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The Correlation between Leukopenia and the Severity of Dengue Hemorrhagic Fever in Pediatric Patients

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Abstract

The aim of this study is to investigate the correlation between leukopenia and the severity of DHF in pediatric patients treated at RSPAL dr. Ramelan Surabaya from January 2021 to December 2022. This analytical observational study employed a cross-sectional design, involving 126 patient records from RSPAL dr. Ramelan Surabaya during the specified period. The sample selection was done using purposive sampling. The variables under investigation were leukopenia on days 2 to 5 and the DHF grade in patients aged 5 to 14 years. The statistical correlation test, Kendall's tau-b, indicated that there is no significant correlation between leukopenia and the severity of DHF in pediatric patients, with a p-value of 0.340, meaning $p > \alpha$ (with $\alpha = 0.05$). The data revealed that out of the patients, 88 (69.8%) had Grade I, 16 (12.7%) had Grade II, 19 (15.1%) had Grade III, and 3 (2.4%) had Grade IV. Leukopenia occurred in 49 (73.1%) Grade I patients, 9 (13.4%) Grade II patients, 7 (10.4%) Grade III patients, and 2 (3%) Grade IV patients. In conclusion, this study found no significant correlation between leukopenia and the severity of DHF in pediatric patients treated at RSPAL dr. Ramelan Surabaya from January 2021 to December 2022.

Introduction

The South East-Asian Region World Health Organization (WHO-SEARO, 2011) states that dengue fever is a dengue virus infection that causes acute fever symptoms. Dengue virus (DENV) has 4 serotypes, namely DENV 1-4 and belongs to the *flavivirus group* (Schaefer *et al* ., 2022) . October 2013, it was discovered that a new serotype (DENV-5) was detected which has the *sylviatic form* of DENV-4 and has some similarities with DENV-2 (Ortiz García, 2013) . This serotype is not yet universally recognized because it is only found in Sarawak and is not transmitted human-to-human (Mustafa *et al* ., 2015) . Female mosquitoes of the species *Aedes albopictus* and *Aedes aegypti* are the primary vectors of the disease . Virus transmission can also occur through blood transfusions, breast milk and organ transplants (Schaefer *et al* ., 2022) . Dengue hemorrhagic fever (DHF) is characterized by an acute onset of high fever such as dengue fever in the initial phase of fever but experiencing a critical phase characterized by plasma leakage which can be seen from blood laboratory tests, platelets < 100,000 cells/mm ^{3} and hematocrit ≥20% . DENV-3 infected 75.9% of the population in Manado compared to other serotypes during the outbreak in 2019 *(Tatura et al.* , 2021) . Asymptomatic initial symptoms create difficulties in early detection which can prevent severity, especially in children (Bodinayake *et al* ., 2021)

In recent decades, there has been a significant increase in cases of dengue fever. Starting in 2000 with 505,430 incidents to 5.2 billion in 2019 (WHO, 2019). It is estimated that more than

50 million cases come from Asian countries, while the death rate is only 2%. Dengue attacks more children in more than 100 countries with a fairly high overall mortality rate of 20 to 25,000 deaths. Globally, increased infectivity is correlated with favorable climatic conditions (Murhekar *et al* ., 2019) . Indonesia is one of the most endemic countries in the world (WHO, 2019). Since the first dengue case was reported in 1968, there have been changes in epidemiology in Indonesia (Setiati *et al* ., 2006) . In 2021, dengue cases are spread across 474 districts with data on 73,518 dengue cases and 705 deaths in 209 districts. The DHF *incidence rate* in 2021 has decreased compared to 2020 to 6,760 (16.8%) but *the case fatality rate* has increased and does not meet the national target of <1% (Ministry of Health, 2021). In 2022, in the 48th week, dengue fever will experience a significant increase with 116,127 cases and 1023 deaths. Shows that 49% of dengue fever attacks children under 14 years of age with 45% of deaths, especially those aged 5-14 years. It is noted that East Java is in second place for cases with 10,751 cases and 127 deaths (Ministry of Health, 2022). The city of Surabaya stated 195 cases of dengue fever (BPS East Java, 2022). In January 2022, 31 cases were reported with the majority aged 5-14 years (Pemerintah Kota Surabaya, 2022). In 2021, dengue fever cases at the Central Naval Hospital dr. Ramelan in pediatric patients reached 71 cases and will increase in 2022 to 204 cases.

The body's defense system against the entry of the dengue virus begins when the dengue virus components come into contact with *host cells* . DENV serotype viruses activate macrophages and human peripheral blood mononuclear cells (PBMC) via *Toll-like receptor* 4 (TLR-4). The virus will enter the dermis so that Langerhans cells and keratinocytes will be infected. Primary viremia begins with the spread of the virus through the blood and infecting immune cells (Martina *et al* ., 2009) . The main targets of DENV infection are immune cells, namely *dendritic cells* , monocytes, macrophages and B cells except CD4+ and CD8+ T cells which will stimulate the apoptosis process (Khanam *et al* ., 2022) . These immune cells will release large amounts of TNF- α type cytokines , which promote inflammation and increase blood vessel permeability in response to apoptosis. (Malavige & Ogg., 2017) . During disease progression, the production of inflammatory cytokines including TNF- α , IL-6 and IL-10 by DENV-infected cells induces the expression of endothelial cell adhesion molecules such as CD62E, CD106 and CD62P, causing inflammation, endothelial damage and plasma leakage resulting in hypovolemic shock resulting in leads to disease severity. (Khanam *et al.* , 2022)

