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Short Communication

Association between tumor necrosis factor-α gene polymorphism and interleukin-6 level with mortality of neonatal sepsis

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Abstract

Sepsis is a systemic infection that significantly causes morbidity and mortality among neonates, which is associated with immature immune response. Variations in the tumor necrosis factor-alpha gene (*TNF-α*) -308G/A may be linked to neonatal sepsis mortality by modulating interleukins (ILs) involved in the immune response cascade, such as IL-6. The aim of this study was to investigate the association between *TNF-α* -308G/A gene variation and IL-6 level with mortality of neonatal sepsis. A cohort of 30 neonates diagnosed with clinical sepsis was recruited. Blood culture was performed for all patients and serum IL-6 levels were examined 24 hours after suspected sepsis. Genetic analysis of *TNF-α* single nucleotide polymorphisms (SNP) -308G/A was conducted using polymerase chain reaction and DNA sequencing. The association was assessed based on bivariate logistic regression. We found that 12 (40%) of 30 patients had blood culture-proven sepsis. Genotype of *TNF-α* -308G/A stratified of the patients was 56.7% for GA and 43.3% for GG. There were no AA variations found in this study. There was no significant association between the *TNF-α* -308 G/A genotype and mortality in neonatal sepsis (p=0.211). Similarly, the allelic model of *TNF-α* -308 gene had no association with mortality (p=0.325). Additionally, there was no association between serum IL-6 level and mortality in neonatal sepsis (p=0.253). In conclusion, SNP of *TNF-α* -308 gene and IL-6 level are not associated with mortality in neonatal sepsis.

Keywords: Genetic variant, IL-6, mortality, neonatal sepsis, TNF-α

Introduction

Sepsis is a life-threatening organ dysfunction caused by immune dysregulation against infection [1,2]. Neonatal sepsis is characterized by the presence of bacteria in body fluids such as blood, bone marrow or urine that occurs in the first month of life [3]. Based on the data from Global Burden of Disease in 2019, the annual incidence of neonatal sepsis was estimated to be 6.31 million cases, marking a 12.79% increase compared to 1990 [4]. The age-standardized incidence and mortality rates of neonatal sepsis reached 97.43 and 3.5 per 100,000 live births in 2019, respectively [4]. A higher incidence rate (2,824 cases per 100,000 live births) was reported in a meta-analysis that included studies published as of May 2019, with an estimated mortality rate of 17.6% [5]. Infants with premature birth and very low birth weight are the most vulnerable group [5]. The threat of infections during the neonatal period is a significant concern, particularly with the rise of multidrug-resistant bacteria and emerging viruses [6-9].

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During the course of sepsis, exaggerated inflammatory reaction of the innate immune system following systemic infection is responsible for organ dysfunction [10]. This response is regulated by cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukins (IL)-6 that act as inflammatory promoters [11]. Previous studies suggested that septic neonates experienced elevated plasma TNF-α and IL-6 levels as compared to those in stable conditions [12,13]. Susceptibility and outcome of sepsis among neonates are believed to be influenced by genetic variations. Single nucleotide polymorphisms (SNP) within TNF-α gene (*TNF-α*), particularly at locus -308 (*TNF-α* -308), has been previously found to be associated with the development and progression of neonatal sepsis [14]. Especially, the SNP has been reported to influence the *TNF-α* promoter activity, thus affecting the TNF-α release. A meta-analysis reported an association between the *TNF-α* G/A genotype with the susceptibility to develop neonatal sepsis [15]. However, the association of this genetic variation with mortality of neonatal sepsis is still underreported, particularly among Indonesian population. The aim of this study was to analyze the genetic profile of *TNF-α* -308 G/A polymorphism and evaluate its association with mortality rates in neonates diagnosed with sepsis. In addition, the present study also identified the association of IL-6 levels with mortality rates.

Methods

Study design and setting

This study employed a prospective cohort design to investigate the association between *TNF-α* -308G/A gene variation, IL-6, and mortality in neonatal sepsis. Neonatal subjects were recruited using a non-probability sampling method. The study population comprised neonates admitted to the neonatal intensive care unit (NICU) of Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia, between September 2023 and April 2024. Sepsis was diagnosed based on clinical symptoms and hematological assessments. Patients were closely monitored from the day of admission until the final outcome, categorized as either survival or mortality.

