International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com

Research Paper

Vol. 1, No. 2, April 2012

VALUE OF C-REACTIVE PROTEIN DETERMINATION IN CEREBROSPINAL FLUID IN CHILDHOOD INFECTIVE MENINGITIS

Khalid Rahim[1], Ashish Prakash[2*], Arun Kumar[3], Meenu Chopra[4]

[1,3]Department of Pediatrics, Yashoda Super-Specialty Hospital Nehru Nagar, Ghaziabad (NCR), Delhi, India
[2]Professor, Department of Pediatrics, Subharti Medical College, Meerut, U.P. India
[4]Department of Pathology, Yashoda Super-Specialty Hospital Nehru Nagar, Ghaziabad (NCR), Delhi, India

[*]*Corresponding author:* ***Ashish Prakash,*** ✉ *Ashprakash@yahoo.com*

ABSTRACT

A prospective study to determine the value of C-reactive protein concentrations in cerebrospinal fluid to differentiate between acute bacterial, partially treated bacterial, aseptic & tubercular meningitis was performed in 51 consecutively observed patients (children), who underwent lumbar puncture due to suspected infective meningitis. Diagnosis included acute or presumed bacterial meningitis (n=11), acute or presumed viral meningitis (n=13), partially treated bacterial meningitis (n=6), tubercular meningitis (n=2) and control group (n=19).The sensitivity, specificity, predictive values and optimum cut-off levels of C-RP in CSF were determined to diagnose these various types of infective meningitis. Determination of C-RP in CSF is a useful additional test on CSF examination in distinguishing between bacterial and aseptic meningitis. CSF-CRP levels>1.3mg/dl is highly suggestive of acute bacterial meningitis.

Key words: Meningitis, C-reactive protein (CRP), Cerebrospinal fluid (CSF).

INTRODUCTION

Meningitis is a significant cause of mortality and morbidity in infancy & childhood. The etiological diagnosis of meningitis remains a problem in clinical practice, as CSF biochemical analysis and cellular response often overlap and CSF gram staining and culture which is the gold standard for determining the causative organism has low positivity especially in developing countries. Moreover culture reports are available after 48 hours. A diagnostic conundrum on the evaluation of children with suspected bacterial meningitis is the analysis of CSF obtained from children already receiving antibiotic therapy.

C-reactive protein (CRP) an acute phase inflammatory response marker is produced by hepatocytes in liver on stimulation by microbial infections and other inflammatory diseases.

The CRP in serum may gain access to CSF by passive diffusion across the highly inflammed meninges in infective meningitis. Several reports have shown an ability of CRP in CSF to discriminate between patients with bacterial meningitis and patients with aseptic meningitis. Nevertheless, a considerable controversy still exists about the actual clinical value of determination of CSF-CRP levels.

The literature reveals that the work done over last few decades on CRP estimation in CSF for distinguishing bacterial from aseptic meningitis is qualitative. Some quantitative studies done out side India have shown significant increase of CRP in CSF but none has derived any **cut off level** to diagnose bacterial meningitis.

Due to availability of new and advanced automated analyzers over the past few years, it has been possible to do an accurate quantitative estimation of CRP in CSF and determine its cut-off levels to diagnose different types of infective meningitis.

SUBJECTS AND METHODS

Clearance was obtained from the institutional ethical committee to conduct this study. The study protocol was fully explained to the parents/guardians, and informed written consent was obtained.

The estimated sample size was calculated according to the formula: $n=t^2 \times p(1-p)/ m^2$ where n is required sample size, p is the estimated prevalence(approximate percentage of total admissions in our hospital suspected of having CNS infection), t is confidence level at 95%(standard value of 1.96) & m is margin of error at 5%(std.value of 0.05), the sample size calculated was $[n=1.96^2 \times 0.04(1-0.04)/0.05^2]$ **60**.Same number of eligible children in the age group of 3 days to 12 years, who were admitted in the Pediatric Department of Yashoda Super Specialty Hospital Ghaziabad from April 2009 to April 2010 with clinical diagnosis of meningitis, were considered for the study. However parents of 5 children did not give consent and in 4 cases lumbar puncture was traumatic. Remaining 51 children included in the study were further divided into 5 groups (table1) according to clinical, biochemical, cytological & bacteriological study of CSF (including PCR for herpes & tuberculosis).

