The use of cerebrospinal fluid C reactive protein and adenosine deaminase as diagnostic markers in differential diagnosis of meningitis

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Abstract
Meningitis is an important public health problem in developing countries with considerable morbidity and mortality. Early categorisation according to the aetiology of meningitis leads to specific treatment with better outcome. Cerebrospinal fluid (CSF) culture is the gold standard but is not easily available and expensive so a rapid but specific test to differentiate types of meningitis is the need of the hour. So this prospective study was carried out in a tertiary care hospital in Eastern India.
50 patients of meningitis based on clinical, radiological and biochemical profile were recruited and serum C reactive protein (CRP), CSF adenosine deaminase (ADA) & CRP were estimated and analysed with respect to conventional parameters. The mean value of serum CRP in pyogenic meningitis was 54.98 which were considerably higher than tubercular and viral counterparts. CSF CRP was also higher in these patients with a mean value 20.35. In tubercular meningitis the mean CSF ADA is 31.16 which were higher compared to other causes. Taking CSF ADA cut off >10IU/L, 17out of 19 patients had higher levels which was quite significant. The sensitivity, specificity, positive predictive value, diagnostic accuracy of CSF CRP is 94.12%, 100%, 100% and 98% respectively and that for CSF ADA is 89.47%, 96.77%, 94.44% and 94% respectively. So CSF ADA & CRP can be used as early and reliable markers to differentiate meningitis.
Keywords: *Meningitis, CSF CRP, CSF ADA, serum CRP.*

Introduction

Infectious diseases constituted the most serious health issue in the world until the beginning of the 20th century when chronic degenerative diseases began to dominate this scenario particularly in developed countries. The prevalence of these diseases and factors influencing it, principally during epidemics formed the dominant political, social, and theological opinion of the different human societies in medieval and modern times which were fundamental in the definition and adoption of many of the pathways that led to civilisation and its progress. Meningitis which is defined as inflammation of the membranes (leptomeninges) surrounding the brain & spinal cord is one of the commonest and deadly

manifestation of infectious pathogens. The magnitude of this problem can be understood from the fact that over 1.2 million cases of bacterial meningitis are estimated to occur worldwide each year. [1] The incidence and case-fatality rates for bacterial meningitis vary by region, country, pathogen, and age group. Without treatment, the case-fatality rate can be as high as 70 percent, and one in five survivors of bacterial meningitis may be left with permanent sequel including hearing loss, neurologic disability, or loss of a limb. [2] The etiological diagnosis of meningitis remains a problem in clinical practice as cerebrospinal fluid (CSF) biochemical analysis & cellular response often overlap. In such circumstances the determination of CSF C- reactive protein (CRP) & CSF adenosine deaminase (ADA) helps in specific diagnosis and choosing the appropriate antimicrobials. This study was done to evaluate the diagnostic utility and accuracy of CSF CRP & CSF ADA in distinguishing the type of meningitis and correlation of these findings with conventional biochemical and cytological parameters.

Methodology

This prospective observational study was carried out in the department of General Medicine in a tertiary care hospital for a period of 1 year. Prior to the study a favourable ethical clearance was obtained from the institutional ethical committee.

The patients were selected by non probability consecutive sampling among those who were admitted in the department presenting with features of acute meningitis clinically like fever, headache, vomiting, photophobia, seizures, alteration of consciousness and focal neurological symptoms. The sample size was calculated from the average admission rate of meningitis patients in the study area which is one of the premier tertiary care centres in West Bengal. Considering the seasonal variation of meningitis the average admission rate is 1 patient/week (p), and the study period being 1 year or 52 weeks (q). So the calculated sample size was n=pXq=1X52=52, on

average 50. The first 50 patients of meningitis determined clinically or biochemically aged <60years were included in this study. Patients with severe chronic co morbidities, encephalitis, brain abscess and meningitis due to fungal or protozoal cause was excluded from the study.

