Section: Biochemistry

Original Research Article

ASSOCIATION OF DIFFERENT INFLAMMATORY MARKERS LIKE INTERLEUKIN-6, C-REACTIVE PROTEIN, PROCALCITONIN IN NEONATES SUFFERING FROM SEPSIS WITH SPECIAL REFERENCE TO COMPLEMENT COMPONENT C3 AND C4

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Abstract

Objective: To find out if c3-c4 hypocomplementemia is present and to observe weather interleukin-6(IL-6), C-reactive protein(CRP), and procalcitonin are associated to these complement components in the cases of neonatal sepsis. **Materials and Methods:** It was Institutional Observational Cross-sectional study conducted in the department of Biochemistry, and Sick new born care unit (S.N.C.U), Dept of Paediatric Medicine, at Medical College & Hospital, Kolkata, West Bengal. A total number of 100 diagnosed cases of neonatal sepsis were enrolled for the study during the period from 01/04/2021 to 31/03/2022. **Results:** In the present study we found, 40 (40.0%) patients were ≤15 days of age, 45 (45.0%) patients were 16-20 days of age and 15 (15.0%) patients were 21-25 days of age. There were increased prevalence of hypocomplementemia as evidenced by fall of C3 and C4 levels. Mean CRP of patients was [27.9800±19.2623 mg/dl], mean IL-6 of patients was [5.1727±2.1163 pg/ml], mean Procalcitonin of patients was [1.6327±1.2498 ng/ml], mean Complement C3 of patients was [.7802±.4049 g/L], mean Complement C4 of patients was [0.1503±.1355 g/L]. Complement C3 & C4 vs CRP, IL-6, Procalcitonin were having negative correlation and they were statistically significant. **Conclusions:** The present study highlights the importance of introducing the studied parameters for early diagnosis of neonatal sepsis. The study findings reveal that inflammatory markers such as CRP, IL6, and Procalcitonin were significantly elevated in neonates with sepsis. Moreover, there were significant negative correlations between complement C3 and C4 parameters against CRP, IL-6, and Procalcitonin. Thus, the estimation of complement C3 and C4 levels along with CRP, IL-6 and procalcitonin is highly recommended for early diagnosis of neonatal sepsis.

INTRODUCTION

Neonatal sepsis is a result of generalized Microbial infection. It may be of early onset(<3 days of birth) or late onset(>3days) entity [1] and is found to be the leading cause of mortality and morbidity in them. So early diagnosis is crucial for starting therapy and reduces mortality. Many studies have included different inflammatory markers like procalcitonin, C-reactive protein (CRP), Interleukin-6 (IL-6), Complement C3 & C4 to clinch to the diagnostic chances of sepsis. Sepsis results from a dysregulated host response to infection leading to uncontrolled inflammation, organ dysfunction and a state known as septic shock. This clinical scenario falls under the multiple organ dysfunction syndrome [2]

So this present study is undertaken to observe the above mentioned markers of sepsis and correlate them against C3 & C4 changes to come to the conclusion regarding their utility to consider them as sepsis panel.

MATERIALS AND METHODS

It was an Institutional, Observational, Cross-sectional study conducted in the department of Biochemistry, and Sick new born care unit (S.N.C.U), Dept of Paediatric Medicine, at Medical

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College & Hospital, Kolkata, West Bengal. A total of 100 diagnosed cases of neonatal sepsis were enrolled for the study which was conducted during the period 01/04/2021 to 31/03/2022..

Inclusion Criteria

1. Previously diagnosed cases of neonatal sepsis.
2. Considered age group up to first 4 weeks of life.

Exclusion Criteria

1. History of autoimmune diseases of mother
2. Genetic and metabolic diseases of new born.
3. Pre-term baby
4. Small for date baby
5. Intra uterine growth retardation (I.U.G.R) baby
6. Mothers treated with steroids

Laboratory investigations

Informed consent was taken from parents of the neonates. 5ml of venous sample was collected from the subjects. The samples were centrifuged, separated and stored at 4 degrees Celsius until analysis. The blood samples were analysed for Procalcitonin, C-reactive protein, Interleukin-6, C3 and C4 levels. All the estimates were done within 8 hrs of collection of specimen and separation of serum.

Ethical Clearance

The study proposal had approval by the Institutional Ethics Committee (IEC) prior to the commencement of the study. (vide Memo No. MC/KOL/IEC/NON-SPON/988/01/2021)

Statistical Analysis

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Unpaired t-tests and Z-test (Standard Normal Deviate) were used to study the significant difference of proportions. Correlation was calculated by Pearson correlation analysis.

