

Serum Interleukin-6 Levels in the Differential Diagnosis of Sepsis and Transient Tachypnea of Newborn

Yenidoğanlarda Sepsis ve Yenidoğanın Geçici Takipnesinin Ayırıcı Tanısında Serum İnterlökin-6 Düzeyleri

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Objective: The aim of this study was to evaluate the role of serum interleukin-6 (IL-6) levels in the differentiation of neonatal bacterial sepsis and TTN.

Material and Methods: The hospital records of 58 newborn infants with respiratory distress who were above 35 weeks of gestational age were investigated. Patients were divided into two groups. The infection group consisted of patients with proven sepsis and clinical sepsis and the other was the TTN group. Clinical findings and white blood cell count, serum C-reactive protein (CRP), IL-6 levels and the ratio of immature neutrophils to total neutrophils count (I/T) were recorded and compared between the two groups.

Results: Serum CRP and IL-6 levels were found higher than the normal limits in both of the groups. However there was no significant difference between them. Duration of respiratory distress was longer and I/T ratio significantly higher in the infection group than the TTN group. The combination of IL-6 and I/T ratio yielded a sensitivity of 80%, a specificity of 48%, a positive predictive value of 44.7%, and a negative predictive value of 80%

Conclusion: The I/T ratio and IL-6 may contribute to the early diagnosis of sepsis with respiratory symptoms in newborn infants but IL-6 alone cannot distinguish between TTN and sepsis.

Key words: Interleukin 6; sepsis; newborn; transient tachypnea.

Amaç: Bu çalışmanın amacı yenidoğanlarda serum interlökin 6 düzeyinin bakteriyel sepsis ve yenidoğanın geçici takipnesinin ayırmada rolünün araştırılması.

Gereç ve Yöntemler: Solunum sıkıntısı olan ve gestasyonel yaşı 35 haftadan büyük olan 58 yenidoğan çalışmaya alındı. Hastalar kanıtlanmış veya klinik sepsisi olanlar ve yenidoğanın geçici takipnesi olanlar olmak üzere iki gruba ayrıldı. Klinik bulgular beyaz küre sayısı, C reaktif protein, serum IL-6 düzeyi, periferik yasmada immature nötrofil sayısının toplam nötrofil sayısına oranı kaydedilerek gruplar arasında karşılaştırıldı.

Bulgular: Her iki grupta da serum CRP ve IL-6 düzeyleri normal limitlerden yüksek bulunmasına rağmen iki grup arasında fark yoktu. Solunum sıkıntısının süresi ve I/T oranı kanıtlanmış veya klinik sepsisi olan grupta belirgin olarak fazla bulundu. IL-6 ve I/T oranı kombine olarak yenidoğan sepsisi erken tanısında birlikte kullanıldığında, duyarlılık %80, özgüllük ise %48 tespit edildi.

Sonuç: IL-6 ve I/T oranı solunum sıkıntısı olan sepsisli yenidoğanların erken tanısı konusunda katkıda bulunurken IL-6 düzeyi tek başına yenidoğanlarda sepsis ve yenidoğanın geçici takipnesinde kullanılamaz.

Anahtar sözcükler: İnterlökin 6; sepsis; yenidoğan; yenidoğanın geçici takipnesi.

INTRODUCTION

Transient tachypnea of the newborn presents at or shortly after birth with grunting, retractions, and an increased respiratory rate. Because the symptoms of TTN are nonspecific and consistent with neonatal sepsis or pneumonia, most infants with TTN are evaluated for infection and are treated with broad-spectrum antibiotics pending a definitive diagnosis. Neonatal septicemia is a life-threatening disease.^[1] Respiratory symptoms are often the first manifestation of septicemia at this age. Such symptoms may mimic the clinical picture of transient tachypnea of the newborn. Some studies have shown that the combination of serum CRP and IL-6 are helpful in newborns evaluated for sepsis. CRP, an acute-phase reactant that increases in the presence of inflammation caused by infection or tissue injury, is synthesized in the liver.^[2] IL-6 is a pleiotropic cytokine involved in many aspects of the immune response.^[3] It is known that IL-6 is an important mediator of host response to stress and infection.^[4,5] There are some promising studies revealing IL-6 alone, or in combination with CRP, as good markers in the diagnosis of early onset neonatal sepsis.^[2,3] However, to our knowledge, there is no study in the literature to date for the differential diagnosis of TTN and neonatal sepsis using CRP and IL-6 combination. This retrospective study aims to investigate CRP and IL-6 levels in neonates with sepsis and respiratory disorders in order to evaluate the usefulness of these parameters in distinguishing between perinatal bacterial infections and TTN in clinical practice in a newborn intensive care unit.

