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THE CORRELATION BETWEEN IgM/IgG DENGUE SEROLOGICAL TEST AND THROMBOCYTE HEMATOCRIT COUNT ON DHF PEDIATRIC PATIENTS AT UNS HOSPITAL

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ABSTRACT

Background: Dengue hemorrhagic fever (DHF) is an infectious disease transmitted by the *Aedes aegepty* mosquito and mostly occurs during the rainy season. This disease can affect all age groups but the occurrence was quite high in children. Supporting examinations and complete blood counts are required to establish the diagnosis of DHF. This study aims to determine the correlation between the results of IgM/IgG dengue serological test and thrombocyte hematocrit count.
Methods: This cross-sectional study was conducted in the UNS Hospital from November to December 2020 and obtained 134 samples that match the inclusion and exclusion criteria. Secondary data were obtained from the medical records of DHF child patients who met the criteria of inclusion and exclusion. Thrombocyte and hematocrit counts were analyzed using chi-square test and Fisher exact test.
Result: This study showed no significant correlation between the serologic test of IgM/IgG dengue and thrombocyte with a value p = 0.451 (p>0.05). There was also no significant correlation between the serologic test of IgM/IgG dengue and hematocrit (p= 0156, p>0.05) on DHF pediatric patients at UNS Hospital.
Conclusion: Thus the serologic test of IgM/IgG of dengue had no correlation with thrombocyte and hematocrit count on DHF pediatric patients at UNS Hospital.

Keywords: *DHF, IgM/IgG dengue, Thrombocyte, Hematocrit*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is an infectious disease transmitted by the *Aedes aegypti* mosquito and mostly occurs during the rainy season. Dengue hemorrhagic fever can affect all age groups.[1] Dengue virus infection (DENV) belongs to the virus family Flaviridae and has four serotypes in recent years. The serotypes are divided into DENV-1, DENV-2, DENV-3 and DENV-4[2]. In October 2013, a new strain of dengue virus was found, namely DENV-5. The life cycle of this strain follows a sylvatic cycle, unlike other strains that follow a human life cycle[3].

Patient diagnosed with dengue hemorrhagic fever fulfill, at least, two of four criteria for clinical symptoms such as persistent fever, hemorrhagic manifestations (positive tourniquet results, petechiae, purpura), liver enlargement, symptoms of shock and laboratory results of thrombocytopenia and/or elevated hematocrit levels .[4]

In patients with dengue hemorrhagic fever, thrombocytopenia and hematocrit increased usually occur on the 3rd or 4th day of illness[5]. Based on a study conducted by Jayashree et al, approximately 80% of patients had clinical manifestations of thrombocytopenia in pediatric DHF/ Dengue Shock Syndrome (DSS) patients aged 0-15 years. Furthermore, one of the supporting examinations used to determine the occurrence of DENV infection is dengue IgM/IgG serological test.[4]

Antibody response to infection will trigger various immunoglobulins (Ig). IgM and IgG serotypes are immunoglobulins that can be found during infection. IgM antibodies are detectable on 3-5 days after disease onset, increase rapidly after two weeks and decrease after 2-3 months.[6] IgG antibodies are detectably low at the end of the first week, increase and then persist over a longer period, years to a lifetime. The IgM/IgG ratio is generally used to detect primary and secondary dengue infections.[7]

The IgM(+)/IgG(-) test results indicate a primary infection, while the IgM(-)/IgG(+) or IgM(+)/IgG(+) test results indicate secondary infection and the IgM(-)/IgG(-) test results indicates the absence of infection. The manifestation of secondary infection often associated with the occurrence of DHF or DSS, while in primary infection, DHF is rare.[8]

Dengue infection is one of the main infectious diseases that occur in children.[9] Children with DHF have lower hematocrit and serum creatinine levels and higher plasma leakage, which increase the risk of DSS and metabolic acidosis.[10] DHF in children also cause an acute liver failure with a contribution of about 18.5% of cases.[11]

In Indonesia, cases of dengue fever are quite high. In 2017 there were 68,407 cases where 38.36% of cases were children aged 5-14 years. The highest number of dengue cases occurred in 3 provinces of Java. In Central Java alone in 2017 there were 3,015 cases with 37 deaths and an increase in 2018 as many as 8,565 cases with 115 deaths[12].

Universitas Sebelas Maret (UNS) Hospital is one of the hospitals that treats DHF patients in Surakarta and surrounding areas. The aim of this study is to determine the relationship between IgM/IgG serological test results with hematocrit and thrombocyte values in pediatric DHF patients at UNS Hospital.