In 2011, WHO classified the severity of dengue fever into 4 *grades* . *Grade* I is fever with bleeding manifestations (positive *tourniquet test*), plasma leakage (platelets < 100,000 cells/mm3 and hematocrit ≥20%). *Grade* II is the symptoms of *grade* I plus spontaneous bleeding . *Grade* III is *grade* I or II plus circulatory failure, weak pulse, narrow blood pressure (≤ 20 mmHg), hypotension and restlessness. *Grade* IV is *grade* III plus sudden shock (no pulsation or blood pressure) . *Grades* III and IV are categorized as *Dengue Shock Syndrome* while *grades* I and II are non-shock DHF (WHO-SEARO, 2011). DHF is clinically divided into 3 phases, namely febrile, critical and healing. The critical phase or *leakage phase* is identified by an increase in body temperature to 37.5 °C to 38 °C accompanied by hematological changes on days 3-7 after the acute fever phase. Significant hematological changes and symptoms in this phase serve to assess the severity of DHF according to the WHO-SEARO classification (2011). Hematological changes are characterized by plasma leakage which results in thrombocytopenia, increased hematocrit and can manifest as fatal symptoms including shock, organ dysfunction, disseminated intravascular coagulation or bleeding (Kalayanarooj, 2011) . Kan and Rampengan (2012) reported that the prevalence of dengue fever associated with shock in Indonesia is 16-40% and the mortality rate is 5.7-50% according to various hospital reports. Research in Denpasar (2021) stated that 29.3% of children experienced *dengue shock syndrome* .

A definite diagnosis of dengue fever is the detection of one of the characteristic characteristics of the virus, namely; serological tests to detect NS1, IgM and IgG antigens in CSF, serum or tissue and molecular tests to detect the dengue virus genome (Mohana & Borges., 2022) . Dengue hemorrhagic fever has bleeding manifestations that can be detected by patient symptoms such as a positive *tourniquet test, petechiae* , ecchymosis, purpura or bleeding from the mucosa, digestive tract, injection site or other locations (WHO, 2011). Evaluation of the course of the disease can be seen from laboratory examinations, especially blood tests. *Complete blood count (CBC)* parameters such as hemoglobin (Hb), hematocrit (Hct), white blood cell count (WBC), differential percentage of SDP and platelet count change in patients infected with dengue fever (Soo *et al* ., 2017) . The main hematological findings in a study conducted in Brazil were leukopenia on day 2 of dengue hemorrhagic fever in 68.3% of patients and thrombocytopenia on day 3 or 4 in 66.5% of patients (De Oliveira *et al* ., 2009) .

Research in Thailand (2008) showed that higher leukocytes were found in patients with mild infections, namely 3,580 cells/µL compared to severe infections such as DHF *grade* II or worse) which amounted to 3,050 cells/µL on day 5. Research at the Lampung District Hospital (2021) also showed different results with an increase in the average number of leukocytes along with increasing DHF *grade* starting with 4,183 cells/µL for *grade* I, 4,754 cells/µL for *grade* II and 6,100 cells/µL for *grade* III . Data from dengue fever patients at Sanglah Hospital, Denpasar (2017) showed leukopenia in dengue fever *grades* I and II on days 4 to 5, while dengue fever *grades* III and IV, which can also be called *dengue shock syndrome,* did not experience leukopenia. This is in contrast to the Denpasar Medical Science Digest (2019) which states that 76.5% of pediatric patients experience leukopenia in DHF *grades* III and IV. Research in Ternate (2020) also stated that DHF with shock, namely *grades* III and IV, showed a severe decrease in leukocyte levels from 9,800 cells/µL to 2,220 cells/µL on day 4, while those who did not experience shock, namely *grades* I and II, did not experience leukopenia.

Based on the explanation above, this study aims to assess the relationship between leukopenia and the severity of dengue fever in pediatric patients treated at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022.

Methods

Research design

The research is an analytical observational study with a *cross sectional approach* which focuses on measuring or observing data at a certain time for the dependent variable and independent variables. The aim is to observe the relationship between one variable and other variables (Wang & Cheng., 2020) .

Research methods

The research design used in this case is quantitative research with analytical observational methods. The research subjects were medical record sheets of patients diagnosed with dengue fever at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022. Then, from all medical record sheets, further analysis was carried out regarding the relationship between leukopenia and the severity of dengue fever in pediatric patients treated at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022.

Population

The target population of this study was pediatric patients diagnosed with dengue fever at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2021. The population covered for this study were all pediatric patients recorded in medical records with a diagnosis of dengue fever at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022 who is undergoing inpatient treatment.

Sample

The sample in this study was selected from an affordable population that met the inclusion criteria and did not meet the predetermined exclusion criteria.

Inclusion criteria

Patients aged 5 to 14 years with a diagnosis of dengue fever at the RSPAL Dr. Ramelan Surabaya from January 2021 to December 2022. Patients who do not have acute infectious diseases from viruses other than dengue (*chikungunya fever* , *Crimean-Congo hemorrhagic fever* , measles, rubella, Epstein-Barr virus, enterovirus, influenza, hepatitis A, hantavirus, COVID-19, rotavirus), bacteria (meningococcemia, leptospirosis, typhoid fever, meliodosis, scarlet fever, urinary tract infections), parasites (malaria). Patients who are not accompanied by systemic diseases (diabetes mellitus and hypertension) and congenital (*congenital heart disease*). Patient data was recorded in the RSPAL medical record, Dr. Ramelan Surabaya from January 2021 to December 2022. The patient's medical record was diagnosed with dengue fever on day 2 to day 5 at RSPAL dr. Ramelan Surabaya from January 2021 to December 2022. There are NS1 test results showing positive or negative anti-IgM and anti-IgG dengue results in the medical records of patients diagnosed with dengue hemorrhagic fever at the RSPAL Dr. Hospital. Ramelan Surabaya from January 2021 to December 2022. There are complete basic hematology laboratory results (Complete Blood Count / CBC) to see the number of leukocytes, platelets, hematocrit in Dr. RSPAL's medical record. Ramelan Surabaya from January 2021 to December 2022.

