SERUM TUMOR NECROSIS FACTOR-ALPHA IN NEONATAL SEPSIS

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ABSTRACT

Tumor necrosis factor-alpha (TNF-alpha) is thought to be an important mediator in the pathogenesis of neonatal septicemia. To assess the role of TNF-alpha in neonatal sepsis, serum levels of TNF-alpha were measured in a group of neonates with septicemia and compared with the levels of gestational-postnatal, age-matched healthy controls. The relationships between severity of infection, the nature of causative microorganisms, and TNF-alpha levels were also investigated in this prospective study. A total of 49 infants (25 full-term, 24 preterm) with proven sepsis and 40 healthy infants (20 full-term, 20 preterm) were included. Serum TNF-alpha levels were measured using the TNF-alpha immunoradiometric assay. The median level of TNF-alpha was found to be significantly higher in infants suffering from sepsis (154 pg/mL) particularly in those with septic shock (242.5 pg/mL) as compared to healthy controls (61.5 pg/mL) ($p < 0.001$). No correlation was found between TNF-alpha and postnatal ages, gestational ages or birth weights of the infants. TNF-alpha levels were not different in surviving and terminal neonates. Although serum TNF-alpha levels were found to be slightly higher in gram-negative septicemia, the difference was not significant. These findings suggest that TNF-alpha plays an important role in the pathophysiology of neonatal septicemia, but its importance as a prognostic factor is not yet clear.

Keywords: Neonatal; sepsis; shock; tumor necrosis factor-alpha

Tumor Necrosis Factor-alpha (TNF-alpha) is reported to be an important mediator of host response to infection. It is a part of the body's normal defense mechanisms but can have toxic effects when produced in excessive amounts.[1] Its primary biological activities include endothelial cell activation, shock, and death. The intravenous injection of TNF-alpha into laboratory animals induces fever and leukopenia; increases hepatic acute-phase protein synthesis; decreases albumin and causes anorexia, sleep loss, capillary leaks, tissue damage, acidosis, hypotension, and death.[2]

Different studies comparing the levels of TNF-alpha in septic and aseptic individuals show contradictory results.[3–6] The serum levels of TNF-alpha are reported to be high in neonates with septicemia and even higher in those with septic shock.[4,5,7,8] On the other hand, results of previous studies showed decreased or unchanged TNF-alpha levels in infants with infectious complications as compared to normals.[3,9] Clinical studies on the relationship between TNF-alpha and septicemia in newborns, particularly in preterm ones, are limited.

The aim of this prospective study was to assess the level of TNF-alpha in neonatal septicemia, which is still an important cause of neonatal mortality and morbidity. The relationships between clinical course, causative microorganisms, and TNF-alpha levels were also investigated.

METHODS AND MATERIALS

This study was performed in the Neonatal Intensive Care Unit of the Çukurova University Medical Faculty, Adana, Turkey. From June, 1992 to July,

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1994, 49 newborn infants (25 full-term, 24 preterm) with proven sepsis and 40 gestational-postnatal, age-matched healthy newborns (20 full-term, 20 preterm) formed the study group (Table 1). Patients with conditions that may lead to elevation of TNF-alpha in the serum (meconium aspiration syndrome, severe asphixia, bronchopulmonary dysplasia, and necrotizing enterocolitis) were not included. Proven sepsis was defined as positive blood cultures of pathogen microorganisms associated with clinical signs of neonatal sepsis. *Staphylococcus epidermidis* was considered to be pathogen if the infant displayed signs and symptoms of sepsis and had at least two blood cultures, on different days, positive for this microorganism.

Eighteen of the septic patients had septic shock, defined as hypotension (systolic blood pressure, more than 2 SD below the mean) in the absence of other causes of shock. In addition, these patients showed clinical signs of shock. Each of the septic infants was treated with broad spectrum antibiotics together with supportive therapy.

The study was approved by the local ethics committee and informed parental consent was obtained in all cases.

