Original Research Article

Altered resistin and IL6 in Neonatal sepsis in patients admitted in a tertiary care teaching hospital at Eastern India

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Abstract

Background: Neonatal sepsis is a clinical syndrome followed by bacteraemia in the first month of life and appears to be one of the primary causes of mortality and morbidity worldwide. The study aim was to detect the levels of resistin, IL-6, CRP and hs-CRP in clinically suspected cases of neonatal sepsis and establish its association with the pathogenesis of the disease. **Materials & Methods:** The case control study consists of 78 neonates of whom 42 were clinically suspected case of sepsis admitted in NICU of Paediatric department and were taken as cases and 36 were normal healthy neonates taken as control subjects in a tertiary care teaching hospital, Durgapur, West Bengal. 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. Two 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, resistin and IL 6. Serum hs-CRP levels was determined with a high-sensitivity nephelometric method while the serum level of IL-6 and Resistin were measured by immunoassay Kits (Raybiotech, USA). **Results:** Serum resistin levels were increased in sepsis cases as compared to controls and were statistically significant (38.96 ± 17.15 vs 15.49 ± 8.54 ng/ml; $p < 0.0001$). It was also observed that serum IL 6 levels were higher in sepsis cases as compared to controls which was statistically significant (58.19 ± 39.97 versus 8.48 ± 3.90 pg/ml; **P** < 0.0001). However, a weak positive correlation was observed between serum resistin with serum IL 6 level (**r** = 0.343; **P** = 0.025) among neonatal sepsis subjects while no correlation was seen in controls (**r** = 0.141; **P** = 0.411). **Conclusion:** The measurement of these sepsis markers is extremely important only in case of neonates with unclear infectious status. We have observed a significant rise in Resistin or IL 6 or hs-CRP which may be suggested as specific marker for the identification of neonatal sepsis. The combination of Resistin or IL 6 or CRP or hs-CRP could therefore be crucial for the diagnosis and would be better predictors of neonatal sepsis and may be crucial in the pathogenesis of the disease.

Keywords: Preterm neonates, neonatal sepsis, mortality and morbidity, serum resistin, interleukin-6 (IL-6), hs-CRP

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Introduction

Neonatal sepsis is a clinical syndrome followed by bacteraemia in the first month of life and appears to be one of the primary causes of mortality and morbidity worldwide [1]. 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 uncertain because of its non-specific clinical presentation and its interference 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 such as tumour necrosis factor- alpha (TNF-alpha), Interleukin-6 (IL-6), Interleukin-8 (IL-8), C-Reactive protein (CRP), and pro-calcitonin (PCT) has been of great interest in potential diagnostic value to detect the progression of the disease [6]. Resistin is a newly discovered proinflammatory hormone which has potential role in adipogenesis and development of insulin resistance secreted by adipocytes in mice and mononuclear cells or macrophage or neutrophils in humans [7, 8]. Few studies showed the role of this hormone in inflammation as well as proinflammatory agent. C - reactive protein (CRP) is an acute phase reactant, produced in the liver bears a half –life of 24 to 48 hours. It is a commonly used marker to diagnose neonatal sepsis but as it takes long time approx 10 to 12 hours responding to an infection, it is not reliable [4]. Further, Interleukin 6 (IL 6), a chemokine produced by the T and B lymphocytes is more sensitive than CRP, but it has limitations to 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 since newborns produces insufficient amounts of acute-phase proteins and thus respond to infection with a smaller increase in CRP compared to adults [9]. Few studies have shown the role of resistin, IL6, CRP and hs-CRP for neonatal but none of them can consistently diagnose sepsis [1]. 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 Resistin, Interleukin-6 (IL-6), C- reactive protein (CRP) and highly sensitive C- reactive protein (hs-CRP) have been estimated in neonates less than 28-day-old as potential early neonatal sepsis markers. The study was aim to detect the levels of Resistin, IL-6, CRP and hs-CRP in clinically suspected cases of neonatal sepsis and establish its association with the pathogenesis of the disease.