Exclusion criteria

Patients under the age of 5 years and over the age of 14 years with a diagnosis of dengue hemorrhagic fever at the RSPAL Dr. Hospital. Ramelan Surabaya from January 2021 to December 2022. Patients with acute infectious diseases from viruses other than dengue (*chikungunya fever* , *Crimean-Congo hemorrhagic fever* , measles, rubella, Epstein-Barr virus, enterovirus, influenza, hepatitis A, hantavirus, COVID-19, rotavirus), bacteria (meningococcemia, leptospirosis , typhoid fever, meliodosis, scarlet fever, urinary tract infections), parasites (malaria). Patients with systemic (diabetes mellitus and hypertension) and congenital (*congenital heart disease*) diseases. Patients who died during the course of dengue fever on days 2 to 5 at RSPAL dr. Ramelan Surabaya from January 2021 to December 2022. Patient data was not recorded in Dr. RSPAL's medical records. Ramelan Surabaya from January 2021 to December 2022. The patient's medical record was diagnosed with dengue fever not on day 2 to day 5 at RSPAL dr. Ramelan Surabaya from January 2021 to December 2022. There are no NS1 test results or tests showing negative or negative anti-IgM and anti-IgG dengue results in the medical records of patients diagnosed with dengue hemorrhagic fever at the RSPAL Dr. Hospital. Ramelan Surabaya from January 2021 to December 2022. There are no complete basic hematology laboratory results (*Complete Blood Count* / CBC) to see the number of leukocytes, platelets and hematocrit in Dr. RSPAL's medical record. Ramelan Surabaya from January 2021 to December 2022.

Sample size formula

The minimum sample size required for this research was obtained from the lameshow formula with a *cross sectional research design.*

$$n = \frac{(\mathrm{Z1} - \frac{\alpha}{2})^{2} x\, P(1-P)}{d^2}$$

Information:

N = desired sample size

$Z_{1-\alpha/2}$ = *critical value* and *standard value* for the 95% *level of confidence = 1.96*

P = *Expected prevalence* or prevalence in previous studies

d = *Margin of error or precision*

q = 1-P

$\alpha = 0.05 \rightarrow {}^{\alpha}/_{2} = 0.025$

$$1 - {}^{\alpha}/_{2} = 0.975 \rightarrow Z1 - {}^{\alpha}/_{2} = 1.96$$

$$Z1 - \frac{\alpha^2}{2} = 3.84$$

$$d = 0.1 \rightarrow d^2 = 0.01$$

P = Percentage of leukopenia with DHF = 0.765

$$n = \frac{3.84\ x\ 0.765\ x\ 0.235}{0.01}$$

$$n = 69$$

Calculations using the lameshow formula show the minimum sample size for this research is 69.

Sampling technique

The research sample will be taken from secondary data originating from medical records using a *purposive sampling technique* where all accessible populations that meet the inclusion criteria and do not meet the exclusion criteria will be taken as the research sample.

Research variable

Understanding

The variables used in this research are: (1) Dependent variable: DHF, severity of DHF; (2) Independent variable: Leukopenia

Operational variables

An explanation of the research variables can be seen in table 4.1.

Table 1. Operational Variables

Variable	Operational definition	Measuring instrument	Measure Results	Data Scale
Leukopenia	The number of white blood cells is less than normal per ml	Medical records of hematology laboratory (CBC) results at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2021	1. <4,000/ml 2. 4,000-11,000/ml (Tigner *et al* ., 2022)	Nominal
dengue fever	An acute infectious disease caused by the dengue virus and characterized by fever and bleeding	Medical records of diagnosis results at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2021	1. Detected NS1, IgM or IgG, plasma leakage which can be indicated by laboratory examination (platelets < 100,000 cells/mm3 and hematocrit ≥20%) and may or may not be accompanied by pleural effusion / ascites. 2. NS1, IgM or IgG is not detected and may or may not be accompanied by plasma leakage (platelets < 150,000	Nominal

			cells/mm3 and hematocrit ≥5-10%) and may or may not be accompanied by pleural effusion / ascites. (WHO-SEARO, 2011)	
Severity level dengue fever	Composition of symptoms and signs of increasingly severe dengue hemorrhagic fever	Medical records of RSPAL pediatric patients , Dr. Ramelan Surabaya for the period January 2021-December 2021	1. *Grade* I: positive ≥*tourniquet test and plasma leak (platelets < 100,000 cells/mm3 and hematocrit* 20%) 2. *Grade* II: spontaneous bleeding 3. *Grade* III: circulatory failure (weak pulse and low blood pressure (≤ 20 mmHg), hypotension and restlessness. 4. *Grade* IV: sudden shock (no pulse or blood pressure found) * *Grades* III and IV are *Dengue Shock Syndrome* (WHO-SEARO, 2011)	Ordinal

Research Tools and Materials

This study used medical records of pediatric patients diagnosed with dengue hemorrhagic fever at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022.

This research will be carried out at RSPAL dr. Ramelan Surabaya data for the period January 2021 - December 2022 in the medical records section. This research was carried out in August 2023 - December 2023.

Data Retrieval or Collection Procedures

Arranging permits for the use of medical records at RSPAL dr. Ramelan Surabaya and ethically sound. Data was obtained by looking at the medical records of pediatric patients diagnosed with dengue hemorrhagic fever at RSPAL dr. Ramelan Surabaya for the period January 2021 - December 2022. Researchers will select samples that match the inclusion criteria and do not have exclusion criteria. Samples that meet the criteria will then have their data recorded according to the research variables. Recording will be continued with data analysis.

Data Management

Data management carried out in this research includes: (1) Verification, namely ensuring that the medical records that will be used as research samples are medical records of patients diagnosed with dengue hemorrhagic fever at RSPAL dr. Ramelan Surabaya for the period January 2021 – December 2022. (2) Cleaning, namely the process of re-examining research samples to ensure that research samples do not include exclusion criteria .

How to Analyze Data

Before data analysis is carried out, data completeness and correctness will be checked. Data analysis consists of Univariate Analysis and Bivariate Analysis (Notoadmojo, 2018).

Univariate analysis aims to interpret and simplify the data and explain how the data is distributed among the sample and research population as shown through the frequency and percentage distribution of each variable. In this study, univariate analysis was carried out to determine the characteristics of the dependent variable, namely the severity of dengue hemorrhagic fever and the independent variable, namely the number of leukocytes.