Blood samples were taken from an antecubital vein as soon as a clinical diagnosis of sepsis was made. The blood was collected in pyrogen-free, sterile tubes and within 1 hr the serum was separated by centrifugation and was kept at −20°C until assay.[4]

Serum TNF-alpha levels were measured in all serum samples using the TNF-alpha immunoradiometric assay (IRE-Medgenix, Belgium) with a sensitivity of 1 pg per mL.[5,10]

Data were reported as median and the significance of the differences between groups was determined with the Mann–Whitney-*U*, Wilcoxon rank sum *W* tests. Relative relations were determined with the Pearson correlation matrix. All *p* values are two-tailed.

RESULTS

The median serum TNF-alpha level of the patients with sepsis was significantly higher (154.0 pg/mL) than that of healthy controls (61.5 pg/mL) ($p < 0.001$). No correlation was found between TNF-alpha and postnatal ages, gestational ages, or weights of the patients ($p > 0.05$).

The median serum TNF-alpha level was also significantly higher in full-term (207.0 pg/mL) and preterm (103.0 pg/mL) newborns suffering from sepsis, as compared to gestational age-matched healthy controls (58.0; 78.5 pg/mL, respectively) ($p < 0.001$). The highest serum TNF-alpha levels were found in infants who suffered from septic shock (median 242.5 pg/mL) (Fig. 1). These levels were significantly higher than the levels of either controls ($p < 0.01$) or septic patients without shock ($p < 0.01$). Sixteen infants with septic shock had an immature/mature neutrophil ratio greater than 0.25 and none has survived.

No significant difference was found between the levels of TNF-alpha in surviving and terminal septic patients (median 194.0; 101.0 pg/mL, respectively) ($p > 0.05$). Also no significant difference was found between serum TNF-alpha levels of preterm and full-term infants either in the septic or control groups ($p > 0.05$).

Taking the third quartile of the TNF-alpha values of healthy newborns (90 pg/mL) as a cutoff value, the calculated values of sensitivity and specificity of serum TNF-alpha as an indicator of sepsis were 72 and 62%, respectively. The positive and negative predictive values using this cutoff were found to be 65 and 70%.

The microorganisms isolated from blood cultures of the infants were mostly gram-negatives (33 of 49, mainly *Klebsiella pneumonia*) followed by gram-positives (14 of 49, mainly *Staphylococcus epidermidis*) and *Candida albicans* (2 of 49). Septic patients with gram-negative infections seemed to have higher serum levels of TNF-alpha than those with gram-positive infections, but the difference was not statistically significant (median 187.0 pg/mL and 74.0 pg/mL, respectively) ($p > 0.05$).

DISCUSSION

Our study showed that serum concentration of TNF-alpha was significantly elevated in septic preterm and full-term newborns, particularly in those who developed septic shock, as compared to healthy controls.

Table 1. Characteristics of the Newborn Infants

	Patients with Sepsis		*Controls*	
	Preterm	***Full-term***	***Preterm***	***Full-term***
Number	24	25	20	20
Sex				
Female	12	15	13	9
Male	12	10	7	11
Gestational age (weeks)	33.2 ±3.3	39.4 ±1.0	33.7 ±2.5	39.9 ±0.8
Birth weight (g)	2112 ±631	3210 ±726	2380 ±737	3415 ±638
Postnatal age (days)	12.8 ±6.8	15.6 ±8.7	11.7 ±7.8	12.8 ±8.0

