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TUMOR NECROSIS FACTOR ALFA (TNF- α) LEVEL IN DENGUE FEVER (DF) AND DENGUE HEMORRHAGIC FEVER (DHF) PATIENTS

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Abstract

Dengue virus infection is still one of the health problems in tropical and subtropical regions. In its pathogenesis, some cytokines play an important role in the natural and adaptive immune response to the severity of dengue virus infection. Replication of dengue virus that activates macrophages will release cytokines for tumor necrosis factor (TNF- α), interleukin-1 (IL-1) and interleukin-12 (IL-12). This study aims to determine whether there are differences in mean TNF- α levels in DF and DHF patients. This study is an analytic observational study using cross-sectional design, where blood samples were taken from 27 people with DF and 27 people with DHF. Serum TNF- α levels were analyzed with ELISA and TNF- α levels were analyzed statistically with Mann-Whitney test. The results showed the mean TNF- α level in DF patients was 76.82 ± 1.20 pg/mL and in DHF patients was 195.15 ± 30.30 pg/mL. This mean differed significantly ($p < 0.05$). Based on the results of the study it can be concluded that there are differences in TNF- α levels in DF and DHF patients.

Keywords: TNF- α , dengue fever, dengue hemorrhagic fever, adult.

Abstrak

Infeksi virus dengue masih merupakan salah satu masalah kesehatan di daerah tropis dan subtropis. Dalam patogenesisnya, beberapa sitokin memegang peranan penting dalam respon imun alamiah dan adaptif pada keparahan akibat infeksi virus dengue. Replikasi virus dengue yang mengaktivasi makrofag akan melepaskan sitokin Tumor Necrosis Factor Alfa (TNF- α), Interleukin-1 (IL-1) dan Interleukin-12 (IL-12). Penelitian ini bertujuan untuk mengetahui apakah ada perbedaan rerata kadar TNF- α pada pasien DD dan DBD. Penelitian ini merupakan penelitian observasional analitik yang menggunakan rancangan cross sectional, dimana sampel darah diambil dari 27 orang penderita DD dan 27 orang penderita DBD. Kadar TNF- α serum dianalisis dengan ELISA kemudian data kadar TNF- α dianalisa secara statistika dengan Uji Mann-Whitney. Hasil penelitian menunjukkan rerata kadar TNF- α pada pasien DD adalah $76,82 \pm 1,20$ pg/mL dan pada pasien DBD adalah $195,15 \pm 30,30$ pg/mL. Rerata ini berbeda secara bermakna ($p < 0,05$). Berdasarkan hasil penelitian dapat disimpulkan bahwa ada perbedaan kadar TNF- α pada pasien DD dan DBD.

Kata kunci : TNF- α , demam dengue, demam berdarah dengue, dewasa.

INTRODUCTION

Dengue virus infection is still one of the health problems in tropical and subtropical regions, including Indonesia, which potentially lead to outbreaks and mortality.⁽¹⁾

Currently, 2.5 billion people or half

of world population are estimated to be at risk of dengue virus infection. World Health Organization (WHO) reports that there are approximately 5 – 10 million dengue cases every year.⁽¹⁾ In 2015, dengue infection cases in Bali was known to be higher than the IR (Incidence Ratio) in previous 4 years. One of the factor associated with this

incidence is the lack of diagnosis classification between dengue fever and dengue hemorrhagic fever.⁽²⁾

Pathogenesis of dengue has not been definitive. Some theories have been proposed to justify the pathogenesis, such as virulence and immunopathology theories, which explain heterologous secondary infection hypothesis. Other theories are endotoxin theory, mediator theory, apoptosis theory, genetic theory, and endothelia theory.⁽³⁾ Those mediators are predicted to be responsible for septic shock, fever, and increased capillary permeability.⁽³⁾

Generally, the first dengue virus infection causes antibody production that will neutralize the virus with the same serotype (homologous). Subsequent dengue virus infection with different serotype will cause bonding between previous antibody and the virus. However it will not neutralize the virus. Non-neutralizing antibody-virus complex will attach to Fc receptors of monocyte/macrophage surface. The virus then enter the macrophage, and virus replication occurs, which simultaneously activates macrophage and releases cytokines: *Tumor Necrosis Factor Alpha* (TNF- α), *Interleukin-1* (IL-1) dan *Interleukin-12* (IL-12).⁽⁴⁾

TNF- α has biological effect, that is, it increases adhesive molecules expression on the surface of vascular endothelial molecules (*intercellular adhesion molecule-1*-ICAM-I and *vascular cell adhesion molecule-1* - VCAM-I). In addition, it also increases the selected ligand expression, as well as increasing integrin expression on the leucocyte surface. Expression of the adhesive molecules will induce vascular permeability and leucocyte migration to the infection sites. Furthermore, TNF- α production in large amount may inhibit myocardial contractility, decrease blood pressure (shock), intravascular thrombosis, and *Tissue Factor* (TF) expression.⁽⁵⁾

A study by Alia et al. 2014⁽⁶⁾ found that TNF- α level was higher significantly in hemorrhagic dengue fever (HDF) patients than in dengue fever (DF) patients, and this cytokine can be used as marker to distin-

guish HDF and non-HDF.

