C-REACTIVE PROTEIN KINETICS IN TERM AND PRETERM BABIES WITH EARLY ONSET SEPSIS AND ITS ASSOCIATION WITH BLOOD CULTURE

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ABSTRACT

Objective: To determine C-reactive protein kinetics in term and preterm babies with early onset sepsis and its association with blood culture.

Study Design: Cross sectional study.

Place and Duration of Study: Neonatal intensive care unit, department of pediatrics, Liaquat College of medicine and dentistry and Darulsehat hospital Karachi, from Jan 2017 to Jan 2018.

Methodology: One Hundred and Two neonates with presumed early onset sepsis (within 72 hours of birth) were enrolled in the study. Blood samples drawn for septic work up including baseline C-reactive protein levels. Blood culture collected with all recommended a-septic measures and is taken as gold standard for proven sepsis diagnosis. C-reactive protein considered positive when >6 mg/dl in preterm and >10mg/dl in term babies.

Results: Out of 102 enrolled neonates, among C-reactive protein positive cases, 42.1% aged <24 hours, 50% were male, 47.5% had low birth weight, 63.2% were term, 36.8% preterm, 42% had positive blood culture, 5.3% observed with less than five thousand WBC, and 44.7% observed with more than twenty-five thousand WBC. Among these parame-ters blood culture and WBC >25000 gives significant association with C-reactive protein (p<0.01).

Conclusion: Despite being septic, premature and low birth weight babies are unable to mount significant C-reactive protein levels/C-reactive protein levels in these neonates do not effectively correlate with infectivity. Our study showed strong association of baseline C-reactive protein with WBC >25000/cumm and blood culture in term babies whose birth weights were appropriate for gestational age. To conclude, prematurity and low birth weight have negative association with C-reactive protein positivity hence in addition to clinical signs and risk factors, other sepsis screen parameters like interleukin, procalciton in should be taken into consideration before decision of antibiotic discontinuation in these neonates with presumed EOS.

Keywords: C-reactive protein, Early onset neonatal sepsis, Preterm, Septic screen, Term.

INTRODUCTION

Despite revolutionary advancement in neonatal care, sepsis in newborn is still challenging for the health care professional because of its variable presentations and unpredictable outcomes. Among the readily available biomarkers for diagnosing neonatal septicemia, C-reactive protein (CRP) plays a key role in humoral immunity against pathogen especially bacteria as one of the acute phase reactants[1]. Bacterial infection stimulates the hepatocytes to produce CRP: a nonspecific immune response, which is a useful clinical marker for the individual host-pathogen interaction. When combined with other septic screen markers like WBC, micro ESR, I/T ratio, interleukin and procalcitonin, the diagnostic accuracy of CRP increases manyfolds[2].

In the immediate postnatal period, very low CRP concentrations have been detected in the cord blood and sera of neonates, therefore it can be presumed that CRP positivity in neonates with clinical signs of sepsis reflects post natal infection[3]. However preterm and low birth weight babies mount lesser increase in CRP owing to the immaturity of hepatic synthetic function.

Prognostic value of CRP is also invaluable during the clinical course of neonatal infection, yet blood culture remains gold standard diagnostic test. Although much research has been done on the role of CRP in early neonatal infection, certain aspects need to evaluated further like

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its response with reference to gestational age and birth weight, effect of non-infectious conditions and subclinical sepsis on CRP levels[4]. Furthermore, less comparative data is available to test its diagnostic accuracy in preterm and lowbirth weight septic babies[5].

Although neonatal care has advanced too much in the recent decade, mortality is still very high and ranges from approximately 1.5% in term to 40% in low birth weight neonates[6]. Clinicians commonly face challenges in diagnosing early neonatal sepsis as clinical signs are subtle and nonspecific and mimic certain non-infectious conditions like hyaline membrane disease/respiratory distress syndrome. Practice of empirical antibiotics administration has led to exposure of these newborns to adverse drug reactions and emergence of resistant pathogenic strains[7]. Therefore need for an effective biomarker for early detection of neonatal sepsis is still on.

Current study is done in order to determine CRP kinetics in term, preterm and low birthweight neoantes with maternal risk factors as well as clinical signs of sepsis in order to plan continuation or discontinuation of antibiotic therapy hence protecting these vulnerable neonates from sepsis complications, development of pathogen resistance and drug side effects. This is especially imperative for resource limited nations like us. Furthermore this study will be helpful for the pediatricians and neonatologists to devise sepsis scoring system based on clinical and laboratory parameters for term and preterm babies separately when compared with recent similar studies.