Bivariate analysis aims to ensure the existence of a statistical relationship and measure the strength of the relationship between the two variables. The severity of dengue hemorrhagic fever (dependent variable) is a nominal scale and the number of leukocytes (independent variable) is a nominal scale and measurements will be carried out once, then the data will be tested using the *Kendall's tau-b correlation test* on a computer.

Data collection will be accompanied by double checking before being combined and categorized according to the variables studied. Statistical analysis of this research uses the *Kendall tau-b correlation* on the computer.

Result and Discussion

Research Implementation

This research was carried out from July to December in 2023 by reviewing medical record data at the Inpatient Installation of the Pediatric Polytechnic Department of RSPAL dr. Ramelan Surabaya in the period January 2021 – December 2022. Data taken are medical record number, age, diagnosis, physical examination, number of days hospitalized, blood pressure, NS-1 results, IgM or IgG results, leukocyte count, platelet count, and hematocrit count. The dengue fever diagnoses included in this study were *grade* I, *grade* II, *grade* III, and *grade* IV dengue fever. Data from medical records of pediatric dengue fever patients in the period obtained 126 pediatric patient data that met the inclusion and exclusion criteria consisting of 88 *grade I patient data, 16 grade II* patient data , 19 *grade III* patient data and 3 *grade IV* patient data reviewed from physical examination. as well as laboratories. The number of leukocytes will be obtained based on the results of the patient's complete blood examination during hospitalization, especially on days 2 to 5. The data obtained will be explained in the form of narratives, tables and pictures.

The incidence of leukopenia in grade I dengue fever in pediatric patients at RSPAL dr. Ramelan Surabaya

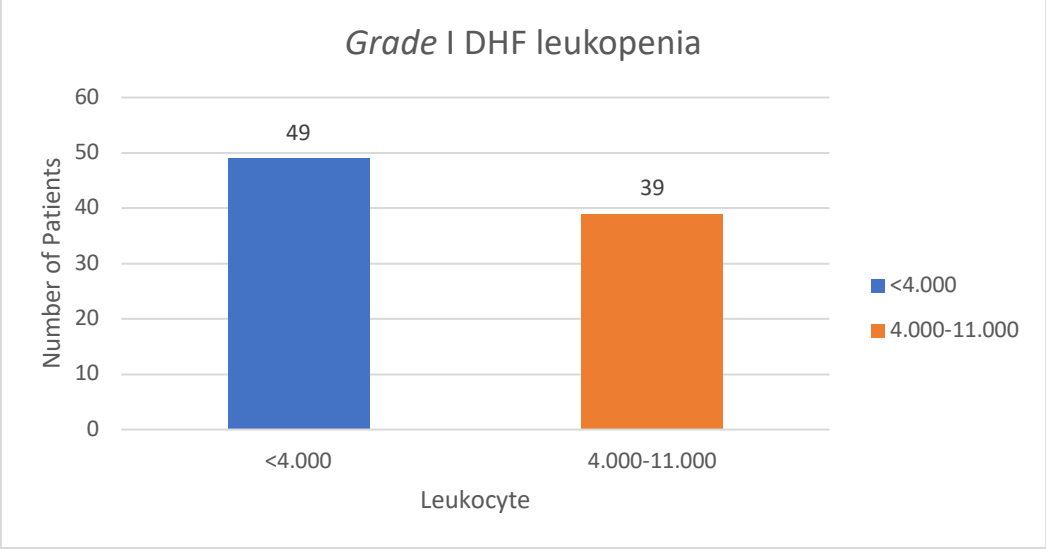

Figure 1. Number of Patients Experiencing Leukopenia in Grade I DHF

The results of the study showed that 49 (55.7%) pediatric patients experienced leukopenia in DHF *grade* I, while 39 (44.3%) did not experience leukopenia. This data can be reviewed from table 2.

The incidence of leukopenia in grade II dengue fever in pediatric patients at RSPAL dr. Ramelan Surabaya

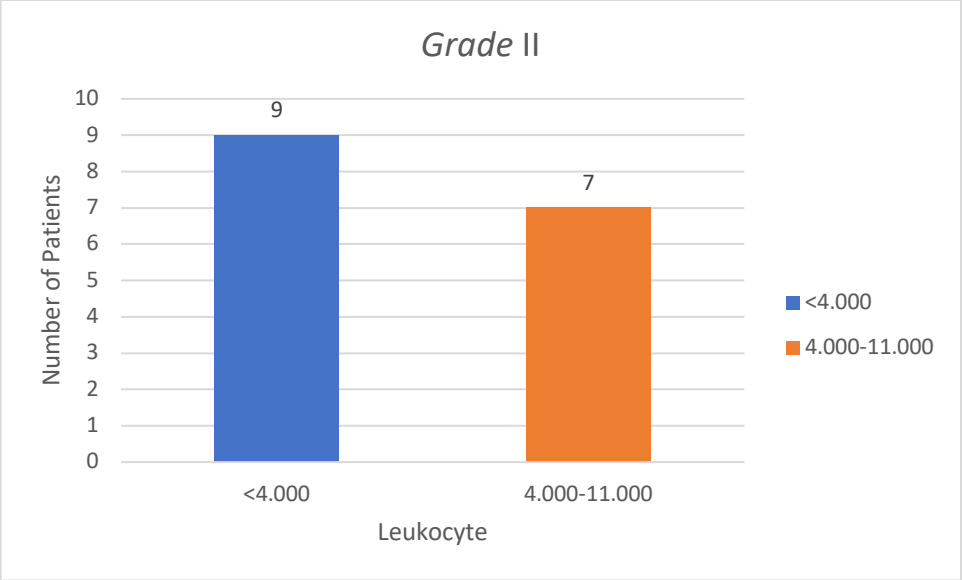

Figure 2. Number of Patients Experiencing Leukopenia in Grade II DHF

The results of the study showed that 9 (56.3%) pediatric patients experienced leukopenia in DHF *grade* II, while 7 (43.8%) did not experience leukopenia. This data can be reviewed from table 3.