In all the 50 patients a non contrast CT Brain was done to exclude brain tumour, encephalitis and look for any features of meningitis. Then complete haemogram, ESR, serum CRP was done after admission. Lumbar puncture was done and CSF analysis was done for cell type, cell count, protein, sugar, CSF CRP, ADA, gram stain, Zeihl Neelsen stain. C-RP was detected by latex agglutination method using commercial kit "Immunoscreen-CRP" by Monozyme. The test utilizes uniform latex particles coated with anti human C-RP which show agglutination in presence of C-RP. The qualitative test was performed on the clinical samples by mixing a drop (40 µL) of specimen with 25 µL of latex reagent on a disposable slide. Both were mixed gently. The test was considered positive if clear agglutination was visible within 2 minutes. Absence of agglutination within 2 minutes indicated negative sample. A positive and negative control was also run in parallel with each test.

The statistical analysis was done by SPSS version 20.0. Categorical variables were expressed as number of patients and percentage of patients and compared across the groups using Pearson's Chi square test for independence of attributes. Continuous variables were expressed as Mean+/- standard deviation and compared the groups using ONE WAY ANOVA test. Sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy of different parameters in predicting different types of meningitis were calculated. An alpha level of 5% was taken i.e. p value less than 0.05 was considered significant.

Results

A total of 50 patients were studied among them 19 patients were tubercular in origin, 17 were pyogenic bacterial meningitis and 14 patients were

of viral aetiology. Regarding the age distribution 16 cases were <30 years, among which 8 were tubercular, 3 were bacterial and 5 were viral. In the age group 31-45 years there were 18 patients among whom 6 were tubercular, 7 were bacterial and 5 were viral. In the age bracket 45-60 years there were 9 patients with 3 cases each of the different types. In the older age group >60 years there were 7 cases among which 2 were tubercular, 4 were bacterial and 1 of viral aetiology. As for gender distribution 21 patients were females and 29 were males. So there was male preponderance with male: female ratio of 1.38:1. In the pyogenic meningitis group 9 patients were males and 8 were females. The skewed sex ratio was most prominent in the viral group with 5 females (35.71%) to 9 males (64.29%). In the tubercular group there were 12 males and 7 females. The patients presented with various clinical manifestations among which fever was commonest present in 98% cases. The frequency of other presenting complaints were headache 90%, vomiting 56%, altered sensorium 44%, photophobia 32%, seizures 16%, focal neurological deficit14%, drowsiness 12%, stupor 6% and comatose in 4% patients.

Serum CRP was estimated in all the patients and mean value was 54.98 in pyogenic meningitis, 25.56 in tubercular and 4.71 in viral meningitis patients. Taking 50 mg/dL as the cut-off level for serum CRP it was seen that among 17 patients of pyogenic meningitis 9 had CRP>50 whereas in 33 cases of other causes only one patient has CRP>50. So the sensitivity of serum CRP as screening test for pyogenic meningitis was 47.06%, specificity was 96.97%, positive predictive value 88.89%, negative predictive value 78.05% and diagnostic accuracy 80%. The average level of CSF CRP in tubercular meningitis was 1.37+/-0.73 mg/dL, in pyogenic meningitis 20.35+/-6.96 mg/dL and in viral meningitis 0.99+/-0.41 mg/dL.

 The comparison of CSF CRP in pyogenic and nonpyogenic meningitis is shown in Table 1 where it is shown the p value is significant <0.001

in pyogenic compared to other causes of meningitis. In routine CSF analysis it was seen that CSF cell count was more when CSF CRP was increased as evident by CRP mean value 11.38 when cells were 0-300 cell/cc which increased to a mean value of 28.92 when cells were >600. Similarly out of 17 cases of pyogenic meningitis 5 cases had CSF protein<100 mg/dL with mean CSF CRP of 12.54+/-3.8 mg/dL, 8 cases had CSF protein 100-200 mg/dL with mean CSF CRP of 20.51+/-2.45 mg/dl, 4 cases had CSF protein >200 mg/dL with mean CSF CRP of 29.8+/-2.12 mg/dl. So with higher CSF protein level more and more cases had CSF CRP in higher range. To study the relation of CSF CRP with CSF glucose level cases of pyogenic meningitis were divided into 3 groups based on CSF to blood glucose level and compared with mean CRP. In the group with CSF/ serum glucose >0.4 the mean was 11.38, in group with 0.2-0.4 the mean was 20.94 and in the group with ratio<0.2 it was 26.60. So group with lower ratio or simply lower glucose levels had higher CSF CRP levels. Taking the cut off value for CSF CRP as 8 mg/dL it was seen that 16 out of 17 patients of pyogenic meningitis had higher values so the sensitivity of CSF CRP was 94.12%, specificity was 100%, positive predictive value was 100%, negative predictive value was 97.06% and diagnostic accuracy was 98%. Although both serum CRP and CSF CRP were increased in cases of pyogenic meningitis but when they were compared among themselves the p value was 0.274 which was not significant statistically.

