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Raised IL-6 and C-reactive protein in neonatal sepsis in Eastern India

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ABSTRACT

Background: Neonatal sepsis (NS) has significant morbidity and mortality rates and it is still difficult to diagnose on presentation. Early diagnosis and treatment decreases the mortality and morbidity of neonatal sepsis (NS). The study's aim was to detect the levels of IL-6, CRP and hs-CRP in clinically suspected cases of neonatal sepsis and establish its association with the pathogenesis of the disease.

Methods: The case control study consists of 70 neonates of whom 36 were clinically suspected case of sepsis admitted in NICU of paediatric department and were taken as cases and 34 were normal healthy neonates taken as control subjects. The cases as well as controls were within 28 days of age. Preterm and term neonates (<28 days of age) of both sexes showing signs of both early and late onset sepsis and also blood culture positive were included in the study. About 4 ml of venous blood was taken for measurement of hs CRP and IL 6. Serum hs-CRP levels was determined with a high-sensitivity nephelometric method while the serum level of IL 6 was measured by immunoassay Kits (Raybiotech, USA).

Results: Serum IL 6 levels were increased in sepsis cases as compared to controls and were statistically significant $(68.94\pm36.32 \text{ versus } 8.26\pm3.82 \text{ pg/ml}; P < 0.0001)$. However, a high positive correlation was observed between ESR with serum IL 6 level (r=0.825; P = <0.001) among neonatal sepsis subjects while no correlation was seen in controls (r=0.279; P=0.098).

Conclusions: It was observed that a significant rise in IL-6 or hs-CRP which may be suggested as specific marker for the identification of neonatal sepsis. The combination of IL-6 or CRP or hs-CRP could therefore be vital for the diagnosis and would be better predictors of neonatal sepsis and may be crucial in the pathogenesis of the disease.

Keywords: C-reactive protein, Erythrocyte sedimentation rate, hs-CRP, Interleukin-6, Neonatal sepsis

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection accompanied by bacteraemia in the first month of life and is known to be one of the leading causes of mortality and morbidity worldwide. The death caused is approximately one million per year in the neonatal period (0-28 days) which are due to

infection, and accounts for over 25% of global neonatal deaths and 10% of all mortality in infants.² The prognosis of neonatal sepsis depends on early diagnosis and efficient antibiotic therapy.³ The early diagnosis of neonatal sepsis is still uncertain because of its non-specific clinical presentation and also interferes with non-infectious conditions like respiratory distress syndrome or maladaptation.^{1,4} Blood culture is gold standard for the

diagnosis of neonatal sepsis but it has limited sensitivity and also time consuming method.⁵ Early detection of neonatal sepsis reduces the inadvertent use of antibiotics, the cost of treatment and also prevents the emergence of drug resistant strains. During recent days acute phase proteins, pro-inflammatory cytokines, adhesion molecules, cell surface markers and chemokines are being established to diagnose neonatal sepsis. The commonly used markers are tumour necrosis factoralpha (TNF-alpha), Interleukin-6 (IL-6), Interleukin-8 (IL-8), C-reactive protein (CRP), highly sensitive C-reactive protein (hs-CRP) and pro-calcitonin (PCT).⁶

C-reactive protein (CRP) is an acute phase reactant, produced in the liver which has a half—life of 24 to 48 hours. It is a commonly used marker to diagnose neonatal sepsis but as it takes 10 to 12 hours to respond to an infection, it is not reliable.⁴ On the other hand, Interleukin 6 (IL-6), a chemokine produced by the T and B lymphocytes is more sensitive than CRP, but it cannot be used as a sole marker of sepsis, as it has a short half-life.⁴

Highly sensitive CRP (hs-CRP) is more sensitive than the conventional CRP, hs-CRP assays measures the CRP levels lower than that measured by the conventional CRP assays. When measured with a high sensitivity analytic method, CRP can be used as a diagnostic marker of neonatal infection. This is because newborns cannot produce sufficient amounts of acute-phase proteins and so they respond to infection with a smaller increase in CRP compared to adults.⁷

Many studies have shown the role of IL6, CRP and hs-CRP for neonatal but none of them can consistently diagnose sepsis. The association between these markers for the prognosis of neonatal sepsis is extremely important to diagnose the disease at early stage.

In this study, levels of Interleukin-6 (IL-6), C- reactive protein (CRP) and highly sensitive C- reactive protein (hs-CRP) have been evaluated in neonates less than 28-day-old by cost-effective methods as potential early neonatal sepsis markers. The study's aim was to detect the levels of IL-6, CRP and hs-CRP in clinically suspected cases of neonatal sepsis and establish its association with the pathogenesis of the disease.