RESULTS

Table 1: Showing Distribution of Age in group among the Patients

Age in group	Frequency	Percent
≤15	40	40.0%
16-20	45	45.0%
21-25	15	15.0%
Total	100	100.0%

In the present study, 40 (40.0%) patients were ≤15 days of age, 45 (45.0%) patients were 16-20 days of age and 15 (15.0%) patients were 21-25 days of age. The value of z is 4.6291. The value of p is < .00001.

Table 2: Showing Distribution of different parameters

Distribution		Frequency	Percent
Sex	Female	51	51.0%
	Male	49	49.0%
CRP (mg/L)	High	66	66.0%
	Normal	34	34.0%
IL6 (pg/ml)	High	51	51.0%
	Normal	49	49.0%
Procalcitonin (ng/ml)	High	53	53.0%
	Normal	47	47.0%
C3 (g/L)	Low	56	56.0%
	Normal	44	44.0%
C4 (g/L)	Low	59	59.0%
	Normal	41	41.0%

In our study, 51 (51.0%) patients were Female and 49 (49.0%) patients were Male. 66 (66.0%) patients had Abnormal(high) and 34 (34.0%) patients had Normal levels for CRP**.** 51 (51.0%) patients had Abnormal (high) and 49 (49.0%) patients had Normal levels for IL6**.** 53 (53.0%) patients had Abnormal (high) and 47 (47.0%) patients had Normal levels for Pro-calcitonin. 56 (56.0%) patients had Abnormal (high) and 44 (44.0%) patients had Normal levels for C3. 59 (59.0%) patients had Abnormal (low) and 41 (41.0%) patients had Normal levels for C4.

Table: 3. Mean & SD value of Age, CRP, Procalcitonin, Complement C3 & Complement C4

	Number	Mean	SD	Minimum	Maximum	Median
Age in Days	100	15.8300	5.3011	5.0000	24.0000	17.0000
CRP (mg/dl)	100	27.9800	19.2623	3.9000	66.3000	22.6000
IL-6 (pg/ml)	100	5.1727	2.1163	1.9800	9.1000	4.9000
Procalcitonin (ng/ml)	100	1.6327	1.2498	0.0200	7.3000	1.1000
Complement C3 (g/L)	100	0.7802	0.4049	0.2000	1.6000	0.8000
Complement C4 (g/L)	100	0.1503	0.1355	0.0100	0.4000	0.0700

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The mean Age of the patients in Days (15.8300±5.3011). The Mean CRP of patients (27.9800±19.2623). The mean IL-6 values of patients. (5.1727±2.1163). the Mean values of Procalcitoninof the patients (1.6327±1.2498). the Mean Complement values of C3 of the patients (7802±0.4049) & the Mean values of Complement C4 of the patients (0.1503±0.1355) respectively.

Table: 4. Showing Correlation of complement C3 (normal range 0.9-1.8g/l) with all parameters

complement C3(normal range 0.9-1.8g/l)			Remarks
CRP(normal Less than 10mg/l)	Pearson Correlation Coefficient (r)	-0.499**	Negative correlation
	p-value	<0.0001	Significant
	Number	100	
IL-6 (normal upto 4.4pg/ml)	Pearson Correlation Coefficient (r)	-0.391**	Negative correlation
	p-value	<0.0001	Significant
	Number	100	
Procalcitonin(normal range less then0.1 ng/ml)	Pearson Correlation Coefficient (r)	-0.475	Negative correlation
	p-value	0.046	Significant
	Number	100	

Table:5. Showing Correlation of: Complement C4 (normal 0.1-0.4g/l) with all parameters

Complement C4(normal 0.1-0.4g/l)			Remarks
CRP(normal Less than 10mg/L)	Pearson Correlation Coefficient (r)	-0.440**	Negative correlation
	p-value	<0.0001	Significant
	Number	100	
IL-6 (normal upto 4.4 pg/ml)	Pearson Correlation Coefficient (r)	-0.295**	Negative correlation
	p-value	0.003	Significant
	Number	100	
Procalcitonin (normal range less then 0. 1 ng/ml)	Pearson Correlation Coefficient (r)	-0.422	Negative correlation
	p-value	0.031	Significant
	Number	100	

DISCUSSION

The diagnosis of neonatal sepsis is very challenging to the clinicians till date due to its nonspecific clinical presentation and role of laboratory is pivotal. The study was conducted in the department of biochemistry and Sick new born care unit (S.N.C.U) Medical College and Hospital, Kolkata after obtaining permission from the Institutional ethics committee (IEC). Study subjects were the diagnosed cases of neonatal sepsis. Since this is a hospital based observational cross-sectional study all cases were selected after obtaining prior consent from parents. 100 patients were included in this study.

