Cerebrospinal Fluid as an Alternative Tool to Blood C-reactive Protein in Laboratory Diagnosis of Bacterial Meningitis in Children: a Comparative Analysis

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ABSTRACT

Introduction: Cerebrospinal fluid (CSF) Gram stain and culture is the gold standard in the diagnosis of bacterial meningitis. However, in culture negative patients, there is no definitive test for diagnosing meningitis. Hence, diagnostic tests to detect bacterial meningitis in these subsets of patients are desired and these tests should be readily available, easy to interpret, and simple to perform. Study aimed to evaluate the diagnostic significance of CRP in CSF and blood as a rapid and simple method of diagnosing Bacterial meningitis in children.

Material and Methods: A total of 90 patients divided into 2 groups, Cases(60) and Control(30) were included from October 2019 to February 2020. Blood and CSF samples were evaluated by latex agglutination test. CSF CRP was also performed by ELISA (XEMA CRP Ultra EIA). CSF Cytopathological parameters such as protein and glucose levels were evaluated. Direct CSF Gram stain was performed and culture(conventional method) was taken as gold standard.

Results: The gram stain and culture were positive in 3.3% and 10% of samples respectively. Serum CRP (38.3%) followed by CSF ELISA(31.7%) and latex agglutination test(25%) was positive. CRP in CSF determined by using ELISA had maximum diagnostic odd's ratio(14.3) followed by blood CRP and latex CSF CRP. Specificity by using CSF was found to be higher than serum. All three tests when compared with gold standard were highly significant.

Conclusions: CSF-CRP can be used as a simple, rapid, accurate and point of care approach for the laboratory diagnosis of Bacterial meningitis, especially in culture negative cases. Larger studies are required to extrapolate the results to entire population.

Key-words: CSF, CRP, ELISA

INTRODUCTION

Importance of C-reactive protein (CRP) and the acute phase inflammatory response as an inflammatory marker were discovered in 1930 by Tillett et al.[1] Inflammation of any organ is invariably associated with rise in CRP levels in serum and body fluids associated with that organ.[2,3] In many countries, attention has been directed to the value of serum CRP measurement.[4] However, routine diagnostic use of cerebrospinal fluid (CSF) CRP in differentiating bacterial and non-bacterial meningitis has been evaluated by very few studies.[2,5] The CSF Gram stain and culture still remains the gold standard in the diagnosis of meningitis, but in patients where these tests are negative, there is no definitive assay for diagnosing meningitis. This is especially necessary in a set up where patients come after getting various antibiotics or where facilities for doing cultures are not readily available. Fatality from meningitis among children less than 15 years ranges from 19.6% to 34%, and many patients who survive are left with permanent disability.[6] Hence, diagnostic tests which are readily available, easy to interpret, and simple to perform are of paramount importance. Being a tertiary care centre, very sick patients with a diagnostic dilemma who are not responding to simple management or atypical disease pattern are generally referred to our hospital. Hence, most patients come after being already started on treatment or come after receiving a course of antibiotics. The present study was designed to evaluate the diagnostic significance of CRP levels in CSF and blood as a rapid and simple method of diagnosing Bacterial meningitis in children in a developing country like India where isolation of organisms is not possible at resource limited setup.

MATERIAL AND METHODS

A total of 90 patients were included in the study which were divided into 2 groups broadly into Case group (n=60) and Control group (n=30). It is a hospital-based study which was conducted from October 2019 to February 2020, in the Microbiology department of VMMC & Safdarjung Hospital in New Delhi, India. This is one of the largest tertiary care hospitals which caters to nearly 10,000 patients daily covering various regions of Northern India. Based on clinical and CSF cytochemistry, cases were divided into bacterial meningitis, tubercular meningitis (TBM).[7] A predesigned patient proforma was designed and after taking consent from parents, data about clinical features and laboratory results, was recorded for each case. All CSF samples received in the microbiology laboratory of our hospital which fulfil the study criteria, were included in this study.