METHODS

This research was an analytic observational study with a cross sectional design. The sample in the study was selected based on the inclusion and exclusion criteria. This study conducted at UNS Hospital from November to December 2020. The population in this study were pediatric patients aged 2-18 years with a diagnosis of DHF at UNS Hospital from January 2018 to December

2019. This study has been approved by health research ethics committee of Universitas Sebelas Maret, Indonesia No.173/UN27.06.6.1/KEPK/EC/202.

The sampling technique used in this research was purposive sampling. In determining the sample size, the formula for the estimation of the proportion with an unknown population size was used. Then, the sample size was calculated by the following formula:

$$n = \frac{Z^2 p(p-1)}{d^2}$$

n = Number of samples
Z = Degree of confidence (usually at 95% level = 1.96)
p = Proportion of a particular case to the population, if the proportion is not known, set 50% (0.50)
d = Degree of deviation from the desired population: 10% (0.10)

Thus, the minimum sample size required was 96 samples and obtained 134 samples that match the inclusion and exclusion criteria.

The independent variable in this study was the results of the dengue IgM/IgG test in pediatric dengue hemorrhagic fever patients aged 2-18 years. The IgM/IgG examination was carried out in one measurement and is carried out simultaneously with a complete blood count. The data used are secondary data obtained from patient medical records at UNS Hospital. The categorical measurement scale is stated as positive on IgM(+)/IgG(-), IgM(-)/IgG(+), or IgM(+)/IgG(+) and negative on IgM(-)/IgG(-) .

The dependent variable in this study was the thrombocyte value obtained from a complete blood count which was carried out simultaneously with IgM/IgG serological tests in DHF pediatric patients aged 2-18 years. The data used are secondary data obtained from patient medical records at UNS Hospital. The categorical measurement scale was expressed as increasing, decreasing and normal based on the normal standards of the WHO guidelines and the hematocrit value obtained from a complete blood count carried out simultaneously with IgM/IgG serological tests in dengue-infected pediatric patients aged 0-18 years. The data used were secondary data obtained from patient medical records at UNS Hospital. The categorical measurement scale was expressed as increasing, decreasing and normal based on the patient's age.

Data were obtained from patient medical records and processed using the Statistical Package for the Social Sciences (SPSS) version 25 computer program which consisted of univariate analysis and bivariate analysis. Univariate analysis consisted of frequency and proportion of each variable studied and bivariate analysis using chi-square test and Fisher exact analysis test.

RESULT

Based on this study, which conducted on the medical records of pediatric patients aged 2-18 years with a diagnosis of DHF at UNS Hospital in January 2018-December 2019, we obtained 134 samples that met the inclusion and exclusion criteria.
The characteristics of the samples were seen based on age, sex and fever at the time of the dengue IgM/IgG examination.

Table 1. The basic characteristic of the samples

Characteristic	Total (%)
Age	
0-5 years	15(11,2)
6-18 years	119(88,8)
Gender	
Male	67(50)
Female	67(50)
Fever period	
Day 3rd-4th	16(11,9)
Day >5th	118(88,1)

Based on table 1, we found that most of the samples aged 6-18 years were 119 samples (88.8%) while the samples aged 0-5 years were only 15 samples (11.2%). Based on gender, the number of samples of men and women is the same. Based on the fever during the dengue IgM/IgG examination, most of the samples were examined when the fever was >5 days, namely 118 (88.1%) samples.

Univariate analysis explained the distribution of each variable, including the results of the dengue IgM/IgG examination as the independent variable, and thrombocyte and hematocrit values as the dependent variable.

Table 2. Distribution of the result IgM/IgG dengue serological test

Result	Total (%)
Positive	113(84,3)
Negative	21(15,7)

Based on table 2, most of the results of the dengue IgM/IgG examination were positive, namely 113 (84.3%) samples, while the results of the IgM/IgG examination were negative in 21 (15.7%) samples.

Table 3. Distribution of platelet and hematocrit count in pediatric patients at UNS Hospital

Group	Total (%)
Trombosit	
Normal	48 (35,8)
Increase	0 (0)
Decrease	86 (64,2)
Hematocrit	
Normal	103 (76,9)
Increase	28 (20,9)
Decrease	3 (2,2)

Based on table 3, there was no increase in the platelet value in the sample. Most of the samples experienced a decrease in thrombocyte values by 86 (64.2%) samples and normal thrombocyte values by 48 (35.8%) samples. Most of the patients' hematocrit values were normal, namely 103 (20.9%) samples, while 28 (20.9%) samples had an increase and 3 (2.2%) samples decreased.

