Advances in Precision Medicine
2022 Volume 7, Issue 1
ISSN: 2424-9106 (Online)
ISSN: 2424-8592 (Print)

IL-6, IL-8, TNF-α, and C-Reactive Protein Levels in the Diagnosis and Prognosis of Neonatal Sepsis

Ruken Yildirim[1]*, Mehmet Celal Devecioğlu[2]

[1]Clinic of Paediatrics, Diyarbakır Children's Hospital, Diyarbakır, Turkey

[2]Department of Pediatrics, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

***Corresponding author:** Ruken Yildirim, rukmay21@hotmail.com

Abstract

Objective: This study aimed to determine the importance and reliability of interleukin-6 (IL-6), IL8, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) levels in terms of diagnosis and prognosis in neonatal sepsis. *Methods:* Thirty newborns who were followed up and treated in the neonatal intensive care unit with a pre-diagnosis of neonatal sepsis and 20 healthy newborns who were born without any problem from mothers without any disease were included in the study. Gender, gestational age, postnatal age, place and mode of delivery, birth weight, IL-6, IL-8, TNF-α, and CRP levels were recorded. *Results:* Of the 30 cases diagnosed with sepsis, 16 (53.3%) were male and 14 (46.7%) were female. In the control group of 20 cases, 11 (55%) were male, and 9 (45%) were female. Of the cases diagnosed with sepsis, 8 were considered early-onset (26.6%) and 22 were considered late-onset (73.4%) neonatal sepsis. The mortality rate in early-onset sepsis was 25%, while this rate was 36.3% in late-onset sepsis cases. The levels of CRP, IL-6, and IL-8 were significantly higher in the sepsis group than in the control group. The difference between the groups in terms of TNF-α levels was not statistically significant. IL-6 ($P = 0.001$) and IL-8 ($P = 0.007$) levels were found to be statistically significantly higher in the deceased cases than in the healing cases. *Conclusion:* CRP, IL-6, and IL-8 levels were found to be useful parameters in the diagnosis of neonatal sepsis, while TNF-α was not found to have diagnostic value. IL-6 and IL-8 levels were found to be significant in the prognosis of neonatal sepsis.

Keywords

Neonatal sepsis
Gestational age
C-reactive protein

1. Introduction

Neonatal sepsis is an important cause of morbidity and mortality characterized by systemic infection findings in the first month of life. Therefore, early diagnosis and treatment are important. According to the time of onset of symptoms, it is divided into early-onset neonatal sepsis (ENS), which is seen in the first 3 days of life, and late-onset neonatal sepsis (GNS), which is seen in the 4^{th}–30^{th} days of life [1]. The presence of a chronic disease in the mother, maternal age above 40 years or below 18 years increases the probability of sepsis compared to normal [1,2]. The incidence of sepsis is 3–10 times lower in term babies compared to premature babies. The risk of ENS is increased approximately 10-fold in the presence of premature rupture of membranes (EMR) (>18 hours) and chorioamnionitis [3]. Maternal rectal and vaginal group B streptococcus (GBS) colonization, presence of fetal distress, multiple pregnancy, and low APGAR increase the risk of early-onset sepsis, whereas invasive interventions such as catheter or catheter placement, reduction of gastric acidity, and the need for surgical intervention increase the risk of late-onset sepsis [4]. Labor and postnatal complications are more frequently associated with ENS [5,6]. Clinical findings and complaints present in neonatal sepsis are mostly non-specific. Cytokines are glycoproteins released from macrophages, lymphocytes, and endothelial cells and have short half-lives. In the development of systemic inflammatory response (SIRS), the balance between proinflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), and anti-inflammatory cytokines (IL-4, IL-10) is very important in the emergence of clinical symptoms. Proinflammatory cytokines are responsible for effective defense against exogenous pathogens. However, their overproduction may be harmful and may cause tissue damage. The relationship between the increase in cytokines (especially IL-1, IL-6, IL-8, and TNF-α) and their related receptors and sepsis has not been fully established and it has been determined that these cytokines are significantly increased in many diseases other than sepsis [7]. This study aimed to determine the reliability and importance of IL-6, IL8, TNF-α, and C-reactive protein (CRP) levels in the diagnosis and prognosis of neonatal sepsis.