METHODOLOGY

This cross sectional study was conducted on 102 neonates with diagnosis of presumed sepsis admittedin NICU of Liaquat College of Medicine & Dentistry (LCMD) Hospital, Karachi, from January 2017 to January 2018. Inclusion criteria being all neonates with presumed early onset sepsis admitted to NICU of LCMD hospital during the study period. Neonates who died soon after admission before collection of hematological samples and neonates with late onset sepsis were excluded from the study. The patients were recruited using non-probability consecutive sampling technique and sample size calculated by WHO calculator using sensitivity of 65% and specificity of 80%. Diagnosis of presumed early sepsis was made on the basis of maternal and neonatal history and neonatal clinical examination (prolong rupture of membranes, maternal pyrexia, home delivery, dai handled case, reluctance to feed, poor sucking, decreased reflexes etc.). NICU of LCMD entertains yearly admissions of around 400 to 450 newborns per year. The major causes of admission to NICU are prematurity, low birth weight, respiratory distress syndrome, neonatal sepsis, birth asphyxia, convulsions and neonatal jaundice. Babies were both term and preterm, had risk factors for early neonatal sepsis and were less than 3 days old. Culture positive babies were defined as confirmed sepsis while babies having negative blood culture are defined as having presumed sepsis.

Two ml of venousblood collected in appropriate tubes after all aseptic measures, 0.5 ml for CRP measurements in lithium heparin green capped tubes. Complete blood count done on hematology analyser. leucocyte count done od coulter counter. Quantitative measurements of CRP done by latex enhanced nephelometry. Values >10 mg /dl were considered positive in term babies while >6mg/dl considered positive in preterm babies.

Empirical antibiotics started on all admitted neonates. After collection of initial lab results, antibiotics stopped in term babies with negative CRP with clinical improvement in signs of sepsis while continued in preterm babies till the availability of bloodculture results.

Data were stored and analyzed using IBM-SPSS version 23.0, count and percentages were reported for the neonatal outcomes, blood culture and WBC results of CRP positive and negative cases, Pearson chi-square test of independence was used to see the association of these parameters with CRP, binary logistic regression analysis was done to estimate the odds ratio and 95% con-

fidence interval of these parameters for CRP positive cases, receiving operating curve was done to estimate the area under the curve for WBC with CRP cases, bar chart also used to give the graphical presentation of the study results, *p*-values >0.05 were considered significant (fig-1).

The current study was approved by the ethics committee of LCMD hospital Karachi. After taking informed consent from parents of admitted neonates, data was collected in a structured questionnaire.

RESULTS

A total 102 neonates were registered in the study. Among the total samples, 56 (54.9%) were males and 46 (45.1%) were females. Mean age of male and female patient was 1.58 ± 0.73 days and 1.63 ± 0.77 days respectively. Table-I reports the association of maternal and neonatal factors and baseline neonatal parameters with CRP, results

