADA test results. Ribera et al[11] had reported that only 2 (1%) out of 213 cases of Non-TBM had ADA values higher than 9 U/l without any explanation. **It is, therefore, imperative that pyogenic meningitis is ruled out by an initial screening, before elevated levels of ADA and LYSA levels are considered for the diagnosis of TBM.** Considering either of the two test definitions, 49% of 61 clinically suspected and bacteriologically negative cases of TBM in the current study had additional supportive evidence in favour of their diagnosis.

The agreement between ADA and LYSA tests in the classification of different groups of meningitis was found to be 97%. The difference between the two tests is not statistically significant (Sign test; $P > 0.2$). **These results suggest that either LYSA or ADA could be applied as an additional test for the diagnosis of TBM.**

Considering the practical aspects of the two tests, LYSA test requires only culture suspension of *Micrococcus lysodeikticus* which can be grown and maintained on nutrient agar. On the other hand, ADA test needs reagents which are

expensive and have a short shelf life i.e.. around 30 days. Also, the assay involves the measurement of ammonia and utmost care should be taken to avoid contamination with tap water. Since LYSA assay is very simple and inexpensive, compared to ADA, it can be performed in laboratories with limited financial support. **Thus, it may be observed that LYSA test is simple to perform and as sensitive and specific as the ADA test in the diagnosis of TBM.**

In conclusion , ADA level of 4 U/l and above and LYSA level of 2 mcg/l and above in CSF could provide additional supportive evidence for the diagnosis of TBM in clinically suspected and bacteriologically negative cases.

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