MATERIAL AND METHODS

Patients

The hospital records of 86 newborn infants who were admitted to our neonatal intensive care unit with respiratory distress from January 2005 to 2006 were evaluated. All of them were above 35 weeks of gestational age. The 28 newborn infants with congenital heart disease, dysmorphic features, a history of hypoxia, and metabolic disorders were excluded. The 58 patients were divided into two groups: the TTN group and the infection group. The infection group consisted of patients with proven sepsis or diagnosis of clinical sepsis. Proven sepsis was defined as clinical suspicion of infection with a positive blood culture. Clinical sepsis was defined as a clinical suspicion of infection (at least 1 symptom from at least 3 categories), elevated serum CRP and increased immature/total neutrophils (I/T) ratio without a positive blood culture.^[3] Clinical symptoms which were used to diagnose sepsis were divided into six categories according to the previously described criteria.^[3] a) Pallor and icterus; b) lethargy, apnea, bradycardia (<100/min), irritability and seizures; c) tachypnea and dyspnea; d) hypotension, tachycardia (>200/min) and compromised microcirculation; e) vomiting and abdominal distension, and f) fever and temperature instability.

The diagnosis of TTN was made according to the previously described criteria: Newborn infants at 6 to 72 hours in their life who had respiratory distress (tachypnea, dyspnea and retraction) and no clinical, X-ray and laboratory findings of sepsis and pneumonia.^[2]

Gestational age, postnatal age, birth-weight, mode of delivery and duration of dyspnea of all enrolled newborn infants were recorded and compared between the two groups.

Blood Samples

Blood samples were taken from all newborn infants at the onset of respiratory distress symptoms. The median sample collection time of both groups was at the 10th hours of life (range 7-18 hours). Blood samples were obtained to determine white blood cell counts and serum CRP, IL-6 levels. Total leukocyte counts were determined with a Sysmex XT-2000i counter (Sysmex, Kobe, Japan). Blood smear was processed by Wright's staining and total 100 leukocyte counted for each patient. Then immature neutrophils and total neutrophil ratio was calculated. An I/T ratio of ≥ 0.20 was used as an indicator of infection.^[6]

CRP Determinations

The CRP concentration in serum was measured by rate nephelometry using the Beckman Array System protein analyser (C-reactive protein reagent set 449760; Beckman Instruments). The lower limit of detection was 4 mg/L. The cutoff values for CRP were revealed as ≥ 8 mg/L (3).

IL-6 Determinations

IL-6 concentrations were measured by a chemiluminescence enzyme immunometric assay (IMMULITE Automated immunoassay system; Immulite DPC, Los Angeles, CA, USA). The lower limit of IL-6 was 6 pg/ml. The cutoff values for IL-6 were revealed as ≥ 20 pg/ml (3).

Statistical Analysis

All statistical analysis was performed using the SPSS program, version 11.5 (SPSS Inc, Chicago, IL, USA). All data were compared between the two groups with the independent samples t-test and Mann-Whitney U test. Parameters are given as mean \pm standard deviation. A p value of less than 0.05 was considered statistically significant.

RESULTS

Fifty eight newborn infants were enrolled in the study. The TTN group consisted of 37 and the infection group consisted of 21 newborn infants. Table 1 lists the descriptive data of the two groups. There was no statistically significant difference in mean gestational age, birth weight, mode of delivery and postnatal age between the groups. In the TTN group cesarean delivery (C/S) indications were as follows: fetal distress in 8 patients (36%), non-vertex presentation in 3 patients (14%), cephalopelvic disproportion in 5 patients (23%),

Table 1. Clinical and laboratory findings of study groups

	Infection group n=21	TTN group n=37	p value
Gestational age (weeks)	37.00±1.64	37.13±1.68	NS
Birth weight(gr)	2763±462	2849± 450	NS
Mode of delivery			
C/S (n, %)	11 (52)	22 (59)	NS
Vaginal (n, %)	10 (48)	15 (41)	NS
Duration of respiratory distress, (hours)	140.85±61.89	27.51±27.49	<0.01*
WBC ($\times 10^3$)	13.40±5.29	15.81±5.27	NS
I/T ratio	0.76±0.43	0.081 ±0.27	<0.01*
CRP(mg/dL)	26.7± 2.3	23.0 ±3.25	NS
IL-6 (pg/ml)	267.5±305.3	126.8±229.8	NS

TTN: Transient tachypnea of newborn, C/S: Caesarean section, NS: Non-significant, WBC: White blood cell, IL-6: Interleukin-6, I/T: Immature/Total rate

* Statistically significant

placenta previa in 2 patients (9%), and oligohydramnios in 4 patients (18%); and in the infection group: fetal distress in 4 patients (36%), cephalopelvic disproportion in 3 patients (28%), acute placental hemorrhage in 2 patients (18%), and oligohydramnios in 2 patients (18%). In the infection group, 6 neonates had positive blood cultures; 4 had Coagulase Negative *Staphylococci*, 1 had *Streptococcus viridans*, and 1 had *Pseudomonas spp*. The remaining 15 neonates had clinical sepsis. The mothers of three newborn infants out of six had early membrane rupture, one newborn's mother had chorioamnionitis and one newborn's mother had a urinary infection. The other newborn infant needed resuscitation in the delivery room because of cardiorespiratory complaints. Only three of six mothers had used prenatal antibiotics.