2. Materials and methods

In this study, 30 patients who were followed up and treated for sepsis in the Neonatal Intensive Care Unit of the Department of Pediatrics, Dicle University Faculty of Medicine in 2008 and 20 newborns who were born healthy to mothers without any disease were included. Ethics committee approval was obtained for the study (12/6/2008 decision no: 614). The diagnosis of neonatal sepsis was based on the presence of risk factors such as EMR, urogenital system infection, home birth, prematurity, and low birth weight in the mother, as well as tachypnoea, nasal breathing, moaning, cyanosis, non-suction, decreased or absent neonatal reflexes on examination. Newborns with findings including tachycardia or bradycardia, peripheral circulatory disorder, hypotension, vomiting, diarrhea, abdominal distension, hypo or hyperthermia, irritability, hypotonia, jaundice, convulsion, cutis marmaratus, and skin rash were diagnosed by differential diagnosis. Töllner sepsis scoring was used for the diagnosis of sepsis [8]. In this scoring method performed according to the clinical and laboratory findings of the cases with sepsis suspicion, a total value below 4 was considered as (-), between 5–10 as suspicious, and above 10 as probable sepsis [8]. Blood culture, peripheral smear, and levels of CRP, TNF-α, IL-6, and IL-8 were obtained from patients with clinically suspected sepsis. In patients with sepsis, a blood culture was obtained before antibiotic treatment. Pediatric BACTEC culture media were inoculated with 0.5–1 mL venous blood. The blood was kept in the blood culture machine (Becton Dıckınson, Phoenix 100, USA) for one week. The microorganisms grown in the culture were stained with gram stain and cultured.

Identification was performed by an infectious diseases specialist and a microbiologist. CRP was analyzed using an immunonephelometer (IMMAGE S/N 2528, Beckman Coulter, USA), and the normal value was accepted as < 3. For cytokines level (TNF-α, IL-6, and IL-8), 2 cc of blood was collected in a gel biochemistry tube. The chemo immunoassay method was performed with an Immulite Automated Analyser, and the TNF-α, IL-6, and IL-8 levels were measured in pg/mL.

Descriptive statistics for continuous variables were expressed as mean and standard deviation (SD), while descriptive statistics for categorical variables were expressed as numbers and percentages (%). Student's *t*-test (independent groups *t*-test) was used to compare group averages in terms of continuous variables. Chi-squared and Fisher Exact tests were used to determine the relationship between groups and categorical variables. The statistical significance level was taken as 5% and the SPSS version 21 statistical package program was used for calculations.

3. Results

The differences between sex, age, postnatal age, and place of birth parameters of both groups were statistically insignificant (**Table 1**). Birth weight (P = 0.002) and gestational age (P = 0.001) were lower in the sepsis group, while EMR (P = 0.011) and mortality (P = 0.003) parameters were higher in the sepsis group. Blood cultures grew in 15 of 30 patients in the sepsis group. Four of these patients were early neonatal sepsis and 11 were late neonatal sepsis. The microorganisms obtained in blood cultures in ENS

Table 1. Demographic characteristics of the groups [n, (%)]

	Sepsis (n = 30)	Control (n = 20)	P value
Gender			
Male	16 (53.3)	11 (55.0)	0.569[b]
Female	14 (46.7)	9 (45.0)	
Age			
0–4 days	8 (26.7)	4 (20.0)	0.425[b]
5–30 days	22 (73.3)	16 (80.0)	
Birth weight (g)			
≤ 2,500 g	13 (43.3)	-	0.002[a]
≥ 2,500 g	17 (56.7)	20 (100.0)	
Birth week			
≤ 38 weeks	11 (36.7)	-	0.001[a]
≥ 38 weeks	19 (63.3)	20 (100.0)	
EMR			
Yes	8 (26.7)	-	0.011[a]
No	22 (73.3)	20 (100.0)	
Postnatal age (days)			
Early	8 (26.7)	4 (20.0)	0.425[b]
Late	22 (73.3)	16 (80.0)	
Place of birth			
Hospital	27 (90.0)	20 (100.0)	0.207[a]
Home	3 (10.0)	-	
Mortality			
Exitus	10 (33.3)	-	0.003[a]
Healing	20 (66.7)	20 (100.0)	

