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UTILITY OF CSF ADA LEVELS IN THE DIAGNOSIS OF TUBERCULOUS MENINGITIS

Pathology

KEYWORDS: Tuberculous Meningitis, Pyogenic Meningitis, Viral Meningitis, CSF ADA.

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ABSTRACT

INTRODUCTION: Tuberculous meningitis (TBM) is the most dangerous form of extra pulmonary TB in many developing countries like India. It occurs in around 7- 12% of the entire TB cases3. Early diagnosis and treatment with antibiotic therapy and active management of the complications are of great importance to prevent the irreversible neurologic sequel and death. AIMS & **OBJECTIVES:** This study was carried out to determine the level of ADA activity in CSF of TBM, Pyogenic meningitis, viral meningitis and non infectious cases. To study the biochemical pattern and to correlate CSF protein and sugar concentration with CSF-ADA in TBM and Pyogenic meningitis, viral and non infectious cases. **MATERIALS & METHODS:** The present study was conducted over a period of 18months from Jan 2014 to June 2015 in the emergency laboratory of a tertiary care hospital in Guntur. A total of 580 CSF samples from all the medicine and the pediatric wards are included in the study. **RESULTS:** The critical cut-off value of ADA levels is calculated as >10 i.e.11 U/L with the help of ROC curve and a sensitivity of 96.77 % and a specificity of 97.30 % is thus seen for the TBM group versus non TBM. As per the statistics, the association between the ADA levels and the lymphocytes, proteins with the ADA levels is also statistically significant ($p < 0.0001$) **CONCLUSION:** In conclusion, ADA level of 11 U/L and above in CSF could provide additional supportive evidence for the diagnosis of TBM in clinically suspected and bacteriologically negative cases.

INTRODUCTION: Tuberculosis is the seventh leading cause of death and disability[2] an estimated 8.8 million new TB cases were recorded in 2005 worldwide, 7.4 million in Asia and sub-Saharan Africa. India accounts for around one third of case and nearly 5 lakh people die of TB each year[1] Tuberculous meningitis (TBM) is the most dangerous form of extra pulmonary TB in many developing countries like India. It occurs in around 7- 12% of the entire TB cases[3]. Tuberculous meningitis (TBM) occurs as a consequence of lymphohematogenous spread of tubercle bacilli during the primary infection. TBM is most common in young children. Initial signs are non-specific including fever, personality changes, listlessness or irritability and loss of developmental milestones. As the meningitis worsens, the flow of CSF is interrupted and signs of raised intracranial pressure develop. With further progression coma and alterations in cardiac and respiratory functions ensue. Diagnosis in the early stages of TBM is usually missed unless suspected.[1] The cytology and biochemistry of cerebrospinal fluid CSF which is the mainstay of diagnosis of different types of meningitis shows considerable overlap and one has to look for other modalities to support the etiological diagnosis

MATERIALS AND METHODS:

A total of 580 CSF samples from all the medicine and the pediatric wards were collected over a period of 18 months from Jan 2014 to June 2015 in the emergency laboratory of a tertiary care hospital in Guntur. CSF tapping is done by lumbar puncture under aseptic precautions. Procedure should be done carefully to avoid traumatic tap. Characteristic findings are clear, colorless fluid. Part of the CSF was processed immediately for microscopic & biochemical examination. For microscopic examination Methylene blue is used as the CSF diluting fluid. Cytocentrifuge the sample at 1000 rpm/min and a smear is prepared from the sediment, the smear is air dried, heat fixed and stained with Wright stain and then seen under oil immersion. For protein levels, Turbidometric method is applied. Normal range for CSF proteins is 15-45mg/dl. Glucose Oxidase method is used for estimation of CSF sugars, normal range is 40-80 mg/dl. For ADA estimation calorimetric method with Berthelot's reagent.

RESULTS AND DISCUSSION:

It is evident that of all the 580 cases studied, a total of 62 cases each are of TBM, and Pyogenic; 91 cases are of viral and 365 cases are of the non infectious group.

Table 1 Comparison of ADA levels in different groups

Diagnosis	Pediatric		Adult	
	No. of cases	Mean ADA, U/L	No. of cases	Mean ADA, U/L
TBM (Tuberculous meningitis)	25	33.80	37	24.70
Pyogenic	26	6.62	36	7.64
Viral	36	4.97	55	5.66
Noninfectious	198	5.78	167	5.43
Total	285	8.21	295	8.16

Amongst the TBM group of 62 cases 25 are children and 37 are adults with a mean ADA levels of 33.8 and 24.70 respectively. Thus 40.32% of the total TBM cases are pediatric ones. Amongst the Pyogenic group of 62 cases 26 are children and 36 are adults with a mean ADA levels of 6.6 and 7.6 respectively. Thus 41.94% of the total pyogenic group cases are pediatric ones. Amongst the viral group of 91 cases 36 are children and 55 are adults with a mean ADA levels of 4.97 and 5.6 respectively. Thus 39.56% of the total viral group cases are pediatric ones. Amongst the non infectious group of 365 cases 198 are children and 167 are adults with a mean ADA levels of 8.21 and 8.14 respectively. Thus 54.25% of the total non infectious group cases are pediatric ones

Table 2 Sex wise distribution of cases

	Males	Females
	No. of cases	No. of cases
TBM	33	29
Pyogenic	35	27
Viral	46	45
Noninfectious	200	165
Total	314	266

From the table 2 it is evident that out of the total 580 cases, sex wise distribution of females in the overall cases is 45.86% i.e., 266. Females show an overall lower incidence which can be attributed to negligent health awareness amongst females and thus less hospital admissions.

