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RESEARCH

NEUTROPHIL/LYMPHOCYTE COUNT RATIO ON DENGUE HEMORRHAGIC FEVER

(Rasio Netrofil/Limfosit pada Demam Berdarah Dengue)

Irmayanti, Asvin Nurulita, Nurhayana Sennang

ABSTRAK

Infeksi virus dengue adalah salah satu masalah kesehatan masyarakat yang menimbulkan dampak sosial dan ekonomi. Pemeriksaan rasio netrofil/limfosit sangat mudah dan cepat dilakukan. Rasio netrofil/limfosit dihubungkan dengan perembesan plasma di pasien DBD. Untuk mengetahui rasio netrofil/limfosit pasien DBD berdasarkan derajat DBD. Penelitian ini merupakan penelitian retrospektif dengan mengambil data rekam medis RSUP Dr. Wahidin Sudirohusodo Makassar masa waktu Juni 2013–Juni 2015. Uji statistik dilakukan dengan uji Mann-Whitney, uji t dan uji kenasaban Spearman. Didapatkan 96 subjek penelitian yang memenuhi patokan kesertaan. Rerata umur subjek penelitian 53,5 tahun (18–89), sebagian besar laki-laki 56,25% dan perempuan 43,75%. Uji Mann-Whitney menunjukkan median leukosit grade I 4,45 (1,10–28,80), grade II 3,25 (1,60–9,20) dengan $p=0,03$. Median netrofil grade I 2,41 (0,47–24,65), grade II 1,16 (0,29–6,50) dengan $p<0,01$. Median trombosit grade I 113,50 (5,00–342,000), grade II 76,50 (3,00–274,00) dengan $p=0,009$. Rasio netrofil/limfosit grade I 2,19 (0,61–17,25), grade II 0,80 (0,18–5,91) dengan $p<0,01$. Uji kenasaban Spearman didapatkan nilai $p<0,001$ menunjukkan kenasaban antara rasio netrofil/limfosit dan derajat DBD bermakna. Nilai kenasaban Spearman sebesar -0,68 menunjukkan hubungan terbalik, semakin rendah rasio netrofil/limfosit, semakin berat derajat DBD. Uji t menunjukkan tidak ada perbedaan bermakna nilai limfosit dan hematokrit pasien DBD grade I dan II. Rasio netrofil/limfosit pada DBD grade I lebih tinggi daripada grade II, semakin rendah rasio netrofil/limfosit semakin berat derajat DBD.

Kata kunci: Rasio netrofil/limfosit, demam berdarah dengue, dewasa

ABSTRACT

Dengue viral infection is one of the community health problems and affects the social and economy aspects. Examination of neutrophil/lymphocyte ratio is very easy and quick to do. Neutrophil/lymphocyte count ratio is associated with plasma leakage in DHF patients. To determine the neutrophil/lymphocyte ratio in DHF patients based on DHF grading. This study was conducted using the medical record datas of Dr. Wahidin Sudirohusodo Hospital Makassar within the period of June 2013–June 2015. The statistical test that had been done were Mann-Whitney test, t-test and Spearman correlation test. We got 96 subjects who fulfilled the inclusion criterias. The average age of the subjects is 53.5 years old (18–89 years old), consisted of 56.25% males for the most part and 43.75% females. Mann-Whitney test showed that median of WBC in grade I was 4.45 (1.10–28.80), median of WBC in grade II was 3.25 (1.60–9.20) with $p=0.03$. Median of neutrophil in grade I was 2.41 (0.47–24.65) and in grade II was 1.16 (0.29–6.50) with $p<0.01$. Median of platelets in grade I was 113.50 (5.00–342.00) and in grade II was 76.50 (3.00–274.00) with $p=0.009$. Neutrophil/lymphocyte ratio in grade I was 2.19 (0.61–17.25) and in grade II was 0.80 (0.18–5.91) with the $p<0.01$. Spearman correlation test we got $p<0,001$ which shows a significant correlation between neutrophil lymphocyte ratio and DHF grading. Spearman correlation value -0,68 shows an inverse correlation. The lower the neutrophil lymphocyte count ratio the worse the DHF grading. The T-test shows there was not significant difference in lymphocyte count and hematocrit between grade I and grade II DHF patients. Neutrophil lymphocyte count ratio was higher in grade I than in grade II DHF patients. The lower the neutrophil lymphocyte ratio the worse the DHF grading.

