Original Article

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Study of ADA Activity in the Cerebrospinal Fluid for the Diagnosis of Tuberculoses Meningitis

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Abstract

Aim: In the present study an attempt has been made to detect the sensitivity of ADA activity in diagnosing Tuberculous Meningitis. *Materials and Methods*: A total of 47 children aged 2 months to 12 years admitted . 21 of these patients had tuberculous meningitis, 10 had bacterial meningitis, another 7 considered to have encephalitis were included and a group of 9 patients who did not have meningitis, but are admitted to pediatric wards for other complaints like epilepsy, febrile seizures and to surgical wards for either inguinal hernia or hydrocele repair who underwent surgery under spinal anesthesia were taken. The patients were divided into four group. *Results:* Adenosine Deaminase activity is measured in cerebro spinal fluid of 21 cases of Tuberculosis Meningitis, 10 Cases of Bacterial Meningitis, 7 cases of Encephalitis and 9 cases of controls. The mean C.S.F ADA level was significantly raised ($p<0.001$) in Tuberculosis Meningitis patients as compared to other study groups. i.e, pyogenic Meningitis Encephalitis and non inflammatory conditions of C.N.S. A cut – off C.S.F ADA level of > 5 LU/L was considered for the diagnosis of Tuberculosis Meningitis and the tests showed a sensitivity and specificity of 95.24% and 88.4% respectively. The ADA activity in TBM cases had significantly correlation with cell count and protein concentration. *Conclusion*: Overall it was found to be a single better test in comprasion to any other tests for the diagnosis of Tuberculous Meningitis.

Keywords: Tuberculous Meningitis; Adenosine Deaminase; Encephalities.

Introduction

Tuberculous meningitis is a major cause of morbidity and mortality in developing countries. It is the most devastating from of TB. Tuberculous meningitis is the infection of the meninges caused by mycobacterium tuberculosis. India is the country with the highest burden of tuberculosis. The WHO statistics for 2015 gave an estimated incidence of 2.2 million cases of tuberculosis for India out of a global incidence of 9.6 million [1-2]. Often it poses a diagnostic problem to the clinicians and the prognosis of disease is closely related to the stage at which the treatment is started. As it is associated with high mortality and morbidity due to sequelae early and correct treatment is essential for a successful outcome in patients of tuberculous meningitis [3].

A definitive diagnosis of tuberculous meningitis depends on the detection of acid fact bacillus in the CFS and culture of mycobacterium tuberculosis. Since culture takes a long time and only less than 5% of TBM cases are positive by smear examination and even the characteristics CFS cytological and biochemical changes are also variable and sometimes even absent there is an urgent need for simple, specific and rapid tests for the diagnosis of TBM.

Several indirect tests such as lactate dehydrogenase level, lactate concentration, bromide partition test and many direct tests such as detection of mycobacterial antigen and presence of tuberculo stearic acid were suggested for early diagnosis of TBM [4,5].

Nevertheless these are complicated inaccessible or too expensive and they lack sensitivity and specificity. In the light of these observations adenosine

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Deaminase activity in the CFS has been found to be a simple and useful investigation in the diagnosis of tuberculous meningities.

This study has been undertaken to conform the high sensitivity and specificity of determining adenosine deaminase activity in the cerebrospinal fluid for the diagnosis of tuberculoses meningitis.

Materials and Methods

A total of forty- seven children aged 2 months to 12 years admitted to institute of child health, KIMS hospital, Secunderabad during 2010 to 2012 were selected for the study. Twenty one (21) of these patients had tuberculous meningitis, ten (10) had bacterial meningitis, another seven (7) considered to have encephalitis were included and a group of nine patients who did not have meningitis, but are admitted to pediatric wards for other complaints like epilepsy, febrile seizures and to surgical wards for either inguinal hernia or hydrocele repair who underwent surgery under spinal anesthesia were taken.

The patients were divided into four group

Group I:

Clinical TBM: This group included patients in whom CSF biochemical abnormalities, positive mantoux/BSG test, Lesion in chest X-ray and response to anti-tubercular therapy.

Confirmed TBM: This group included patients with CSF smear positive for mycobacterium tuberculosis or CSF profile as in meningitis along with biopsy evidence from any other site.

Group II

Bacterial and partially treated Bacterial Meningitis: This group included patients with clinical history, positive CSF culture and/or gram strain, CSF showing polymorpho nuclear leukocytosis, low sugar, high protein with or without positive blood culture and response to antibiotics.

Group III

Encephalitis: This group includes patients with clinical history, normal CSF or pleocytosis and or elevated protein.

Group IV

Controls: This group included patients with febrile seizures, Epilepsy, etc, having normal CSF biochemistry, negative culture/ grames staining with no prior antibiotic therapy and uneventful subsequent coarse.

The cause of Meningitis was established by the following investigations.