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Definitions

Bacterial meningitis, was defined by a CSF leukocyte count of 100–10,000/ mm3 with polymorphonuclear neutrophils (PMNs) of >50%, a CSF glucose level <2/3 blood sugar level, and a CSF protein level of 100–500 mg/dl.

Tubercular meningitis (TBM), was defined as those with a history of contact with a sputum positive tuberculosis (TB) case, clinico-radiological findings consistent with TB, and a positive reaction (>20 mm in duration) to 5 tuberculin units (TU) of purified protein derivative (PPD), and a CSF pleocytosis level of 10–500/mm3 with predominant lymphocytes and a high CSF protein (100–3000 mg/dl), or in cases where a CSF culture and/or Ziehl-Neelsen staining have revealed acid-fast bacilli.

Control group, included those with a fever with convulsions but no meningitis. These convulsions were caused by epilepsy or febrile convulsions.

Inclusion criteria

All children aged 1 month to 15 years were included in the study.

Exclusion criteria

Patients diagnosed with fungal meningitis or concomitant illnesses such as human immunodeficiency virus (HIV). Patients presenting with convulsions due to non-infectious conditions like rheumatic disorders, malignancies, and tissue injury were excluded from this study.[8]

Laboratory methods

Blood and CSF samples were also sent for CRP assay by latex agglutination test. CSF CRP was also performed by highly sensitive ELISA (XEMA CRP Ultra EIA) as per manufacturer instructions. Other laboratory investigations included an assessment of cytopathological parameters such as protein and glucose levels in the CSF. A CRP titre of 0.6 mg/L and greater by latex agglutination test was considered positive in this study. Sensitivity of ELISA (XEMA CRP Ultra EIA) as per kit literature was taken as 0.05 mg/L. A CSF Gram stain was performed for each sample as soon as the sample was received. Culture of CSF(done by conventional method and enrichment done in Brain heart infusion broth) was taken as gold standard testing. CSF and serum CRP were then evaluated against this gold standard.

STATISTICAL ANALYSIS

Sensitivity, Specificity and diagnostic odds ratio was calculated for each method using culture as gold standard. The test applied for statistics was Chi-square test and a P value of <0.05 was taken as statistically significant.

RESULTS

The CSF from 90 children (60 cases and 30 controls) was analysed. Table 1 shows the demographic distribution in cases. The highest number of meningitis cases were noted to be higher in neonates despite being completely immunised for age. Males were greater in number than females [Table 1].

Total culture confirmed cases i.e confirmed bacterial meningitis were 6(10%). Organisms isolated in CSF were *E. coli* (02); *Enterococcus spp.* (01); *Staphylococcus aureus* (02); *Pseudomonas* spp. (01).

Table 2 shows the pathological parameters. Polymorphs were raised maximally. Protein was moderately raised in 10% of cases whereas mild and high rise of protein was almost equal. Sugar was decreased in 18.3% of cases.

Clinical correlation and differentiation of different types of meningitis was done in table 3. Maximum number of cases were of pyogenic bacterial meningitis followed by tubercular meningitis.

Microbiological investigations are shown in table 4. The gram stain and culture were positive in 3.3% and 10% of samples in case group. Serum CRP gave the maximum positivity (38.3%) followed by CSF ELISA (31.7%) and latex agglutination test (25%).

Statistical tests are depicted in table 5 and 6. CRP in CSF

Characteristics		Number	Percentage
Age	Neonate	33	55.0
	Pediatric	23	38.3
Sex	Male	32	53.3
	Female	26	43.3
Prior antibiotic administration		3	5
Underlying condition		5	8.3
Completely immunized		45	75
Seriously ill at admission		12	20

Table-1: Demographic distribution of cases presenting with meningitis

Parameter	Positivity	Percentage (%)
Total count increased	4	6.7
Differential count (raised)		
Polymorph	15	25
Lymphocyte	5	8.3
Protein		
Highly (800-600)	5	8.3
Moderately (80-200)	6	10
Mild (60-80)	5	8.3
Sugar		
Low	11	18.3
Very low	1	1.7