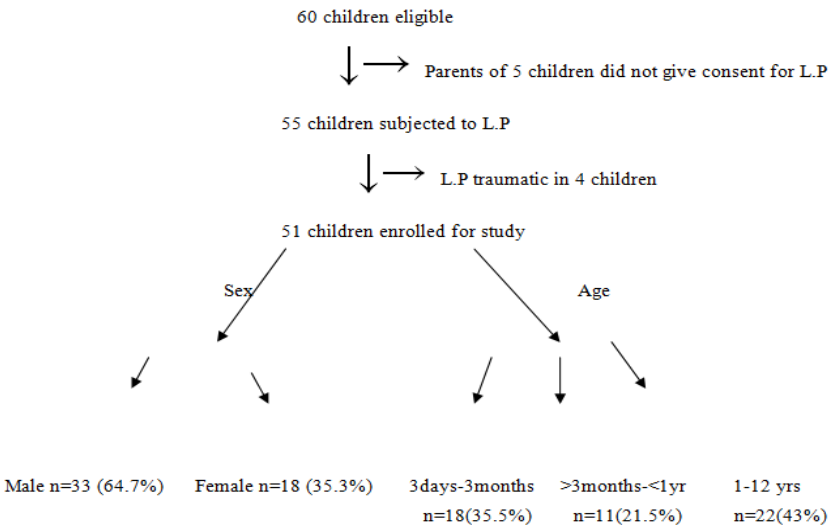


Fig 1. Study Flow Chart with Sex & Age Distribution.

Criteria for diagnosis of various types of meningitis on CSF examination were taken as follows**: [1, 2]**

For bacterial meningitis: CSF- WBC count>100cells/cu.mm predominantly polymorphs; protein>45mg/dl (>120mg/dl in neonates); CSF sugar<75% of serum sugar.

For aseptic meningitis: WBC count>5cells/cu.mm (>30 in neonates) predominantly monocytic/lymphocytic; protein>45mg/dl (>120mg/dl in neonates), CSF sugar normal.

For tubercular meningitis: WBC count>10cells/cu.mm predominantly lymhocytic, protein>100mg/dl, CSF sugar<75% of serum sugar.

For partially treated bacterial meningitis: WBC count>5cells/cu.mm (30cells in neonates) with predominance of polymorphs or ambiguous cytology, protein>100mg/dl, CSF sugar<75% of serum sugar.

Besides CSF exam, patients were subjected to other investigations like Hb, TLC, DLC, Platelet Count, Blood Culture, Montoux Test, Radiology (CXR, CT, MRI) wherever indicated to aid in diagnosis.

CRP estimation in CSF was done by a fully automated biochemistry analyzer **Spectra-XL by Vital Scientific N.V. Netherlands** and the reagent kit CRP – **Turbilatex from Spinreact, S.A.V. Spain** was used.

The principle of this analysis is that the latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance range, dependent upon the CRP contents of the patient's sample. The reagent absorbance is analyzed by the analyzer based on photometric measurements.

Statistical analysis was done by using Mann-Whitney test (non-parametric) by comparing CRP levels in CSF in each group against the **control i.e. 'No CNS Infection' group.** P value < 0.05 was considered to be significant. Software used was SPSS of 16.0 versions.

RESULTS

The median value and range of CRP concentrations in CSF were as given in table1.

It was observed that CRP levels rise significantly in bacterial meningitis as compared to the cases who were having normal CSF examination(control group), **p=0.000.** Significant rise was also seen in partially treated bacterial meningitis (p=0.002) and tubercular meningitis (p=0.022), however number of cases in tubercular meningitis was low (n=2).

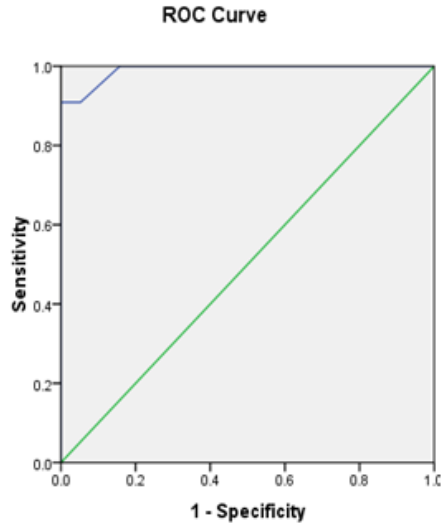
There was no significant difference in CRP levels between aseptic meningitis group & control group. But significant CRP levels were seen in three cases of aseptic meningitis who were having brain parenchymal lesions documented by MRI (median value of 1.3mg/dl (range 1.0 – 2.0mg/dl, p=0.011).