Serum levels of CRP were higher than normal in the infection and TTN groups but there was no statistically significant difference between them (26.7±2.3 mg/dL, 23.0 ±3.25 mg/dL, respectively, p>0.05).

In addition, serum IL-6 levels were high in both the infection and TTN groups but no significant difference was observed between them (267.5±305.3 mg/dL, 126.8±229.8 mg/dL respectively p>0.05). We found the specificity of IL-6 ≥20 to range from 16% to 47% and the sensitivity ranged from 68% to 0.93%.

We found a higher I/T ratio in the infection group than the TTN group (0.76±0.43, 0.081±0.27 respectively p<0.01). We found the specificity of I/T to range from 34% to 67% and the sensitivity ranged from 57% to 93%. The combination of IL-6 and I/T ratio yielded a sensitivity of 80%, a specificity of 48%, a positive predictive value of 44.7%, and a negative predictive value of 80%. The duration of dyspnea was also longer in the infec-

tion group than the TTN group (140.85±61.89 hours, 27.51±27.49 hours respectively p<0.01).

DISCUSSION

This retrospective study has shown that the IL-6 concentration at onset of symptoms may not distinguish between TTN and proven sepsis and clinical sepsis. Nor might initial CRP levels exclude newborn infants with TTN. These results are not in concordance with findings reported in clinical studies.^[2,3] This may be explained by the alteration of the diagnostic value of IL-6 at birth by the physiologic severity and risk indexes. IL-6 mediates a nonspecific inflammatory response in tissue injuries and infections and catecholamines and epinephrine in particular have been shown to increase IL-6 production.^[5,7,8] IL-6 may not be a good parameter for excluding the possibility of sepsis and thereby eliminating the need for antibiotics. The sensitivity of IL-6 in initial determinations for the diagnosis of early onset neonatal sepsis has been reported to be consistently increased in newborn infants, with specificities ranging from 0.88 to 0.93.^[4] However, in our study the IL-6 specificity was found to be lower than previously reported studies.

CRP alone is of limited value at the onset of symptoms. Although CRP is highly sensitive, it had a relatively low sensitivity on day 0.^[9] Previous studies have sought to identify which pre and perinatal complications would mimic or mask alterations in the CRP response caused by infection but have yielded conflicting results.^[10] Some maternal factors such as premature rupture of the membranes (PROM), perinatal asphyxia, and other problems not resulting from infection are associated with increased CRP.^[11,12] In a neonatal intensive care unit (NICU) population with wide ranging differences in age, CRP was increased in those with a history of PROM, fetal distress, and chorioamnionitis.^[11-13]

The maximum CRP response occurs around 24 hours after onset (day1). CRP should therefore be used mainly in serial determinations. In our study, CRP was unable to distinguish between the TTN and infection groups. Although all of the patients were admitted to the NICU on the first day of their lives, serial measurements of inflammatory markers were not performed. Also, ten patients in the TTN group (27%) had fetal distress that may have influenced the results of CRP.

The high I/T ratio is a well known and simple method for predicting infection in newborn.^[14] In this study we found higher I/T ratios in newborn infants with sepsis than with TTN. The I/T ratio is a very simple bedside method when compared to measurement of IL-6. Ang et al. have reported that the specificity of I/T range from 75% to 91%, and the sensitivity ranges from 22% to 47%.^[15] These studies demonstrate that the I/T ratio is less sensitive but more specific in the diagnosis of neonatal sepsis. This emphasises the use of multiple indicators for detection of sepsis. In our study, the I/T ratio was consistent with previously published studies.

The duration of respiratory distress was found longer in newborns with sepsis in this study. This is not a surprising finding because of the natural course of TTN. However, these clinical findings are meaningful 3 days after birth. Therefore, it cannot preclude overdiagnosis of sepsis and unnecessary use of antibiotics.

All measurements in this study were carried out on day 1 of life. The absence of serial measurements of serum IL-6 and CRP levels is a limitation of this study. A prospective study that includes serial IL-6 and CRP level measurements may give more precise results.

In conclusion, this study showed that IL-6 cannot distinguish between sepsis and TTN in newborn infants. We suggest that IL-6 should be used in combination with other clinical and laboratory findings in the early diagnosis of neonatal sepsis.

Conflict of Interest

No conflict of interest declared by the authors.

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