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Original Article

Tumor necrosis factor-alpha and interleukin-6 in early-onset neonatal sepsis

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Abstract

Background Neonatal sepsis remains a major cause of mortality and morbidity in newborns. Early-onset neonatal sepsis occurs in infants under the age of 72 hours, while late-onset neonatal sepsis occurs in infants over the age of 72 hours and may be due to nosocomial infection. Diagnosing neonatal sepsis is a challenge, as its clinical symptoms are not clear. Corroborating tests include routine blood, C-reactive protein (CRP), serology, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) examinations.

Objective To compare the TNF-α and IL-6 levels in patients with proven and unproven early-onset neonatal sepsis (EONS)

Methods This case-control study was done in the Perinatology Unit, Abdul Moeloek Hospital, Lampung. Subjects were under the age of 72 hours with risk factors and clinical symptoms of sepsis. They underwent routine blood tests and blood cultures. Infants with positive cultures were considered to have proven sepsis (26 subjects) and infants with negative blood cultures were considered to have unproven sepsis (26 subjects). All subjects underwent serological examinations of TNF-α and IL-6.

Results There were no differences in the basic characteristics of subjects between the two groups. Levels of TNF-α in the sepsis group were significantly higher than in the unproven group [(28.30 *vs.* 10.96 pg/mL, respectively (P=0.001)]. Furthermore, Il-6 was significantly higher in the proven sepsis group than in the unproven sepsis group [(28.3 *vs.* 9.69 pg/mL, respectively) (P=0.006)].

Conclusion Levels of TNF-alpha and IL-6 are significantly higher in infants with proven than unproven early-onset neonatal sepsis. **[Paediatr Indones. 2016;56:15-8.]**

Keywords: *TNF-α, IL-6, neonatal sepsis*

One out of four newborns die due to infection.[1,2] Bacterial infection in newborns may rapidly evolve into generalized sepsis. This condition has a gradual and subtle onset, with nonspecific symptoms that may severely compromise the infant's clinical state if untreated, and have life-threatening consequences.[2] Sepsis in neonates hospitalized in the neonatal intensive care unit (NICU) is a global problem and a significant contributor to morbidity and death. The incidence of neonatal sepsis in developed countries is 1 to 5 per 1,000 live births, while that in developing countries is higher, from 1.8 to 18 per 1,000 births of life.[1] Cipto Mangunkusumo Hospital, Jakarta, reported that 13.68% of newborns from all live births had infections,with a mortality rate of 14.18% in 2005.[3]

The diagnosis of neonatal sepsis is challenging, as the clinical symptoms of neonatal sepsis are not easily recognized. Investigations used to support a sepsis diagnosis include routine blood tests and examinations of CRP, serology, TNF-α, and IL-6.[3,4] Little is understood

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about the roles of TNF–α and IL-6 in neonatal sepsis.[5-9] As such, we aimed to assess the levels of TNF-α and IL-6 in patients with early-onset neonatal sepsis (EONS), either proven or unproven.

Methods

This case-control study was conducted from January to July 2014 in the Perinatology Unit of Dr. H. Abdul Moeloek General Hospital, Lampung. The inclusion criteria were newborns under the age of 3 days with risk factors and clinical symptoms for sepsis. Subjects' parents provided informed consent. Exclusion criteria were congenital abnormalities and critical illness, other than sepsis. Risk factors for neonatal sepsis were maternal fever, smelly amniotic fluid at birth, or rupture of membranes more than 12 hours prior to delivery. The clinical symptoms of neonatal sepsis included lethargy, poor sucking ability, irritability, vomiting, bloating, respiratory disorders, seizures, temperature instability, and delivery in unhygienic environments.

The minimum sample size was calculated to be 52 subjects, consisting of 26 proven sepsis and 26 unproven sepsis patients. Proven sepsis was defined as a positive blood culture taken at the age of less than 72 hours. We used a consecutive sampling method to collect participants. Data collection began with the selection of infants with risk factors and clinical symptoms of sepsis. Infants who qualified then underwent routine blood tests and blood cultures. Measurements of TNF-α and IL-6 were performed by ELISA method.[2] Possible correlations between TNF-α and IL-6 levels and EONS were analyzed by univariable test. Results with P values < 0.05 were considered to be statistically significant.

Results

Fifty two neonates <72 hours of age were enrolled, consisted of 26 neonates with proven and 26 neonates with unproven sepsis. Blood specimens were taken at <24 hours of age. Characteristics of subjects in the the proven and unproven sepsis groups did not significantly differ in terms of body weight (P=0.617), body length (P=0.700), Apgar score at 5 minutes (P=0.339), hemoglobin level (P=0.661), leukocyte count (P=0.351), or platelet count (P=0.533) (**Table 1**).

