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A STUDY ON ADENOSINE DEAMINASE AND C-REACTIVE PROTEIN IN CEREBROSPINAL FLUID OF MENINGITIS PATIENTS OF DIFFERENT TYPES.

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ABSTRACT **Introduction:** The three commonest types of Meningitis are Bacterial, Viral and Tubercular; they differ in their patho-physiology and management. That is why early differentiation between them is important. Bacterial meningitis have heavy fatality rates whereas Tubercular meningitis inflicts severe neurological sequalae.

Aims & Objectives: To determine whether ADA and CRP can be used successfully to predict the type of Meningitis affection

Methodology: This is a Hospital based cross-sectional observation study which was undertaken in Biochemistry dept. Medical College, Kolkata. Eighty cases of meningitis were taken. ADA and CRP was measured along with Protein, Sugar and Differential staining cell counting. The cases were divided into three groups (Bacterial, Viral, and Tubercular) on the basis of microscopic and Biochemical Examination.

Results & Analysis: ANNOVA test was done along with Bon Ferronis Test which revealed that there was significant difference in mean of ADA & CRP in the three different groups.ROC curve for CRP between Reactive and Non-reactive meningitis revealed that if CRP values are more than 11.0, 95% of the cases were reactive whereas ROC curve for ADA between Tubercular and Non Tubercular forms showed when the concentration was more then 9.0, 95% of the cases were of Tubercular origin.

Conclusion: When taken together ADA and CRP successfully predict the type of Meningitis. It is also helpful in diagnosing those cases when the Cell count and type was equivocal.

KEYWORDS : ADA, CRP, Meningitis.

INTRODUCTION

Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges [1]. The most common symptoms are fever, headache and neck stiffness. The three commonest types of Meningitis are Bacterial, Viral and Tubercular; they differ in their patho-physiology and management. That is why early differentiation between them is important. Bacterial meningitis have heavy fatality rates whereas Tubercular meningitis inflicts severe neurological sequalae [2] . Adenosine Deaminase (also known as adenosine aminohydrolase, or ADA) is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. Its primary function in humans is the development and maintenance of the immune system. However, the full physiological role of ADA is not yet completely understood. Adenosine Deaminase (ADA) is an endogenous tissue enzyme which is released in the serum of the patients with different types of malignancies and infections including viral hepatitis, Infectious Mononucleosis and Tuberculosis[3]. In pleural and CSF fluids elevated ADA levels are very commonly associated with tuberculosis.

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex[4].

MATERIALS AND METHODS

This is a Hospital based cross-sectional observation study which was undertaken in Biochemistry dept. M.C.H-Kolkata from Jan 2017 to Jan 2018 after obtaining ethical clearance from the institution. Eighty cases of meningitis within the age group of 10 to 75 years were included in this study. ADA, CRP was measured along with Protein and Sugar in CSF and Differential staining cell counting. The cases were divided into three groups (Bacterial, Viral, and Tubercular) on the basis of microscopic and Biochemical Examination.

Written informed consent was taken from the patients as per proforma Demographic data; detailed History and Clinical findings and other laboratory work up were recorded.

Study Group patients are those suffering from meningitis as per clinical suspicion and presentation of the patients as per ESCMID (European Society for Clinical Microbiology and Infectious Diseases). Diagnosis of the type of Meningitis was done on the basis of clinical evaluation supported by relevant diagnostic work up.

Patients with history of auto-immune disorder, suffering from HIV and other immune-deficient conditions. Cases of brain and spinal tumors causing blockage of CSF flow / xanthochromia, of Intra Cranial Hemorrhage, Metastatic Brain Tumours, Traumatic spinal tap and with high Bilirubin levels in serum pertaining to liver Pathology were excluded from the study.

Statistical Analysis:

Statistical Analysis of the study was done by SPSS Software version 20.0 and Microsoft Excel 2013 notepad.

Principle

The ADA assay is based on enzymatic deamination of adenosine to ionosine.

One unit of ADA is defined as the amount of ADA that generates one µmole of Ionosine from Adenosine per minute.

Reference Range:

The ADA activities in 60 healthy human serum samples were found in the range of 0-15 u/l. For Pleural fluid values were found to be in range of 0-24 u/L, for CSf values were found in the range of 0-5 u/L, for pericardial fluid values were in the range of 0-44 u/L and for Ascitic fluid 0-30u/L.

Assay Procedure:

ADAZYME is an enzymatic assay system that can be used for both manual and automated chemistry analyser and measured at 546 nm,37˚c.[5] As much as possible of CSF is to be collected in a syringe, clean skin with alcohol inside out in a spiral manner before aspirating the specimen.