EMR: Premature rupture of membranes; a. Fischer's Exact test; b. Chi-squared test

patients were *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus lugdunensis*, *Staphylococcus hominis*, *Staphylococcus aureus* in 2 patients with ENS, *S. epidermidis* in 2 patients with GNS, and the others were *S. hominis*, Klebsiella, *Staphylococcus saprophyticus*, *Streptococcus cristatus*, *Acinobacter baumannii*, Shigella, and *Macrococcus caseolyticus*.

TNF- α, IL-6, IL-8, and CRP levels were higher in the patient group. According to the results of the difference analysis, IL-6 (191.5 ± 319.5), IL-8 (1161.9 ± 1853.0), and CRP (30.0 ± 44.7) levels were found in the patient group while IL-6 (19.1 ± 27), IL-8 (175.2 ± 457.8), and CRP (2.9 ± 2.6) levels were found in the control group. The difference between the groups was statistically significant. The difference in TNF-α levels between the groups was not statistically significant **(Table 2**). The differences in TNF-α, IL-6, IL-8, and CRP levels between early and late sepsis groups were not statistically significant. The differences in these parameters between positive and negative culture groups were not statistically significant. IL-6 (454.4 ± 447.1) and IL-8 (2,415.6 ± 2,659.0) levels in the sepsis group with high mortality rates were statistically significantly higher than IL-6 (60.1 ± 81.3) and IL-8 (535.0 ± 841.3) levels in the group of patients with sepsis resulting in healing (**Table 3**).

4. Discussion

In this study, the IL-6, IL-8, and CRP levels were higher in the sepsis group and there was a positive correlation between high IL-6 and IL-8 levels and mortality rate.

In sepsis, while the lipopolysaccharides in the microorganism were initially thought to be the cause, it has been found that many mediators such as TNF-α, IL-1, IL-6, and IL-8, which occur with the stimulation of lipopolysaccharides, are involved in the formation of sepsis [2,6].

Many studies evaluated CRP in the diagnosis of neonatal sepsis. The sensitivity and specificity of CRP were reported to be 74% and 62%, respectively, by

Burstein *et al.* [9], and 95.7% and 82.4%, respectively, by Bunduk and Adu-Sarkodie [10]. Morad *et al.* reported that CRP levels were significantly higher in the sepsis group than in the sepsis-suspected group [11]. The diagnostic value of CRP for neonatal sepsis was found to be 88.0% by Ye *et al.* [12], and 70.07% by Hisamuddin *et al.* [13]. Meanwhile, a study by Adib *et al.* showed that the sensitivity of CRP in the early diagnosis of sepsis was 45%, and specificity was 95% in their study [14].

Table 2. IL-6, IL-8, TNF-α, and CRP levels in the sepsis and control groups (mean ± SD)

	Sepsis (*n* = 30)	**Control (*n* = 30)**	***P* value**
IL-6 (pg/mL)	191.5 ± 319.5	19.1 ± 27.9	0.024
IL-8 (pg/mL)	1,161.9 ± 1,853.0	175.2 ± 457.8	0.029
TNF-α (pg/mL)	58.3 ± 73.0	41.69 ± 31.2	0.343
CRP (mg/L)	30.0 ± 44.7	2.9 ± 2.6	0.010

IL-6: Interleukin-6; IL-8: Interleukin-8; TNF-α: Tumor necrosis factor-α; CRP: C-reactive protein

Table 3. Relationship of IL-6, IL8, TNF-α, and CRP levels with prognosis in patients with sepsis

	Exitus (*n* = 10)	**Healing (*n* = 20)**	***P* value**
IL-6 (pg/mL)	454.4 ± 447.1	60.1 ± 81.3	0.001
IL-8 (pg/mL)	2,415.6 ± 2,659.0	535.0 ± 841.3	0.007
TNF-α (pg/mL)	94.2 ± 111.6	40.4 ± 34.9	0.055
CRP (mg/L)	48.9 ± 62.1	20.6 ± 30.7	0.103

