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Original Article

Correlation between tumor necrosis factor-alpha and septic shock in children

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Abstract

Background The crucial role cytokines play in the pathophysiology of sepsis is widely accepted. Infection stimulates the production of cytokines in various cell types. Tumor necrosis factor-alpha (TNF-α) is one of the most extensively investigated cytokines in experimental and clinical sepsis. Tumor necrosis factor-alpha has been shown to mediate lethality in experimental sepsis.

Objective To evaluate for a possible correlation between TNF-α level and septic shock in children.

Methods This cross-sectional study was conducted in Manado from June to September 2011. A total of 40 patients with a recent diagnosis of sepsis or septic shock were included. Plasma specimens were collected from subjects for measurement of TNF-α concentration. Logistic regression analysis was used to assess the correlation between TNF-α level and sepsis, as well as the probability of shock in children with sepsis, with $P<0.05$ as statistically significant.

Results There was a strong positive correlation between TNF-α level and the probability of shock in children with sepsis (regression coefficient = 0.78, P = 0.002).

Conclusions There is a strong positive correlation between TNF-α level with the probability of shock in children with sepsis. Higher plasma level of TNF-α is associated with higher probability of septic shock. **[Paediatr Indones. 2013;53:1-5.]**

Keywords: *TNF-α, sepsis, septic shock*

Sepsis is one of the major causes of mortality in critically ill patients and develops as a result of the host response to infection. Sepsis may be defined as a generalized inflammatory response of the entire organism, and often manifests it self as the systemic inflammatory response syndrome (SIRS).[1,2] The progression of SIRS usually leads to life-threatening multiple organ dysfunction, culminating in multiple organ failure (MOF).[3] The most severe hemodynamic manifestation of sepsis is a hyperdynamic shock characterized by increased cardiac output and loss of peripheral resistance.[4] The pathogenesis of sepsis is a result of a complex network of events.[1,5] Components of the Gram-negative bacteria cell wall (endotoxins) are the predominant molecules responsible for the initiation of sepsis. Endotoxins, in addition to other bacterial molecules, trigger a generalized response that involves both cellular and humoral pathways with the generation of pro- and anti-inflammatory mediators.[6-8]

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TNF-α was the first circulating, pro-inflammatory cytokine to be widely evaluated in septic patients.[7,9] Previous studies found that TNF-α was a good marker in the diagnosis of sepsis and also valuable in following the effectiveness of treatment, as well as determining the prognosis of disease.[10,11] We aimed to investigate the relationship between serum TNF-α level and septic shock in children.

Methods

This cross-sectional study was conducted in the Prof. Dr. R.D. Kandou Hospital, Manado, North Sulawesi, from June to September 2011. Admitted patients were considered for inclusion in this study if they had a clinical diagnosis of sepsis, based on the parameters proposed by International Pediatric Sepsis Consensus Conference 2005.[12] This definition of sepsis is the presence of at least two of the following criteria: temperature > 38°C or < 36°C , tachycardia, tachypnea, and leukocytosis or more than 10% immature forms, in addition to a focal infection. Septic shock was defined as severe hypotension that last more than 1 hour, despite adequate fluid replacement. We excluded patients with severe malnutrition, immunodeficiency, or malignancy.

Blood samples for TNF-α measurement were collected at the time of hospital admission, after clinical evaluation, and obtaining informed consent. Blood was collected in sterile tubes, centrifuged at 3,000 rpm for 10 minutes, and stored at -20°C until processed further. All measurements were done at a reference laboratory. Tumor necrosis factor-alpha level were determined by standard enzyme linked immunosorbent assay (*Quantikine HS TNF-α/ TNFSF1A immunoassay*).

Logistic regression analysis was used to evaluate a correlation of TNF-α level to septic shock in patients with sepsis. We considered results to be statistically significant for $P<0.05$. Statistical analysis was performed using the *Statistical Product and Services Solutions* (SPSS) version 17.0. This study was approved by the Ethics Committee at Prof. Dr. R.D. Kandou General Hospital.

Results

Characteristics of subjects are shown in **Table 1**. Forty children were initially included in the study, 27 males and 13 females. Septic shock was diagnosed in 5 males and 4 females. Subjects ranged in age from 0.1 year to 12.2 years. Mean age of sepsis subjects was 3.63 (SD 2.73) years, while the mean age of septic shock subjects was 3.43 (SD 3.88) years. Foci infections that we found were bronchopneumonia (17/40), acute diarrhea (10/40), encephalitis (5/40), meningitis (2/40), and acute abdomen (6/40). Mean TNF-α plasma level of the sepsis group was 12.83 (SD 11.13) pg/mL, while that of the septic shock group was 37.55 (SD 23.06) pg/mL.

Logistic regression analysis revealed a highly significant positive correlation between TNF-α level and probability of shock in sepsis children ($r=0.78$ and $P=0.002$) (**Figure 1**).