Mix well and incubate for 5 mins at 37˚c. Read the initial absorbance A1. Measure the change in absorbance per minute (Δ/min) for next 3 mins.

Calculation: ADA activity in IU/m= ΔAT/ ΔAC x Concentration of the calibrator

ΔAT: Mean absorbance per minute of the test sample
ΔAC: Mean absorbance per minute of the Calibrator

Linearity: The linearity of the procedure is from 0 to 200 U/L

C-REACTIVE PROTEIN AUTOMATION Manual Procedure by Immunoturbimetic method[6] Read optical density of calibrators, controls and samples at 340 nm. Generate a reference curve by successive 1:2 dilutions of Calibrator High in saline or use the ready for use calibrator set. Use saline as zero point. Calculate ΔOD's, plot a calibrator curve and read the concentration of controls and samples. Reference Values 0 – 10 mg/l (IFCC), resp. 0-1 mg/dl.

RESULTS

Table 1. Distribution Of The Study Participants According To Sex

Sex	Frequency	(%)
Male	50	62.5
Female	30	37.5
Total	100	100

62.5% of them were male subjects and the rest were female.

Table 2. Distribution Of Study Participants According To Age

Age in years	Frequency	(%)
0-15	21	26.3
16-30	21	26.3
31-45	15	18.8
46-60	16	20.0
61-75	5	6.3
more than 75	2	2.5
Total	80	100

The main bulk of the subjects in this study have age range from 10 to 60 yrs. This shows a camel hump appearance. The majority of the cases lie in two distinct age groups. 10 to 30yrs consisting of children and young adults then again at 46 to 60 i.e. early elderly age group.

Table 3. Distribution Of Study Participants According To Type Of Meningitis

		Frequency	Percent
Valid	bacterial	21	26.3
	tubercular	13	16.3
	viral	46	57.5
	Total	80	100.0

Categorization of the type of affection shows highest for asceptic or viral meningitis and lowest is tubercular meningitis.

Table 4. Comparison Of Mean CRP Among Different Categories Of Meningitis

Descriptives								
CRP : Kit Reference Interval 0 to 10 Iu/ml								
Clinical assumption of the Type	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
viral	46	6.2324	2.25159	.33198	5.5638	6.9010	2.00	12.00
tubercular	13	10.3077	6.66314	1.84802	6.2812	14.3342	2.00	25.00
bacterial	21	16.4286	5.96298	1.30123	13.7143	19.1429	10.00	37.00
Total	80	9.5711	6.14115	.68660	8.2045	10.9378	2.00	37.00

Table 5. Anova Test showing significant difference in mean CRP among three types of meningitis grouped as 1. Asceptic, 2. Bacterial, 3. Tubercular [7]

ANOVA					
CRP					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1507.337	2	753.669	39.423	.000
Within Groups	1472.046	77	19.117		
Total	2979.383	79			

Post Hoc Analysis (Bon Ferroni test) to show significant difference in mean CRP among categories.

Table 6. Bonferronis Correction For CRP

(I) DiagCategory	(J) DiagCategory	MeanDifference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
viral	tubercular	-4.07530*	1.37338	.012	-7.4365	-.7141
	bacterial	-10.19618*	1.15150	.000	-13.0144	-7.3780
tubercular	viral	4.07530*	1.37338	.012	.7141	7.4365
	bacterial	-6.12088*	1.54303	.000	-9.8973	-2.3445
bacterial	viral	10.19618*	1.15150	.000	7.3780	13.0144
	tubercular	6.12088*	1.54303	.000	2.3445	9.8973

*. The mean difference is significant at the 0.05 level.

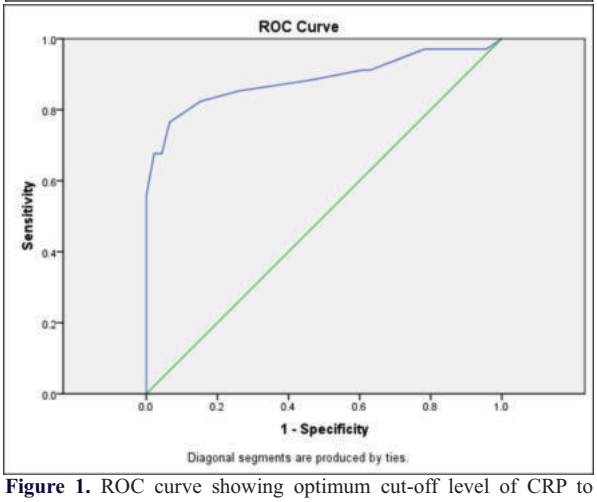

Figure 1. ROC curve showing optimum cut-off level of CRP to differentiate between viral and reactive (bacterial and tubercular) meningitis

Table 7. Area Under ROC Curve For CRP

Area Under the Curve				
Test Result Variable(s):CRP				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.885	.044	.000	.800	.970
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Coordinate points to determine optimum cut off of CRP to differentiate between viral and reactive (bacterial and tubercular) meningitis.