Key words: Neutrophil lymphocyte count ratio, dengue hemorrhagic fever, adult

INTRODUCTION

Dengue virus infection is one of the public health problems resulting in social and economic impacts. The prevalence of dengue virus infection increases every year with the wide endemic area. DHF is mostly found in tropical and sub-tropical climates, especially in urban areas. Indonesia is an endemic area, spreading all over the country. The incidence of dengue virus infection has increased significantly in recent decades. Two-fifths of the world's population is currently at risk for dengue infection. World Health Organization (WHO) states that every year the number of dengue infection cases is 50–100 millions worldwide.¹ According to the Indonesian Ministry of Health, the percentage of death rate in 37 cases of dengue infection per 100,000 populations in Indonesia in 2012 was 0.90%.²

Dengue virus infection first discovered in Indonesia was in Surabaya in 1968 and then in Jakarta in 1969. In DKI Jakarta in 2007, there were 27,959 cases with a Case Fatality Rate (CFR) of 1.59%.³ In Makasar, there were 988 cases of DHF from January 2013 to June 2015, consisted of 473 pediatric patients and 515 adult patients at the Dr. Wahidin Sudirohusodo Hospital.⁴

Establishing a diagnosis of DHF is needed to detect the presence of specific antibodies against dengue virus in the serum, namely IgM antidengue and IgG antidengue.² Thrombocytopenia and leukopenia levels can also be considered as accurate parameters for the diagnosis of dengue infection after the third day of fever. Besides, NS1 antigen examination is very necessary, especially on the first and second day of fever, while IgM antidengue examination is recommended on the fifth day of fever.⁵

Moreover, an increase in atypical lymphocytes can be detected from the third day of fever to the eighth day of fever in DHF cases. Suvatte and Longsaman⁶ call this condition as transformed lymphocytes.⁶ Similarly, the high percentage of transformed lymphocytes is also reported on buffy coat suspension in DHF cases, about 20–50%. This percentage is very typical for DHF case since its proportion is very different from other viral infections' (0–10%). An in-depth research conducted by Sutaryo⁷ also argues that transformed lymphocytes can also be called as blue plasma lymphocytes (BPL).⁷ A serial BPL examination on peripheral blood preparations shows that BPL in dengue infection reaches peak on the sixth day of fever. Therefore, from the fourth day to the eighth fever, there is a significant difference in the proportion of BPL in dengue fever. However, from the second day to the ninth day of fever, there is no significant difference in the proportion of

BPL in DHF, either with shock syndrome or without shock syndrome.⁸

Furthermore, increased neutrophils and decreased lymphocytes are physiological immune responses of leukocytes to stress, such as in severe trauma, major surgery, tissue damage and sepsis. Ratio of the percentage of neutrophils to the percentage of lymphocytes in the blood can also be considered as a sign of stress on the immune response, known as Neutrophil Lymphocyte Stress Factor (NLSF). The neutrophil/lymphocyte count ratio will be increased in systemic inflammation or severe infection; as a result, it can be used as a clinical evaluation in patients with systemic inflammation.⁹

The neutrophil/lymphocyte count ratio less than 5 in physiological state (normal value of 75% neutrophil and lymphocyte >15%) can become higher than 6 in pathological state, e.g. due to systemic inflammation or severe infection. Zahorec⁹ says that there was a correlation between an increase in the neutrophil/lymphocyte count ratio and the disease severity in oncologic patients in ICU.⁹ Unlike Zahorec, a research conducted by Wonoagung¹⁰ finds that the neutrophil/lymphocyte count ratio in dengue fever was lower than that in typhoid fever and tended to decrease in further dengue virus infections.¹⁰ Meanwhile, a research conducted by Raeza Sendy¹¹ argues that increased number of lymphocytes could indicate increased the clinical degree of DHF since there was a significant correlation between the number of lymphocyte cells and the clinical degree of DHF.¹¹

Thus, laboratory examination on leukocyte level, especially neutrophil and lymphocyte levels can be used as markers of the infection process in the body. The examination of neutrophil and lymphocyte levels even are not expensive, but fast and very easy to apply. As a result, this research aimed to determine the neutrophil/lymphocyte count ratio, especially in dengue hemorrhagic fever.