Risk factors and clinical symptoms of neonatal sepsis are shown in T**able 2**. Risk factors were not significantly different between groups in terms of maternal fever during delivery (P=0.773), rupture of the membranes >12 hours prior to birth (P=0.087), and infant lethargy (P=0.126). The comparison of TNF-α and IL-6 levels between the proven and unproven sepsis is shown in **Figure 1**.

The mean TNF-α levels were significantly higher in the proven sepsis (mean 28.3 (SD 21.71) pg/mL) compared the unproven sepsis group (mean 10.96 (SD 9.69) pg/mL) group. Likewise, the mean IL-6 levels

Table 1. Characteristics of subjects by group

Characteristics	Proven sepsis (n=26)	Unproven sepsis (n=26)	P value
Mean body weight (SD), g	2,630 (791)	2,519 (793)	0.617
Mean body length (SD), cm	48.19 (1.74)	47.96 (2.49)	0.700
Mean Agpar score at 5 minute (SD)	6.96 (1.61)	7.35 (1.23)	0.339
Mean hemoglobin level (SD), g/dL	14.29 (2.55)	14.63 (3.08)	0.661
Mean leukocyte count (SD), 10^9/L	14.16 (5.73)	12.87 (3.98)	0.351
Mean platelet count (SD), billion/L	190 (104)	207 (92)	0.533

Table 2. Risk factors and clinical symptoms of neonatal sepsis

Risk factors	Proven sepsis	Unproven sepsis	OR	P value
Maternal fever, n	19	33	1.18	0.773
Rupture of membranes >12 hours prior to birth, n	26	26	2.56	0.087
Infant lethargy, n	35	17	2.44	0.126

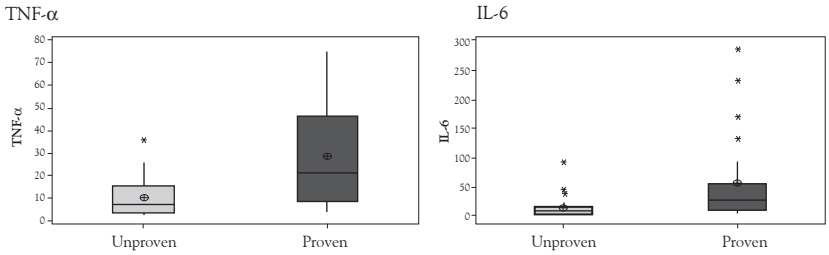

Figure 1. TNF-α and IL-6 levels in proven and unproven sepsis

were significantly higher in the proven sepsis than in the unproven sepsis group (P=0.006)].

Discussion

In this study we showed that neonates with proven sepsis had higher TNF-α and IL-6 levels compared to those with unproven sepsis.

Bacterial infection continues to be the major cause of morbidity and mortality in newborns. Because the prognosis for sepsis largely depends on early identification and treatment, these newborns are subjected to extensive diagnostic evaluation and empirical, systemic antibiotic treatment, pending laboratory results. The definitive diagnosis of sepsis is made by a positive blood culture, which requires a minimum of 48–72 hours, yielding a positive result in only 30–70% of cases.[1,3,4,10] The underdeveloped immune system predisposes newborns to infection. Sepsis and endotoxins activate monocytes, macrophages, lymphocytes, fibroblasts, and endothelial cells that produce and secrete TNF-α, IL-6, and other proinflammatory cytokines. IL-6, stimulated by TNF-α, IL-1, and endotoxins of viral and bacterial infections, acts as a T-cell activation indicator, induces antibody secretion by human B-cells, causes differentiation of cytotoxic T-cells, and also inhibits TNF-α production. Moreover, IL-6 is the major stimulant in hepatic protein synthesis, that is, CRP and fibrinogen, during acute phase responses.[3,4]

We found significantly higher levels of TNF-α in the proven EONS group, compared to the unproven neonatal sepsis group. We also found signifcantly higher IL-6 levels in the proven sepsis group compared to the unproven sepsis group.Similarly, previous study showed increased serum TNF-α and IL-6 in patients with neonatal sepsis compared to neonates without proven neonatal sepsis.[4] Moreover, another study showed that the concentrations of some proinflammatory cytokines, especially TNF-α, IL-6, and IL-8, in systemic circulation increased during severe infection and septic shock. They found that serum IL-6, IL-8, and TNF-α levels were higher in septic than in non-septic newborns.[5,11]

In conclusion, both TNF-alpha and IL-6 levels are significantly higher in patients with proven early-onset neonatal sepsis than in those with unproven sepsis. The potential diagnostic role of these biomarkers needs to be further studied.

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