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Albumin globulin ratio in children with dengue virus infection at Prof. Dr. R. D. Kandou Hospital, Manado Indonesia

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ABSTRACT

Backgrounds: Dengue virus infection (DVI) consists of mild-to-severe manifestations. The increasing hematocrit of 20% is used for detecting leakage of plasma, but it is a single parameter that is easily influenced by certain factors. It is necessary to examine the ratio of albumin/globulin which is more stable. The purpose of this study is to find out the ratio of albumin/globulin against various degrees of DVI.

Methods: This is an analytic observational study with a cross-sectional and prospective cohort. The study was conducted at the Prof. Dr. R.D. Kandou Hospital, Pancaran Kasih Hospital, and R.W. Monginsidi Hospital from March 2013 to October 2014. The sample is obtained from at least 38 children for each group of DVI. Data were analyzed using SPSS 15, with significant value at $p \leq 0.05$.

Results: There were 164 children with DVI during the study, but we analyzed only 39 patients with DF, 39 patients with DHF, and 41 patients with DSS. All patients with DF had a albumin/globulin ratio ≥ 1.2, whereas 4 DHF patients and 5 DSS patients had a albumin/globulin ratio ≥ 1,2.

Conclusions: There were significant differences in the ratio of albumin/globulin between DF, DHF, and DSS groups. It is emphasized that in the evaluation of plasma leakage using the ratio of albumin/globulin the changing levels of albumin should be considered. We should keep in mind also that there are other factors that can affect the synthesis of albumin.

Keywords: Ratio of albumin/globulin, Dengue virus infection, DF, DHF, DSS

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INTRODUCTION

Dengue virus infection (DVI), notably dengue hemorrhagic fever (DHF), is still a health threat in the world because it is a cause of serious morbidity and mortality, particularly in tropical and subtropical regions, including Indonesia.[1,2] Each year an estimated 50 million cases of DVI with 500,000 cases of dengue and at least 24,000 cases were reported dead.[3,4]

In Asia, DHF/dengue shock syndrome (DSS) is currently the main cause of patients admitted to hospital, with a high mortality rate ranging from 0.5 to 5%.[5,6] In Indonesia, the incidence of DHF has increased prominently from $37.11^{0}/_{00}$ in 2004 to $60.06^{0}/_{00}$ in 2008.[7]

Dengue virus infection can cause a spectrum of clinical manifestations, varying from self-limited fever, namely dengue fever (DF), to the severe form, DHF, and can lead to shock or death.[3] Theoretically, DF is a relatively mild disease whereas DHF is a disease that can be life-threatening if it is not detected or addressed adequately.[8] The increasing of vascular permeability and vasculopathy is a sign of DHF and DSS and what distinguishes it from DF.[9] The state of plasma leakage that occurs in DHF with shock within no more than 48 hours and generally after 48 hours will be followed by immediate and spontaneous improvement.[10]

Based on the protocol of the World Health Organization (WHO) in 1997, DHF can be distinguished from the DF base on increasing hematocrit, pleural effusion, or ascites.[8] Other way to detect the leakage of plasma is by chest X-ray, ultrasound, and hypoalbuminemia. The leakage of plasma can happen when albumin levels ≤ 3.5 g% or there are changes in albumin level of 0.5 g%.[11] Nevertheless, all the means mentioned above is a single parameter that can be influenced by certain factors, such as hematocrit values found normal in DVI patients with anemia, bleeding, and fluid therapy; therefore, there is a need to have more stable parameters that can be assessed using the ratio of albumin/globulin.[12] A study using the ratio of albumin/globulin was conducted on patients with systemic inflammatory response syndrome (SIRS) and on patients who suffered from burns and sepsis. The normal value of the ratio of albumin/globulin is 1.2–1.5.[13,14] A research on the assessment of ratio of albumin/globulin conducted on people with burns were associated with severity of disease, but the research on the DVI has never been reported. Besides, it has huge clinical significance in the early detection of plasma leakage in DVI.[13,14]

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MATERIALS AND METHODS

This is an analytical observational study with a cross-sectional and prospective cohort. The study was conducted at Prof. Dr. R.D. Kandou Manado Hospital, PancaranKasih Hospital and R.W. Monginsidi Hospital from March 2013 to October 2014. The examination of blood samples was carried out in the laboratory department of Prof. Dr. R.D. Kandou Manado Hospital, Manado Clinical Laboratory and Prodia Jakarta Laboratory. The samples were obtained from at least 38 children for each study group (DF, DHF, DSS) with $\alpha = 0.05$, power 80%, and $r = 0.4$.[15]

The inclusion criteria were patients aged 1–15 years diagnosed with DVI (DF, DHF, DSS), which is based on WHO criteria in 1997 as well as the child and parent/guardian who agreed to provide *informed consent*, while the exclusion criteria on patients suffering from a viral illness or other bacteria is based on clinical examination and laboratory. However, patients who received corticosteroids and suffered poor nutrition and obesity as well as DSS patients who were referred and having hospital admission showing the signs of shock were not found. There is also criteria of drop-out, namely a blood sample lysis, wherein patients whose parents refused to do a second blood sampling (48 hours after the first blood sample/in case of worsening) or third blood sample (48 hours after the deterioration).