Materials & methods

The case control study consists of 78 neonates of whom 42 were clinically suspected case of sepsis admitted in NICU of Paediatric department and were taken as cases and 36 were normal healthy neonates taken as control subjects in a tertiary care teaching hospital, Durgapur, West Bengal between July 2018 to Decemeber 2018. 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, 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 parents 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. Two 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, Resistin and IL 6. Serum hs-CRP levels was determined with a high-sensitivity nephelometric method while the serum level of IL 6 and Resistin were 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. Statistical analysis of different biochemical parameters was performed by Students' *t*-test. All variables were expressed as mean ± 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 Table 1. 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. hs-CRP levels 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.

Serum resistin levels were increased in sepsis cases as compared to controls and were statistically significant (38.96 ± 17.15 vs 15.49 ± 8.54 ng/ml; $p < 0.0001$) (Figure 1A). It was also observed that serum IL 6 levels were higher in sepsis cases as compared to controls which was statistically significant (58.19 ± 39.97 versus 8.48 ± 3.90 pg/ml; **P** < 0.0001) (Figure 1B). However, a weak positive correlation was observed between serum resistin with serum IL 6 level (**r** = 0.343; **P** = 0.025) among neonatal sepsis subjects while no correlation was seen in controls (**r** = 0.141; **P** = 0.411) [Figure 2].

Discussion

Neonatal sepsis is an obvious cause of death in neonates owing to late onset of symptoms and non-specific clinical presentation [10]. The blood culture is the gold standard for the diagnosis though it is time consuming and might be negative due to inadequate sample collection or administration of antibiotics intra-partum or intermittent [5]. However, in this study we have taken only the blood culture positive neonates to study serum resistin, IL-6 and frequently used acute phase reactant CRP or hs-CRP. These markers were assessed 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 statistically significant difference between the cases and control groups. The most common organisms isolated were *Klebsiella* species and Coagulase negative staphylococcus (CONS) in most of the neonates. Few studies also observed that *Klebsiella* and CONS species were the most common organisms 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 gives strength to our results and supports the fact [11]. Our study reveals that the serum resistin levels was found to be higher in neonatal sepsis which was statistically significant with the control group (Figure 1A). Further a weak correlation was observed between serum resistin and serum IL 6 levels in neonatal sepsis which was statistically significant (Figure 2). This suggests that the proinflammatory protein has a potential role in acute as well as chronic inflammation. Further, it may be suggested that the activation of inflammatory cascade triggered by cytokines and characterised by the influx of neutrophils and myriad of IL 6 trans-signalling play an important role in the pathogenesis of neonatal sepsis [12-15]. Moreover, IL-6 increases the production of CRP by the liver and resistin expression on peripheral blood mononuclear cells [16]. One study depicted resistin as an effective biomarker in adults with sepsis and resistin levels were elevated in inflammatory bowel disease which was in correlation with TLC levels, CRP and disease activity [17]. A recent study reported resistin as an indicator of neonatal sepsis and further showed that resistin may be a biomarker of neonatal sepsis with the same efficacy as other markers such as CRP, IL6, and procalcitonin [18]. However, another study showed that resistin levels were higher in neonates with PPROM than in those without PPROM [19]. Furthermore, another study reported that the basal serum resistin levels were significantly lower than the pretreatment and follow up levels in both EOS and LOS groups and was significantly correlated with CRP levels in premature infants with neonatal sepsis [20]. In our study, the serum C-reactive protein (CRP) level was significantly elevated in the clinically suspected neonatal sepsis groups than the control groups which is consistent with fewer studies [4, 21, 22]. Other studies showed that CRP has high specificity and is a better indicator of severe bacterial infection in neonates which may be presumed by our results [10]. Moreover, CRP is the most extensively studied and commonly available laboratory test used for the prognosis and diagnosis of neonatal sepsis [1]. A study depicted that serial CRP measurement along with other markers such as interleukins advances the diagnostic accuracy of

neonatal sepsis [1]. Other studies also confirms that the CRP level is a good predictor of severe bacterial infection in neonatal sepsis, but the increase in CRP level is slightly lower in sepsis due to the hindrance of CONS infection which is depicted in our study [23].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 measures very low levels of CRP. A study showed the hs-CRP level was significantly higher in the sepsis group than the control group and intensified that it 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 [24]. In our study hs-CRP was assayed along with IL6 or resistin 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 or IL 6 or resitin were better predictors and dependable markers of neonatal sepsis.Moreover, our study showed the serum level of 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, 10, 25, 26]. A study revealed that serum IL 6 level showed a highly significant value even in umbilical cord blood in septic neonates [27]. Another study also showed similar sensitivity in culture positive and clinical sepsis neonates [27]. Furthermore, a study reported 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 [28]. The measurement of these sepsis markers is extremely 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 Resistin or IL 6 or hs-CRP which may be suggested as specific marker for the identification of neonatal sepsis. The combination of Resistin or IL 6 or CRP or hs-CRP could therefore be crucial for the diagnosis and would be better predictors of neonatal sepsis and may be crucial in the pathogenesis of the disease.