METHODS

This study was conducted retrospectively by taking secondary data of dengue hemorrhagic fever patients diagnosed at the Dr. Wahidin Sudirohusodo Hospital from period June 2013 to June 2015. Inclusion criteria were patients aged more than 18 years old who were diagnosed with DHF based on NS1 antigen and/or IgM/IgG examinations together with routine blood laboratory examination at the Dr. Wahidin Sudirohusodo Hospital in Makassar. DHF, according to WHO (2011), can be classified based on the degree of

severity, such as grade I DHF and grade II DHF caused by dengue virus diagnosed based on NS1 antigen and/or IgM/IgG examinations.

NS1 antigen examination is an examination of non-structural-1 dengue (NS1) antigen to detect dengue virus infection earlier than using dengue antibody examination. On the other hand, dengue-specific IgM/IgG examination is an examination to detect the presence of Immunoglobulin M and Immunoglobulin G antibodies against dengue virus. Thus, NS1 antigen and IgM/IgG examinations were performed using Immunochromatographic method (rapid test), expressed in positive and negative results.

Next, the neutrophil/lymphocyte count ratio was measured. The neutrophil/lymphocyte count ratio is the result of the absolute number of neutrophil distribution divided by the absolute number of lymphocytes in dengue hemorrhagic fever patients in a unit of $10^3/\mu\text{L}$. The neutrophil/lymphocyte count ratio was obtained from routine blood examination results using K3EDTA blood samples, measured by using flowcytometry method with Sysmex XT-2000i tool. Afterwards, the data collected were analyzed statistically to assess whether there was a difference in the neutrophil/lymphocyte count ratio between grade I DHF patients and grade II DHF patients. If normally distributed, the data then were analyzed using t test. But, if abnormally distributed, the data then were analyzed using Mann-Whitney test and Spearman correlation test.

RESULTS AND DISCUSSION

This research was conducted on ninety-six subjects with dengue hemorrhagic fever diagnosis. The characteristics of those research subjects can be seen in Table 1. The research subjects consisted of 52 patients with grade I DHF and 44 patients with grade II DHF. The majority of the research subjects were male (56.25%). The percentage of the research subjects experiencing leukopenia was 53.12%, while 7.29% experiencing leukocytosis. Twenty-five percent of the research subjects even still had normal platelet count at the time of taking the data (Table 1).

The data collected then were analyzed using Mann-Whitney test. The results of the Mann-Whitney test showed that there were significant differences of leukocyte ($p=0.03$), neutrophil ($p=0.01$), platelet ($p=0.009$) and the neutrophil/lymphocyte count ratio ($p<0.01$) between grade I DHF patients and grade II DHF patients. Next, the data were analyzed using Spearman correlation test. The results of the Spearman test showed that there was an inverted correlation between the neutrophil/lymphocyte count ratio and the severity degree of dengue with a p value of <0.001 ($r=-0.687$). It means that the lower the neutrophil/lymphocyte count ratio was, the more severe the degree of dengue was. T test then was performed. The results of the T test showed that there was no significant difference in lymphocyte and hematocrit levels between grade I DHF patients and grade II DHF patients (see Table 2).