Routine History taken , Clinical examination, CBP/ E.S.R, Chest X-ray and Mantoux test

Specific Test Like

1. C.S.F for appearance/ cell count/ protein/ Glucose.
2. C.S.F for smear examination after Ziehl-Neelson's stain and gram stain.
3. C.S.F for culture on Lowenstein- Jensens medium for Mycobacterium Tuberculosis.
4. Histopathological examination of Lymph nodes.
5. CT scan of Brain.
6. The C.S.F Adenosine Deaminase Assay in all Groups.

The Human Erythrocytes have high ADA content (Muller Beissen Hietz and keller 1966) and could lead to high ADA value in the fluids. Hence blood stained specimens are excluded from this study.

The ADA assaywas performed according to the sensitive calorimetric method of Galante and Giusti principles. C.S.F is collected by traditional Lumbar puncher method. Blood stained C.S.F due to traumatic tap is discarded. The samples should not be stored for longer than 48-72 hours at 4 C. Storage of sample for more than 5 days at 4 C results in realease of Ammonia, even if microbial contamination is avoided. This gives high blank values.

Results

By analyzing the results from the data collected it can be firmly inferred that the mean ADA activity C.S.F was significantly higher in Tuberculosis Meningities when compared to bacterial meningitis, Encephalitis, Controls.

The Mean value of ADA activity in cerebrospinal fluid of Tuberculous Meningitis cases was 14± 4.24 while in Encephalities it was 4.2±1.8 In Bacterial Meningitis, the mean value was 3.1±1.7 and in normal controls the value was 4.1±1.1.

Out of 21 cases of Tuberculous Meningitis, 20 had ADA levels >5 U/L where as only 3 out of 26 non-Tuberculous Meningities cases had values above this

level.

By taking a cut – off ADA level of > 5 LU/L for the diagnosis of Tuberculous Meningitis, the test had sensitivity and specificity of 95.24% and 88.4% respectively.

The Mantoux test was found to be positive in 42.85% of cases, X-ray chest suggestive of pulmonary Tuberculosis was found in 2 cases and military mottling in 3 cases.

It was observed that Adenosine Deaminase activity has significant positive correlation with other C.S.F parameters especially proteins concentration in cerebro spinal fluid.

Table 1: Results of diagnostic procedures in patients with bacterial meningitis

Age	Sex	CSF cell count/cu.mm neutrophilia	CSF sugar mg %	CSF ADA protein mg%	CSF ADA u/l activity	CSF gram stain/culture
8/12	M	500	30	200	6.2	Klebsiella
3/12	F	750	20	150	4.5	Pneumococci
4	M	200	35	100	4	Stph.aureus
2	F	100	48	84	4	-
2	M	1000	10	60	3.5	E.coli
5	F	100	40	50	3	-
6	M	150	38	60	2	Pneumococci
8	M	200	35	55	2	-
10	f	300	20	60	0.34	-
3	f	90	28	75	1.5	-

Table 2: Patients with encephaltis

Age	Sex	CSF cell count/cu.mm	CSF protein	CSF sugar	CSF ADA activity u/l
1	F	56	30	60	
1 1/2	F	790	26	40	5
3	F	60	44	50	4
4	M	28	80	60	4
6	m	15	40	42	6
5	M	60	30	44	0.5
4	F	50	40	45	5.6

Table 3: CSF ADA levels in different study groups (n:47)

Group	No of Cases	CSF ADA u/l mean+_sd	CSF ADA u/l range
Tuberculous Meningitis	21	14.2_+4.24	4.8-20
Bacteial Meningitis	10	3.1_+1.7	0.34-62
Encephalittis	7	4.2+_1.8	0.5-6
Controls	9	4.1+_1.1	2.2-6

Discussion

Tuberculosis Meningitis remain undiagnosed in the intial stages. Laboratory findings may not confirm to the so called typical results. In addition the microscopic demonstration of Tubercle Bacilli in C.S.F in tropical countries despite careful attempts is possible only in 10-20% of cases. Repeated negative cultures have been demonstrated in autopsy proved cases.

Adenosine Deaminase Assay is a cheap, simple and useful one among the numerous tests available. For determining the diagnosis of Tuberculous meningitis some of the other diagnostic tests for patients with TBM includes- identification of Mycobacterial products (Detection of Tubercular Stearic acid) – a fatty acid present in Mycobacterial cell wall, Detection of Mycobacterial antigen immune assay, particularly Elisa, Antibodies to Mycobacterial antigen, polymerase chain reaction (PCR).

Nevertheless these methods these methods are complicated, inaccessible and too expensive. The initial lumbar puncher with C.S.F examination is not diagnostic is most patients with Tuberculous Meningitis. Repeated Lumbar puncture is important. Slowly raising C.S.F protein levels associated with a decreasing glucose concentration and gradual shift in differential count from Neutrophils or Lymphocytes over a period of days to weeks suggests the diagnosis. Classically the C.S.F examination in T.B.M is clear color less and may show a pellicil or cobweb clot on standing. There is moderate degree of pleocytosis usually not exceeding 500 cells/ cu.mm.