METHODS

The case control study consists of 70 neonates of whom 36 were clinically suspected case of sepsis admitted in NICU of paediatric department and were taken as cases and 34 were normal healthy neonates taken as control subjects in ICARE Institute of Medical Sciences and Research, Haldia. The cases as well as controls were within 28 days of age. Preterm and term neonates (< 28 days of age) of both sexes showing signs of both early and late onset sepsis and also blood culture positive were included in the study. The clinical signs and symptoms of sepsis includes abdominal distension, temperature

instability, dyspnoea, tachypnoea (>70/min), feeding intolerance, hepatosplenomegaly, lethargy, irritability, tachycardia (HR>190bpmin), bradycardia (HR<90bpmin). Neonates born with congenital anomalies, type 1 diabetes or those neonates who have undergone any surgical procedure were excluded from the study. Routine biochemical parameters were done for both cases as well as controls. Informed consent was taken by either parent in both the groups. The study was approved by the Institution Ethics committee.

About 4 ml of venous blood was taken by arm venous puncture in sterile vials. 2 ml of blood was collected without anticoagulant and serum was separated by centrifugation at 3500 rpm for 15 - 20 mins and was used for measurement of hs CRP and IL 6. Serum hs-CRP levels were determined with a high-sensitivity nephelometric method while the serum level of IL 6 was measured by immunoassay Kits (Raybiotech, USA). For the detection of CRP in serum, CRP kit was used which is a rapid latex agglutination test. 0.5-1 ml of blood was injected into the BACTEC culture vial under complete aseptic conditions.

Positive vials were Gram stained and sub cultured and incubated in appropriate temperature and atmospheres according to established methods. Full identification of organisms was done with standard bacteriological and biochemical methods. The rest of the blood sample was collected in sterile tube containing potassium-EDTA anticoagulant for measurement of ESR by Wintergreen method.

Statistical analysis

Statistical analysis of different biochemical parameters was performed by Students' t-test. All variables were expressed as mean \pm SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed "t"-test and for nonparametric Mann-Whitney U "t" test. To find out the correlation between two variables, Pearson's product moment correlation coefficient was used. A value of P < 0.05 was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA).

RESULTS

The demographic and biochemical profile of the sepsis subjects and healthy controls is presented in Table1. There was no significant difference in age, sex distribution or BMI in either of the two groups between sepsis cases and control subjects (Table 1). All the neonatal subjects included in the study were blood culture positive. ESR and hs-CRP were elevated in sepsis cases as compared to controls which were found statistically significant (Table 1). CRP positive cases were seen in sepsis neonates as depicted in Table 2.

Table 1: Demographic and biochemical profile of the subjects.

	Control (n=34)	Cases (n=36)
Gestational age (weeks)	34.6±3.3	35.2±2.4
Sex (M/F)	20/14	24/12
Weight (gm)	2198±903	2282±732
FPG (mg/dl)	78.23±7.52	82.7±10.47
ESR (mm/h)	14.2±3.24	92.24±39.31*
hs-CRP (mg/L)	0.72 ± 0.14	2.76±1.3*

FPG, fasting plasma glucose; ESR, Erythrocyte sedimentation rate; hs-CRP, highsensitivity-C Reactive Protein. Age, BMI, and serum levels of biochemical parameters were expressed as the means±SD. Statistically significant, *p<0.001 vs Control.

Table 2: Serum CRP levels (mg/liter) of patients with neonatal sepsis and controls.

G	roups	Control (n=34)	Cases (n=36)
No	o. of cases	Nil	32
po	sitive for CRP		

Serum IL 6 levels were increased in sepsis cases as compared to controls and were statistically significant (68.94±36.32 versus 8.26±3.82 pg/ml; P<0.0001) (Figure 1).

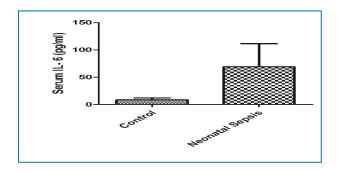


Figure 1: Correlation of serum IL 6 levels in sepsis cases as compared to controls and were statistically significant (68.94±36.32 versus 8.26±3.82 pg/ml; P<0.0001).

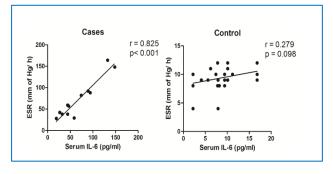


Figure 2: Correlation between ESR with serum IL 6 level (r=0.825; P=<0.001) among neonatal sepsis subjects while no correlation was seen in controls (r=0.279; P=0.098).

However, a high positive correlation was observed between ESR with serum IL 6 level (r=0.825; P= <0.001) among neonatal sepsis subjects while no correlation was seen in controls (r=0.279; P=0.098) (Figure 2).

