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Interleukin-6 and Nitric Oxide Levels in Neonatal Sepsis*

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Aim: The objectives of this study were to compare interleukin-6 (IL-6) and nitric oxide (NO) levels in newborns with culture-proven sepsis with levels in age- and gestational age (GA)-matched controls, and to investigate the correlation between mediator levels and circulatory functions in the septic group.

Materials and Methods: Samples for IL-6 and NO levels were obtained from newborns with blood culture-proven sepsis before the beginning of antibiotic therapy and on the 3rd day of treatment. Heart rate, blood pressure and other treatment modalities including inotropic support were also recorded. Age- and GA-matched newborns without infection were included as controls. Minitab 13.0 was used for statistical analysis.

Results: Eighteen septic and 23 control patients were included in the study. IL-6 and NO levels were significantly higher in septic patients, and low blood pressure as a sign of the circulatory effects of sepsis was found to be negatively correlated with IL-6 levels on the 3rd day of treatment in newborns with septic shock. No correlation was found between clinical findings and NO levels.

Conclusions: IL-6 and NO levels were high in septic newborns as expected; however, circulatory findings were only correlated with IL-6 levels on the 3rd day of treatment in the septic shock group, suggesting that systemic inflammatory response syndrome (SIRS) is an ongoing process during infection in this subgroup. NO probably plays its major role much earlier as a defense mechanism in the complex chain of events during SIRS.

Key Words: Nitric oxide, IL-6, newborn, sepsis

Sepsisli Yenidoğanlarda İnterlökin 6 ve Nitrik Oksit Düzeyleri

Amaç: Bu çalışmanın amacı kan kültüründe üreme olan sepsisli yenidoğanlarda interlökin 6 (IL6) ve nitrik oksit (NO) düzeylerini ölçerek gebelik yaşı ve postnatal yaşı hasta grubuyla benzer olan kontrol grubuyla karşılaştırmak ve sepsisli bebeklerdeki mediatör düzeyleri ile kardiyovasküler sistem bulguları arasındaki ilişkiyi araştırmaktır.

Yöntem ve Gereç: Sepsisli yenidoğanlardan tedavi öncesinde ve tedavinin 3. gününde IL6 ve NO düzeyleri için kan örnekleri alınmıştır. Ayrıca hastaların kalp atım hızları, kan basınçları, almakta oldukları dolaşım desteği tedavileri kaydedilmiştir. Kontrol grubu olarak , hastaların gebelik yaşı ve postnatal yaşına benzer özellik gösteren ve enfeksiyon dışı nedenlerle izlenen yenidoğanlar alınmıştır. İstatistiksel analiz için Minitab 13.0 programı kullanılmıştır.

Bulgular: Çalışmaya 18 sepsisli, 23 kontrol alınmıştır. Hasta grubunun IL6 ve NO düzeyleri kontrol grubuna göre yüksek bulunmuştur. Sepsisin yol açtığı dolaşım bozukluğunun bir göstergesi olarak alınan kan basıncı tedavinin 3. gündeki IL6 düzeyi ile negatif korelasyon göstermiştir. NO düzeyleri ile klinik bulgular arasında korelasyon bulunmamıştır.

Sonuç: IL6 ve NO düzeyleri sepsisli yenidoğanlarda kontrol grubuna göre yüksek bulunmuştur.Ancak kısa sürede düşmesi beklenen IL6 düzeyleri septik şoklu hastalarda 3. günde halen yüksek kalıp kan basıncı ile negatif korelasyon göstermiştir. Bu durum septik şoklu hastalarda sistemik inflamatuvar yanıt sendromunun (SIRS) halen devam ettiğini göstermektedir.Öte yandan NO klinik etkilerini hastalığın çok daha erken döneminde kompleks bir mekanizma içinde gösteriyor olabilir.

Anahtar Sözcükler: IL6, NO, yenidoğan, sepsis

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Introduction

In the pediatric population, the definition of sepsis and related clinical conditions have been revised and modified usually parallel to changes in definitions in the adult population. According to the recent literature, pediatric *sepsis* is defined as systemic inflammatory response syndrome (SIRS) with infection, *severe sepsis* as sepsis together with organ dysfunction (hypoxia, acidosis, hypoperfusion, oliguria), and *septic shock* as sepsis together with hypotension-hypoperfusion despite fluid resuscitation (1).

