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The Relationship between TNF- Levels and Platelet Counts in Dengue Hemorrhagic Fever Patients in RSUD DR. Soedjono Selong, East Lombok Regency, West Nusa Tenggara Province

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A B S T R A C T

Dengue hemorrhagic fever is an infectious disease caused by the dengue virus (DENV) and transmitted through the Aedes aegypti mosquito.Macrophage infection by dengue virus causes activation of T-helper and T-cytotoxic so that lymphokines and interferon gamma are produced. Interferon gamma will activate monocytes so that various inflammatory mediators are secreted such as TNF-, IL-1, IL-6 which results in endothelial cell dysfunction and plasma leakage. Vascular disorders that occur are characterized by a decrease in the number of platelets. This study aims to determineThe relationship between TNF- levels and the number of platelets in patients with dengue hemorrhagic fever at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province. Analytical observation with cross-sectional design. This research was conducted in RSUD DR. Soedjono Selong, the time of the study was April-September 2021. The sample of this study was dengue hemorrhagic fever patients who were hospitalized at RSUD DR. Soedjono Selong as many as 70 people. This research uses the method*consecutive sampling,* with the independent variable in the form ofTNF- and the dependent variable in the form of platelets. The platelet count was checked withThe Sysmex XN 550 autoanalyzer uses the hydrodynamic impedance counting method (sheath flow DC method) and checks the levels ofTNF-α with ELISA kit. Data analysis was carried out using SPSS, namely univariate test, bivariate test with Chi-square. There were 27 (38.6%) males and 43 (61.4%) females with a total of 70 patients. Withthe age of the youngest patient was 1 year and the age of the oldest patient was 66 years with a mean age of 24 years ± 20 years. All 70 dengue hemorrhagic fever patients had normal TNF- levels. There were 30 (48.6%) with normal platelet counts and 36 (51.4%) with abnormal platelet counts. And found a non-significant relationship between levels ofTNF- with the number of platelets with a value (p = 0.908). There is no relationship between levels ofTNF- with platelet count in dengue hemorrhagic fever patients in RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province.

BACKGROUND

Dengue hemorrhagic fever is an infectious disease caused by the dengue virus (DENV) and is transmitted through infected Aedes aegypti and Aedes albopictus mosquitoes. This disease is a global health problem and is a disease with high morbidity and mortality in children and adults. This disease is spread throughout tropical and subtropical countries including Indonesia (Sudoyo, 2015, Candra, 2013). The World Health Organization (WHO) estimates that about 2.5-3 billion people currently live in dengue transmission zones. Dengue hemorrhagic fever is an acute febrile disease triggered by infection with the dengue virus (DENV). DENV is a positive single-stranded RNA flavivirus, a member of the family Flaviviridae. This virus has four main serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) (Wang et al., 2020). Dengue fever can appear throughout the year and can affect

all age groups. DHF cases annually reach 390 million people (Runge et al., 2014; Ebi & Nealon, 2016).

DHF patients in Indonesia were reported in 2017 as many as 59,047 cases with 444 deaths, thus the IR (Incidence Rate) of DHF in 2017 was 22.55 per 100,000 population and the Case Fatality Rate (CFR) was 0.57% (Ministry of Health, Republic of Indonesia). , 2018). Dengue Hemorrhagic Fever (DHF) in NTB Province is still a public health problem. It was reported that 2,697 cases of DHF out of which 21 people died, with an IR (Incidence Rate) of 50.9 per 100,000 population and a Case Fatality Rate (CRF) of 0.8%. East Lombok Regency is one of 10 regencies/cities in NTB Province. East Lombok has the largest population compared to other districts in NTB Province. The number of dengue cases in East Lombok in 2021 reached 484 cases (NTB Provincial Health Office, 2021).

Dengue hemorrhagic fever can cause various clinical manifestations, namely high fever, joint pain, and vascular damage. Dengue hemorrhagic fever can be fatal when it reaches the condition of Dengue Shock Syndrome (DSS). This condition can cause death for the sufferer (Sudoyo, 2015). The occurrence of macrophage infection by dengue virus causes activation of T-helper and T-cytotoxic so that lymphokines and interferon gamma are produced. Interferon gamma will activate monocytes so that various inflammatory mediators are secreted such as IL-1, IL-6 and TNF-α which result in endothelial cell dysfunction and plasma leakage. Vascular disorders that occur are characterized by decreased platelet count, plasma leakage, increased hematocrit, and damage to vascular endothelial cells.

Research on TNF- levels and platelet counts in cases of dengue hemorrhagic fever has been widely carried out in Indonesia. However, research on TNF- levels and platelet counts in cases of dengue hemorrhagic fever, especially in NTB Province, has not been carried out. Therefore, researchers are interested in knowing the relationship between TNF- levels and platelet counts in patients with dengue virus infection at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province.