The other parameter of interest in the study was CSF ADA which was measured in all the cases. Patients with tubercular meningitis had average CSF ADA 31.16 IU/L which was higher than that in pyogenic meningitis 2.03 IU/L, and viral meningitis 2.46 IU/L. Taking the upper level of CSF ADA as 10 IU/L the different types of meningitis were compared the results are shown in Table 2.

As regarding the CSF analysis ADA increased with the increasing cell count, mean ADA was found to be 58.76 when cells>200/cc whereas it

was 20.74 when the cell count was 100-200/cc and 13.2 when cell count was 0-100/cc. Similarly in groups with increased CSF protein the ADA level was also increased like mean ADA was 54.97 when cell>200, 19.31 when cell count was 101-200 and 13.20 when cell count was 0-100. However there was no statistically significant correlation between CSF glucose and CSF ADA with the p value being 0.122 during comparison between groups. So the patients with tubercular meningitis had a raised ADA compared to nontubercular counterparts as shown in Table 3.

In this study the sensitivity of CSF ADA as a diagnostic tool was 89.47%, specificity was 96.77%, positive predictive value was 94.44%, negative predictive value was 93.75% and diagnostic accuracy was 94%. The final outcome of the study subjects in tubercular meningitis were 15.79% patients expired while the rate was lower in pyogenic meningitis where 11.76% patients died. All the patients of viral meningitis recovered. But there was no statistical correlation with CSF ADA &CRP with the mortality and outcome of the patients.

Table 1: p value in different types of meningitis taking 8 mg/dL as cut off value of CSF CRP

		Diagnosis			Total	P Value		
		Tubercular Meningitis (A)	Pyogenic Meningitis (B)	Viral meningitis (C)		AvsB	AvsC	BvsC
CSF CRP	<8	19(100)	1(5.88)	14(100)	34(68)	<0.001	NA	<0.001
	>8	0(0)	16(94.12)	0(0)	16(32)			
Total		19(100)	17(100)	14(100)	50(100)	Significant	Not Significant	Significant

Table 2: P value in different types of meningitis taking 10 IU/L as cut off value of CSF ADA

		Diagnosis			Total	P value		
						AvsB	AvsC	BvsC
		Tubercular meningitis (A)	Pyogenic meningitis (B)	Viral meningitis (C)				
CSF ADA	<10	2(10.53)	17(100)	13(92.86)	32(64)	<0.001	<0.001	0.263
	>10	17(89.47)	0(0)	1(7.14)	18(36)			
total		19(100)	17(100)	14(100)	50(100)	Significant	Significant	Not Significant

Table 3: CSF ADA in tubercular meningitis taking 10 IU/L as a cut off value

		Etiology		Total
		Not tubercular meningitis	Tubercular meningitis	
CSF ADA	<10	30	2	32
	>10	1	17	18
Total		31	19	50

Discussion

Acute infections of the nervous system are among the most important problems in medicine because early recognition, efficient decision making and rapid instituition of therapy can be lifesaving. These distinct clinical syndromes include meningitis, encephalitis, brain abscess, subdural empyema and infectious thrombophelibitis.