In sepsis, depending on the activation of the complement, contact activation, and the intrinsic coagulation systems, many mediators like interleukin-6 (IL-6), nitric oxide (NO), and tumor necrosis factor-α (TNF-α) are released (2) and have been shown to play different roles in the clinical course of the disease in a time-dependent manner.

IL-6 plays many roles in the inflammatory response in relation with the pathogenesis of bacterial infections. Studies have shown that plasma IL-6 levels increase by 64-100% in cases with sepsis (3,4). It is the major regulator of acute protein response. In liver cells, it stimulates the synthesis of C-reactive protein (CRP), serum amyloid A, α1-acid glycoprotein, α1-antitrypsin, and fibrinogen, and is also responsible for myocardial depression (5).

NO is synthesized from oxygen and arginine by NO synthase. During septic shock, endothelium releases abundant NO as a response to endotoxin, platelet activating factor (PAF), IL-1, and TNF-α (6-8), resulting in vasodilatation, inhibition of platelet aggregation, inhibition of smooth muscle cell proliferation, and decreased endothelial expression of proinflammatory mediators.

NO production i) increases in sepsis and septic shock of the newborn and pediatric patients (9,10), ii) is correlated with the severity of disease and the degree of inflammation (9,11,12), and iii) increases with the initiation of sepsis and decreases in the recovery period (9). The objective of this study was to measure IL-6 and NO levels in patients with culture-proven sepsis before treatment and 48 hours into treatment and assess the correlation between mediator levels and clinical findings.

Materials and Methods

Newborns who had clinical signs of sepsis were considered eligible for the study. In addition to sepsis work-up consisting of whole blood count, CRP, and blood cultures, which were performed in all, an additional 1 cc venous sampling was obtained for determination of IL-6 and NO levels. The same studies were repeated at the 3rd day of antibiotic treatment (at the end of the 48th hour). Infants who had positive blood cultures remained in the study group and those whose blood cultures were negative were excluded. Healthy newborns of the same gestational and postnatal age who were hospitalized for reasons other than infection were included as the control group and 1 cc of blood was drawn for determining IL-6 and NO levels. The study was approved by the hospital ethics committee and informed parental consent was obtained. All blood samples were centrifuged at 3500 rpm (revolutions per minute) for 5 minutes, and serum was separated and stored at -70°C.

According to the clinical course, patients were divided into three groups as sepsis, severe sepsis and septic shock, and intergroup differences were recorded.

Measurement of IL-6 Levels

IL-6 measurements were performed with sandwich ELISA method by BioSource International, Inc. hIL-6 kit.

Serum samples, standard solutions and control samples were introduced to the medium enclosed with specific IL-6 antibody and biotinized monoclonal IL-6 antibody was added to the top. After incubation at room temperature for 2 hours, the liquid formed on the top was removed and the plate was washed and streptavidin-peroxidase enzyme complex was added to every measurement space. After incubation at room temperature for 30 minutes, the plate was washed again in an appropriate manner and chromogen was added to every measurement space and blue color formation was observed. After waiting at room temperature for 30 minutes, stop solution was added to every measurement space and in 2 hours they were read by ELISA reader at 450 nm wavelength.

Measurement of NO Levels

The amount of total nitrate in the test samples was determined by a modification of the procedure described by Brahman and Hendrix (13), using the purge system of

a Sievers Instruments Model 280A Nitric Oxide Analyzer. Plasma samples were deproteinized using chilled 96% ethanol (plasma/ethanol = 1/2 vol/vol) before use. A saturated solution of VCL_3 (Vanadium III chloride) in 1M HCL (800 mg/dl) was prepared and filtered before use. Five milliliters of this reagent were added to the purge vessel and purged with nitrogen gas for 10 minutes prior to use. The purge vessel was equipped with a cold water condenser and a water jacket to permit heating of the reagent to 95 ºC using a circulating water bath. The HCL vapors were removed by a gas bubbler containing 15 ml of 1M NaOH. The gas flow rate into the chemiluminescence detector was controlled using a needle valve.

Samples were injected into the purge vessel to react with the VCL_3/HCL reagent, which converted nitrate to NO. The NO produced was stripped from the reaction chamber and detected by ozone-induced chemiluminescence in the chemiluminescence detector. Ppb (parts per billion) values detected and NO levels in samples were calculated from the peak values. A standard curve was constructed using various concentrations of NO_3^- (10mM to 100µM). Nitrate levels in samples were calculated by using the standard curve.

