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Correlation Between Leukocyte and Platelet in Patients with Degree 1 and 2 Dengue Fever Ajibarang Hospital

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Abstract

Background: Dengue Hemorrhagic Fever (DHF) is characterized with main clinical features including fever, bleeding, hepatomegaly, abdominal pain, vomiting and hypothermia, and severe plasma leakage. Leukopenia, thrombocytopenia, and increased hematocrit values can be found as abnormal blood profile. Leukocytes are often ignored even though viral infections are usually accompanied by leukopenia. Therefore, researchers wanted to know whether leukopenia could be used as a predictor of worsening of thrombocytopenia in dengue fever patients.

Aim: The purpose of this study was to determine the relationship between leukocytes count and platelets count in DHF grades 1 and 2 patients at the Ajibarang Regional General Hospital.

Method: Analytical observational quantitative research method with a cross-sectional study design in patients with Dengue Hemorrhagic Fever grades 1 and 2 at the Ajibarang Regional General Hospital was carried out in March-May 2022. The variables of leukocyte count and platelet count were obtained from complete blood count by Sysmex XS series. Spearman's test was used to analyze the relationship between two variables.

Result: There was significant difference in hemoglobin, hematocrit, and thrombocyte level between DHF Grade 2 and Grade 1 ($p<0.05$). Spearman Test showed p= 0,833 between leukocytes and platelets count ($p<0.05$).

Conclusion: There is no relationship between leukocyte count and platelet count in patients with dengue hemorrhagic fever grades 1 and 2.

Keywords: Leukocyte Count, Platelet Count, Grade 1 dengue hemorrhagic fever, Grade 2 dengue hemorrhagic fever

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BACKGROUND

Dengue Hemorrhagic Fever (DHF) still becoming a major global health problem worldwide. Approximately 28 countries in worldwide were infected and almost 4 million people live in areas at risk of Dengue transmission[1]. Indonesia is a tropical country where the two main species of Dengue Virus (DENV) mosquito vectors, *Aedes aegypti* and *Ae. Albopictus* were lived[2]. Over the last 50 years, Indonesia has experienced an increase in the incidence of dengue hemorrhagic fever. The incidence rate is cyclic, peaking approximately every 6-8 years. Java Island contributes the highest average number of cases of dengue hemorrhagic fever every year. In recent years, Bali and Kalimantan have had the highest incidence while Papua Island, the easternmost region of the Indonesian archipelago, has had the lowest incidence[3]. High rainfall, especially above 200 mm, causes dense populations of Aedes mosquitoes, especially after rainy season. This is related to the increasing number of natural places for Aedes to breed outside the home[4]. Previous studies have shown that climate greatly influences DENV and dengue vectors[5].

DHF has four clinical features including severe fever, bleeding, often accompanied by hepatomegaly and circulatory failure[6]. Some infected individuals may experience hypovolemic shock resulting from severe plasma leakage. DHF is also characterized by several other signs such as pleural effusion, leukopenia (<4,000/μL), thrombocytopenia (<100,000/μL), increased hematocrit values, fatigue, abdominal pain, vomiting and a sudden decrease in body temperature. If dengue fever did not treat adequately it can end in death[7].

Based on WHO, DHF is classified into 4 criteria, where grades III and IV are considered dengue shock syndrome (DSS). The presence of thrombocytopenia with hemoconcentration events differentiates grade I and II DHF from Dengue Fever (DD) cases[8]. Grade 1 has a clinical symptom namely fever accompanied by another not typical symptoms. the only signs of bleeding that appear are a positive tourniquet test and/or easy bruising. Grade 2 with symptoms similar to grade 1 accompanied by spontaneous bleeding, usually forming in the skin or other bleeding. Meanwhile, grade 3 symptoms are like grade 2 accompanied by circulatory failure as indicated by weak and rapid pulse, narrowing of pulse pressure, hypotension, cold acral, and easy fatigue. Grade 4 shows symptoms like grade 3 accompanied by an unpalpable pulse and blood pressure.

Leukopenia usually can be found in dengue fever patients. Decreasing leukocytes count in DHF patient occurred especially on days 3 to 8, starting with progressive leukopenia. Research from Brazil shows that the average leukocyte counts in patients infected with dengue is $4.6 \times 109/L$ with the lowest number being $0.7 \times 109/L$ and the average leukopenia that can occur in dengue infection probably due to DENV infection reaching bone marrow stromal cells, causing suppression of hemopoiesis thus ending with a decrease in the number of leukocytes production[9].

DHF patients not only experience leukopenia, but also experience severe thrombocytopenia. Severe thrombocytopenia is a significant sign related to dengue fever prognosis. Thrombocytopenia occurs in the final phase of fever and the initial leakage phase due to DENV infection which induces a decrease in the number of platelets, making patients susceptible to spontaneous bleeding. Mechanism of this decrease is still not clear, but it probably due to immunological effects caused by DENV-2 infection which triggers platelet activation which has been shown to be attacked and phagocytosed by immune cells. Previous studies also suggest that Dengue Virus Nonstructural Protein 1 (NS1) can induce humoral immunity[10,11].