The C.S.F protein level usually ranges between 100 to 300 mg/dl. During the initial stages but with time it may exceed 1000 mg/dl as the disease progresses. C.S.F glucose concentration is below 40 mg/dl or 50% of a simultaneous blood glucose for measurement in 50-85% of patients on admission. It trends to decline steadily in untreated cases.

The raised ADA activity under antigenic stimulation shows its importance in rapid proliferation of cells in order to prevent the accumulation shows its importance in rapid proliferation of cells in order to prevent the accumulation of toxic metabolites and thus reflects good cell mediated immunity [6].

The results of present study showed that mean ADA level in TBM patients was significantly raised ($p<0.001$) as compared to Bacterial Meningitis, Encephalitis and controls a finding a similar to that of various other studies published on same issue. The mean C.S.F ADA levels in TBM cases of pediatric age group have been reported. To be ranging between 11.6 to 13.6 IU/L. In previous studies a relatively higher mean C.S.F ADA values (15.7-21.3 IU/L) have been observed in adults patients.

These results showed that ADA secretion by T-Lymphocytes in r4esponse to Mycobacterial antigen vary and lower activity is observed in C.S.F of pediatric TBM patients. It may be due to difference in immunological reactivity to Tubercular antigen in children as compared to adults.

In prevent study a cut off C.S.F ADA level of >5 LU/L was considered for the diagnosis of TBM with sensitivity and specificity of 95.24% and 88.4% respectively. Overall it was found to be a better diagnostic test in comparison to any other single tests like C.S.F biochemistry Mantoux test, C.S.F smear and culture positivity for Mycobacterium Tuberculosis.

A study by Corral et al [7] found that CSF ADA level cut-off point of 8.5 IU/L for the diagnosis of tuberculous meningitis had 57% sensitivity and 87% specificity; however, another study by Machado et al [8] found that elevated CSF ADA levels are nonspecific for diagnosis of TBM in HIV positive patients. Ribera et al [9] demonstrated similar findings in their study of adults TBM patients. Mishra et al [10] demonstrated that mean CSF ADA activity in tuberculous meningitis was 9.4U/L which was significantly elevated as compared to partially treated bacterial meningitis.

Malan et al [11] found overlapping ADA levels between TBM and pyogenic meningitis in pediatric patients and authors concluded that it is of lesser help in the diagnosis of childhood TBM In prevent study only three cases had false positive levels in the Non- TBM group, moreover the study confirm the usefulness of C.S.F ADA activity for the diagnosis of TBM in children. A significantly higher CSF ADA activity has been observed in confirmed TBM as compared to clinical TBM cases ($P< 0.05$)a finding in accordance to the observation of Selvakumar et al [5]. In contrast, no significant difference between the two groups has been reported by other workers. The higher enzymatic level in confirmed TBM may be due to persistent antigenaemia leading to more pronounced immunological response. This is further supported by the observation of Ribera et al [9]. who showed an increase in CSF ADA level during the first 10 days of anti-tuberculous therapy and the authors pointed out that increase in enzymatic activity may be because of greater stimulation of T-cells by the release of antigen due to bactericidal effect of chemotherapy.

The mean CSF ADA activity in TBM patients correlated well with C.S.F count and protein concentration. This was in agreement with data of Malan et, al [10], Prasad et al [6]. The increase in C.S.F ADA activity with increasing C.S.F ADA activity with increasing C.S.F cell count in these patients. Thus indicate local immune response due to Lymphocyte proliferation in response to antigen. Alternatively the raised enzymatic levels may be because of seepage across damaged blood-brain barrier, permitting ADA to enter the C.S.F from blood or adjacent cerebral tissue.

In o non-tuberculous etiology Baheti et al [12]found that CSF ADA level of 6.5IU/L may differentiate tuberculous from other ethiology. Bindu TH et al [13] study mean cerebrospinal fluid adenosine deaminase levels in tuberculous meningitis patients was significantly higher than non-tuberculous meningitis patients with $P <0.01$.

Thus it is evident that C.S.F ADA activity determination is a useful test for the early diagnosis of TBM since it is simple relatively inexpensive and takes less time to perform. It can be considered as one of the rapid diagnostic tests in children.

Conclusion

As the ultimate usefulness of various investigations employed in diagnosis of TBM relies mainly on early and specific diagnosis of TBM to prevent the unwanted. Dangerous complications and sequelae, any investigation which can satisfy our need may be of utmost help. From the results above obtained in present study it can be firmly and affirmatively inferred that measurement of C.S.F ADA activity can

be taken as a reliable diagnostic aid for a simple, early rapid and specific diagnosis of Tuberculosis Meningitis.

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