Original Article

Role of CSF C-Reactive Protein for the Differentiation of Bacterial Meningitis from Aseptic Meningitis in Children

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Summary:

Background: Acute bacterial meningitis (ABM) is one of the common causes of morbidity and mortality in children. Easy and early diagnostic tool is required for rapid detection of acute bacterial meningitis to reduce mortality and morbidity.

Objectives: The study was conducted with the aim to identify the importance of cerebrospinal fluid C- reactive protein (CSF-CRP) to establish the diagnosis of ABM, and to measure the specificity, sensitivity, positive and negative predictive values of CSF-CRP in the diagnosis of Acute Bacterial Meningitis.

Methods: This prospective study was carried out in Dhaka Shishu (Children) Hospital during the period of December 2004 to April 2005. Children admitted between age 0-12 years with fever and convulsion were screened. Patients were divided into three groups on the basis of CSF findings- bacterial meningitis, aseptic meningitis and no meningitis control group. Only CSF culture proven cases were selected as ABM. CSF CRP was measured in all cases along with CSF cytology, biochemistry and culture-sensitivity. Complete were blood count, random blood sugar and other tests also done to treat all cases. All patients were treated adequately (if culture positive according to sensitivity) and were monitored as long as they stayed in hospital. Outcome was assessed clinically during discharge.

Results: Twenty patients had acute bacterial meningitis, 15 aseptic meningitis, and 15 cases CSF findings normal and taken as control. CSF-CRP was positive (>6mg/L) in 35% of the cases of ABM but it was found negative in all aseptic meningitis and in the control groups. Sensitivity of CSF -CRP was low (35%) but specificity was high (100%). The positive predictive value of CSF-CRP was 100% and negative predictive value 53.6%. Among the organisms isolated from acute bacterial meningitis, H. influenzae was the leading pathogen (40%) in infancy followed by S. pneumoniae (35%) and N. meningitidis (5%). Out come of the treatment of CSF-CRP positive ABM cases was found poor (p= 0.035).

Conclusion: It is concluded from the study that significant(>6mg/L) level of CRP in CSF is highly specific for diagnosis of ABM and ruled out aseptic meningitis. But negative CSF-CRP could neither exclude pyogenic meningitis nor did it rule out aseptic meningitis.

Key Words: *C-reactive protein, Cerebrospinal fluid, Bacterial Meningitis, Aseptic meningitis*

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Introduction

Meningitis is one of the most potentially serious infections occurring in infants and older children and is an important cause of morbidity and mortality[1]. Case fatality rates for bacterial meningitis range from 4.5% in developed countries to15–50% in developing countries[2,3,4]. A further 15–20% of survivors sustain neurological sequelae[5,6]. The mortality from

meningitis is close to 100% in untreated individuals and can still be up to 40% in children who received appropriate antibiotic therapy in developing countries[4]. Most of these fatalities occur within 72 hours of admission to the hospitals[1].

Rapid and accurate diagnosis coupled with early appropriate therapy is of utmost importance in reducing morbidity and mortality of the patients[7]. Culture and sensitivity, Gram stain, cytology and biochemistry of cerebrospinal fluid (CSF) sample are traditionally being done to diagnose and to differentiate pyogenic from aseptic meningitis. Proper culture is affected by prior antibiotic therapy, delay in transportation and inoculation. It takes more than 24 hours to isolate the organism. Gram stain lacks specificity and has interpretative errors[8]. Possible causes of false positive result of Gram stain include contamination of tubes from lumber puncture trays, glass slides and or Gram's reagents. Further more probability of visualization of bacteria on Gram stain is dependent upon the number of organism present. The overall sensitivity of Gram stain to detect bacterial meningitis was 67% with a positive predictive value of 60%. Most patients without bacterial meningitis have negative Gram stain(specificity 99.9%) with a negative predictive value of 99.9% [9].

Because of limitation of Gram stain regarding sensitivity and specificity and also culture of the CSF sample especially in partially treated cases, several rapid diagnostic tests have been developed to aid in the diagnosis of ABM[8].

Latex agglutination test and other rapid diagnostic test are available but costly and present only in selective area. Detection of nuclear polymorph leukocytes in the CSF is a fairly reliable indicator of pyogenic meningitis. Leukocyte count in bacterial meningitis may be elevated to greater than 1000/mm^3 and typically there is neutrophilic predominance (75–95%)[10].