Among all these CNS meningitis is quite important in regard to prevalence and chance of cure with a rapid diagnosis and early initiation of therapy. Investigations like PCR & ELISA although specific are exorbitant and not readily available so in these circumstances CSF ADA & CRP may add a new dimension to management of meningitis. C reactive protein or CRP is an acute phase serum globulin formed by the body in response to stimuli such as infections, tissue necrosis or neoplasm. CSF CRP is one of the most reliable and early indices to differentiate bacterial from non bacterial meningitis because of passive diffusion of the protein along the inflamed meninges.[3,4] The results of our study corroborated the findings from previous and contemporary work in similar clinical setting. Fever and persistent headache was the common presenting symptoms. This was similar in studies carried out in adult patients such as Carpenter *et al* although the symptoms were different in paediatric populations.[5] In HIV affected individuals due to depressed cellular immunity the clinical response is inadequate so these cases were not included in the study. The mean value of serum CRP in pyogenic meningitis is 54.98 with a standard deviation of +/-30.82 which is increased compared to meningitis due to other causes. Similar results were found in a study by De Beer *et al* where patients with bacterial meningitis had CRP concentration from 41-400 mg/L with a median value of 260 mg/L, which was significantly higher (P<0.001) than patients with viral meningitis who had a median value of 10 mg/dl. [6] Taking 50 as a cut off value for serum CRP the sensitivity as a screening test in the detection of bacterial meningitis was 47.06% and specificity was 96.97%. Similar results were shown in a study by Abramson JS *et al* where the C-RP test was able to detect bacterial meningitis with a sensitivity of 97% (72 of 74), a specificity of 86% (139 of 161), a positive predictive value of 77% (72 of 94), and a negative predictive value of 99% (139 of 141). [7] In a much earlier study published in 1959 by Jansoon E *et al* similar results were found. [8] It was also seen that patients who had higher CSF CRP had increased CSF cell count, protein levels and an inverse ratio with CSF to blood glucose. This was consistent with previous studies. [9-12] The mean CSF CRP level of pyogenic meningitis, tubercular meningitis and viral meningitis were 20.35+/-6.96 mg/dl, 1.37+/-0.73 m/dl and 0.99+/-0.41 mg/dl respectively. The difference between the mean level of CSF CRP between pyogenic and non pyogenic meningitis was statistically significant (p<0.001). The sensitivity and specificity of CSF CRP was 94.12% and 100% when the cut off was taken as >8 gm/dl. But there was no significant correlation between CSF CRP and serum CRP levels. This may be due to initial hospital admission CRP levels were far above the upper limit of normal (19 mg/l) in all patients with bacterial meningitis, regardless of the duration of illness, the age of the patient, the bacterium involved, fever, the erythrocyte sedimentation rate, or the cerebrospinal-fluid cell count.[13] Adenosine deaminase or ADA is an enzyme of purine catabolism catalyzing hydrolytic deamination of adenosine to inosine. It is released by T cells during cell mediated immune response to the tubercle bacilli so it can be considered as a marker of mycobacterial infection. The source of raised ADA is the damaged blood brain barrier permitting ADA to enter CSF from blood or adjacent cerebral tissues or as a result of lymphocyte-macrophage proliferation indicating local response.[14] When CSF ADA was used as diagnostic tool for detection of tubercular meningitis in our study it had good specificity and diagnostic accuracy. This was also seen in similar studies by Ribera E *et al,* Malan C *et al,* Mishra OP *et al* and Eintracht S. [15-17]

But either test done in isolation will cause confusion as there may be overlap of ADA levels between tuberculous and bacterial meningitis like Malan C *et al.* Gambhir IS *et al.*[15,18] Also the cell type in initial stages of tuberculosis may be neutrophil rich, and partially treated bacterial meningitis may have lymphocyte predominance.

Elevated ADA levels are suggestive of tubercular origin but they can't distinguish bacterial from viral meningitis as both show low ADA levels. So it is essential to do both these tests simultaneously to improve the diagnostic accuracy. Nowadays many new markers for differentiating meningitis are in vogue like CSF lactate, procalcitonin and some more are on the anvil like Lipocalin 2, soluble triggering receptor expressed on myeloid cells-1 (Strem-1), CD 163, high mobility growth box-1, TNF alpha, IL-1 beta, IL 6, IL 8, lipopolysaccharide binding protein, myeloperoxidase, lactotransferrin etc. So the search for a sensitive, easily reproducible, cost effective marker to distinguish types of meningitis is ongoing and an avenue for research.