It was found that, 66% of the patients had high CRP whereas normal CRP values were found in 34%, the difference being statistically significant (p< .00001) (z=4.5255)

Higher number of patients had Abnormal (Raised) IL6 (51.0%) followed by Normal IL-6 in 49.0% We also found that, most of the patients had Abnormal (Raised) Procalcitonin in 53.0% and 47% in whom it was normal.

In our study, mean CRP of patients was [27.9800±19.2623 mg/dl], mean IL-6 of patients was [5.1727±2.1163 pg/ml] and mean Procalcitonin of patients was [1.6327±1.2498 ng/ml]

Study by **Sharma S et al (2019)** showed the role of serum procalcitoninas a diagnostic biomarker in sepsis and compared it with other sepsis markers (IL-6, CRP) in patients of suspected sepsis. A total of 80 patients were included in this study from ICU and each patient was investigated for serum Pro-calcitonin, Interleukin-6 and C-Reactive Protein levels. The study showed elevation in the level of Pro-calcitonin, IL6 and CRP among the sepsis group [3]. Study by **Rashwan, Hassan et al** found significant higher serum levels of hsCRP and Procalcitonin among the proven neonatal sepsis subgroup versus neonates with probable sepsis [4].

Our present study also showed elevation in the level of Pro-calcitonin, IL-6 and CRP among 53%, 51% and 66% of the neonates with sepsis respectively. Study conducted by **Rasool KH et al.** showed higher levels of serum IL6 than controls (p<0 .05)[5]. This is in accordance with the results of **Hotoura et al., (2011)**[6] which found an increase in IL-6 in the group of neonates with sepsis as compared to the values in healthy control neonates with no signs of infection. Study further showed serum IL-6 to be a more sensitive and specific index for early diagnosis of sepsis. In a prospective study done at the neonatology in intensive care unit of Cairo University, 96 full term neonates were studied: IL-6 was found to be significantly higher in the sepsis group than in the control group. IL-6 > 90 pg/ml was an excellent marker with high sensitivity and specificity (Mostafa and Mona , 2012)[7].

The findings of **Hotoura et al. (2011)** confirm those of earlier researchers who considered IL-6 to be a very precise early marker of neonatal infection .[6]

We showed that, most of the patients has abnormal (low) C3 [56 (56.0%)] followed by Normal C3 (44%) which was statistically significant (p=.02914) (z=1.6971). Most of the patients had Abnormal (Low) C4 [59 (59.0%)] followed by normal C4 (41.0%) which was statistically significant (p=0.01078) (z=2.5456), mean Complement C3 of

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patients was [0.7802±.4049] mean Complement C4 of patients was [0.1503±0.1355]

Our study found low levels of serum C3 and C4 among 56% and 59% of neonates with sepsis respectively. These finding however was not agreed with the study of **Singh et al. (1990)** [8] who found no statistically significant depression in the levels of complement components C3 and C4 in infected babies.

We also found that, complement C3 vs CRP, IL-6 and Procalcitonin were having negative correlation which was statistically significant as described : The value of Pearson Correlation Coefficient (r) was (-0.499) for CRP. The P-Value was <0.0001 the result was statistically significant. Negative correlation was found between IL-6 vs complement C3, P-Value was <0.0001. The result was statistically significant. The value of Pearson Correlation Coefficient (r) was -0.475 for IL-6. Negative correlation was also found between Procalcitonin vs Complement C3. The P-Value was 0.046 and significant statistically (table no 4).

In this study, observation was that, Complement C4 vs CRP, IL-6 and Procalcitonin were having Negative correlation and the results were statistically significant (table no 5).

These findings observed in neonatal sepsis points out to the finding of low complement C3, & C4 levels and increased levels of inflammatory markers namely Procalcitonin, CRP and IL6.

Thus, the results show that serum CRP, IL6, Procalcitonin, C3 and C4 parameters show significant changes in sepsis. Several other studies have documented such changes during the first month of neonatal life. Thus, a combination of several sepsis biomarkers may be of value in the early diagnosis of sepsis and these parameters can be accepted as sepsis panel markers.

CONCLUSION

The present study highlights the importance of early diagnosis of neonatal sepsis for appropriate management. The study findings reveal that inflammatory markers such as CRP, IL6, and Procalcitonin were significantly elevated in neonates with sepsis. Moreover, there was a significant negative correlation of complement C3 and C4 with CRP, IL-6, and Procalcitonin. Thus, the estimation of complement C3 and C4 along with other inflammatory markers as stated here is highly recommended as sensitive markers in neonatal sepsis.

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