A CSF leukocyte count < 250/mm^3 may be present in as many as 20% of patients with bacterial meningitis[10]. Pleocytosis may be absent in patient with severe overwhelming sepsis and is a poor prognostic sign. Pleocytosis with a lymphocytic predominance may be present during the early stage of acute bacterial meningitis; conversely, neutrophilic pleocytosis may be present in patients during the early stages of acute viral meningitis. Use of antibiotics makes the gram stain and culture negative and may alter the CSF cytology from neutrophilic to lymphocytic predominance. Empirical antibiotic therapy is often given.In such circumstances the detection of C-reactive protein in CSF appears to provide a new dimension to the diagnosis of meningitis[11].

In young children with meningitis, blood or CSF analysis cannot differentiate all cases of aseptic from bacterial meningitis. Consequently patient with aseptic meningitis generally received expensive antibiotic for prolong duration causing financial burden to poor parents and lengthening of hospital stay[12].

Serum CRP is an acute phase-reactant that has been utilized clinically to aid in the diagnosis of neonatal sepsis, urinary tract infection, pneumonia, meningitis[13-20]. Carrol et al detected CSF C-reactive protein by latex slide agglutination test which was 100% sensitive and 94% specific in differentiating bacterial meningitis from aseptic meningitis[20,21]. CRP estimation can help in diagnosing cases of ABM more effectively than culture[22].

Bangladesh is a developing country, with limited resources and skilled manpower particularly in peripheral set up. An easy and comprehensive test to diagnose ABM would be an alternative tool to diagnose ABM. Routine use of CSF CRP in diagnosing ABM could be a reliable and easy method and can be done for rapid diagnosis of meningitis. It is not an alternative of CSF culture, cytology and biochemistry, but for initial quick assessment it can be considered as first line of investigation for suspected meningitis to differentiate ABM from aseptic cases in rural or remote area where investigation facilities are limited. The test does not require much expertise to conduct and interpret the result.

So this study was conducted with the objective to measure the specificity, sensitivity, positive and negative predictive values of CSF-CRP in the diagnosis of bacterial meningitis.

Material and Methods

This prospective study was conducted in Dhaka Shishu (Children) Hospital, a 500 bedded tertiary

hospital, from December 2004 to April 2005. During this period all children admitted in the age group of 0-12years with fever and convulsion were screened.CSF samples were collected from suspected cases of meningitis. Children who got antibiotic for more than 24 hours before CSF study or had congenital central nervous system abnormality or suffering from any chronic illness were excluded.

Patients were divided into three groups on the basis of CSF findings- bacterial meningitis, aseptic meningitis and no meningitis control group. The no meningitis control group was finally diagnosed as case of febrile convulsion as their age was less than 6years and CSF finding was normal.

Clinically suspected cases of meningitis patients were selected from emergency room or inpatient department. CSF samples were collected before starting antibiotic. After admission patients' history, physical findings were recorded in a developed questionnaire. Decision of doing lumber punctures was taken by physicians of the respective wards. CSF samples were collected during lumber puncture and were send to laboratory for estimation of CRP, culture and sensitivity, cytology, bacteriology and biochemistry. Blood sample were send simultaneously to estimate completed blood count and random sugar. All patients were treated adequately (if culture positive according to sensitivity) and were monitored as long as they stayed in hospital. Outcome was assessed clinically during discharge. Cure means patients of ABM got improved without any obvious damage. Not completely cured means patients have some form of post meningitis sequelae or death during or at the end of treatment.

The parents were individually explained about the study and informed written consent were taken.

Case definition:

Bacterial meningitis were defined when bacteria were Isolation from CSF culture.

Aseptic meningitis: CSF WBC count 50 to <500 cells/ mm^3 with lymphocytic predominance (>50%), and mildly elevated protein (>40 mg/dl), normal or slightly reduced sugar concentration with negative CSF bacterial culture and Gram stain[24,25,26].

No meningitis (control group): Clinically suspected meningitis patient whose CSF examination yielded negative bacterial culture, negative Gram stain and normal CSF cytology and biochemistry[25].

CRP determination:

Two ml of CSF from each patient was aseptically collected in two sterile screw capped tubes. 1 ml in tube no.1 (marked previously) for culture, 1 ml in tube no.2 (marked previously)for biochemistry, cytology, Gram staining and CRP. Qualitative (slide agglutination) and semi quantitative determination of CRP in CSF and serum were done. By semi quantitative method CRP result can be expressed as 6, 12,18mg/L etc. and level below 6 mg/L cannot be detected.