At CRP level ≥ 11.5 there is 98% specificity that the type of meningitis will be reactive.[8]

Table 8. Comparison Of Mean ADA Among Different Categories Of Meningitis

Descriptives								
ADA: Kit reference Range 0 to 5 IU/ml								
Clinical assumption of the Type	N	Mean	Std. Deviation	Std. Error	95%Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
viral	46	3.7950	1.96527	.28976	3.2114	4.3786	1.10	8.00
tubercular	13	20.9431	9.09413	2.52226	15.4476	26.4386	7.30	43.00
bacterial	21	4.1643	2.51458	.54873	3.0197	5.3089	1.10	10.00
Total	80	6.6785	7.50792	.83941	5.0077	8.3493	1.10	43.00

Table 9. ANOVA Test Showing There Is Significant Difference In ADA Level Among Three Types

ANOVA
ADA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3160.433	2	1580.217	94.126	.000
Within Groups	1292.702	77	16.788		
Total	4453.135	79			

Table. 10 Post Hoc Analysis Bon Ferroni Test

Multiple Comparisons						
ADA Bonferroni						
(I) DiagCategory	(J) DiagCategory	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
viral	tubercular	-17.14808*	1.28700	.000	-20.2979	-13.9983
	bacterial	-.36929	1.07908	1.000	-3.0102	2.2717
tubercular	viral	17.14808*	1.28700	.000	13.9983	20.2979
	bacterial	16.77879*	1.44598	.000	13.2399	20.3177
bacterial	viral	.36929	1.07908	1.000	-2.2717	3.0102
	tubercular	-16.77879*	1.44598	.000	-20.3177	-13.2399
*. The mean difference is significant at the 0.05 level.						

It shows there is significant difference in mean ADA level between viral and tubercular meningitis. There is also significant difference in mean ADA level between bacterial and tubercular meningitis. But the difference of mean ADA between viral and bacterial is not significant.[8]

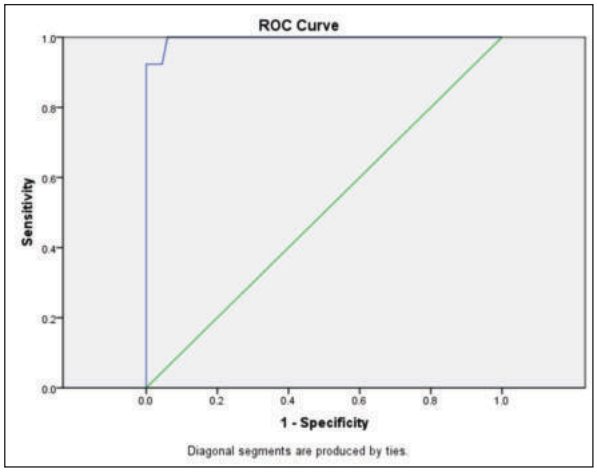

Figure2. ROC Curve Showing Optimum Cut-off Level Of ADA To Differentiate Between Tubercular And Non-tubercular Meningitis

Table 11. Area Under the ROC Curve for ADA				
Test Result Variable(s):ADA				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.996	.005	.000	.000	1.000
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Coordinate points to determine optimum cut of ADA to differentiate between tubercular and non-tubercular meningitis.

At ADA value≥ 9.9 there is 98.5% specificity that the type of meningitis will be tubercular.[8]

Table 12. Table For Conclusion

CRP Level	ADA level	Possibility
-	-	Viral
+	-	Bacterial
-	+	Tubercular

(-) less than cut-off value, (+) higher than cut off value

Considering the CRP and the ADA values of the samples, prediction of the type of Disease can be most accurately made from the chart presented in the table no 12 above showing the verities of the values of these two parameters.

DISCUSSION

Meningitis is a fatal disease with world-wide distribution and demands most urgent diagnosis and treatment to save life. Biochemical measurement of protein and sugar was undertaken. Chloride has been supplemented with two more parameters i.e ADA & CRP.

This study was undertaken with an aim of finding the special significance of ADA & CRP in the CSF of suspected meningitis sufferers and studying the pattern of their elevations in the particular type of meningial affection.