Study populations and sample size

The study utilized a consecutive total sampling approach, employing a non-probability sampling technique. Sample selection was carried out by determining subjects who met the research criteria until a certain period of time so that the required number of samples was achieved. Subjects in this study were neonates admitted to NICU of Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia, with suspected sepsis based on clinical symptoms and hematological examinations. Only neonates within the first 28 days of life were included. Subjects with gestational age <34 weeks, birth weight <1500 gram and congenital anomalies were excluded. Moreover, we only included singletons that did not have any histories of metabolic disorders or other severe illnesses not related to sepsis. A sample size of 30 neonates was selected due to resource constraints and the availability of patients meeting the inclusion criteria within the study period. This sample size was considered appropriate based on the recommendations outlined in the methodology literature [16]. Additionally, the chosen sample size was consistent with previous studies on neonatal sepsis and gene polymorphism [17]. A flow diagram depicting the subject recruitment in the initial phase and follow-up period is presented in **Figure 1**.

Blood sampling

Blood samples were collected during 24 hours of clinical sepsis and before antibiotic therapy. Sampling was performed with the infant at a stable temperature (in an incubator). In addition, 3–5 ml samples were collected from venous blood using 24–22 gauges IV catheter. The sample is then placed in a yellow tube. The collected blood sample is then centrifuged at 3000 rpm for 15 minutes to separate the serum and plasma. In addition, routine blood tests, IL-6 and *TNF-α* -308 G/A polymorphism profiles were performed. The blood samples used were non-hemolyzed and frozen serum.

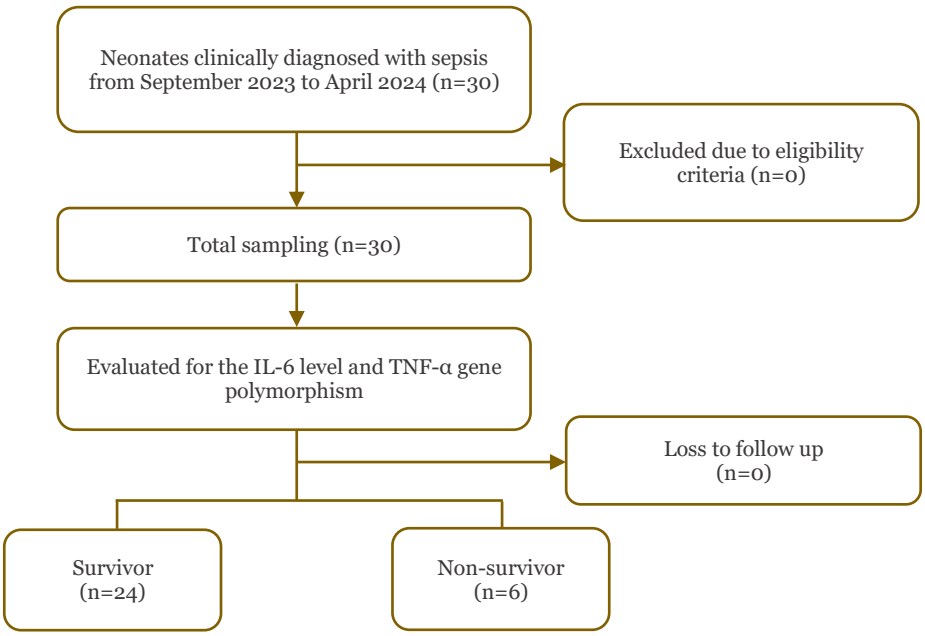

Figure 1. The flow diagram for observational studies illustrating the number of subjects recruited and included in the analysis.