SUBJECT AND METHOD

Study Design, Population and Sample

Cross-sectional study. This research was conducted in RSUD DR. Soedjono Selong, East Lombok Regency. This research was conducted in April-September 2021. The target population is all patients with dengue hemorrhagic fever in RSUD DR. Soedjono selong. The source population was dengue hemorrhagic fever patients who were hospitalized in April-September 2021. The sampling technique was consecutive sampling. Sample size based on formula*Lameshow*ie 70 samples.

Research variable

The dependent variable in this study is platelets while the independent variable is TNF-α. TNF- is a very potent pro-inflammatory cytokine that plays an important role in the pathogenesis of various diseases. Platelets are the number of blood platelets examined in patients with dengue hemorrhagic fever. Examination of platelet count with Autoanalyzer Sysmex XN 550 using hydrodynamic impedance counting method (sheath flow DC method) and examination of TNF-α levels with ELISA kit.

Research Flow Summary

The sample in this study were patients who were hospitalized and were willing to sign the consent form to become respondents.

Blood and serum for examination were obtained from dengue hemorrhagic fever patients who were hospitalized and brought to the Clinical Pathology Laboratory of RSUD DR. Soedjono Selong. Serum was obtained from venous puncture, centrifuged at 5000 rpm for 10 minutes, at room temperature. The supernatant was taken carefully, and if resuspension occurred, it was centrifuged again. The supernatant taken can be used immediately or stored at -20 0C.

Examination of the platelet count with the Autoanalyzer Sismex XN 550 using the hydrodynamic impedance counting method (sheath Flow DC method) in a way that is before using the tool, quality control and background are carried out first, then the results of quality control and background must fall within the reference value range of QC and Background. Then 25 L of the EDTA sample was aspirated by the apparatus. Furthermore, it will be processed in the tool, then the sample will be processed in the CBC 5 differential count tool. The results are issued by the tool, verification and validation are carried out with units of X 103/ L.

Level checkTNF-α with the ELISA method was carried out according to the examination procedure in the Elisa kit. Results are read with Elisa Reader.

Data analysis

All data analysis was performed using SPSS® version 25 (IBM Corp., Chicago). The data obtained on each variable are presented descriptively in tabular form. Data were analyzed using Chi-Square test with a significance level of $p<0.05$. To find out the relationship between levelsTNF- with platelet count in dengue hemorrhagic fever patients in RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province.

Research Ethics

The protocol of the study was approved by the medical and health research ethic committee Faculty of Medicine, Al-Azhar Islamic university with certificate No. 09/EC/FK-06/UNIZAR/III/2021, on 9th of March 2021.

RESULTS

Sample Characteristics

The sample in this study were dengue hemorrhagic fever patients who were hospitalized at RSUD DR. Soedjono Selong numbered 70 people. There were 27 (38.6%) males and 43 (61.4%) females. With the youngest age of 1 year and the oldest age 66 years with an average age of 24 years ± 20 years. From a total of 70 patients with dengue virus infection had normal TNF- levels and 34 (48.6%) normal platelet counts and 36 (51.4%) abnormal platelet counts were found.

Table 1. Sample characteristics

Characteristic	Category	Frequency	Percentage
Gender	Man	27	38.6%
	Woman	43	61.4%
TNF-α	Normal	70	100%
	Abnormal	0	0%
Platelets	Normal	34	48.6%
	Abnormal	36	51.4%

Table 2. Baseline characteristics

Variable	mean	SD	Min.	Max.
Age (Years)	24.60	20.22	1	66

Tabel 2
Kategorisasi *Burnout*

Kategori	Rentang Skor	Frekuensi	Persentase (%)
Tinggi	35 ≤ X	45	68,2%
Sedang	25 ≤ X < 35	16	24,2%
Rendah	X < 25	5	7,6%
Total		**66**	**100%**

Bivariate Analysis

Bivariate analysis in this study was to find the relationship between TNF- levels with platelet counts, the results showed that there was no relationship between TNF- levels and platelet counts.(p<0.908).

Table 3. Relationship of TNF-α with Platelets (Chi Square analysis)

Variable	Platelets				p
	Normal		Abnormal		
	n	%	n	%	
TNF-α					
Normal	34	48.6	36	51.4	0.908
Abnormal	34	48.6	36	51.4	

DISCUSSION

This research was conducted in April-September 2021, at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province. The purpose of this study was to determine the relationship between TNF- levels and platelet count in patients with dengue hemorrhagic fever. This type of research is analytic observational with a cross sectional design. Sampling was done by consecutive sampling. The number of samples is 70 people.