Table 1. Characteristics of subjects with sepsis or septic shock

Characteristics	Groups	
	Sepsis n=31	Septic shock n=9
Mean age (SD), years	3.63 (27.3)	3.43 (3.88)
Male gender, n	22	5
Foci infections:		
Bronchopneumonia, n	12	5
Acute diarrhea, n	8	2
Encephalitis, n	4	1
Meningitis, n	1	1
Acute abdomen, n	6	-
Mean TNF-α (SD), pg/mL	12.83 (11.13)	37.55 (23.06)

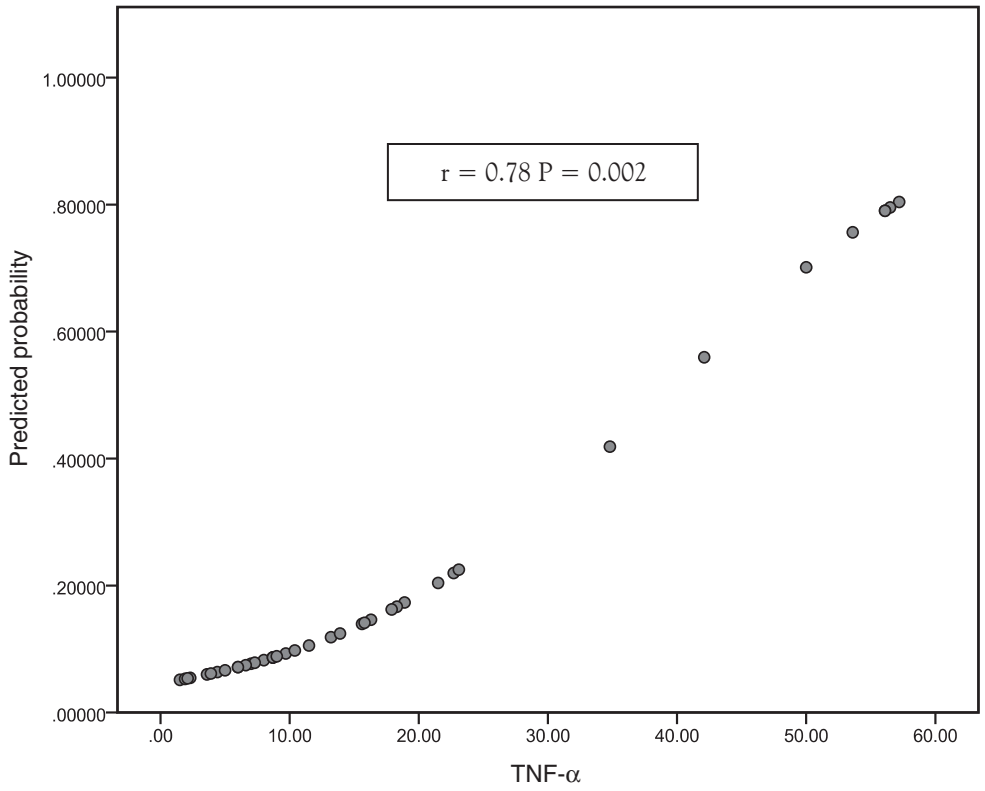

Figure 1. Correlation between TNF-α plasma level and probability of shock in children with sepsis.

Discussion

This study was conducted in the pediatric intensive care unit at Prof. Dr. R.D. Kandou Hospital in Manado. Subjects were recruited by consecutive sampling and consisted of 27 boys and 13 girls. Similar to previous studies, we observed that sepsis was more prevalent in boys than in girls.[13-15]

The most common foci of infections in our study were bronchopneumonia, followed by acute diarrhea, similar to previous studies.[14,16] However, Kumar *et al*.[17] found the second largest foci of infection to be meningitis.

TNF-α was the first circulating, pro-inflammatory cytokine to be widely evaluated in septic patients.[7,9] Previous studies found that TNF-α was a good marker in the diagnosis of sepsis and also valuable in following the effectiveness of treatment and determining prognosis of the disease.[10,11] Moderate quantities of TNF-α contribute to systemic inflammation, while high quantities play a crucial role in septic shock.[18,19]

We found that the mean TNF-α level in the sepsis group was 12.83 (SD 11.13) pg/mL, compared to the septic shock group of 37.55 (SD 23.06) pg/mL. Kocabas *et al*.[10] found a lower mean TNF-α level (> 7.5 pg/mL) that can be used to diagnose a patient with sepsis. Oberholzer *et al*.[20] also found a lower mean TNF-α level, 9.3 pg/mL in sepsis patients and 29.5 pg/mL in septic shock patients. In contrast, Kumar *et al*.[17] reported a higher mean TNF-α level, 47 pg/mL in the sepsis group and 59 pg/mL in the septic shock group. Furthermore, Heper *et al*.[21] also found a higher mean TNF-α level, 76.41 pg/mL in the sepsis group and 123 pg/mL in the septic shock group. These differences might be due to the timing of blood sample collection in different phases of illness.

Logistic regression analysis revealed a highly positive, significant correlation between TNF-α level and the probability of shock in children with sepsis ($r = 0.78$, $P = 0.002$). We found that higher TNF-α level were associated with higher probability of shock during sepsis. This result was consistent with a study

by Bozza *et al*.[11] that showed plasma level of TNF-α in septic shock patients to be significantly higher than those in sepsis patients. Furthermore, Heper *et al*.[21] in Turkey suggested that TNF-α level in patients who died were higher than TNF-α level in patients who recovered (P=0.032).

A limitation of this study was our not performing blood cultures. Also, we only collected one blood sample from each patient at the time of hospital admission, for the purposes of measuring plasma TNF-α level. Since plasma TNF-α level peak several hours after a Gram negative bacteria infection, we may not have accurately assessed maximum TNF-α level in our subjects.

In conclusion, we find increased TNF-α level in children with septic shock and a strong positive correlation between TNF-α level and the probability of shock in children with sepsis, with higher TNF-α level associated with higher probability of shock during sepsis. We suggest that plasma TNF-α concentration can be used as a laboratory examination to help evaluating the probability of shock in sepsis patients. Further study is needed to determine a cut off TNF-α level for predicting the occurrence of shock in sepsis patients.

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