The incidence of leukopenia in grade III dengue fever in pediatric patients at RSPAL dr. Ramelan Surabaya

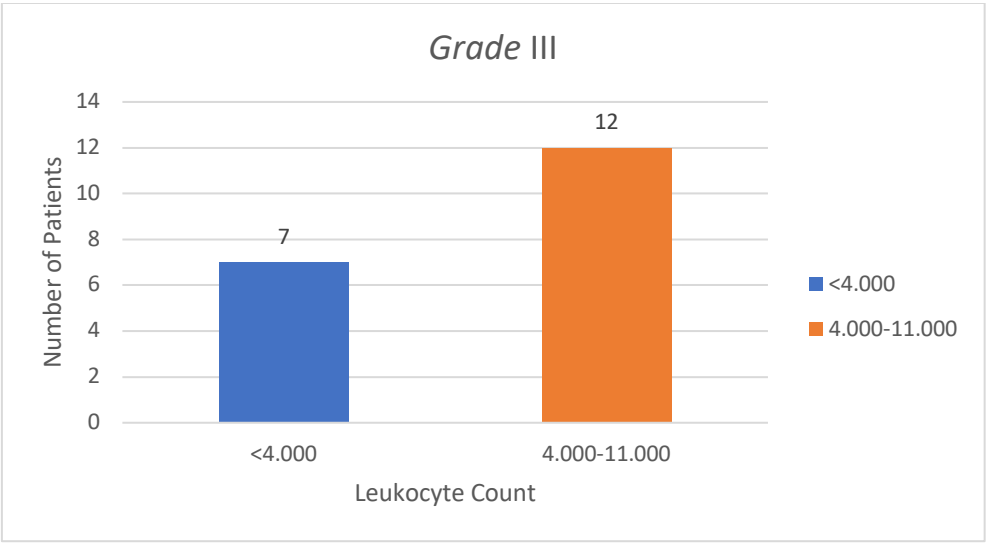

Figure 3. Number of Patients Experiencing Leukopenia in Grade III DHF

The results of the study showed that there were 7 (36.8%) pediatric patients who experienced leukopenia in DHF *grade* III, while 12 (63.2%) did not experience leukopenia. This data can be reviewed from table 4.

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The incidence of leukopenia in grade IV dengue fever in pediatric patients at RSPAL dr. Ramelan Surabaya

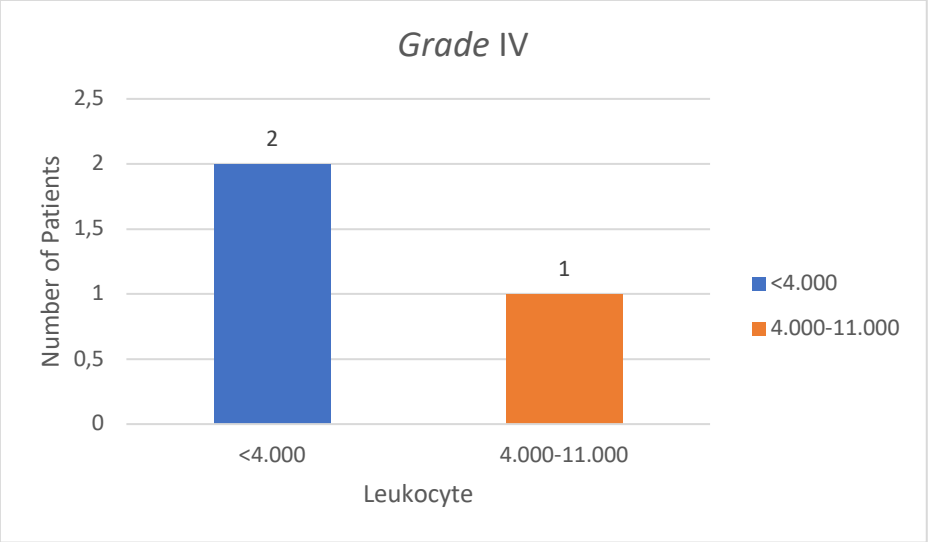

Figure 4. Number of Patients Experiencing Leukopenia in Grade IV DHF

The results of the study showed that there were 2 (66.7%) pediatric patients who experienced leukopenia in DHF *grade* IV, while 1 (33.3%) did not experience leukopenia. This data can be reviewed from table 5.

The patient incidence rate is based on DHF grade I-IV in pediatric patients at RSPAL dr. Ramelan Surabaya

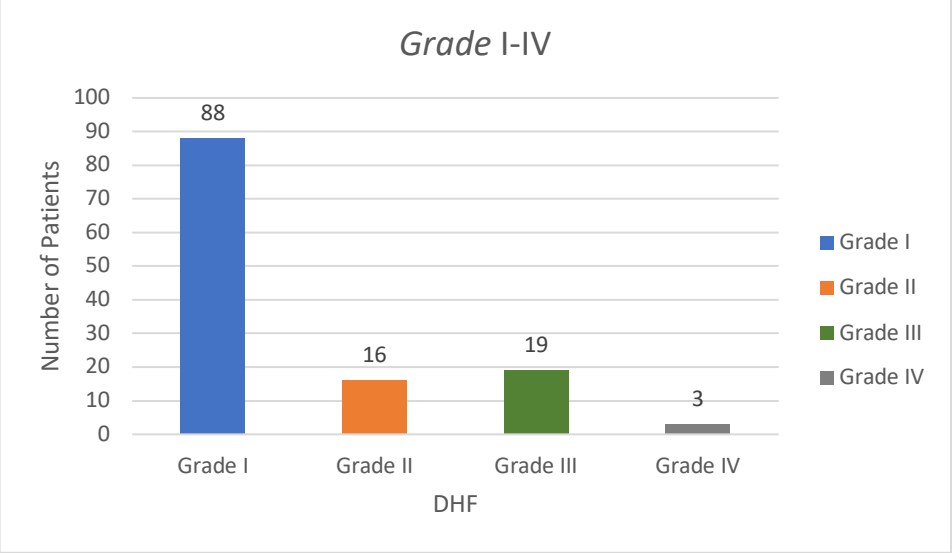

Figure 5. Patient Numbers Based on DHF Grade

The data shows that from a total of 126 data, there were 88 (69.8%) of *grade I dengue fever,* 16 (12.7%) of *grade II, 19 (15.1%) of grade III* , and 3 (2.4%) of *grade* IV. Low dengue infections have the highest frequency of hospitalization at RSPAL dr. Ramelan Surabaya. The research results can be reviewed from table 6.