SNPs genotyping

Blood was drawn from a vein using a 3 mL syringe and collected into EDTA tubes. Blood was taken before the administration of any pharmacological therapy, such as antibiotics. The extraction reagent used was Genomic DNA Mini Kit (Blood/Cultured Cell), (Geneaid Biotech Ltd., New Taipei City, Taiwan). The PCR master mix used was TaqMan GTX press Master Mix (Applied Biosystems, CA, USA). The thermocycler used was the CFX96 Touch Real-Time PCR System (Bio-Rad, CA, USA). Genotyping of *TNF-α* -308 G/A (rs1800629) polymorphism was performed by analyzing rs1800629 using TaqMan® SNP Genotyping Assay (ID: C_7514879_10) (Applied Biosystems, CA, USA). The results were confirmed by sequencing, which was performed by the 1st BASE DNA Sequencing Division, Apical Scientific Laboratory, Selangor, Malaysia.

Statistical analysis

The distribution normality was assessed using the Shapiro-Wilk test. Continuous data with normal distribution were presented in mean values and standard deviation (mean±SD), while those with abnormal distribution were presented in median (interquartile range, (IQR)). Categorical data were presented as frequencies. Bivariate logistic regression analysis was performed to calculate the odd ratio (OR) and its 95% confidence interval (CI). Significant differences in IL-6 levels between genotypic or allelic groups were observed through the Mann-Whitney U test. A *p*-value of less than 0.05 was considered statistically significant. All statistical analysis was performed on Jamovi 2.3.28 (The Jamovi Project, Sydney, Australia).

Results

Characteristics of patients

The characteristics of the recruited neonates are presented in **Table 1**. Most of the patients were male (n=18; 60%) and weighed ≥2.5 kg at birth (n=25; 83.3%). The median age was 10.5 (14.5) days. All patients exhibited warm extremities, where only 5 (16.7%) and 11 (57.9%) had pale and jaundice symptoms, respectively. The mean temperature, heart rate, and respiratory rate of the patients were 36.78±0.15°C, 134.2±11.51 bpm, and 43.1±2.37 time per minute (tpm), respectively. The median values of hemoglobin, hematocrit, erythrocyte, and platelet were 12.8 (4.27) gr/dL, 29.5 (15%), 5.1 (2.97) 10^6 cells/µL, and 153.0 (327.1) 10^3 cells/µL, respectively. Six patients (60%) did not survive during the end of the follow-up period, while 24 (80%) survived. The mean leukocyte count was 12.04±5.110^3 cells/µL.

Table 1. Characteristics of septic neonates recruited in this study

Variable	Frequency (%)
Sex	
Male	18 (60)
Female	12 (40)
Birth weight (kg)	
≥2.5	25 (83.3)
<2.5	5 (16.7)
Age, median (IQR) (days)	10.5 (14.5)
Warm extremity	
Yes	30 (100)
No	0 (0)
Pale	
Yes	5 (16.7)
No	25 (83.3)
Jaundice	
Yes	11 (57.9)
No	19 (42.1)
Vital sign	
Temperature, mean±SD (°C)	36.78±0.15
Heart rate, mean±SD (beat per minute)	134.2±11.51
Respiratory rate, mean±SD (time per minute)	43.1±2.37
Blood parameters	
Hemoglobin, median (IQR) (gr/dL)	12.8 (4.27)
Hematocrit, median (IQR) (%)	29.5 (15)
Erythrocyte, median (IQR) (10^6 cells/ µL)	5.1 (2.97)
Platelet, median (IQR) (10^3 cells/µL)	153.0 (327.1)
Leukocyte, mean±SD (10^3 cells/µL)	12.04±5.1
Outcome	
Dead	6 (20)
Survived	24 (80)

Distribution of *TNF-α* -308 gene variation

Among all patients, most of the genetic variant had homozygous GG genotype (43.3%), with heterozygous GA genotype occupying 56.7% of the total patients. None of the patients had AA genotype for the *TNF-α* -308 gene (0%).

Factors associated with neonatal sepsis mortality

Sex (p=0.710), age (p=0.263), birth weight (p=0.124), and having proven or unproven blood culture (p=0.710) were not associated with mortality in neonatal sepsis (**Table 2**). Genotypic model of *TNF-α* -308 gene variation (GG versus GA) showed no significant association with neonatal sepsis (OR:0.30; 95%CI: 0.04–1.98; p=0.211). In the allelic model (G versus A), the association was also not observable (OR: 0.44; 95%CI: 0.09–2.26; p=0.325). The serum IL-6 level was not associated with mortality (OR: 1.05; 95%CI: 0.96–1.15; p=0.253) (**Table 2**).