Table-2: Pathological parameters of cases presenting with meningitis

Clinical Diagnosis	Number (%)	Serum CRP	CSF latex	CSF ELISA
Bacterial Meningitis	8 (13.3)	6	4	5
TBM	3 (5)	2	1	1
Control Group	30 (33.3)	8	2	1

Table-3: Clinical correlation and differentiation of meningitis along with the CRP levels assessed by different methods

Isolation of organism		
Gram stain	2	3.3
Culture (conventional)	6	10
Test	**Number**	**Percentage**
Latex CSF CRP	15	25
CSF CRP ELISA	19	31.7
Serum CRP	23	38.3

Table-4: Microbiological tests done for the patients presented with meningitis

Test	**Sensitivity**	**Specificity**	**Diagnostic odd's ratio**
Latex CSF CRP	66.7%	79.6%	7.8
ELISA CSF CRP	83.3%	74.1%	14.3
Serum CRP	83.3%	66.7%	10.0

Table-5: Statistical analysis of CRP levels of different methods taking culture as gold standard

Serum CRP	**Culture Positive**	**Culture Negative**	**P value**
Test Positive	5	18	0.0269
Test Negative	1	36	
CSF latex CRP	Positive	Negative	P value
Positive	4	11	0.0298
Negative	2	43	
CSF ELISA CRP	Positive	Negative	P value
Positive	5	14	0.0101
Negative	1	40	

Table-6: Comparative analysis taking culture as gold standard

determined by doing ELISA had maximum diagnostic odd's ratio followed by blood CRP and latex CSF CRP. However, specificity by using CSF was higher than by using serum. All the three tests were compared with conventional culture technique as gold standard. The p value for all the three tests was highly significant.

DISCUSSION

The diagnosis of Bacterial meningitis poses the biggest challenge as the clinical and biochemical picture is often masked because of prior antibiotic use. This becomes even more difficult in a population like ours where patients first attend multiple private practitioners and receive a course of antibiotic. They are then referred to our hospital. In such a scenario, the isolation of the organisms from blood or CSF becomes very difficult. Moreover, CSF cultures for pyogenic organisms are positive in only 30–60% of cases, according to various researchers.[9] In many places, facilities to isolate blood or CSF borne organisms is lacking and, even if it is available, it takes a long time for culture reports to come. The lack of a single test to diagnose the aetiology of meningitis promptly and accurately, makes it more important to find a quick and reliable method for early bedside diagnosis. Our results suggest that CSF CRP can serve as an ideal method in situations where it traditionally has been difficult to isolate organisms to aid with diagnosis.

Passive diffusion across the highly inflamed meninges would be a reasonable explanation as to how CRP gains access to CSF.[10] Only 6 samples, 10% of the 60 cases displayed organisms that had been isolated in CSF cultures by conventional culture method (Enterococcus [2], Pseudomonas [1], E. coli [1]). In our study, the frequency of bacterial isolates in CSF was lower(10%) than that observed in other studies where it was 36% but the isolated organisms were similar to ours.[11] In another study, the CSF cultures were positive for the presence of organisms in only 16% of cases.[12] The low positivity can be explained by the fact that ours is a tertiary care centre where majority of the patients come after receiving a course of antibiotics. Most of the time patients are admitted in critical situation where empirical course has to be started before taking the samples.

The total white blood cell count in CSF was raised in 5% of the cases and the neutrophil count was increased in 25% of cases. The glucose level in CSF was the low in most (18.3%) of the cases while it was decreased significantly in only 1/60 case. Similarly, CSF lymphocyte levels were increased in 8.3% and protein levels were increased in 5% of cases. Similar findings were reported by Abro et. al.[12] These significant parameters may also be helpful in differentiating different types of meningitis. Levels of CRP in serum and CSF increase as a result of invasive central nervous system infection.[4] Increased CRP production is an early and sensitive response to most forms of microbial infections and the value of its measurement in the diagnosis of various infective conditions was established in previous studies done by Deeber et. al and McCarthy et. al.[13,14]

In our study, increased blood CRP levels was noted in 38.3% of cases, with a sensitivity of 83.3% and specificity of 66.7%. The diagnostic odd's ratio was 10. When compared with CSF culture, the test was found to be statistically significant ($P<0.05$ or $= 0.026$). Similar findings have been reported by Singh et. al.[15] However, Prasad et. al noted different findings in their study where serum CRP sensitivity for pyogenic meningitis was lower (76%) and specificity was higher (68%) than in our study.[16] Blood CRP with a high sensitivity can be used as a screening test for different types of meningitis, but since the specificity was low in our study, its diagnostic accuracy has yet to be established.