Statistical Analysis Results

Hypothesis testing

The leukopenia variable is a nominal scale while the severity of dengue fever is an ordinal scale, so the test used in this research is the *Kendall's tau-b test* with the following hypothesis:

H0: There is no relationship between leukopenia and the severity of dengue fever in pediatric patients treated at RSPAL dr. Ramelan Surabaya for the period January 2021 - December 2022.

H1: There is a relationship between leukopenia and the severity of dengue fever in pediatric patients treated at RSPAL dr. Ramelan Surabaya for the period January 2021 - December 2022.

The relationship between leukopenia and the severity of dengue fever

The relationship between leukopenia and dengue fever grade can be seen from table 5.6. Patients with *grades* I, II and IV experienced more leukopenia.

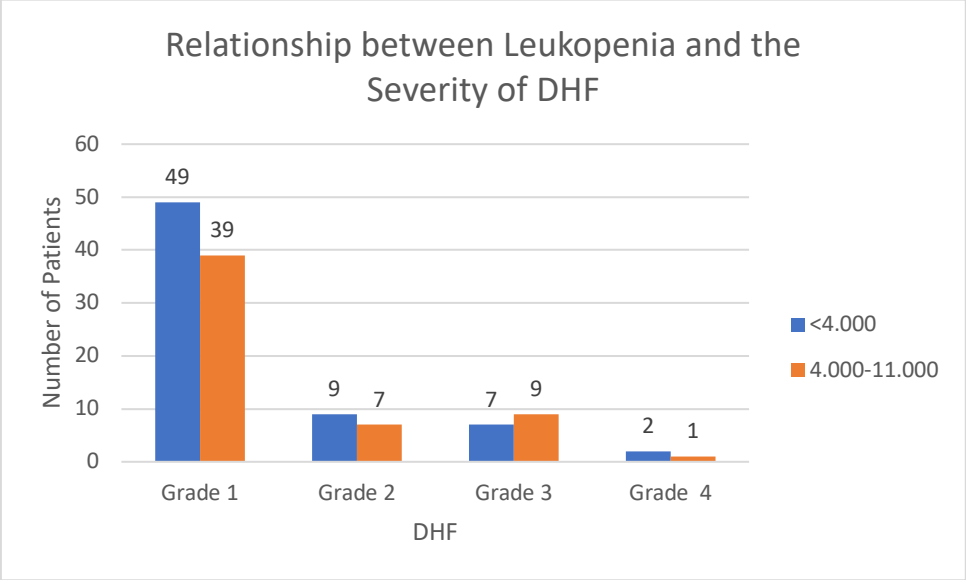

Figure 6. Relationship between Leukopenia and the Severity of DHF

The relationship between leukopenia and dengue fever grade can be seen from table 7. Research data shows that the total number of patients who experienced leukopenia was 67 with grade I of 49 (73.1%), grade II of 9 (13.4%), grade III of 7 (10.4%) and grade IV of 2 (3%). The total number of patients who did not experience leukopenia was 59 with grade I of 39 (66.1%), grade II of 7 (11.9%), grade III of 12 (20.3%) and grade IV of 1 (1, 7%).

Based on the results of computer tests using *Kendall's tau-b,* it shows a significance value: P=0.34 (P>0.05), which means H0 is accepted. Thus, there is no relationship between leukopenia and the severity of dengue fever in pediatric patients treated at RSPAL dr. Ramelan Surabaya for the period January 2021 - December 2022. The results of significant values can be seen in attachment 6 .

Leukopenia in Pediatric Patients Diagnosed with *Grade* I DHF

The DENV serotype virus enters the host's body through the bite of an infected *Aedes mosquito* (Schaefer *et al* ., 2022) . Macrophages and human peripheral blood mononuclear cells (PBMC) are activated via *Toll-like receptor* 4 (TLR-4) (Martina *et al* ., 2009) . Primary viremia begins when the virus enters the blood and infects immune cells, namely *dendritic cells* , monocytes, macrophages and B cells which will stimulate apoptosis. Then, apoptosis of these immune cells results in the release of cytokines and inflammatory mediators namely TNF-α, IL-6 and IL-10 which induce the expression of endothelial adhesion molecules such as CD62E, CD106 and CD62P. The results of this molecular induction increase vascular permeability so that it can manifest as plasma leakage (Khanam *et al* ., 2022) . The apoptosis process that occurs results in a decrease in the number of leukocytes. Leukopenia is a decrease in leukocyte values below the threshold, namely less than 4,000/ ^{mm3} (Tigner *et al* ., 2022) . The resulting symptoms are

classified as *grade* I, which is still mild and there is no shock (WHO, 2011). In research that has been conducted, *grade* I patients experienced more leukopenia than other *grades* . Similar results were obtained in research in Bali (2017) which showed an average leukopenia number of 3.12 ±1.927 thousand per mm3 compared to other *grades* . Research in Lampung (2017) showed that the number of *grade* I patients who experienced leukopenia was the same as those who did not experience leukopenia. This difference is due to differences in the age range of research subjects, namely 0 to 46 years.