Table 2. Factors associated with mortality of neonatal sepsis

Variable	Frequency (%)		OR (95%CI)	p-value
	Survivor (n=24)	Non-survivor (n=6)		
Sex				
Male	15 (62.5)	5 (83.3)	Ref	Ref
Female	9 (37.5)	1 (16.7)	0.70 (0.11–4.59)	0.710
Age, median (IQR) (day)	13.5 (14.3)	7 (4.5)	1.07 (0.95–1.20)	0.263
Birth weight, mean±SD (kg)	2.975±0.371	2.625±0.319	1.00 (0.99–1.01)	0.124
Blood culture				
Proven	11 (45.8)	3 (50.0)	Ref	Ref
Unproven	13 (54.2)	3 (50.0)	0.70 (0.11–4.59)	0.710
IL-6, median (IQR) (pg/mL)	13.9 (20)	14.1 (15.3)	1.05 (0.96–1.15)	0.253
TNF-α -308 SNP				
Genotype				
GG	9 (37.5)	4 (66.7)	Ref	Ref
GA	15 (62.5)	2 (33.3)	0.30 (0.04–1.98)	0.211
Allele				
G	33 (68.75)	10 (83.33)	Ref	
A	15 (31.25)	2 (16.67)	0.44 (0.09–2.26)	0.325

Association between IL-6 and *TNF-α* -308 gene variation

The difference in serum IL-6 levels based on the genotypes or alleles of the *TNF-α* -308 gene is presented in **Figure 2**. For genotype GG versus genotype GA, the median (IQR) of IL-6 levels were 20.5 pg/mL (13.9 pg/mL) and 13.8 pg/mL (16.1 pg/mL), respectively. As for allele G versus allele A, the median values were found to be 14.1 pg/mL (16.6 pg/mL) and 13.8 pg/mL (16.1 pg/mL), respectively. No significant association was found, either in genotypic (p=0.917) or allelic group (p=0.928).

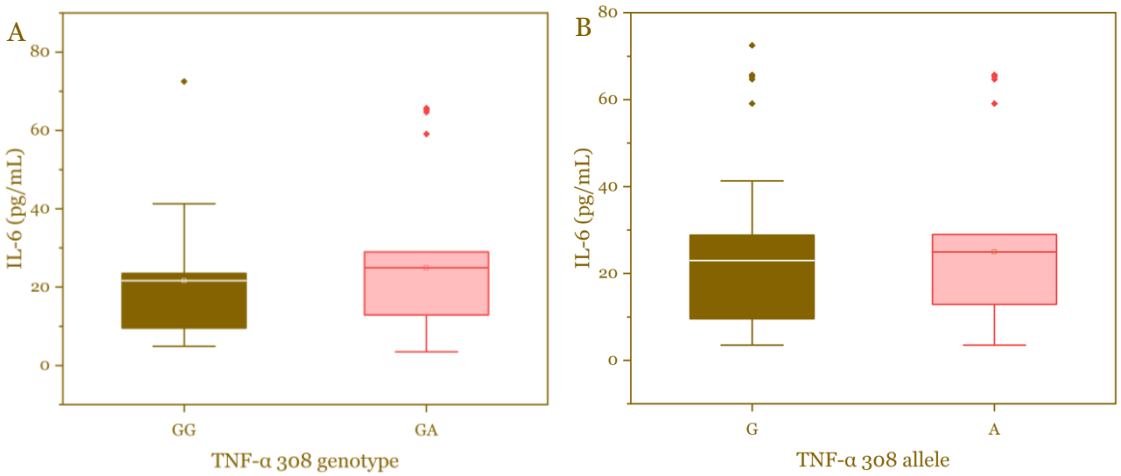

Figure 2. Distribution of serum IL-6 levels according to genotypic (A) and allelic (B) models of *TNF-α* -308 SNPs. The IL-6 levels between groups were not significantly different at $p<0.05$ based on Mann-Whitney U test.