Table 1. Basic characteristics of the research subjects

Characteristics	n (People)	%	Median	Min-Max
Age (years)	96		53.5	18–89
Sex				
Males	54	56.25		
Females	42	43.75		
DHF				
Grade I	52	54.16		
Grade II	44	45.83		
Leucocytes (103/uL)				
Leukopenia	51	53.12		
Normal	38	39.58		
Leukocytosis	7	7.29		
Platelets (103/uL)				
Normal	24	25		
Thrombocytopenia	72	75		

*Source: Secondary Data

Table 2. Results of analysis based on the classification of dengue virus infection degree

	Median	Min-Max	Mean	p*
Leucocytes (103/ μ L)				
Grade I	4.45	1.10–28.80	6.06	0.03*
Grade II	3.25	1.60–9.20	3.74	
Neutrophil (103/ μ L)				
Grade I	2.41	0.47–24.65	3.68	<0.01*
Grade II	1.16	0.29–6.50	1.37	
Lymphocytes (103/ μ L)				
Grade I	1.12	0.30–2.81	1.27	0.66**
Grade II	1.64	0.35–3.39	1.67	
Hematocrit (%)				
Grade I	38.50	19.00–51.00	38.07	0.70**
Grade II	38.00	27.00–54.00	39.31	
Platelets (103/ μ L)				
Grade I	113.50	5,00–342,000	124.96	0.009*
Grade II	76.50	3,00–274,000	79.11	
Neutrophil/Lymphocyte Ratio				<0.01*
Grade I	2.19	0.61–17.25	3.20	p<0.001***
Grade II	0.80	0.18–5.91	0.98	r=-0.687***

*Mann-Whitney Test, **T Test, *** Spearman Test

- Grade I : If there are fever, hemorrhage manifestation, plasma permeation, thrombocytopenia ($<100 \times 10^3/\mu\text{L}$) and increased hematocrit level ($\geq 20\%$)
- Grade II : If there are grade I DHF syndrome and spontaneous bleeding¹²

In this research, moreover, the lowest age of the research subjects was 18 years, while the highest age was 89 years. This suggests that dengue virus infection can affect all adult age groups. The number of the male research subjects was also higher (56.25%) than females (43.75%). Similarly, a research conducted by Diana¹³ at the Dr. Kariadi Hospital in Semarang finds that there were more males suffering from DHF than females.¹³ The majority of male patients with DHF may be caused by a fact that most of males work outside, so they can be more susceptible to *Aedes aegypti* mosquito bites.¹³

Furthermore, Table 1 shows the total number of the research subjects was 96 patients who met the inclusion and exclusion criteria. Those research subjects then were divided into two groups based on the degree of severity, namely 52 patients with grade I DHF and 44 patients with grade II DHF. However, there were none of the research subjects classified into grade III DHF and grade IV DHF.

In addition, the results of the Mann-Whitney test in this research illustrated that there were significant differences in levels of leukocytes, neutrophils, lymphocytes, hematocrit, and platelets, as well as the neutrophil/lymphocyte count ratio between grade I DHF patients and grade II DHF patients. It

is also known that the median level of leukocyte in grade I DHF patients was 4.45 ($10^3/\mu\text{L}$), higher than in grade II DHF patients only about 3.25 ($10^3/\mu\text{L}$) with a p value of 0.03. It indicates that there was a statistically significant difference in leukocyte count. A research conducted by Yenni¹⁴ using multivariate logistic regression analysis shows the research subjects suffering from DHF with leukopenia had a risk of Dengue Shock Syndrome (DSS) 2.9 times greater than the research subjects without leukopenia.¹⁴ Leukopenia in dengue virus infection occurs due to bone marrow suppression, either due to direct viral infection or indirect one, through the production of proinflammatory cytokines suppressing the bone marrow.¹⁵

In this research, the median level of neutrophil in grade I DHF was 2.41 ($10^3/\mu\text{L}$), higher than in grade II DHF only about 1.16 ($10^3/\mu\text{L}$) with a p value of <0.01. It indicates that there was a significant difference in the level of neutrophils between grade I DHF patients and grade II DHF patients. In grade II DHF patients, the number of leukocytes and neutrophils reduced. In the body, neutrophils will migrate to the area of infection or tissue injury and then will circulate for 7–10 hours before migrating to the infection/tissue area.¹⁶

The levels of platelets and hematocrit also are usually used as indicators of the disease besides of the clinical condition of the hospitalized DHF patients. In this research, the median level of platelets in grade I DHF patients was 113.50 (103/ μ L), higher than that in grade II DHF patients only about 76.50 (103/ μ L) with a p value of 0.009. It means that there was a statistically significant difference.