Leukopenia in Pediatric Patients Diagnosed with *Grade* II DHF

The infection process begins when the virus infects host cells to activate macrophages and human peripheral blood mononuclear cells (PBMC) via *Toll-like receptor* 4 (TLR-4) (Martina *et al* ., 2009) . Immune cells, namely *dendritic cells* , monocytes, macrophages and B cells that are infected, will die through apoptosis. The response to cell death will trigger the release of TNF-α, IL-6 and IL-10 which increase blood vessel permeability. Cytokines produced by DENV-infected cells induce the expression of endothelial cell adhesion molecules (CD62E, CD106 and CD62P) which cause inflammation, endothelial damage and plasma leakage which begins with increased permeability (Khanam *et al* ., 2022) . At this stage, the severity of endothelial dysfunction causes coagulopathy, resulting in spontaneous bleeding and leading to a state of hypovolemia (WHO, 2011). The death of immune cells infected by viruses results in low values in the number of leukocytes. A decrease in white blood cells or leukopenia is characterized by a leukocyte value below 4,000/mm ^{3} (Tigner *et al* ., 2022) . Platelet values below 10,000/microliter can cause epistaxis, *petechiae* , bleeding gums and *intracranial bleeding* that is not accompanied by trauma (Noé & Jadoui., 2018) . However, at this level of severity it is still not included in the DSS. In this study, there were 9 out of 16 patients who experienced leukopenia in *grade* II DHF. These results are similar to the Lampung study (2017) which found more patients with leukopenia than those without leukopenia for DHF *grade* II with a total of 56.3%. Similar results were also found in the Bali study (2017) with the average leukopenia in *grade* II amounting to 3.35 ±2.071 thousand per mm ^{3}.

Leukopenia in Pediatric Patients Diagnosed with *Grade* III DHF

Starting with DENV entering host cells to activate macrophages and human peripheral blood mononuclear cells (PBMC), which are the body's immune cells, via *Toll-like receptor* 4 (TLR-4) (Martina *et al* ., 2009) . Infected immune cells, namely dendritic cells, monocytes, macrophages and B cells, will undergo apoptosis, which can cause a cytokine response which results in more severe endothelial damage and plasma leakage (Martina *et al* ., 2009) . The plasma leakage process is based on inflammatory cytokines (TNF-α, IL-6 and IL-10) which induce the expression of endothelial cell adhesion molecules such as CD62E, CD106 and CD62P (Khanam *et al* ., 2022) . Apoptosis experienced by these immune cells can result in leukopenia which is characterized by a leukocyte value of less than 4,000/mm3 (Tigner *et al* ., 2022) . At this stage, increased vascular permeability results in hypovolemic shock due to *disseminated intravascular coagulation* (DIC) (WHO, 2011). DIC is based on excessive use of coagulation factors and increased fibrin deposition. Consumption that is too high can cause a deficiency in coagulation factors, causing bleeding, while the spread of fibrin deposition causes obstruction of the microvasculature which manifests as organ failure. Both processes result in low intravascular blood volume resulting in decreased *venous return* which is related to *cardiac output* which manifests in *hypovolemic shock* (Venugopal, 2014) . The clinical signs are hypotension, narrowed pulse pressure and circulatory failure so it is classified as DSS (WHO, 2011). There were fewer *grade III* patients who experienced leukopenia than those without leukopenia in this study. This research is supported by research at Abdul Wahab Sjahranie Hospital Samarinda (2017) which shows the average of normal leukocytes at *grade* III, namely 6587.5. Research in Bali (2017) also said the same thing with an average number of leukocytes of 5.86 ±4.021 thousand per ^{mm3} .

Leukopenia in Pediatric Patients Diagnosed with *Grade* IV DHF

The DENV virus will enter the host's cells through the bite of the *Aedes mosquito* (Schaefer *et al* ., 2022) . The same process occurs when the virus infects immune cells to kill or induce apoptosis of these cells. Infected immune cells are *dendritic cells* , monocytes, macrophages and B cells through *Toll-like receptors* (TLR-4) which will stimulate apoptosis (Martina *et al* ., 2009) . Signs of apoptosis can be seen from low leukocyte values. Apart from that, this process can release inflammatory cytokines, namely TNF-α, IL-6 and IL-10. These inflammatory cytokines will stimulate the endothelial cell adhesion molecules CD62E, CD106 and CD62P which results in increased endothelial permeability (Khanam *et al* ., 2022) . The higher the grade of dengue fever, the higher the resulting damage. At this level of severity, the spread of intravascular coagulation can cause severe bleeding in organs such as the heart, brain and others, leading to fatal conditions (WHO, 2011). *Hypovolemic shock* conditions cause insufficient oxygen delivery to tissues due to reduced blood volume. As compensation from the body, cells in organs use anaerobic metabolic components such as lactic acid *(Taghavi et al.* , 2023) . Decreased consciousness is a manifestation of reduced perfusion in the brain while in the heart it can result in decreased contractility which can result in *Pulseless Electrical Activity* . This condition is a malfunction in the heart's electrical system, which can result in an irregular rhythm and is an early condition of *asystole* (Oliver *et al* ., 2023) . Clinical signs that can be found at this level of fragility are cold acral, no pulsation and irregular blood pressure (WHO, 2011). Decreased white blood cells caused by viral effects can be characterized by leukopenia (Tigner *et al* ., 2022) . There were 2 out of 3 *grade IV* dengue fever patients who experienced leukopenia in this study. This is different from research by Rosdiana et al. (2017) where the average number of leukocytes was 5874. Research in Bali (2017) also stated that the average number of leukocytes in patients experiencing grade IV dengue fever was normal, namely 5.21 ±2.583 thousand/ ^{mm3} . This difference could be caused by the varying number of research subjects.

Incidence Rate of *Grade* I DHF in Pediatric Patients

Symptoms of *grade* I DHF include DF with bleeding manifestations characterized by a positive *rumple leed* or *tourniquet test* and plasma leakage as assessed by platelet values < 100,000 cells/mm3 and hematocrit ≥20% (WHO, 2011). In this study, *grade* I was the highest level of severity experienced by 88 (69.7%) pediatric patients. Research at Felson et al. (2007) also had the largest population for *grade* I with 56 (58.9%). *Grade* I is also the highest level of severity of dengue fever with 36 (49.3%) patients in research conducted. The same results were reported by Saputra et al. (2021) research (2017) with 12 (30.8%) patients experiencing *grade* I. Differences were found in research conducted at RSUD dr. Soetomo Surabaya (2020) who said that *grade* I was ranked second at 11 (9.5%) of the severity of other dengue fever with a vulnerable age group of 1 to 18 years (Maesarah, 2020) .