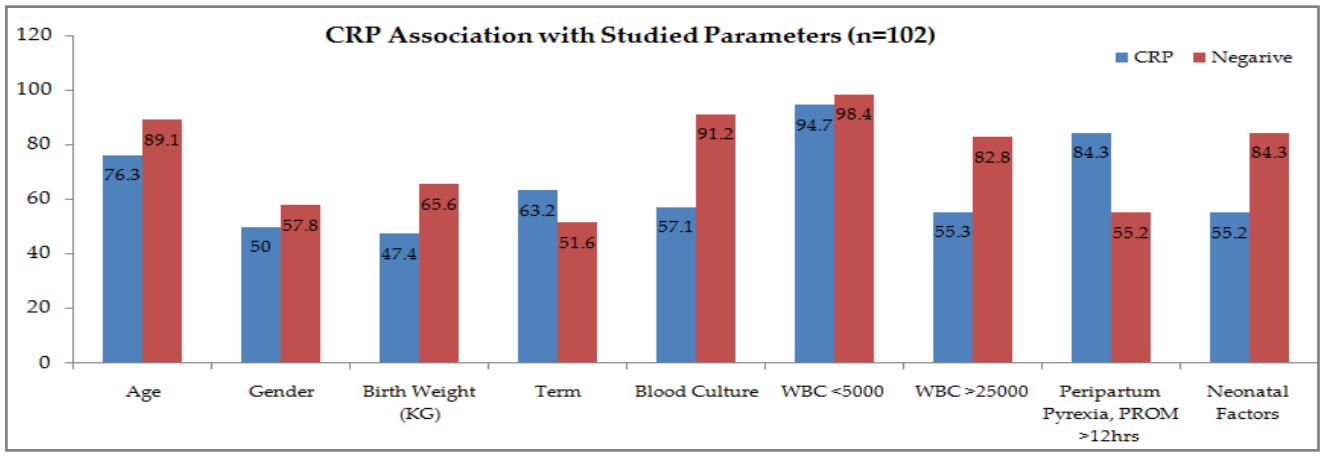

Figure-1: Bar chart showing the percentages of outcomes in CRP cases.

Table-I: Association of CRP with baseline studied neonatal parameters.

Parameters		CRP		*p*-value
		Negative, n (%)	Positive, n (%)	
Age of neo-nates (days)	1 day	40 (62.5)	16 (42.1)	0.093
	2 days	17 (26.6)	13 (34.2)	
	3 days	7 (10.9)	9 (23.7)	
Gender	Male	37 (57.8)	19 (50)	0.44
	Female	27 (42.2)	19 (50)	
Birth Weight (Kg)	≤2.5 kg	42 (65.6)	18 (47.4)	0.07
	>2.5 Kg	22 (34.4)	20 (52.6)	
Term	Term	33 (51.6)	24 (63.2)	0.25
	Pre-Term	31 (48.4)	14 (36.8)	
Blood Culture	Negative	52 (91.2)	16 (57.1)	<0.01*
	Positive	5 (8.8)	12 (42.9)	
WBC <5000	No	63 (98.4)	36 (94.7)	0.28
	Yes	1 (1.6)	2 (5.3)	
WBC >25000	No	53 (82.8)	21 (55.3)	<0.01*
	Yes	11 (17.2)	17 (44.7)	
Peripartum pyrexia, PROM >12hrs.	Yes	22 (34.4)	20 (52.6)	0.07
	No	42 (65.6)	18 (47.4)	
Neonatal factors	Apnea, Vom-iting, Feed Reluctancy	21 (55.2)	54 (84.3)	<0.01*
	Hypothermi, APGAR <6	17 (44.7)	10 (15.6)	

**p-value <0.05 was considered significant using Pearson Chi Square test*

shows among CRP positive cases, 42.1% of neonates presented on day 1 of life, 50% were males, 47.5% observed with less than or equal to 2.5 kg birth weight, 63.2% were term, 36.8% preterm, 42.% had positive blood culture, 5.3% observed with less than five thousand WBC, and 44.7% observed with more than twenty-five thousand WBC. Among these parameters blood culture and WBC >25000 gives significant association with CRP (p<0.01).

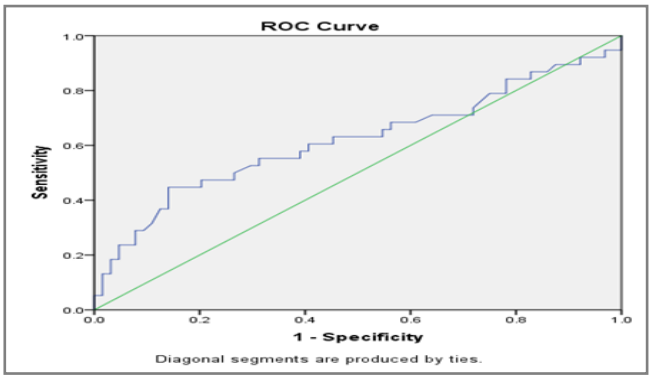

Figure-2: ROC for WBC with CRP.
Area under the curve was 61.8% and considered significant with p-value 0.046

Table-II: Estimation of CRP odds ratio using binary logistic regression model.

Parameters	Odds Ratio (95% C.I)	*p*-value
Age (Days)	1.81 (1.05-3.13)	0.03*
Female Baby	0.73 (0.33-1.64)	0.44
Birth Weight ≤2.5	0.48 (0.21-1.07)	0.07
Term	1.62 (0.71-3.67)	0.25
Positive Blood Culture	7.8 (2.39-25.5)	<0.01*
WBC <5000	3.5 (0.31-39.97)	0.31
WBC >25000	3.91(1.57-9.71)	<0.01*

**p<0.05 was considered significant for odds ratio*

ROC was done for WBC with CRP positive cases showed; there was 61.8% area under the curve with significant *p*-value 0.046 (fig-2).