Initial blood sampling was taken when patients were diagnosed with DF, DHF, and DSS; the second blood sampling was performed when patients experienced worsening within a duration ≥ 48 hours after the initial/first blood sampling. The third blood sampling was done within a period ≥ 48 hours after the second blood sampling. This study was approved by the Department of Biomedical Research Ethics Committee Prof. Dr. R.D. Kandou Manado Hospital. We compared the ratio of albumin/globulin from first blood samples between DF, DHF, and DSS. We then compared the ratio at the beginning from the first blood sampling to second/third blood sampling. There was a leakage in plasma when the ratio of albumin/globulin <1.2. Data were analyzed using a statistical software package, version 15 (SPSS 15); with chi-square test and one way anova test. It is said to be significant if the p value ≤ 0.05.

RESULTS

During the study period, there were 164 children with DVI treated in hospital. After anamnesis and

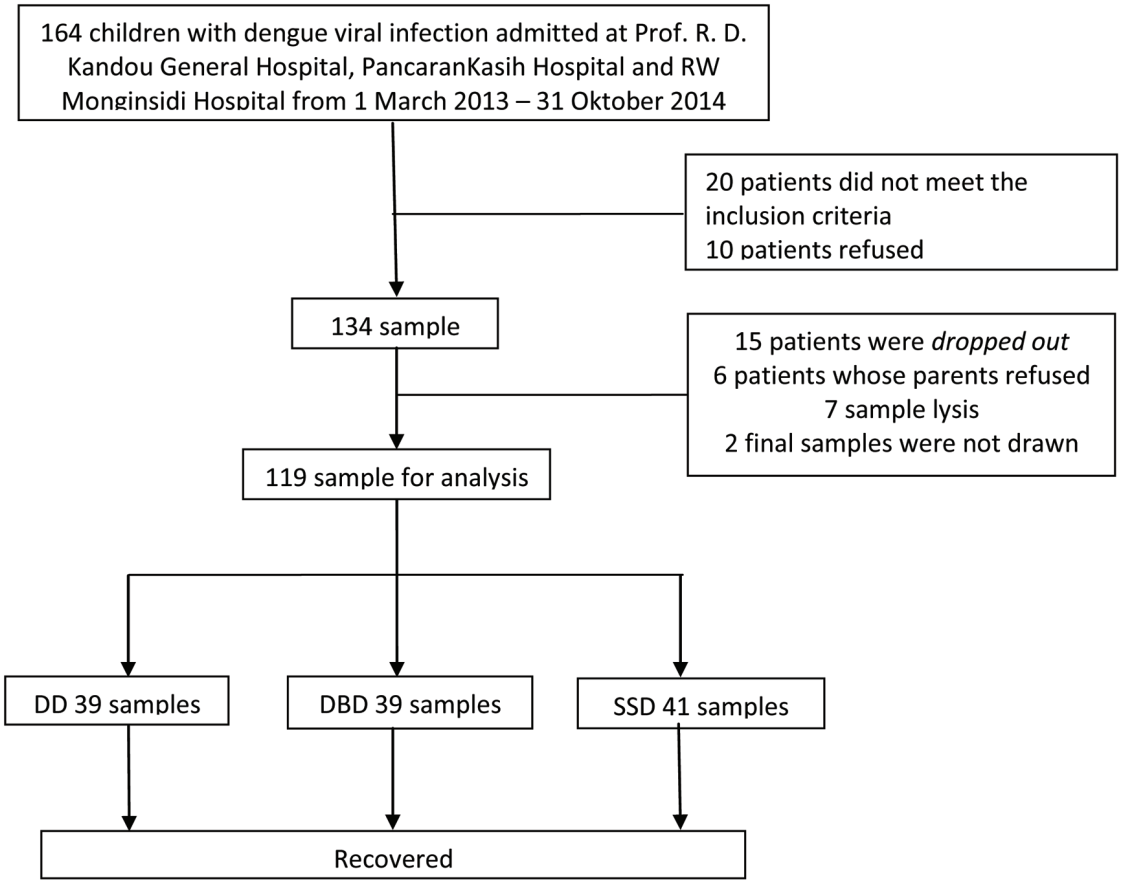

Figure 1 The study population

further inspection, there were 20 children who did not meet the inclusion criteria and 10 children whose parents who did not agree; therefore, the remaining samples of the study were 134. Furthermore, 15 samples were dropped out since 6 parents/guardians refused, 7 samples were lysis, and 2 blood samples were both unsuccessfully taken so that the remaining 119 samples were divided into 3 groups consisting of 39 patients with DF, 39 patients with DHF, and 41 patients with DSS for analysis. At the end of the study, all the subjects of the study were healed (Figure 1).

In the study planned, the blood sampling was taken 3 times from the subject in case of clinical deterioration from DF to DHF or from DHF to DSS. During the study period, there were no subjects who experienced clinical deterioration, so that the samplings of all study subjects were only taken 2 times, namely initial blood samples diagnosed for DVI (DF, DHF, DSS) and ≥ 48 hours later.