A critical phase of dengue virus infection resolves within 48-72 hours and followed by quick recovery phase without remaining symptoms, strongly suggest the roles of mediators or cytokines.⁽⁷⁾

The aim of this study is to determine the difference in mean of TNF- α level between hemorrhagic dengue fever (HDF) patients and dengue fever (DF) patients.

METHOD

The study design was a cross sectional analytical study to measure the mean levels of TNF- α in group of patients with dengue fever (DF) and in group of patients with dengue hemorrhagic fever (DHF). Inclusion criteria were blood samples of DF and DHF patients aged 26 – 45 years old which were obtained from January – June 2017 by Biology Molecular Laboratory, Warmadewa University. Whereas, exclusion criteria were lysed blood samples and also blood samples of patients who had coinfection with acute disease.

Blood sample examination was carried out by ELISA method using Bioassay Technology Laboratory kit at Biomolecular Laboratory of Warmadewa University.

Table 1. Subjects' characteristics

Characteristics	DF (n=27)	DHF (n=27)	p
Age (year)			
Mean \pm SD	34.11 \pm 1.26	34.03 \pm 1.17	0.931 ^{ns}
(Min – maks)	26 – 45	26 – 44	
Gender, n (%)			
Men	15 (55.6)	15 (55.6)	0.608 ^{ns}
Women	12 (44.4)	12 (44.4)	
Headache, n (%)	12 (44.4)	24 (88.9)	0.001*
Myalgia, n (%)	6 (22.2)	12 (44.4)	0.074 ^{ns}
Loss of appetite, n (%)	10 (37)	12 (44.4)	0.0391*
Fever duration (day)			
Mean \pm SE	3.48 \pm 0.21	4.07 \pm 0.24	0.079 ^{ns}
(Min – maks)	2 – 5	2 – 7	
Lowest thrombocyte (10 ³ / μ L)	119.5 \pm 7.54	42.89 \pm 3.99	0.000*
Mean \pm SD			
Hematocrit elevation (%)	10.15 \pm 0.68	28.84 \pm 0.87	0.000*

Characteristics comparison of subjects between DF and DHF groups were analyzed by Mann Whitney test and chi square test with significance level of 0.05. Age, gender, myalgia, and duration of fever between the groups being compared were not significantly different. Meanwhile, there were significant differences in term of headache, loss of appetite, the lowest thrombocyte level, and the percentage of increase in hematocrit in the two groups (<0.05).

A comparison of TNF- α in DF group and DHF group is shown by Table 2.

Table 2. Mean (\pm SE) TNF- α level in DF and DHF patients

Group	Mean TNF- α level (pg/mL)	P
DF	76.82 \pm 1.20	0.000*
DHF	195.15 \pm 330	

Based on the statistical analysis with Mann-Whitney test ($\alpha=0.05$), there was significant difference in mean of TNF - α between DF group and DHF group. The mean of TNF- α level in DF group was lower compared to that in DHF group.

DISCUSSION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are infectious diseases caused by dengue virus. This infection has clinical manifestations like fever, myalgia and/arthritis accompanied by leucopenia, rash, and thrombocytopenia.⁽¹⁾

This study showed that the age did not differ significantly between DF patients and DHF patients (Table 1). This indicates that the risk of developing DHF is the same in adults. The age of subjects involved in this study was of adults with the youngest age was 26 years old and the oldest age was 45 years old. The results also showed that men and women have the same risk of getting DF and DHF infections.

Immunopathogenesis mechanism plays role in dengue infection, and cytokines are among them. TNF- α (*Tumor Necrosis Factor α*) is one of the cytokine types majorly produced by mononuclear phagocyte, which activates and stimulates endothelial to produce aggregation molecules. Dengue virus infection invokes immune responses (monocyte, T helper-1, and T helper 2), which trigger the amplification of TNF- α , *Interleukin-1 β* and *Interleukin-6* and subsequently become stressors to endothelial cells. TNF- α is a solvent factor that plays an important role in the elevation of vascular permeability.⁽⁸⁾

CONCLUSION

There was significant difference in TNF- α mean between DF patients and DHF patients.

RECOMMENDATION

It is suggested to conduct a following research on the levels of severity in dengue virus infection related to TNF- α and other cytokines such as IL-1, IL-6, etc.

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