Statistics

Minitab statistics program version 13.0 was used for statistical analysis.

Differences between sepsis and control groups were determined with Mann- Whitney U test and $P < 0.05$ was accepted as statistically significant. Differences within the sepsis group on days 1 and 3 were determined by Wilcoxon Signed Rank test and $P < 0.05$ was considered significant.

In the sepsis group, within days, the relations between clinical findings and IL-6 and NO levels were evaluated using Pearson correlation analysis, the linear relation of the investigated parameters was tested previously, and values with appropriate correlation coefficients were accepted as significant if P was determined <0.05. Patients with sepsis, severe sepsis, and septic shock were analyzed within the same group by Wilcoxon Signed Rank test and Pearson correlation. Mann-Whitney U test was used for group comparisons.

Results

Eighteen babies with positive blood cultures were included in the sepsis group. The microorganism species found in positive blood cultures were as follows: *Klebsiella* spp. (n = 7), coagulase negative staphylococcus (CNS) (n = 3), *E. coli* (n = 2), *Serratia* spp. (n = 2), *Acinetobacter* spp. (n = 2), *Candida non-albicans* (n = 1), and *α-hemolytic streptococcus* (n = 1).

Twenty-three babies with gestational and postnatal ages similar to those of the 18 septic babies and without any findings of infection were included in the control group. Gestational age (GA), birth weight (BW) and postnatal age of babies in the sepsis and control groups are shown as mean ± SD (median) in Table 1 together with the IL-6 and NO levels. IL-6 and NO levels were higher in the patient group than in the control group on both the 1st and 3rd days of sepsis.

Table 1. Septic and control newborns.

	Sepsis (n = 18) mean ± SD (median)	Control (n = 23) mean ± SD (median)	P
GA (week)	31.56 ± 4.42 (30)	33.39 ± 3.27 (33)	0.14
BW (gram)	1637 ± 702 (1540)	2146 ± 831 (1690)	0.03
Postnatal age (days)	15.28 ± 15.38 (9)	13.04 ± 10.02 (10)	0.89
IL-6 pg/ml	263 ± 222.5 (157.4) (day 1)	12.95 ± 14.07 (7.8)	<0.0001
IL-6 pg/ml	179.7 ± 213.2 (58.7) (day 3)	12.95 ± 14.07 (7.8)	0.0005
NO (µM/L)	53.43 ± 38.87 (47.34) (day 1)	33.8 ± 10.75 (32.04)	0.0044
NO (µM/L)	49.65 ± 30.37 (39.6) (day 3)	33.8 ± 10.75 (32.04)	0.11

GA: Gestational age. BW: Birth weight. IL-6: Interleukin-6. NO: Nitric oxide.

In the sepsis group on the 1st day of treatment, 10 patients were on inotropic support. At the end of the 48th hour of therapy (3rd day), 9 patients were on inotropic support. For inotropic support, dopamine was given to 9 patients, while dopamine + dobutamine combination was applied in 3 patients according to preference of the attending physician. When patients with sepsis were analyzed within themselves, 4 were diagnosed as only sepsis, 5 as severe sepsis, and 9 as septic shock.

As findings in the severe sepsis and sepsis groups were very similar, they were combined for statistical analysis. Maximum heart rate, minimum blood pressure, and IL-6 and NO levels on the 1st and 3rd days of treatment in patients with septic shock and those with severe sepsis and only sepsis are shown in Table 2.

In patients with sepsis and severe sepsis, there was no correlation between IL-6 and NO levels and vital findings.

In patients with septic shock, IL-6 levels on the 3rd day were found to be negatively correlated with the lowest blood pressure on the same day (P = 0.033, correlation coefficient: -74.8%). There was no relation between NO levels on the 1st and 3rd days and inotropic support or vital findings on the same days.

Discussion

Despite the advances in newborn intensive care, the incidence, morbidity, and mortality of neonatal sepsis are still high. Sepsis can progress to severe sepsis and septic shock depending on the degree of severity. Septic shock is the most important cause of death from sepsis.

In this study, performed with the aim of investigating the role of mediators on clinical findings during the course of sepsis in newborns, serum IL-6 levels in septic patients on the 1st day were approximately 20 times higher than in the control group. Although the IL-6 levels of the 3rd day were decreased when compared with the 1st day, they were still significantly higher than in the control group. In the sepsis group, there was no statistically significant difference between the IL-6 levels of the 1st and 3rd days, which shows that the SIRS findings in the patient group persisted.