IL-6: Interleukin-6; IL-8: Interleukin-8; TNF-α: Tumor necrosis factor-α; CRP: C-reactive protein

Forrest *et al.* concluded that CRP would be more useful in frequent measurements of diagnosis and treatment and that it was an important criterion in the termination of antibiotic therapy [15]. In this study, CRP positivity was present in 90% of the patients at the time of diagnosis and was compatible with other studies. The difference between the CRP levels of the control group and the sepsis group was statistically significant. On the other hand, variables affecting the CRP level should also be taken into consideration in the diagnostic value of CRP. Vasiljević *et al.* reported that gestational week and body weight had a significant effect on CRP level in patients with early sepsis [16], while Gyllensvärd *et al.* reported that neonatal sepsis could be diagnosed more easily with CRP and clinical symptoms [17].

The presence of any relationship between TNF-α release in patients with sepsis has been the subject of research for years. Rukmono *et al.* found that TNF-α levels were higher in patients with sepsis than in patients with suspected sepsis [18]. Tracey *et al.* encountered sepsis and septic shock clinic in many cases in which they applied TNF-α in their animal experiments [19]. In a study conducted in adult patients, it was observed that TNF-α serum level was increased in 32 of 37 patients diagnosed with sepsis [20]. Studies on the role of cytokines in neonatal sepsis are fewer than studies on sepsis in adults. Hibbert *et al.* reported that TNF-α serum levels did not differ significantly in preterm and term neonatal sepsis groups [21]. In this study, a slight increase was found in TNF-α levels of the sepsis group as compared to the control group, despite it is not statistically significant.

In many neonatal sepsis studies, the IL-8 level was found to be high. In the study conducted by Hack *et al.* [20], serum IL-8 level was found to be high in 89% of adult sepsis patients. Friedland *et al.* suggested that there was a positive correlation between the increase in IL-8 level and mortality rate [22]. Since there are not enough studies on the role of cytokines in neonatal sepsis, the present results in this study will be compared with the results found in adult sepsis. Circulating IL-8 levels are not affected by the gestational week and postnatal age of the baby [23]. The fact that the half-life of IL-8 in the blood is around 60 hours has created the impression that it may be more useful in the diagnosis and follow-up of sepsis as compared with other cytokines. However, further studies are required. In this study, IL-8 levels of the sepsis group appeared to be higher as compared to the control group, and the mortality rate was higher in patients with very high IL-8 levels. Morad *et al.* found that IL-6 levels were significantly higher in patients with sepsis [11]. Ye *et al.* reported the diagnostic value of IL-6 in neonatal sepsis as 98% in their study [12]. Rukmono *et al.* found that IL-6 level was higher in patients with sepsis than in patients with suspected sepsis [18]. On the other hand, Kocabaş *et al.* reported that the specificity and sensitivity of IL-6, IL-8, and CRP parameters were lower than procalcitonin and TNF-α parameters [24]. Hack *et al.* also found that there was a directly proportional relationship between elevated IL-6 levels and mortality rate in their study of adult sepsis cases [20], which is similar to the results Sullivian *et al.* obtained in children [25]. In this study, IL-6 was significantly elevated in the sepsis group and there was a direct correlation between this elevation and mortality rate. However, it is difficult to determine the exact time of onset of sepsis even though blood samples were taken from the patients when sepsis was suspected.

5. Conclusion

TNF-α was not as important as other parameters in the diagnosis and mortality of neonatal sepsis. However, although IL-6, IL-8, and CRP have an important role in the diagnosis of neonatal sepsis, IL-6 and IL-8 were found to be more significant in determining the severity and mortality of infection.

Disclosure statement

The authors declare no conflict of interest.

Ethical consent

The ethical appropriateness of the study was approved by the Ethics Committee of the Faculty of Medicine (date: 12/6/2008, decision no: 614). This study was conducted in accordance with the Helsinki Declaration.

Financial support

No financial support was received from any organization or individual for this study.

Author contributions

Data Collection and Processing: RY; Analysis and Interpretation: RY, MCD; Literature Review: RY, MCD; Writing: RY.

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