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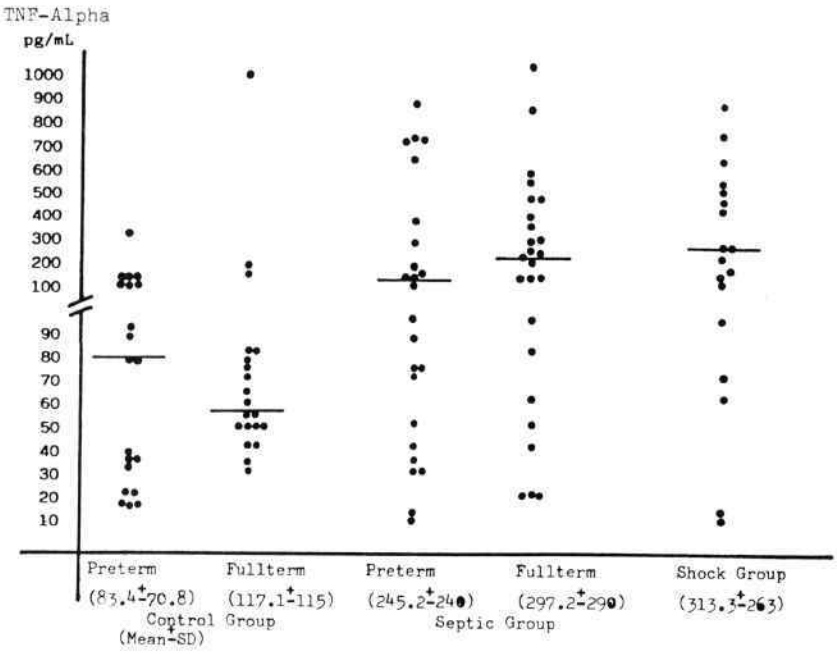


Figure 1. Median Serum TNF-alpha levels in the different groups of patients.

These data confirm the results of studies by Girardin et al.,[4] Roman et al.,[5] de-Bont et al.,[7] and Özdemir et al.,[8] showing that serum TNF-alpha was much higher in neonatal systemic infection, particularly in septic shock. On the contrary, our findings vary from the results of Miller et al.[3] and Harris et al.[9] who reported lower or unchanged levels of TNF-alpha in infants with perinatal infectious complications as compared to healthy infants.

Normal values of TNF-alpha in healthy neonates have not yet been established. The mean serum concentrations of 4.4–89.9 pg/mL have been considered to be normal in different studies.[3–5] In our study, the median level of serum TNF-alpha concentration in healthy newborns was 61.5 pg/mL.

TNF-alpha secretion from monocytes of full-term babies was recently found to be significantly increased as compared to that of monocytes from preterm babies.[11] However, this has not been confirmed in more recent studies.[4] We could not find any difference in serum TNF-alpha levels in preterm and full-term infants either in the septic or control groups. Because the immune functions of extremely premature infants (birth weight <1000 g) are significantly different and these babies have had different serum levels of TNF-alpha, they are not evaluated in this study.

TNF-alpha has been reported to be a sensitive and specific prognostic factor in determining the patients with the potential for developing septic shock and the highest levels of TNF-alpha were found in those who died of sepsis.[5,12–14] Our findings partly confirm this. We found significantly higher levels of TNF-alpha in patients who suffered from septic shock but no difference was found between surviving and terminal septic neonates.

The serum level of TNF-alpha has been investigated in gram-positive and gram-negative septicemia in different studies and no difference has been found.[5,14] We also could not find any relationship between TNF-alpha and the species of the causative microorganisms.

In conclusion, our study shows that the serum level of TNF-alpha is increased in preterm and full-term neonates with septicemia, particularly in those suffering from septic shock. These findings suggest that TNF-alpha plays an important role in the pathophysiology of neonatal sepsis. Low serum levels of TNF-alpha in an infant, born to a mother who received antibiotics intrapartum for infectious reasons, would decrease cost of unnecessary hospitalizations and antibiotic use from undiagnosed infections. However, the importance of TNF-alpha in predicting the prognosis and the outcome is not yet clear. But a high TNF-alpha level in combination with an immature/mature neutrophil ratio greater than 0.25 in a septic neonate with shock seems to be highly suggestive for poor outcome. More studies with repeated determinations of TNF-alpha every few days over a period of time in infants treated for septicemia are needed and may be more informative.

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