Table 3. Comparison of CSF ADA levels with CSF proteins

CSF proteins (mg/dl)	TBM		Pyogenic		Viral	
	No of cases	Mean ADA	No of cases	Mean ADA	No of cases	Mean ADA
0-50	-	-	-	-	29	6.27
51-100	8	12.37	24	5.37	56	4.92
101-150	13	18.76	13	5.53	6	5.33
151-200	20	25.5	9	6.8	-	-
201-250	10	33.2	6	10.83	-	-
251-300	7	37.71	2	6	-	-
301-350	0	-	4	15.5	-	-
351-400	1	50	-	-	-	-
>401	3	86.66	4	11.25	-	-
Total	62		62		91	

It is clear from this table that there is a positive correlation of CSF ADA levels with that of proteins. With increasing levels of proteins there is a simultaneous rise in the ADA levels from table 1. It also becomes clear that the association is statistically significant (p <0.0001). This significance is seen in the tbm and the Pyogenic groups but not in the viral and the non infectious groups.

Table 4 CSF- ADA level according to the lymphocyte count in TBM cases

Lymphocyte count per mm^3	TBM	
	No of cases	Mean ADA
0-50	-	-
51-100	21	16.33
101-150	22	25.9
151-200	15	35.73

From the above table it is clear that ADA levels increase with an increase in the total lymphocyte count. Thus a positive correlation is established between the two. It is also clear that the association is statistically significant (p<0.0001) in the TBM group.

Table 5. Correlation of CSF ADA levels with polymorphs

Polymorphs count per mm^3	TBM		Pyogenic	
	No. of cases	Mean ADA	No. of cases	Mean ADA
0-25	61	28.18	29	5.27
26-50	1	40	14	5.71
51-75	-	-	10	9.7
76-100	-	-	2	9
>100	-	-	7	14.14

It is clear from the above table that there is some amount of dissociation between the CSF ADA levels and the total polymorphs count .There is slight increase in the total ADA levels with increase in the polymorphs count. This could be attributed to the relatively low percentage of polymorphs in the very high total count and a higher percentage of lymphocytes. This is also evident in the statistical analysis that the association is insignificant.

Table 6. comparison of CSF ADA levels with CSF sugar

CSF sugar(gm/dl)	TBM		Pyogenic	
	No. of cases	ADA	No. of cases	ADA
<40	12	31	54	7.42
>40	50	27.74	8	5.75

There is no correlation between the CSF ADA levels and the sugars and this is also statistically insignificant.

Table8: Correlation between Various Continuous Variables:

	Lymphocytes -ADA	Proteins-ADA	Polymorph-ADA	Sugars-ADA
	Pearson's correlation coefficient (p)	Pearson's correlation coefficient (p)	Pearson's correlation coefficient (p)	Pearson's correlation coefficient (p)
ALL patients	0.93 (<0.0001)	0.74 (<0.0001)	0.08 (<0.05)	-0.09 (0.03)
TB	0.99 (<0.0001)	0.99 (<0.0001)	0.28 (0.03)	-0.08 (0.53)
Pyogenic	0.20 (0.13)	0.41 (<0.001)	0.30 (0.02)	-0.16 (0.21)
Non-infectious	0.56 (<0.0001)	0.13 (0.02)	NA	0.02 (0.71)
Viral	0.23 (0.03)	-0.13 (0.23)	-0.04 (0.74)	0.09 (0.41)

It is evident that there is a strong positive correlation between lymphocytes and the ADA levels which is also statistically significant (p <0.0001) for the overall patients, TBM and non infectious groups. There is but at the same time it shows no significance for the Pyogenic, viral groups. It is also evident that the association between proteins and the ADA levels is statistically significant (p<0.0001) for the overall patients, TBM and Pyogenic groups but at the same time it shows little significance for the viral& non infectious groups. Similarly there is no statistical significance observed between the ADA levels and the polymorphs count, and the sugar levels in any of the groups.