On the provided slide a drop or 50µl of test material (CSF) were mixed with CRP reagent, agglutination reactions were seen after two minutes, presence of which were considered as positive result[11]. A smooth homogenous milky suspension indicates a CRP concentration of less than 6 mg/L which was considered as negative result. Agglutination indicates a CRP content more than or equal to 6mg/L. For quantitative CRP determination samples were diluted with 0.9% sodium chloride solution. Approximate CRP concentrations were obtained multiplying the titer by the limit of sensitivity 6 mg/L.

Statistical analysis was done by SPSS version 12.0 for Window. For significance of test Pearson c^2 test was done. A p-value of < 0.05 was considered as statistically significant.

Results

During the study period, total 109 patients with fever and convulsion were admitted in DSH out of which 58 patients could not enrolled as they received antibiotics and one due to presence of meningomyelocele. Total 50 patients who met the inclusion criteria according to the case definition were enrolled into the study and finally analyzed. The study population consisted of 20 children with bacterial meningitis, 15 children with aseptic meningitis, 15 children without meningitis were taken as control. Three-fourths 75% (n=15) of the ABM cases were infants (1–12m), 20% (n=4) of the cases were between 1–12 years, and 5%(n=1) less than 1 month of age. Among the aseptic meningitis 73.3% (n=11) were 1–12 years and 26.7%(n=4) were infants. In the control group 53.3% (n=8) were 1–12 years of age, 40%(n=6) were infants and 6.7%(n=1) were less

than 1 month (Table-I).Among the organisms isolated from acute bacterial meningitis, *H. influenzae* was the leading pathogen (40%) in infancy followed by *S. pneumoniae* (35%) and *N. meningitidis* (5%). Below 1 month *E. coli* (5%) and above 12 months of age *S. pneumoniae* (10%) & *N. meningitidis* (5%) were the pathogens isolated (Table-II). In the CSF of bacterial meningitis, the mean leukocyte count was 4064/mm^3; PMN 85%, mean protein value 316 mg/dl, and mean glucose was 23mg/dl. In aseptic meningitis, the mean leukocyte count was 148/mm^3; PMN 19%, mean protein value 104mg/dl, and mean glucose was 53 mg/dl. (Table-III).

Among the acute bacterial meningitis patients, 35% of the cases were CSF-CRP positive. In aseptic and control group all the patients had CSF- CRP negative. For the diagnosis of Acute Bacterial Meningitis, sensitivity of CSF -CRP was found 35% but specificity was 100% (Table-IV). Here, the positive predictive value of CSF-CRP was 100% and negative predictive value was 53.6% (Table-IV).

Outcome of the treatment of ABM between the CSF-CRP positive and negative groups showed that in CSF-CRP negative group 10 patients were complete recovered, 3 patient not cured(Table-V).Where as in CSF-CRP positive group had 2 patients who recovered completely and 5 others did not (Table-V). The difference between the groups were found statistically significant (P=0.035).

Table - I

Age distribution of studied cases (n=50)

Age in Months	Acute Bacterial Meningitis (n=20)	Aseptic Meningitis (n=15)	Control (n=15)
<1 month	1	0	1
1 – up to 12 month	15	4	6
12 – up to 144 month	4	11	8

Table-II

Bivariate distribution of aetiopathogens of ABM according to age.

Organisms	Age		
(n=20)	0 – 1months n (%)	1 – 12months n (%)	12 – 144 months n (%)
S. pneumoniae (n=9)	0	7 (35)	2 (10)
H. influenzae (n=8)	0	8 (40)	0
N. meningitidis (n=2)	0	1 (5)	1 (5)
E. coli (n=1)	1 (5)	0	0
Total	1 (5)	16 (80)	3 (15)

Table-III

Laboratory Characteristics of CSF in studied patients.

Parameters	Bacterial meningitis n=20	Aseptic meningitis n=15	Control n=15
Total WBC (mm^3) Range (Mean)	60 – 20,000 (4064)	58 – 440 (148)	0 – 4 (2)
PMN (%) Range (Mean)	60 – 95 (85)	0 – 60 (19)	0 (0)
Protein (mg/dl) Range (Mean)	100 – 600 (316)	42 – 300 (104)	15 – 40 (24)
Glucose (mg/dl) Range (Mean)	9 – 65 (23)	36 – 72 (53)	48 – 78 (59)
CRP (Positive)	7(35%)	0	0

Table -IV
Sensitivity, specificity of CSF C-Reactive Protein in study subjects

CSF CRP test result	True diagnosis(ABM) Present (ABM)	Absent (Aseptic meningitis)
CRP-Positive	7	0
CRP-Negative	13	15
Total	20	15