Table 1: Demographic and biochemical profile of the subjects

	Control (n = 42)	Cases (n = 36)
Gestational Age (weeks)	34.8 ± 2.9	35.4 ± 2.2
Sex (M/F)	32/10	24/12
Weight (gm)	2284 ± 926	2392 ± 752
FPG (mg/dl)	82.23 ± 7.22	84.7 ± 9.36
ESR (mm/h)	15.2 ± 4.24	84.64 ± 49.44*
hs-CRP (mg/L)	0.84 ± 0.18	3.26 ± 1.2*

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

Groups	Control (n = 36)	Cases (n = 42)
No. of Cases positive for CRP	Nil	40

Fig. 1

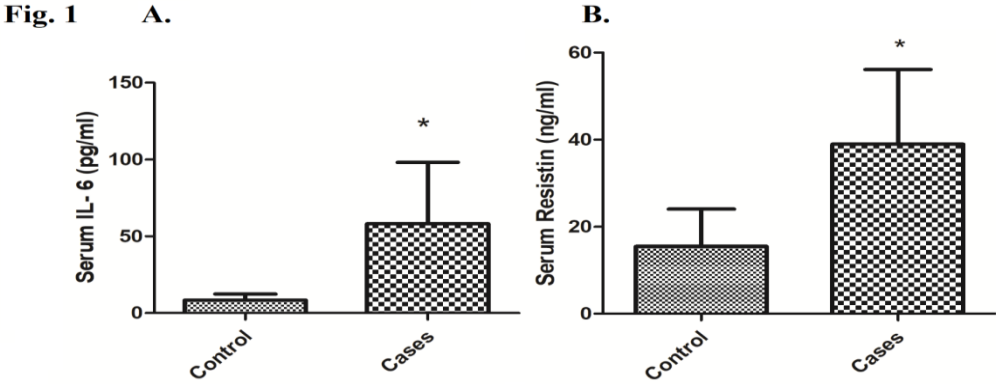

Fig 1A: Serum resistin levels were increased in sepsis cases as compared to controls and were statistically significant (38.96 ± 17.15 vs 15.49 ± 8.54 ng/ml; p < 0.0001).
Fig 1B: Serum IL 6 levels were higher in sepsis cases as compared to controls which was statistically significant (58.19 ± 39.97 versus 8.48 ± 3.90 pg/ml; P < **0.0001).**

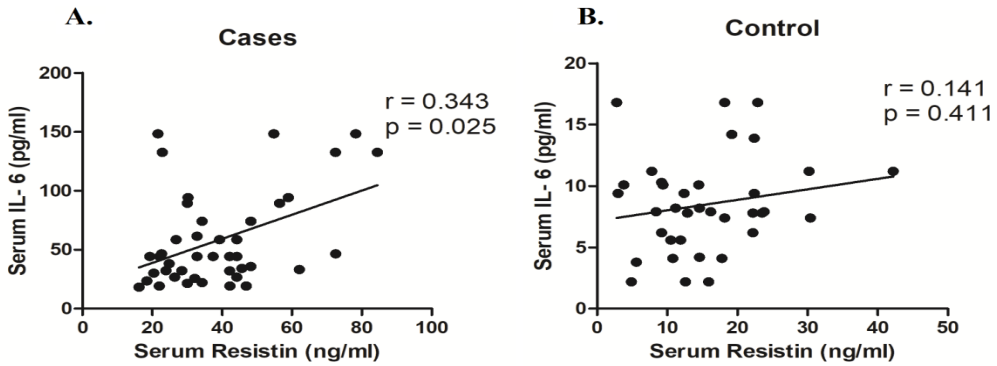

Fig 2: A weak positive correlation was observed between serum resistin with serum IL 6 level (r = **0.343;** P = **0.025) among neonatal sepsis subjects while no correlation was seen in controls (**r = **0.141;** P = **0.411)**

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