Thrombocytopenia, moreover, also has an important role in the pathogenesis of dengue virus infection. In patients with dengue virus infection, thrombocytopenia begins on the third day until on the seventh day, while platelet count will return to normal one on the eighth day or on the ninth day.¹⁷ Thrombocytopenia in dengue virus infection occurs through destruction and shortening of platelet lifetime as well as suppression mechanism of bone marrow.¹⁸ According to WHO, thrombocytopenia and plasma leak presence characterized by hemococccinent are important indicators in diagnosing DHF.¹² Schexneider *et al.*¹⁹ says that bleeding does not correlate well with platelet count.¹⁹ In contrast, a research conducted by Khan *et al.*²⁰ in Lahore shows that there were 14.9% of dengue patients with thrombocytopenia experiencing bleeding.²⁰ Like the research conducted by Khan *et al.*²¹, a research conducted by Ong *et al.*²¹ argues that the ratio of mortality in DHF patients who had bleeding manifestation were 3–4 times greater than in patients without bleeding manifestation.²¹

Many literatures, furthermore, cite that a reduction in the number of leukocytes and neutrophils together with lymphocytosis relatively occurs at the time of fever. The change in the number of leukocytes is known as the ratio of neutrophils to lymphocytes (neutrophils < lymphocytes), which can be used to predict critical periods due to plasma infiltration.²² A research conducted by Zahorec⁹ shows that there was a correlation between severity of the disease and an increase in the neutrophil/lymphocyte count ratio in oncology patients hospitalized in ICU.⁹ Unlike Zahorec, Wonoagung¹⁰ mentions that the neutrophil/lymphocyte count ratio in dengue fever was lower than that in typhoid fever, but tended to decrease in further dengue virus infections.¹⁰

In this research, the neutrophil/lymphocyte count ratio in grade I DHF patients was 2.19, higher than in grade II DHF patients, only about 0.80. The results of the Mann-Whitney test showed that there was a significant difference with a p value of <0.01. The results of the Spearman test, furthermore, illustrated that there was a significant correlation between the neutrophil/lymphocyte count ratio and

the severity degree of DHF with a p value of <0.001. The Spearman's correlation value was -0.68 indicating an inverted correlation. It means that the lower the neutrophil/lymphocyte count ratio is, the more severe the degree of DHF is. This is consistent with a theory stating that the neutrophil/lymphocyte count ratio corresponds to plasma permeation. Unfortunately, there were still some limitations in this research. For instance, there were none of the research subjects classified into grade III DHF and grade IV DHF. Consequently, it is still not known whether there is a significant difference in the neutrophil/lymphocyte count ratio between all severity degrees of DHF.

Finally, the results of the T test showed that there was no significant difference in lymphocyte level (p=0.66) and hematocrit level (p=0.70) between grade I DHF patients and grade II DHF patients. Similarly, a research conducted by Valentino²³ at the Dr. Kariadi Hospital in Semarang and a research conducted by Syumarta²⁴ at the M. Jamil Hospital in Padang show that there was no correlation between the number of hematocrit and the clinical degrees of DHF severity. In contrast, a research conducted by Raeza Sendy¹¹ argues that the higher the number of lymphocytes was, the higher the clinical degrees of DHF severity was since there was a significant correlation between the number of lymphocytes cells and the clinical degrees of DHF severity.¹¹

CONCLUSION AND SUGGESTION

It can be concluded that the neutrophil/lymphocyte count ratio in grade I DHF patients is higher than that in grade II DHF patients. It is also known that the higher the neutrophil/lymphocyte count ratio is, the lighter the degree of DHF severity is. Therefore, further researches are necessary to focus more on other factors that may affect dengue virus infection as well as to perform with more stringent inclusion criteria. Further researches are also essential to focus on the neutrophil/lymphocyte count ratio in children with DHF.

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