Sensitivity =7/20 x100= 35%, Specificity = 15/15 x100 = 100%

Table -V
Comparisn of CSF- CRP statatus in relation to outcome

	Negative	Positive
Not cured	3	5
Cured	10	2
Total	13	7

X2'=4.432, df = 1, p =0.035

Discussion

Bacterial meningitis is a potentially life threatening illness. Prompt recognition and early appropriate treatment is essential to reduce mortality and morbidity. The result of this hospital based study showed that infants were most vulnerable for acute bacterial meningitis. The etiopathogenes of bacterial meningitis in children (1 month – 144 months) were *S. pneumoniae* (45%), *H. influenzae* (40%), and *N. meningitidis* (10%). Laboratory characteristics of CSF in patients with ABM showed neutrophilic leukocytosis. As expected, in aseptic meningitis CSF pleocytosis was at lower range with lymphocytic predominance and in the control group all the values were found within normal range. CSF protein was elevated more in ABM than aseptic meningitis. Expectedly, CSF glucose was much lower in ABM than that of aseptic meningitis as well as the control groups. These findings were consistent with the findings of Prober CG[7].

CSF-CRP was detectable in seven of twenty ABM cases (35%). Not a single case was detected to have raised CRP in the CSF of aseptic meningitis and in the control group. CSF- CRP was measured in this study by semi quantitative latex agglutination method where cut off value was equal or more than 6 mg/L for observation of agglutination[11]. But in other studies different methods and different cutoff values were used[27]. In different studies CSF-CRP was positive in 66% and 85% where cut off values for positive were at the level of >1mg/L and >0.5mg/L respectively[28,29]. Despite their lower level of cut off values they also observed negative response in all the cases of aseptic meningitis in their study, which again was very consistent with our study observations.

No clear cut explanation of CRP migration to CSF was available in the literature. CSF-CRP concentrations were several folds lower than those of serum. This difference was explained by direct hepatic release of CRP into plasma which then undergoes ultra filtration to form CSF[27]. Diffusion of serum albumin and globulin across the inflamed meninges has been demonstrated and it seems feasible that CRP may cross from serum to CSF in a similar fashion. In suspected meningitis who subsequently demonstrates detectable CRP in CSF should be declared as bacterial meningitis[21]. As per Pemde HK et al,[11] CRP in CSF is specific for bacterial meningitis which is not detectable in aseptic meningitis, a fact also observed in our study. In viral meningitis, the tissue response is chiefly due to T cells, macrophage and necrotic tissues of caseous nature. These might be responsible for the binding of larger quantities of CRP molecules thereby permitting only a few of them to appear in CSF. This can be a probable explanation of undetectable level of CRP in CSF of viral meningitis. On the other hand, in bacterial meningitis the chief cells are polymorphs lacking the site for binding of CRP molecules in the inflamed tissues allowing more CRP to accumulate in CSF which could be detected by CRP test[11].

Negative CSF slide agglutination test for CRP could not exclude pyogenic meningitis. Routine CSF examination can differentiate between ABM and Aseptic meningitis but lacks precision. For better differentiation between ABM and aseptic meningitis, predictive values were calculated between these two groups. In this study the negative predictive value of CSF-CRP in ABM was 53.6% which was too insensitive (sensitivity-35%) to be useful for routine application for the diagnosis of ABM. On the contrary, positive predictive value was 100% (100% specific). It signifies that the presence of CRP in CSF strongly suggested the diagnosis of ABM and ruled out aseptic meningitis. This observation was also familiar with the finding of Pemde HK et al[11].

CSF- CRP positive patients demonstrated significantly higher mortalities and morbidities whereas; CSF- CRP negative patients had much higher recovery. This thoroughly highlighted the importance of the CSF-CRP level in ABM to be used as a bad prognostic criterion.

The determination of CSF-CRP have significant role in differentiating bacterial meningitis from aseptic meningitis, sensitivity 35% and specificity 100%. Its presence significantly favored, in this study, the diagnosis of acute bacterial meningitis and predicted the possibility of poor outcome of the treatment[30].

Conclusion

The study concluded that –

- The presence of significant level (>6mg/L) of CSF-CRP strongly suggested the diagnosis of ABM and ruled out aseptic meningitis (positive predictive value 100%). However, negative result could not rule out acute bacterial meningitis or aseptic meningitis (negative predictive value 53.57%).
- Presence of reactive CRP in CSF could be considered as a bad prognostic criteria predicting a poor outcome of the treatment.

Limitations

CSF-CRP was estimated by semi quantitative method. Therefore, exact levels of CRP could not be detected. With better laboratory facilities detection of the exact CRP values could minimize our study limitations.

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