Table 10: Sensitivity Specificity Calculations for Different Groups for Diagnosis of Tuberculous Meningitis by ADA test (at critical cutoff value of >10)

	All (N = 580)	TBM v/s Pyo (N = 124)	TBM v/s Viral (N = 153)	TBM v/s Non (N = 427)
Sensitivity	96.77 %	96.77 %	96.77 %	96.77 %
Specificity	97.30 %	88.71 %	97.80 %	98.63 %
Positive Predictive Value	81.08 %	89.55 %	96.77 %	92.31 %
Negative Predictive Value	99.61 %	96.49 %	97.80 %	99.45 %

Figure1:

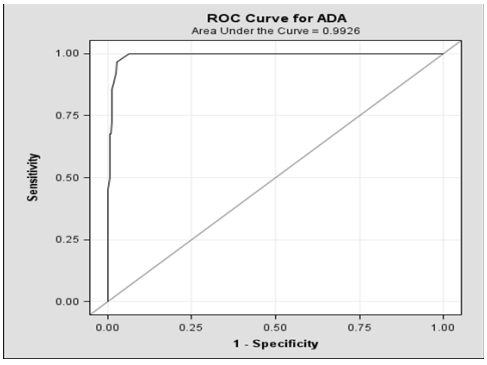

FIG 1. The figure shows the ROC curve drawn to show the accuracy of CSF ADA in the diagnosis of TBM from non TBM group of patients. The area under curve is 0.99 comparing the ADA activity between the tuberculous meningitis cases (n=62) and non tuberculous meningitis cases (n=518), the ROC curve identified a CSF ADA level of 11.0 U/l as the best cut-off value to differentiate between the two, with a sensitivity of 96.77 % a specificity of 97.30%, with an area under the curve of 0.99.

The mean CSF ADA activity was found to be significantly higher in CSF of TBM patients 28.37±16.69, than in the CSF from non-TBM infectious meningitis 5.77±3.17, (p<0.0001) in this study. Kashyap et al[45] did one of the largest studies on 117 patients of TBM and showed that the mean CSF ADA activity was found to be significantly higher in CSF of TBM patients, 14.31±3.87, than in the CSF from non-TBM infectious meningitis, 9.25± 2.14. Malan C et al[31] showed that in both bacterial and TBM groups, the mean ADA level in the CSF was significantly higher than in aseptic meningitis (p<0.0001). Similar results were noted in our study where the mean ADA value in viral meningitis was 5.38±2.41 which was well below the cut-off value. In our study, we found that the mean value of CSF ADA was 7.20 ± 5.98 in Pyogenic meningitis. Some studies have reported a lower efficacy of this test and show an overlap between Tuberculous meningitis and Pyogenic meningitis.[44] So we used the higher cut-off value of 11 U/l in order to increase the specificity of ADA and to overcome the above lacuna as suggested by Selvakumar[34]. ROC curve has been used in the study to calculate the cut off value as done by . Chotmongkol et al.[42]

In our study, ADA levels ranged from 10-120U/l in TBM with a mean levels of 33.8U/L with a cut off value as 11U/L in pediatric populations, with a range of 10-120U/L. Malan et al[29] and Coovadia et al[9] 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[30] in adults. These results show that ADA secretion by T-lymphocytes in response to mycobacterial infection is similar in different ethnic groups. Malan et al[29] reported a value of 6U/l as the cut off point for classification; Coovadia et al[9] had a cut-off point of 10 U/l for differentiation while in adults Ribera et al[30] 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[34]. In this study, 5(1.3%) of 365 Non-infectious patients and 7 of 62 (11.29%) Pyogenic cases had ADA values more than 10 U/l. Similarly, Malan et al[29] reported that 13 (31%) of 42 patients with Pyogenic meningitis had values higher than 9 U/l. Coovadia et al[9] observed that 14 of 16 Non-t TBM patients had ADA levels more than 10U/l. It is, therefore, evident that the CSF from patients with

Pyogenicmeningitishadalsoelevated ADA levels. This aspect should be takenintoconsiderationwhileinterpretingADAtestresults. Ribera et al [30] 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 are considered for the diagnosis of TBM.

CONCLUSION:

Smear and/or culture for AFB, smear and culture for bacteria, India ink preparation, latex agglutination for antigens of bacteria or cyptococci remain the gold standard for diagnosis of various etiologies of meningitis. However, some of these tests especially for TBM and Pyogenic meningitis can take time and may turn out negative. This is when physicians are in a diagnostic dilemma. Many patients are needlesslyreceivingantituberculoustreatmentandantibioticsinhigh dosesonerroneousinterpretationofCSF.

Early confirmatory diagnosis and aggressive management can help prevent serious CNS complications and at the same time reduce unwarrantedorharmfultherapyforpatients..ADAactivityisnotedtobe higher in tuberculous meningitis. The levels of ADA are low in viral meningitis. The tests for ADA in CSF are simple and can be carried out in a centrallaboratorywitharapiddiagnosis,thusreducingunwarrantedor harmful therapy for patients. However, these tests should be interpreted judiciously in the light of the patients' clinical manifestations and the CSF characteristics.

This study re emphasizes the need to raise the awareness about using the rapid test ADA for diagnosing meningitis and to encourage more studies with larger sample size in this regard.

In conclusion, ADA level of 11 U/L and above in CSF could provide additional supportive evidence for the diagnosis of TBM in clinically suspected and bacteriologically negative cases.

The laboratory method for measuring ADA is inexpensive, relatively simple to perform and can also be adapted to an autoanalyzers. It may thus be useful in laboratories with limited resources, especially in underdeveloped and developing countries like India where the incidenceoftuberculosisisveryhigh.

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