Table 1: **Group distribution & CSF-CRP levels in study groups**

Group	Final diagnosis	No.of cases (%)	CRP levels in CSF(mg/dl) Median (range)	P-value(significant<0.05)
I	Bacterial meningitis	11(19.6)	3.40 (1.0-7.7)	**0.000**
II	Aseptic meningitis	13(21.6)	0.40 (0.2-2.0)	**0.209**
III	Tubercular meningitis	02(3.9)	3.55 (2.8-4.3)	**0.022**

IV	Partially treated bacterial meningitis	06(11.8)	1.25	(0.8-2.2)	**0.002**
V	No CNS Infection	19(37.2)	0.40	(0.1-1.1)	

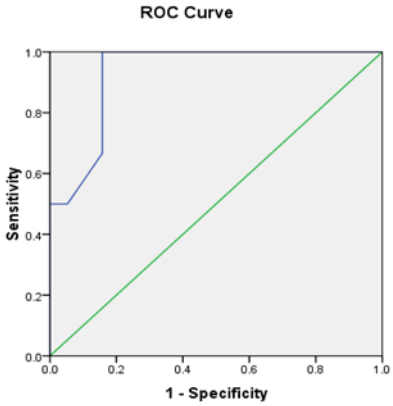
On ROC curve analyses, when optimum cut-off level of CSF-CRP in **bacterial meningitis** was taken as **1.3mg/dl** the sensitivity and specificity was **90.9%** and **100%** with a PPV and NPV of 100% & 95% respectively. The diagnostic accuracy determined by area under curve (AUC) was 99 %.(Fig.2)



Cut-off level of CSF-CRP	Sensitivity	Specificity	PPV	NPV
1.3 mg/dl	90.9%	100%	100%	95%

Fig.2.Sensitivity,Specificity,PPV & NPV of CSF-CRP in Diagnosis of Acute Bacterial Meningitis when Cut-off level was taken as 1.3 mg/dl on ROC Analysis

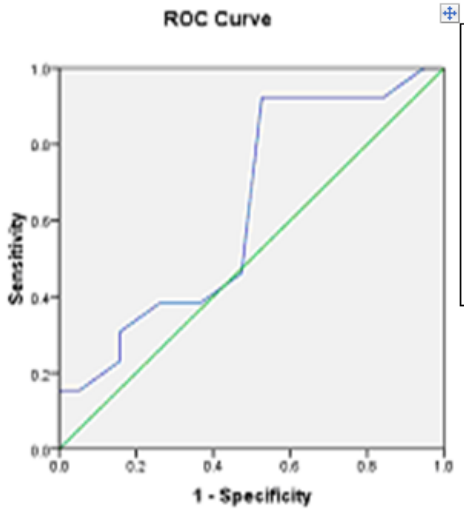
On taking optimum cut-off level of CSF-CRP as 0.805mg/dl in partially treated bacterial meningitis group the sensitivity & specificity of the test was 100% & 84.2% respectively with a PPV & NPV as 66.6%& 100% with a diagnostic accuracy(AUC) of 93%.(Fig.3).



Cut-off level (CSF-CRP)	Sensitivity	Specificity	PPV	NPV
0.805mg/dl	100%	84.2%	66.6%	100%

Fig.3. Sensitivity, Specificity, PPV & NPV of CSF-CRP in Diagnosis of Partially Treated Bacterial Meningitis when Cut-off level was taken as 0.805 mg/dl on ROC Analysis.

In case of Aseptic meningitis when optimum cut-off level of CSF-CRP was taken as 0.45mg/dl,the sensitivity & specificity was 46.15% & 52.63% and PPV & NPV 40% & 58.8% respectively with a diagnostic accuracy of 63.2%.(Fig.4)



Cut-off level (CSF-CRP)	Sensitivity	Specificity	PPV	NPV
0.45 mg/dl	46.15%	52.63%	40.0%	58.82%

Fig 4. Sensitivity, Specificity, PPV& NPV of CSF-CRP in Diagnosis of Aseptic Meningitis when Cut-off level was taken as 0.45mg/dl on ROC Analysis

In view of low number of cases in tubercular meningitis ROC curve analyses was not done in this group.