Dengue Virus Nonstructural Protein 1 (NS1) that is part of DNV can cross-react with human platelets and endothelial cells, thereby inducing endothelial cell damage and apoptosis. DENV NS1 protein has been reported to cause disruption of endothelial monolayer integrity through activation of macrophages and human peripheral blood mononuclear cells (PBMC) via Toll-like receptor 4 (TLR4)[12]. In addition, binding of DENV NS1 to Toll-like receptor4 (TLR4) on platelets can trigger platelet activation and subsequently aggregate and then attach to the endothelium and be phagocytosed by macrophages, which ultimately causes thrombocytopenia and bleeding.

Research conducted by Triana D, *et al.* discussed the relationship between hematocrit levels, platelet count and leukocyte count on the severity of dengue fever, but there is no relationship was found between leukocyte count and platelet count on the severity of dengue fever[13]. Research conducted by Ugi D, *et al.* also produced similar values where there was no relationship between the number of leukocytes and the severity of DHF[14]. However, based on previous theory, severe dengue fever is associated with high cross-reaction between the endothelium and antibodies, resulting in massive plasma seepage. Endothelial cross-reaction is directly proportional to the cross-reaction that occurs in platelets, causing platelet damage due to increased antibodies, resulting in severe thrombocytopenia. The high number of antibodies formed is also directly proportional to the number of circulating DENV infections. Higher DENV titer showing severe the bone marrow stromal cell infection that can occur, resulting in suppression of hemopoiesis and ending in a decrease in the number of leukocyte production.

Based on this description, this study aims to find the relationship between the number of leukocytes and the number of platelets and dengue hemorrhagic fever infection degrees 1 and 2.

METHOD

This research is a quantitative type of research with an analytical observational approach and uses a cross-sectional research design.

Data Collection and Analysis Techniques

Respondent from this search was obtained using the consecutive sampling method. Data used in this study are primary data that obtained from the results of measuring the number of leukocytes and platelet counts in venous blood and secondary data regarding the diagnosis of DHF based on the medical records of patients at Ajibarang Regional Hospital.

Univariate analysis was carried out to obtain the average value (mean), standard deviation, median value, minimum value, maximum value, frequency and percentage of each research variable.

Normality analysis was carried out using the Shapiro Wilk Test. Bivariate analysis was carried out to find the relationship between the number of leukocytes and the number of platelets and the incidence of grade 1 and 2 dengue hemorrhagic fever using the Spearman test.

Research procedure

Individuals who meet the inclusion and exclusion criteria and are willing to take part in this study are become respondent. Gender, age, vital signs and duration of symptoms since the onset of fever were collected from respondent. Respondent from this study had 3 cc of venous blood taken. Measurement of the number of leukocytes and number of platelets using a Sysmex series XS Hematology analyzer machine. The data that has been obtained will be coded and analyzed using statistical software.

RESULT

This study was carried out in the laboratory and medical records of Ajibarang Regional Hospital by taking a total sample of 37 people suffering from DHF Grade 1 and 2. This number exceeded the sample size calculation to strengthen the significance value. The 37 research subjects consisted of 20 patients with grade 1 DHF and 17 patients with degree 2 DHF. Table.1 Data on the characteristics of each group of research subjects for degree 1 and 2 dengue fever showed that hemoglobin and hematocrit levels increased even though they were still within the normal range, which were suspected of having more severe hemoconcentration in degree 2 compared to degree 1.

Table 2. showed that there was not significant correlation between the number of leukocytes and the number of platelets in grade 1 and 2 dengue fever patients overall (p = 0.833).

Table 1. Characteristic Respondent

Parameter	Median (min-max) / Mean ± SD (min-max)		p
	DHF Grade 1	DHF Grade 2	
Age (year)	28 (19-59)*	35.88 ± 11.12 (19-55)	0.427
Hemoglobin (g/dL)	13.6 ± 1.2 (11.6-15.9)	15.05 ±2.36 (10.4-18.6)	0.015*
Leukocyte (x 10^3/µl)	3.30 (1.35-9.70)	4.79 (1.34-13.9)	0.094
Thrombocyte (x 10^3/µl)	86.5 (20-171)	23 (4-189)	0.002*
Hematocrit (%)	39.3 ± 2.92 (32.8-45.8)	43.42 ± 6.57 (31.7-54.7)	0.002*
Erythrocytes (x 10^6/µl)	4.76 ± 0.62 (3.78-6.39)	5.25 ± 0.75 (3.94-6.34)	0.180
MCV (Fl)	83.8 (56-95)*	82.56 ± 3.28 (75-88)	0.542
MCH (Pg)	29.7 (19-33)*	28.68 ± 1.22 (26-30)	0.200
MCHC (%)	34.61 ± 1.08 (33-36)	34.75 ± 0.91 (33-37)	0.423