Incidence Rate of *Grade* II DHF in Pediatric Patients

Grade II is the clinical grade limit where there is no shock. The symptoms experienced by *grade* I are accompanied by spontaneous bleeding (WHO, 2011). Spontaneous bleeding includes epistaxis, petechiae, bleeding gums and intracranial bleeding that is not accompanied by trauma (Noé & Jadoui, 2018) . Observations in this study showed that the number of *grade* II results was 16 (12.7%). Samarinda (2017) also stated that *grade* II was not the clinical grade with the largest population in the study with 26 (27.4%) patients. This is different from Surabaya (2020) and Lampung (2017) which stated that *grade* II was ranked first with the number of patients being 15 (45.4%) and 24 (61.6%).

Incidence Rate of *Grade* III DHF in Pediatric Patients

This clinical grade is included in the DSS group which is characterized by circulatory failure, weak pulse, narrow blood pressure ((≤ 20 mmHg), hypotension and anxiety (WH0, 2011). This study received 19 (15.4%) patients and was ranked second most for level of severity of dengue fever. The same position was obtained in research in Bali (2015) with 13 (17.8%) patients for *grade* III. Research conducted at RSUD Wajab Sjahranie Samarinda had 8 (8.4%) patients and was ranked third most. This is also in line with research in Surabaya (2020) which received 6 (5.2%) patients. Research in Lampung (2017) received the fewest patients, namely 3 (7.6%) for *grade* III. This can be influenced by the speed treatment to prevent the occurrence of DSS.

Incidence Rate of *Grade* IV DHF in Pediatric Patients

DSS occurs when dengue virus infection causes a sharp drop in blood pressure and damage to vital organs (Sudulagunta et al., 2016) . *Grades* III and IV are classified as clinical degrees of DHF that involve shock. The difference lies in *grade* IV which shows signs of shock such as cold acral, no pulsation and irregular blood pressure (WHO, 2011). *Grade* IV was rarely encountered in this study with the smallest number being 3 (2.4%) patients. The same results were also reported in research in Samarinda (2017) with 5 (5.3%) patients and Surabaya (2020) with 1 (0.9%) patient.

Relationship between Leukopenia and Severity of DHF in Pediatric Patients

Leukopenia is a reduction in white blood cells in the blood circulation with a number of less than 4,000 (Mart *et al* ., 2022) . Dengue virus infection enters the body through the bite of the Aedes *aegypti* or *albopictus mosquito* (Schaefer et al., 2022) . Viruses attack the body's target cells, especially immune system cells. The immune system, especially white blood cells such as B cell lymphocytes, macrophages, monocytes and dendritic cells, are targets that will be damaged, disrupting the production of new white blood cells. The dengue virus can cause white blood cells to apoptotic, but the body's immune response also causes a decrease in white blood cells. Inflammatory cytokines (TNF-α, IL-6 and IL-10) by DENV-infected cells bind to *Toll-like receptor* 4 (TLR-4) in macrophages. Then it induces the expression of endothelial cell adhesion molecules such as CD62E, CD106 and CD62P causing inflammation, endothelial damage and plasma leakage which are the main signs of DHF (Khanam *et al* ., 2022) . Plasma leakage is a sign of the critical phase which lasts 24-48 hours on day 3. The result of plasma leakage is a rapid decrease in the number of platelets, an increase in hematocrit, the patient can experience leukopenia before there is a decrease in the number of platelets. This endothelial dysfunction can manifest as severe symptoms including *hypovolemic* shock, organ dysfunction, disseminated intravascular coagulation or bleeding (Schaefer *et al* ., 2022) .

This research is supported by research at Abdul Wahab Sjahranie Hospital, Samarinda by Rosdiana et al. (2017) regarding the relationship between the results of leukocyte, platelet and hematocrit examinations with the clinical grade of dengue fever in pediatric patients. At Abdul Wahab Sjahranie Hospital, Samarinda, it shows the same results as this research. . The results of the Spearman test for the number of leukocytes with dengue *grade* showed a value of 0.439, which means p > a (a = 0.05). The average data shows that the number of leukocytes from patients is within normal limits so that there is no significant relationship between the number of leukocytes and *the grade of* DHF (Rosdiana *et al* ., 2017) . Research by Maneuver (2019) regarding the number of leukocytes and clinical grade of dengue infection sufferers at RSUD Dr. H. Abdul Moeloek Lampung found that there was no significant relationship between the clinical degree of dengue infection and the number of leukocytes. The significance value obtained was p = 0.462, which means p > a (a = 0.05). In this research, the research subjects used were 0 to 11 years old, numbering 7 (17.9%).

This is different from research conducted in Bali (2017) which stated that there was a significant correlation between the number of leukocytes and the clinical degree of dengue fever in pediatric patients at Sanglah General Hospital, Denpasar with a value of p = 0.000, which means P < a (a = 0.05). The research subjects consisted of 36 patients in *grade* I, 10 patients in *grade* II, 13 patients in *grade* III and 14 patients in *grade* IV.

Insignificant research results are often caused by limited existing data. Data limitations include various aspects such as too small a sample size, imbalance in the sample group and incomplete data.

Conclusion

Based on the results and discussion, it can be concluded that the incidence rate of leukopenia in pediatric patients diagnosed with DHF grade I at Dr. Ramelan General Hospital Surabaya from January 2021 to December 2022 was 73.1% (49 patients). The incidence rate of leukopenia in pediatric patients diagnosed with grade II DHF at Dr. Ramelan General Hospital Surabaya from January 2021 to December 2022 was 13.4% (9 patients). The incidence rate of leukopenia in pediatric patients diagnosed with grade III DHF at dr. Ramelan General Hospital Surabaya from January 2021 to December 2022 was 10.4% (7 patients). The incidence rate of leukopenia in pediatric patients diagnosed with grade IV DHF at Dr. Ramelan General Hospital Surabaya from January 2021 to December 2022 was 3% (2 patients.

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