DISCUSSION

Neonatal sepsis is obvious a major cause of death in neonates owing to late onset of symptoms and nonspecific clinical presentation.8 The blood culture is the gold standard for the diagnosis although it is time consuming and it may be negative due to inadequate sample collection or administration of antibiotics intrapartum or intermittent.⁵ However, in this study we have taken only the neonates who were blood culture positive to study the cytokine IL-6 and the most commonly used acute phase reactant CRP or hs-CRP or ESR. These markers were evaluated to find an association out which of the above markers alone or in combination would be dependable and a better predictor of neonatal sepsis. The markers showed significant difference between the cases as well as control group. The most common organisms isolated were Klebsiella species and Coagulase negative staphylococcus (CONS) in most of the neonates. Previous studies showed that Klebsiella and CONS species was the most common organism in case of late-onset neonatal sepsis and its association with the biofilm forming strains inhibits the host immune system in counteracting the infection which is in confirmation with our results.9

In present study, the serum C-reactive protein (CRP) level was significantly raised in the clinically suspected neonatal sepsis groups than the control groups which is consistent with other studies. 4,10,11 Fewer studies showed that CRP has high specificity and is a better indicator of severe bacterial infection in neonates which may be assumed by our results.8 CRP is the most extensively studied and commonly available laboratory test used for the prognosis and diagnosis of neonatal sepsis.¹ A study by Hofer et al., dictated that serial CRP measurement along with other markers such as interleukins advances the diagnostic accuracy of neonatal sepsis. 1 Other studies suggests CRP level is a good predictor of severe bacterial infection in neonatal sepsis, but the increase in CRP level is low in case of sepsis due to CONS infection which correlates with present study.¹²

The serum level of highly sensitive C-reactive protein (hs-CRP) showed significantly higher in the clinically suspected sepsis group than in the control group and it is also known that hs-CRP is more sensitive than the conventional CRP as it can measure very low levels of CRP. A study by Abdollahi et al., also observed the hs-CRP level was significantly higher in the sepsis group than the control group and proposed that hs-CRP may be used in combination with other sepsis markers like interleukin(IL-6) and procalcitonin (PCT) would be a better predictor of neonatal sepsis than using it alone. ¹³ In present study hs-CRP was assayed along with IL6 and it shows a marked rise in neonates than in control groups

and may be of good value than the other markers in the diagnosis of neonatal sepsis. Thus, the above results obtained from our study suggest that the CRP and IL-6 were better predictors and reliable markers of neonatal sepsis.

Moreover, our study showed the serum level of interleukin-6 (IL-6) is significantly higher in the clinically suspected cases of neonatal sepsis group than the normal healthy controls which is in confirmation with other studies. 6,8,14,15 A study by Smulian et al, reported that IL-6 level showed a highly significant value even in umbilical cord blood in septic neonates. 16 Another study done by Buck et al., also showed similar sensitivity in culture positive and clinical sepsis neonates.¹⁷ Another study done by van Dissel et al, suggests that the serum levels of TNF-α and IL-6 when measured together for the diagnosis of neonatal sepsis it would provide a good sensitivity and specificity.¹⁸ Present results also showed that ESR would be crucial for the prognosis of neonatal sepsis as a marked rise in seen in ESR in neonatal sepsis and furthermore, a good correlation was also observed between the ESR and IL6 levels.

The measurement of these sepsis markers is of utmost important only in case of neonates with unclear infectious status. The diagnosis of neonatal sepsis is still a challenge for both the laboratory as well as clinicians due to, absence of standardized cut-off values for sepsis markers and non-specific clinical presentation. Despite these we have observed a significant rise in IL-6 or hs-CRP which may be suggested as specific marker for the identification of neonatal sepsis. The combination of IL-6 or CRP or hs-CRP could therefore be vital for the diagnosis and would be better predictors of neonatal sepsis and may be crucial in the pathogenesis of the disease.

CONCLUSION

The diagnosis of neonatal sepsis is still a challenge for both the laboratory as well as clinicians due to, absence of standardized cut-off values for sepsis markers and non-specific clinical presentation. So, a reliable test is needed to diagnose neonatal sepsis. IL-6 is a very early marker of neonatal infection. IL-6 was mostly positive within 24 hours of onset of sepsis in comparison with other tests. So, IL-6 is more useful than other markers for early detection of neonatal sepsis.

CRP estimation does have a role in the diagnosis of neonatal sepsis but the test is not specific enough to be relied upon as the only indicator. From this study, we conclude that the IL-6 is the highly sensitive marker and CRP is the more specific marker for the identification of neonatal sepsis. hs-CRP is a less reliable marker. The combination of IL-6 and CRP has the high sensitivity and negative predictive value when compared to other markers. Therefore, a combination of markers i.e. IL-6 and CRP would be the better predictors of neonatal sepsis.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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