CSF CRP has been reported to be one of the most reliable and early indices to diagnose bacterial meningitis.[10,17] Corral et al. found positive CSF CRP in 24/32 patients with culture-proved bacterial meningitis, while only 2/32 children with non-bacterial meningitis had CSF which was positive for CRP. Corral et al. has shown that CSF- CRP (latex agglutination test) was positive in all patients with bacterial meningitis, which 100% sensitivity and 94% specificity was observed in comparison to control group.[2] In study by Bengershom et al. on children with suspected meningitis, CSF-CRP level Cut off was obtained as 0.4 mg/L and the sensitivity and specificity values were 94% and 100% respectively.[18] Komorowski et al. reports that CSF-CRP was effective indicator for rapid diagnosis of meningitis in 60% of adults.[19] In a study by Gray et al. CRP levels were greater than 100 ng/ml, identified in 95% of bacterial meningitis.[20]

Singh et al. reports that CRP level was positive in 84% of bacterial meningitis, and it was negative in all cases of meningitis.[15]

According to Corral et al., CSF CRP was a more sensitive test for diagnosing bacterial meningitis than any other laboratory test for CSF.[2] Their study demonstrated that CSF CRP levels are also useful in diagnosing partially treated cases of meningitis. Our results also correlate with their findings. CSF CRP done by latex/ ELISA were positive in 15/19 cases, giving it a sensitivity and specificity rate of 66.7/83.3% and 79.6/74.1%, respectively. Similar sensitivity for meningitis was also reported by Singh et. al.[15] The diagnostic odd's ratio was 7.8/14.3. Both the tests when compared with CSF culture, the tests were highly significant ($P<0.05$ or $= 0.0298/0.0101$). For pyogenic meningitis, a sensitivity of 84% and 94%, and a specificity of 100% have been reported by Corral et. al and Singh N et. al.[2,12] In another study by Macfarlane et. al, a sensitivity of 97%, which was slightly greater than our study, and a specificity of 98% was observed.[21] Similarly, a sensitivity of 97% and specificity of 86% for bacterial meningitis was also reported by Abramson et. al.[22] In our study, CSF as well as blood CRP sensitivity was high (CSF CRP was equal to blood CRP) when performed by ELISA. This indicates that CSF as well as blood CRP can be a good screening test for bacterial meningitis. The specificity was higher in CSF CRP than blood CRP (79.6% versus 66.7%).

A high specificity of 94% in CSF CRP was noted by Gaur et. al which can be used as a diagnostic marker of bacterial meningitis.[6] This indicates that CSF CRP is a better marker than serum CRP. A similar view has been expressed by other researchers such as John et. al who found raised levels of CRP in CSF to be a better indicator of bacterial meningitis. It also served to distinguish bacterial meningitis from tubercular infections, and other central nervous system disorders.[23]

This study had few limitations. Our study had a small sample size. Thus, while concluding that CSF CRP levels can serve as a useful screening test for bacterial meningitis, a further study with a larger population is required to validate our study. Measuring CRP levels before and after antibiotics would also give a better interpretation.

CONCLUSION

It can be concluded that, in addition to current conventional diagnostic methods (biochemistry, cultures and gram stain), CSF-CRP is a simple, rapid, accurate and bed side approach for the laboratory diagnosis of Bacterial meningitis, especially in culture negative cases. Larger sample of meningitis cases are needed to validate the present CSF CRP cutoff values in future studies. Larger studies are needed to extrapolate the results to entire population.

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