Based on the calculation of the expected count between the results of the dengue IgM/IgG serological test and the thrombocyte value, there was no expected count value of less than 5. Thus, both variables meet the chi-square test requirements. Besides, the expected count calculation between the results of the dengue IgM/IgG serological test and the the hematocrit value was found that there were 3 cells that had an expected count value of less than 5, which this variable would use Fisher's exact analysis test.

Table 4. Bivariate Test Results Correlation between Dengue IgM/IgG Serological Test Results on Thrombocyte and Hematocrit Values

Result of serological test	p
Thrombocyte count	0,451
Hematocrit count	0,156

Based on table 4, we found that the p value of the relationship between the results of the dengue IgM/IgG serological test and the thrombocyte value was 0.451 ($p>0.05$), which indicates that there was no significant relationship between the two variables. Based on the Fisher exact test which was carried out on the results of the dengue IgM/IgG serological test with a hematocrit value, $p = 0.156$ ($p>0.05$), thus there was no statistically significant relationship between the two variables.

DISCUSSION

DHF is a disease caused by dengue virus infection which is transmitted by the *Aedes aegypti* mosquito.[13] In establishing a diagnosis of DHF, one of the supporting examinations is needed in the form of a dengue IgM/IgG serological test and a complete blood count to see the value of platelets and hematocrit.[14] Hematologic and biochemical changes can be used to detect plasma leakage and prevent morbidity and mortality from developing DHF.[15]

Based on the results of the study in table 4, there was no significant relationship between the results of the dengue IgM/IgG serological test and the platelet value in pediatric patients with DHF at UNS Hospital. This result was consistent with studies that have been conducted that the mean platelet volume of dengue-infected patients does not have a significant correlation with the results of serological tests.[16] DHF has several hematological manifestations, one of which is thrombocytopenia. Thrombocytopenia was reported in 79% of cases of DHF.[17]

Thrombocytopenia occurs due to an inflammatory process due to dengue virus infection, which causes the use of platelets during the coagulopathy process, activation of the complement system and increased sequestration in the periphery[18]. Decreased platelet value can be used as an indicator in predicting the occurrence of complications from DHF. However, complications from DHF can also occur in patients with normal platelet values.[19]
In pediatric patients aged 5-12 years with a diagnosis of DHF, most of the decline in platelet values occurs on the 4-5th day after the appearance of symptoms.[15] Based on this study, the average thrombocyte count did not show a significant correlation with patient severity, serological test results and treatment results so that the average thrombocyte value has not become an important prognostic factor in dengue hemorrhagic fever.[16]

Based on the results of the study in table 4, we found that there was no significant relationship between the results of the dengue IgM/IgG serological test and the hematocrit count in pediatric patients at UNS Hospital. These results were supported by previous study conducted by Ramdhani et al that showed no significant relationship between the hematocrit value and the development of DHF.[20] The increase of percentage in the hematocrit value was used to assess the presence of increased vascular permeability and plasma leakage.[21] In pediatric patients aged 5-12 years with a diagnosis of DHF, an increase in hematocrit could be detected on day 4 and indicated the patient in a critical phase.[15] Increased hematocrit was common in DHF patients who experience shock.[22] Increased hematocrit values in patients with DHF phase 1 and 2 were rare.The average hematocrit value in children infected with DHF was 44.1 and had no significant correlation with DHF disease.[23] The dengue IgM/IgG examination with the ELISA method had a sensitivity of 40.05% while the PCR examination was 71.94% and the NS-1 examination was 68.37%. Examination of IgM/IgG had a high level of sensitivity in the late phase of dengue disease, whereas in the early phase it had not been detected.[24] Based on the study conducted by Lee et al, which comparing six commercial diagnostic test kits for dengue IgM/IgG, they found that there was a possibility of false positive results on the IgG examination so that other tests are needed to confirm the results of the dengue IgM/IgG examination.[25] The combination of NS-1 examination with dengue IgM/IgG could increase the specificity of serological tests.[26]

The limitations of this study were the omission of confounding variables, non-uniform time blood sampling and the large age characteristics of the sample.

CONCLUSION

Based on this study regarding the relationship between the results of the dengue IgM/IgG serological test with the thrombocyte and hematocrit values in children with DHF at the UNS Hospital, we concluded that there was no significant relationship between the results of the dengue IgM/IgG serological test with the platelet and hematocrit values in DHF pediatric patient at UNS Hospital.

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