Note: *Significant for Difference Test (p<0.005)

Table 2. Result of Correlation Test

Parameter	Median (min-max)	r	p
Leukocytes (x 10^3/µl)	66 (4-189)	0.036	0.833
Thrombocyte (x 10^3/µl)	3.46 (1.34-13.90)		

DISCUSSION

Respondent in this study were divided into two group based on their grading severity of DHF. Total respondent in this study was 37 patients with DHF, 20 patients in classified as Group DHF Grade 1, and 17 patients classified as Group DHF Grade 2. Characteristic respondent from this study (Table.1) showing that there are no significantly age differences between two group. All respondents are adult at productive age. Hemoglobin, Erythrocyte, MCV, MCH, and MCHC that were usually used as biomarker for anemia condition still in normal range[16]. Result from this study indicate that there are no significant different all of anemia biomarker between two group. Result in this study in line with previous theory that describe that the degree of DHF does not use hemoglobin levels as parameter, but only uses clinical criteria and laboratory criteria consisting of hematocrit levels and platelets level[15]. Hemoglobin level generally change in severe cases accompanied by massive and long-lasting bleeding, unlike DHF which is characterized by spontaneous bleeding[17].

Hematocrit of DHF patients in this study was still within normal limits even though there was any significant difference between DHF Grade 1 dan DHF Grade 2. HCT forms the most important indicator in recognizing dengue complications. Elevated HCT indicate hemoconcentration and possibly impending shock. This is caused by fluid leakage from capillaries that are usually seen in DHF Grade 3 and 4[18].

Differences test between two group in this study showing that there is no significant difference Leukocyte count. Result from this study also indicate that both Leukocyte count respondent in two group were decrease. This result in line with previous study that describe that DHF patient usually experience mild leukopenia to moderate leukocytosis, thrombocytopenia, and normal or increased hematocrit values[19]. Leukopenia will appear between the 1st and 3rd day of fever in 50% of mild dengue cases. This is mostly caused by the degeneration of mature PMN cells and the formation of young PMN cells. Granulocyte concentration decreases between days 3 and 8[20]. During fever, the number of leukocytes and neutrophils begins to decrease, accompanied by relative lymphocytosis. Leukopenia reaches its peak before fever and returns to normal 2 - 3 days after the body temperature decreases to normal[21]. The leukopenia process occurs due to bone marrow depression by the dengue virus. This emphasis process goes through several phases as follows.

Leukopenia occurs due to bone marrow depression caused by direct viral infection process or an indirect mechanism through the production of pro-inflammatory cytokines which suppress the bone marrow[22]. Changes in total Leukocyte count (≤5,000 cells/mm3) and neutrophil to lymphocyte ratio (neutrophil<lymphocyte) are useful for predicting the critical period of plasma leakage[23,24]. Previous study showed that leukopenia (<4,000/mm3) was observed in 96 (55.17%) study cases, while 26 cases (14.94%) had leukocytosis (>11,000/mm3)[22]. Normal leukocyte counts were observed in 52 (29.89%) cases with counts between 4,000-11,000 cells/mm^3 [23]. Another study also showing that 44 DHF patient from total 99 patient had leukopenia[25].

Decrease in platelets count was found in this study. DHF Patient from two group had low platelets count level (<100,000/mm^3). Decrease Platelets count from normal range commonly referred as thrombocytopenia. It occurs due to increased destruction of platelets in the blood, decreased thrombopoiesis, and impaired platelet function. In dengue fever, immune complexes are found on the surface of platelets which are thought to cause platelet aggregation which will then be destroyed by the reticuloendothelial system. When the platelet count is <100,000/mm^3, the function of platelets in hemostasis is disrupted so that vascular integrity is reduced and causes vascular damage. Thrombocytopenia led to bleeding manifestation which can cause shock and worsen the degree of dengue fever [21]. This is in accordance with the results of this study. Difference test show that there are significantly different platelets count from two group. DHF Grade 2 group having lower platelets count than DHF Grade 1 group ($p<0.05$). Patients with a higher DHF grade will have a lower platelet count caused of severity vascular damage and more severe bleeding.

Spearman rank test was performed to analyzed the correlation between leukocyte and thrombocyte count in all grade DHF patient in this study. Result from statistical analysis show that there is no significant correlation between Leukocyte and Thrombocyte in this study. This result in line with previous study. Positive correlation was found between Leukocyte and Thrombocyte but not statistically significant ($p>0.05$)[26,27]. Correlation test was not significant probably cause of limited respondent in this study and it became our study limitation.

CONCLUSION

Result from this study describe that all DHF patient are not experienced anemia condition that it can be seen from Hemoglobin and Eritrosit level. Leukocyte and Thrombocyte respondent from two group in this study decline but only thrombocyte count that has significant difference between DHF Grade 1 and DHF Grade 2. There is not correlation between Leukocyte and Thrombocyte in all respondent in this study.

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None of the authors has any conflict of interest.
The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Informed consent was obtained from all the patients included in the study.

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