Biochemical Findings-

CSF ADA:-

Table-15 shows the comparison of mean ADA values of different samples. It is found to be highest (20.94) in the clinically suspected tubercular variety. The viral group had a mean value of 3.7 and the Bacterial group had the value of 4.1. It clearly demarcates the tubercular variety of the other two types[8] .It also highlights that the bacterial and viral types of meningitis cannot be demarcated from this ADA value which are very closely similar and overlapping This is also in conformity previous work. Analysis of significance of these values was assessed by statistical methods named ANOVA followed by Bon Ferronis Correction. The Table no 16, 17- clarifies the picture to distinguish clearly between the three sets of results obtained in order to point out to the causative nature of the disorder.

This parameter will help us to differentiate Tubercular Meningitis from other Non-Tubercular types. The means of ADA with the other two groups were significantly different statistically. Hence we can safely assume that the overlap of value distribution of ADA for the tubercular and non-tubercular variety will be much less. This helps us to use ADA as a marker of Tubercular meningitis safely. From the figure no-18 showing ROC Curve states that ADA values above 9.9 IU/ml predicts tubercular variety with 98% specificity[8]. This also is very similar to the previous study by other researchers. This cut off value gave a good sensitivity and specificity in differentiating it from non-TBM.

CSF CRP:-

Table no 10 showing the results of CRP Levels states that the suspected group with bacterial meningitis showed highest mean value of 16.43 mg/dl, Tubercular variety had a mean value of 10.30mg/dl whereas the level was smallest in Viral group and it was 6.23 mg/ml. The results were similar and in agreement with previous studies.[9]

Statistical analysis of significance was presented in the tables. Analyses of variants (ANOVA) of these results were also done in the table. This clearly shows that there was significant difference between the means of different groups which could be utilized to predict the type of disease with confidence and also to distinguish between the Bacterial from the other two groups. In regards to ascertain that ROC curve was plotted and it also pointed out that a safe differentiation between these two groups can be made with 98.5% specificity when the cut off value of CRP is more than 11.5 mg/dl. Previous study found that when the CRP values were above 16.5 the chances of bacterial Meningitis increased significantly[10].

CONCLUSION

The study gives us a confidence to identify the cause of Meningitis with certainty by utilizing two parameters ADA & CRP levels in the CSF samples. Tubercular etiology is found to be associated with predominantly raised ADA; Bacterial (Pyogenic) Meningitis is related to a predominant rise in CRP where as normal values of the both parameters are found in the Viral group. The other parameters traditionally being followed as a primary investigation of the CSF in such patients include Protein and Glucose. Chloride estimation in CSF was not considered in this study due to lack of facility. The results obtained from the traditionally used parameters as mentioned above were also utilized to help in grouping of the clinically suspected cases and analysis of these different groups on the basis of their different results are obtained. These results are in conformity with the observations found by different researchers during last 30 years and thus can be offered to the clinical community as an additional diagnostic tool to identify the etiology of meningitis for treatment.

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Permission From IRB: Yes

REFERENCES

1. Harrisons Textbook of a medicine 12th edition
2. GBD 2013 Mortality Causes of Death Collaborators (January 2015). "Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013". *Lancet*. 385 (9963):117–71. doi:10.1016/S0140-6736(14)616822PMC4340604PMID 2 5530442.
3. Ribera E, Martinez-Vazquez JM, Ocaña I,et.al. Activity of adenosine deaminase in cerebrospinal fluid for the diagnosis and follow-up of tuberculous meningitis in adults. J Infect Dis. 1987 Apr; 155(4):603-7.
4. R Idker PM, Buring JE. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events. Circulation 2003; 107:3917.
5. Guisti G, Galanti B: Adenosine deaminase: colorimetric method. In Methods of Enzymatic Analysis. 5th edition. Edited by: Bergmeyer HU. Weinheim ,Verlag Chemie, 1984:315-323.
6. Teitz, N. W. (Ed)., Textbook of Clinical Chemistry, W.B. Saunders, (1986),
7. Hansson LO, Axelsson G, Linné T, et.al. Serum C-reactive protein in the differential diagnosis of acute meningitis. Scand J Infect Dis. 1993; 25(5):625-30.
8. Gautam N, Aryal M, Bhatta N, et.al " Comparative study of Cerebrospinal Fluid adenosine Deaminase activity in patients with meningitis. Nepal Med Coll 2007; 9:104-6
9. Belagavi AC, Shalini M.Cerebrospinal Fluid C Reactive Protein and Adenosine Deaminase in Meningitis in Adults. J Assoc Physicians India 2011 Sep; 59:557-60
10. Mishra OP , Loiwal V, Ali Z,et.al Cerebrospinal fluid Adenosine deaminase activity and C-Reactive Protein in tubercular and partially treated Bacterial meningitis. Indian Pediatrics .1995 Aug; 32(8):886-9.