Table-II reports the odds ratio for CRP Cases, with 95% confidence interval, age of babies gives significant positive association with CRP positive cases, female babies were less likely to observed with CRP positive cases as compared to male babies, birth weight under 2.5 kg or less gives negative association with CRP positive cases, samples with positive blood culture, WBC <5000 and WBC >25000 are more likely to found with CRP positive, *p*-values gives the evidence that, age of baby, blood culture and WBC more than 25000 gives the significant association with CRP cases.

DISCUSSION

In our study majority of neonates presented with clinical signs of sepsis on day 1 of life, an alarming observation to closely monitor maternal and perinatal contributing factors for this finding. Furthermore male predominance observed, a finding consistent with similar studies by Hisamuddin *et al*[8]. The reason is two fold; 1st is that, in our population, males babies are given priority in seeking urgent health care as compared to female; 2nd, it is a poorly understood phenomenon related to the presence of single X chromosome in males involved in immunoglobulin synthesis[9].

Positive CRP levels observed in neonates who presented with clinical signs like apnea, vomiting and reluctancy to feed while weak association seen in babies presented with hypothermia and APGAR <6 mins. The reason behind these findings need to evaluated further in another such study. Considering maternal risk factors, positive association seen in newborns delivered to mothers having PROM >13 hours with foul smelling amniotic fluid and peripartum pyrexia. These findings are in concordance with previous studies by Mathai *et al* in Tamil[10,11].

Regarding sensitivity and specificity of CRP for neonatal infection, area under the curve was 61.8% and considered significant with *p*-value 0.046. For diagnosis of EOS, different studies showed variable results of sensitivities (ranging from 29 to100%) and specificities (6 to 100%)[12-14].

The relationship of maturity (term neonates), WBC >25000/cumm and 1st day of life at presentation with positive CRP was statistically significant[15].

Although kept at a lower threshold, preterm babies were unanble to show significant CRP positivity, owing to the immaturity of hepatic synthetic function. This is similar to the study done

by Turner *et al*[16]. He studied relevance of CRP levels with postnatal age since 1 week of life. He demonstrated higher CRP responses in term and appropriate birth weight babies than in preterm and low birth weight.

In the current study, 44.1% of the study population were preterm and among them 36.8% showed CRP positivity, the reason being already discussed in the forementoined paragraph. Yet inference of results will be more generalized if similar studies will be conducted in multicenter neonatal intensive care units where large study population of premature septic babies can be taken into consideration.

As per study done by Hengst *et al* in 2003, risk for neonatal sepsis substantially increase with decreasing gestational age[17]. The presumed cause of this is linked to the fact that these neonates are immunologically immature and usually require lengthy hospital stay and more invasive interventions[18]. In the current study the incidence of Neonatal sepsis is more in Neonates weighing >2.5 kg i.e. 34.4% as compare whose weight is >2.5 kg i.e. 65.5%. It is because of lung immaturity and lack specific and non-specific antibodies in the preterm neonates.

Thus, CRP can be considered as one of the key diagnostic parameter for EOS but its diagnostic accuracy is enhanced many folds when considered in conjunction with clinical signs of sepsis and other sepsis biomarkers (WBC, micro ESR, procalcitonin, interleukin)[19,20]. Elevated CRP levels aids diagnosis of sepsis and may be used to guide management, however the cut-off levels of CRP may vary and should supplement other biomarkers in the diagnosis of neonatal sepsis[21-23].

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CONCLUSION

Despite being septic, premature and low birth weight babies are unable to mount significant CRP levels/CRP levels in these neonates do not effectively correlate with infectivity. Our study showed strong association of baseline CRP with WBC >25000/cumm and blood culture in term babies whose birth weights were appropriate for gestational age. To conclude, prematurity and low birth weight have negative association with CRP positivity hence in addition to clinical signs and risk factors, other sepsis screen parameters like I/T ratio, interleukin and procalcitonin should be taken into consideration before decision of antibiotic discontinuation in neonates with presumed EOS.

Furthermore, when taken into consideration certain clinical signs and perinatal factors are specifically significant in suspecting septicemia in this vulnerable population of neonates. Further studies on larger scale are needed to identify sepsis marker reference ranges for term and preterm septic neonates in order to devise a sepsis scoring system which could be readily applicable and trustable.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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