There was a nearly significant difference between the IL-6 levels in patients with septic shock and sepsis/severe sepsis on the 3rd day (P = 0.053). The highest IL-6 levels were seen in patients with gram-negative sepsis and in these patients IL-6 levels were high even after the 48th hour of therapy.

Table 2. Results of patients with septic shock and sepsis/severe sepsis.

	Septic shock (n = 9) mean ± SD (median)		P	Sepsis/ Severe sepsis (n = 9) mean ± SD (median)		P
	1st day	3rd day		1st day	3rd day	
Maximum heart rate/minute	172.89±15 (168)	156.88±19.74 (151)	0.09	165.63±10.64 (164)	154.38±19.8 (159)	0.23
Minimum blood pressure (mmHg)	37.11±9.21 (37)	44.63±11.59 (46.5)	0.72	45.5±11.54 (46)	46±7.33 (46)	0.86
IL-6 (pg/ml)	358.1 ± 214.8 (500)	271.2 ± 226 (230.1)	0.52	167.9 ± 196.8 (85.6)	88.1 ± 163.3 (20.5) *P = 0.053	0.01
NO (µM/L)	43.4 ± 13.52 (39.45)	48.81 ± 22.31 (45.57)	0.63	63.5 ± 52.9 (48.5)	50.5 ± 38.2 (37.5)	0.34

IL-6: Interleukin-6. NO: Nitric oxide.

*P: Nearly significant difference between the IL-6 levels in patients with septic shock and those with sepsis/severe sepsis on the 3rd day of the disease.

In patients with septic shock, the lowest blood pressure on the 3rd day showed a negative correlation with IL-6 levels. Despite studies suggesting that TNF-α and IL-1b are responsible for the myocardial depression in sepsis, a study performed in England including patients with meningococcal septic shock using genetic analysis revealed that IL-6 is the primary responsible cytokine in myocardial depression (5). The higher IL-6 levels in our patients with septic shock and the relation determined between these high levels and a decrease in blood pressure support that this cytokine is responsible for the myocardial dysfunction in sepsis.

Nevertheless, in this study, serum nitrate levels were measured and NO levels in the sepsis group before treatment were found to be statistically higher than in the control group ($p = 0.04$). Wong et al. (10) compared the nitrite and nitrate levels of children with sepsis syndrome and critically ill children and found that nitrite and nitrate levels were higher in cases with sepsis and hypotension. In 1996, Doughty et al. (12) showed the relation between the increase in IL-6 levels and the increase in nitrite and nitrate levels in cases with sepsis and organ failure and hypothesized that increased nitrite and nitrate levels in sepsis reflected excessive proinflammatory response.

In this study, there was no difference in 3rd day NO levels between the sepsis and control groups. Furthermore, there was no difference in NO levels between the sepsis, severe sepsis and septic shock patients. In patients with septic shock, there was no relation between the NO levels of the 1st and 3rd days and the lowest blood pressures or degree of inotropic support. In addition, there was no relation between serum IL-6 and NO levels in patients with septic shock. This can be attributed to the SIRS mechanisms being more complex in the newborn when compared with other pediatric age groups. Especially in the patient group principally consisting of premature patients, these results can be attributed to some cytokine mechanisms yet being in the maturation period, and other interactions not yet being resolved.

In conclusion, in studies performed in newborns to date, IL-6 and NO levels were demonstrated to increase in sepsis, and these levels were also increased in our study in accordance with the literature. It has repeatedly been reported in the literature that IL-6 increases rapidly during the onset of sepsis followed by a rapid decline within 24 hours, which therefore makes it a less useful marker during the course of the disease (14-16). There have been discussions as to whether high cytokine levels are secondary to the inotropes given to support circulation; however, Oberbeck et al. (17) reported that IL-6 levels in sepsis are not increased by dopamine. In our study, IL-6 was found to be negatively correlated with low blood pressure on the 3rd day of the disease in the septic shock group. This might suggest that in a subgroup of patients, IL-6 levels remain high, which may be a sign that SIRS continues in this group, maintaining its adverse effects on hemodynamics. If this subgroup of patients can be identified, perhaps with serial IL-6 measurements, additional individualized supportive treatment modalities might be developed.

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