DISCUSSION

CSF culture and gram staining was positive in 18% cases (2 out of 11). Organisms isolated were Strep. pneumoniae (n = 1) and Staph aureus (n = 1).

Blood cultures was positive in 3 patients of partially treated bacterial meningitis (E. coli in 2 cases & pseudomonas in 1) and total leukocyte counts & differential counts were taken into consideration in the remaining 3 cases in support of CSF findings in this group to differentiate them

from aseptic meningitis cases .PCR for Herpes was positive in 2 cases.

CSF culture positivity is low in our study as compared to that reported by various workers from India, which is around 30 – 60% **[3]**. One reason for low positivity in our study could be the use of human blood agar instead of sheep blood agar in our microbiology lab. Although S.pneumoiae, S.aureus, S.pyogenes grow on HuBA, the colony size tends to be very small, the morphology often varies from that present on animal blood agar, & hemolysis is minimal. As colony size, colony morphology & hemolysis are all critical to the identification of these organisms, there is a much greater chance that these organisms from human specimens cultured on HuBA will be overlooked or misidentified**. [4]**

In our study we observed that CSF-CRP rises significantly in acute bacterial meningitis. Taking **1.3mg/dl** of CRP in CSF as optimum **cut-off level**, we were able to diagnose acute bacterial meningitis with a **sensitivity = 90.9%; specificity = 100%**; PPV = 100% & NPV = 95%.

CSF-CRP rise was also significant in partially treated bacterial meningitis and tubercular meningitis. However the number of cases in tuberculous group was too low (n=2) to draw the conclusion.

On taking optimum cut-off level of CSF-CRP in partially treated bacterial meningitis as 0.805mg/dl, sensitivity & specificity were 100% & 84.2% respectively.

It was noticed that CSF-CRP does not rise significantly in aseptic meningitis, however there was significant rise of CRP in CSF in cases that were having brain parenchymal lesion documented by radio-imaging. The potential explanation for this observation could be local production of CRP in central nervous system by the affected tissues**. [5]**

Based on these observations of our study, we conclude that CRP in CSF is a useful additional test for distinguishing between bacterial meningitis from aseptic meningitis. This is true even with prior antibiotic therapy.

CSF-CRP level **> 1.3mg/dl** is highly suggestive of bacterial meningitis. However if CRP levels in CSF are raised significantly and other biochemical & cytological findings on CSF examination is suggestive of aseptic meningitis, then one should actively search for brain parenchymal lesion by doing neuro-imaging.

Limitations of our study were small sample size, low culture positivity, less number of cases in tubercular meningitis group and despite taking into consideration of all evidences there might have been overlap of cases between aseptic and partially treated bacterial meningitis on the basis of CSF cytology & biochemistry.

Contributors

Concept and design of the study was provided by AP, which was carried out by KR. Data analysis and interpretation of results were done by KR and finally revised and approved by AP & AK. Laboratory assays were done by MC. The final manuscript was approved by all authors.

Funding

Partial funding by Yashoda Hospital Ghaziabad.

Competing interests

None stated.

REFERENCES

1. Prober CG. Central nervous system infections. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, eds.Nelson textbook of pediatrics.18th ed. Philadelphia: Elsevier Saunders; 2007.p.2513 table 602-1.

2. Haslam RA. Neurologic Evaluation. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, eds.Nelson textbook of pediatrics.18th ed. Philadelphia: Elsevier Saunders; 2007.p.2440-41.

3. Kumar L, Chitlengiya S, Ayyagiri A. The current status of pyogenic meningitis in children. Indian Pediatr 1980; 17:438-440.

4. F.M.Russell, S.S.N.Biribo, G.Selvaraj, F.Oppedisano, J.R.Carapetis. As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in lab. of developing countries Clin Microbio.2006 Sep,44(9):3346-3351.

5. Yasojima K, Schwab C, McGee. Human neurons generate C-reactive protein & amyloid P: up regulation in Alzheimer disease. Brain Res 2000; 887:80-89[Medline].

6. Tillett, W.S. and Fracis, t. Jr. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus, J. Exper. Med, 1980; 52: 561-571.

7. Hanley JA, McNeil BJ. The meaning and use of area under a receiver operating characteristic curve.Radialogy1982;143:29-36