Based on the results of research that has been carried out, it is found that the most patients with dengue hemorrhagic fever are women, namely 43 people compared to 27 male patients. The results of this study are in accordance with research conducted by Candra (2013), which found that there were more female patients with DHF, namely 16 people and 11 men. The results of this study also obtained dengue hemorrhagic fever patients with the youngest age 1 year and the oldest patient age 66 years with an average age of 24 years.± 20 years.

The results of this study obtained TNF- levels for all patients with dengue hemorrhagic fever, which amounted to 70 people had normal TNF- levels. The average value of TNF- level was 31,244 pg/ml with the lowest TNF- level of 27,345 pg/ml and the highest level of TNF- of 32.164 pg/ml. According to Supit, 2015 that normal TNF- levels are 10-100 pg/ml.

TNF- is the main cytokine in the acute inflammatory process (Baratawidjaja, 2012). Normal levels of TNF- as a cytokine are very low, namely 1x10-9 (Abbas et al., 2012). Severe infections can trigger the production of large amounts of TNF- and cause systemic reactions. At low levels, TNF- acts on leukocytes and endothelium, inducing acute inflammation. At moderate levels, TNF- plays a role in systemic inflammation and at high levels, TNF- can cause pathological abnormalities of septic shock.

Increased levels of TNF- in cases of DHF can be caused by the presence of more dengue virus viremia in the body and continues to increase according to the severity, which causes infected macrophages to produce more TNF- cytokines. At the same time, the levels of other anti-inflammatory cytokines are still low and cannot inhibit the production of TNF- cytokines, causing pathological effects (Aliah, 2011). This condition known as cytokine storm has been believed to be the basic pathogenesis of the clinical manifestations of more severe viral infections. Cytokine storm effects directly on vascular endothelial cells by increasing capillary

permeability and causing plasma leakage (Rathakrishnan et al., 2012).

In this study also found patients with dengue hemorrhagic fever with normal platelet counts as many as 34 (48.6%) people and patients with abnormal platelet counts as many as 36 (51.4%) people. The average platelet count was 167,401.43 cells/mm3 with the lowest platelet count at 2000 cells/mm3 and the highest platelet count at 577,000 cells/mm3 with an average of 167401 ± 136615 cells/mm3. When dengue virus infection occurs, platelets undergo several changes, namely suppression of production in the bone marrow and increased destruction in the periphery, resulting in thrombocytopenia (Dewi, 2015).

Dengue virus infects macrophages in the human body, macrophages produce several cytokines as a result of the infection, namely TNF-, Interleukin-1, and interleukin-12. TNF- showed a significant increase in patients infected with dengue fever compared to TNF- levels in patients infected with hemorrhagic fever (Siskayani et al., 2018). In line with research by Meena et al., (2019) stated that there was an increase in TNF- levels that paralleled a decrease in platelets. An increase of one TNF- unit was associated with a decrease of 160 platelet units.

The results of this study indicate that there is no relationship between TNF- levels and the number of platelets in dengue hemorrhagic fever patients at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province. The results of this study can be influenced due to several factors, namely not grouping between DHF with shock and DHF without shock. TNF- levels in DHF with shock are higher than DHF without shock and the platelet count is lower in DHF patients with shock than DHF without shock (Candra, 2013). Meanwhile, according to Siskayani et al., (2018),TNF- levels will increase when the condition of dengue hemorrhagic fever is in shock, which is twice as much as in the condition of dengue hemorrhagic fever without shock. Swhile in this study, the sample was DHF patients who did not experience shock because TNF- levels were all within normal limits with an average TNF- value of 31,244 pg/ml. Another factor may be due to the inclusion criteria of the patient's age, there is no age limit because toddlers have a different immune response from adults who may also have a different TNF-α response. In addition, there are differences in the instruments or kits used to check TNF- levels so that they can give different values.

The limitation of this study is that there is no control group that can be used as a comparison with the group of patients with dengue virus infection in measuring TNF- levels and platelet counts and measuring TNF- levels done once, namely when the patient is admitted to the hospital. In this study, there was no age limit for patients with dengue hemorrhagic fever. In addition, dengue virus infection can be in several categories where there are slight differences in pathophysiology at that stage, this is a limitation of the study due to limited data and not being followed up (no paired sera).

CONCLUSION

Based on the results of the study, it can be concluded that there is no relationship between TNF- levels and the number of platelets in patients with dengue fever at RSUD DR. Soedjono Selong, East Lombok Regency, NTB Province.

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CONFLICT OF INTEREST

There was non conflict of interest in this study

AUTHOR'S CONTRIBUTION

Herlinawati contributed in making proposals and data collection, Resna Hermawati contributed in data collection, Fahriana Azmi contributed in data processing. Sabariah contributed in data processing.

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