Discussion

Patients recruited in the present study were predominantly males and those with a birth weight over 2.5 kg, with a median age of 10.5 (14.5) days. Despite warm extremity was observed in all patients, only a few patients had pale and jaundice symptoms. In previous studies, the total sample was predominated by males, where this group was found to be more susceptible to neonatal sepsis [18,19]. Characteristics of the patients in this study also shared similarities with previous studies performed in different countries [20-22]. The inflammatory response in sepsis often leads to signs of shock which may compromise peripheral circulation, resulting in changes that indicate worsening of the patient. A previous report suggested that clinical manifestations of neonatal sepsis may include unstable body temperature, hypotension, poor peripheral perfusion (pallor), tachycardia or bradycardia, respiratory distress, spontaneous bleeding, jaundice, seizures and acid-base balance abnormalities [23]. Respiratory distress occurrence is common in the previous study [24]. Clinical manifestations in sepsis infants are diverse across populations, where some may require confirmation through supporting tests such as hematology and other sepsis markers such as IL-6.

In the present study, we found that the *TNF-α* -308 gene variation was not associated with mortality in neonatal sepsis, and neither did the IL-6 level. This finding aligns with previous research that reported no association between *TNF-α* -308 G/A genotype variant with the development and severity of neonatal sepsis [25]. Moreover, a meta-analysis suggested the absence of association between *TNF-α* -308G/A polymorphisms and sepsis-related mortality [15]. In contrast, other studies suggested a significant association between this genetic variant with the development and mortality of neonatal sepsis [26,27]. The *TNF-α* -308 polymorphism with at least one allele A has been witnessed to reduce the risk of sepsis susceptibility and mortality [26]. Allele A is believed to have a protective role against sepsis progression into septic shock, consequently leading to reduced mortality [26,27]. As for IL-6, previous studies suggested that IL-6 is a significant indicator for predicting mortality among septic neonates [28,29]. Differences of these studies may be attributed to differences in the characteristics and ethnicity of the subject and study population. It also might cause by the sample size and differences in study design.

Neonates who develop sepsis may exhibit increased levels of circulating TNF-α and IL-6 cytokines in plasma when compared to healthy infants [30]. These cytokines are classified as early-phase biomarkers of early-onset sepsis [31, 32]. TNF-α plays an important role in the acute inflammatory response by inducing the release of various inflammatory mediators and activating immune and structural cells [33]. Genetic variations in the gene that expresses TNF-α can affect its circulating levels [26]. For instance, allele A of the *TNF-α* -308 G/A polymorphism is associated with higher TNF-α expression [26]. Meanwhile, interleukin-6 is released during the early response to infection before the increase of C-reactive protein and release of TNF-α. IL-6 is synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, decidua, chorion, amnion and trophoblast immediately after the stimulation of microbial infection [33]. The diagnostic sensitivity of IL-6 for neonatal sepsis can reach 100% [31].

The findings of the present study may be limited by the size of the research sample taken over a period of time and tend to be a minimal sample. This study obtained samples from neonates admitted to the NICU of Dr. Zainoel Abidin Hospital and tended to be of the same ethnicity and not diverse. This study included infants who met the inclusion criteria for clinical sepsis. However, not all cases were confirmed by blood culture. Future studies should recruit a larger, more diverse sample and include control groups with varied ethnicity, birth weight, and maternal history to achieve more representative results.

Conclusion

Both genotypic (GG versus GA) and allelic models (G versus A) of the *TNF-α* -308 gene variation were not associated with mortality in neonatal sepsis patients in this present study. Similarly, no association was found between mortality and serum IL-6 level. The genotypic and allelic variations of *TNF-α* -308 gene were also not associated with the serum IL-6 level. Further investigation with a larger sample size is warranted.

Ethics approval

The study was approved by the ethical committee for health research of Dr. Zainoel Abidin Hospital Banda Aceh, Indonesia number: 132/ETIK-RSUDZA/2023.

Acknowledgments

Authors have nothing to declare.

Competing interests

Authors have no known conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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