Conclusion

The serum CRP level was noticeably higher in bacterial meningitis compared to non bacterial although not statistically significant. CSF CRP level was also higher in this cases which correlated with conventional markers of cell count and biochemical parameters. CSF ADA was higher in tuberculous meningitis compared to non tuberculosis cases. However there was no correlation between CSF ADA & CRP with clinical presentation and outcome. Further studies are needed for validation of the findings and to identify new specific affordable markers for meningitis.

Reference

1. Laboratory methods for the diagnosis of meningitis 2nd edition. Centre for disease control and prevention
2. Mauricio L Barreto, Maria Glória Teixeira, and Eduardo Hage CarmoInfectious diseases epidemiologyJ Epidemiol Community Health. 2006 Mar; 60(3): 192–195.
3. Elden J, Yolken RH. C-Reactive Protein and limulus amoebocyte lysate assay in diagnosis of bacterial meningitis. J Paediatr 1986, 108: 423-426
4. Singh UK. Cerebrospinal fluid C-reactive protein in the diagnosis of meningitis in children. Indian Paediatr 1994, 31: 939-942
5. Carpenter RR, Petersdorf RG. The clinical spectrum of bacterial meningitis. Am J Med 1962;33:262-275
6. F C de Beer, G F Kirsten, R P Gie, N Beyers, and A F Strachan. Value of C reactive protein measurement in tuberculous, bacterial, and viral meningitis. Arch Dis Child. 1984 Jul; 59(7): 653–656.
7. Abramson JS, Hampton KD, Baba S, Wasilaukas BL, Marion MJ. The use of C-reactive protein from cerebrospinal fluid for differentiating meningitis from other central nervous system diseases. J Infect Dis. 1985 May;151(5):854-8.
8. Jansoon E, Jalava L, Wager O. C-reactive protein in bacterial meningitis. Ann Med Exp Fenn 1959, 37: 371-373
9. Stearman M, Southgate HJ. The use of cytokine and C-reactive protein measure-ments in cerebrospinal fluid during acute infective meningitis. Ann Clin Biochem. 1994 May; 31 (Pt 3):255-61.
10. Tankhiwale S S, Jagtap P M, Khadse R K, Jalgaonkar S V. Bacteriological study of pyogenic meningitis with special reference to C-reactive protein. Indian J Med Microbiol 2001;19:159-60
11. Goran Rajs, Zvezdana Finzi-Yeheskel, Andrea Rajs, Michael MayerC-Reactive Protein Concentrations in Cerebral Spinal Fluid in Gram-positive and Gram-negative Bacterial Meningitis. 2002. Clinical chemistry 48:591-592.
12. Clause et al of Denmark. CRP in CSF. Dan. Med. Bull.1962; 9.
13. Vaishnavi C, Dhand UK, Dhand R, Agnihotri N, Ganguly NK. C-reactive proteins, immunoglobulin profile and mycobacterial antigens in cerebrospinal fluid of patients with pyogenic and

tuberculous meningitis. J Hyg Epidemiol Microbiol Immunol. 1992; 36(3):317-25.

14. Ribera E, Martinez-Vazquez JM, Ocaña I, Segura RM, Pascual C. 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.

15. Malan C, Donald PR, Golden M, Taljaard JJ. Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. J Trop Med Hyg. 1984 Feb; 87(1):33-40.

16. Mishra OP, Loiwal V, Ali Z, Nath G, Chandra L. Cerebrospinal fluid adenosine deaminase activity for the diagnosis of tuberculous meningitis in children. J Trop Pediatr. 1996 Jun; 42(3):129-32.

17. Eintracht S, Silber E, Sonnenberg P, Koornhof HJ, Saffer D. Analysis of adenosine deaminase isoenzyme-2 (ADA(2)) in cerebrospinal fluid in the diagnosis of tuberculosis meningitis. J Neurol Neurosurg Psychiatry. 2000 Jul; 69(1):137-8.

18. Gambhir IS, Mehta M, Singh DS, Khanna HD. Evaluation of CSF-adenosine deaminase activity in tubercular meningitis. J Assoc Physicians India. 1999 Feb; 47(2):192-4.