Characteristics of the study sampling based on the severity of DVI (DF, DHF, DSS) can be seen in Table 1 below.

Table 1 Characteristics of the study sampling based on the severity of the dengue viral infection (DF, DHF, DSS)

Variable	*DF* (*n* = 39)	DHF (*n* = 39)	DSS (*n* = 41)	*P*
Gender (%)				
Man	17 (43.6)	15 (38.5)	19 (46.3)	0.385*
Women	22 (56.4)	24 (61.5)	22 (53.7)	
Nutritional status (%)				
Under weight	7 (17.9)	7 (17.9)	8 (19.5)	0.978*
Good nutrition	32 (82.1)	32 (82.1)	33 (80.5)	
Age (year)				
Average	7.76	8.95	7.06	0.011**
SD	3.00	2.51	2.87	
95% CI	6.68-8.63	8.14–9.77	6.15–7.97	
Median	7.92	8.92	6.5	
Span	1.42–12.92	4–13.3	2.08–12.3	
Interval of fever (day)				
Rerata				
SD	2.6	4.1	4.4	<0.001**
95% CI	0.60	0.8	0.7	
Median	2.37–2.76	3.84–4.37	4.2–4.63	
Span	3.00	4	4	
Laboratorium	1–3	3–6	4–6	
Blood sample I				
Hematokrite, %	36.99 (3.43)	42.59 (2.99)	45.35 (3.44)	
Albumin, g/dl	3.88 (0.41)	3.39 (0.51)	2.63 (0.54)	
Globulin, g/dl	3.06 (0.37)	3.79 (0.84)	3.51 (1.23)	
Albumin/Globulin	1.31 (0.15)	0.94 (0.26)	0.85 (0.32)	
Blood sample II				
Hematokrite, %	36.76 (3.35)	35.79 (2.8)	35.34 (2.77)	
Albumin, g/dl	3.89 (0.48)	3.81 (0.4)	3.51 (0.54)	
Globulin, g/dl	3.12 (0.54)	3.14 (0.67)	3.05 (0.81)	
Albumin/Globulin	1.29 (0.32)	1.25 (0.28)	1.21 (0.31)	

Ket: SD = standard deviation * = X^2 test (Chi Square) ** = one way ANOVA test

Table 1 shows that there were no significant differences in gender distribution and nutritional status ($p > 0.05$). Of the entire samples obtained, there were 51 boys who were classified as follows: 17 in the DF group, 15 in the DHF group, and 19 in the DSS group. More females were found than males in each group, with 22 children in the DF group, 24 in the DHF group, and 22 in the DSS group. Based on nutritional status, we found that the research samples were dominated by good nutrition (81.5%). The distribution of good nutritional status based on group was obtained, with 32 children in DF and DHF group, respectively, and 33 children in the DSS group.

All the subjects were in the age range of 1.42–13.3 years, with the mean age of patients with DF at 7.76 (SD 3.0) years, mean age of patients with DHF at 8.95 (SD 2.51) years, and the mean age of patients with DSS at 7.06 (SD 2.87) years. The ANOVA test results of the patients' age show that there is a significant difference between the DF, DHF, and DSS groups, with $p = 0.011$ ($p < 0.05$), followed by Benferroni test. Those experiencing DHF were older than those who have DF or DSS. In the age group of DF and DHF, and DF and DSS, there are no significant differences, with $p = 0.128$ and $p = 1.000$ ($p > 0.05$), respectively, while in the age group of DHF and DSS there was a significant difference, with $p = 0.009$ ($p < 0.01$).

Results of the ANOVA for duration of fever showed there was a very significant difference between the DF, DHF, and DSS groups, with $p < 0.001$ ($p < 0.01$), followed by Benferroni test. There was a very significant difference with $p < 0.001$ ($p < 0.01$) in duration of fever in DF and DHF group, and DF and DSS group, whereas in DHF and DSS group there was no significant difference, with $p = 0.147$ ($p > 0.05$).

Table 1 shows that in the initial/first blood sampling at DF group, there was no hemoconcentration based on hematocrit level whereas in the DHF and DSS group there was hemoconcentration which was equal to the ratio of albumin/globulin in the DF group, which was ≥1.2 and for the DHF and DSS group the majority ratio of albumin/globulin was <1.2. In the second blood samples obtained, there was no hemoconcentration from hematocrit or the ratio of albumin/globulin on DF, DHF, or DSS group.

Based on the above results, we found that there was no difference in the ratio of albumin/globulin in the second blood samples of the patients with DF, DHF, and DSS; therefore, the comparative analysis of the ratio of albumin/globulin between the first and second blood sample in DF, DHF, and DSS group was not conducted. However, analysis of the ratio of albumin/globulin with varying degrees of

Table 2 **Analysis of the relationship of albumin/globulin <1.2 and ≥1.2 with clinical degree of dengue viral infection (DF, DHF, DSS)**

The degree of clinical	Albumin/globulin		p^*
	<1.2 (*n* = 71)	≥ 1.2 (*n* = 48)	
DF	0	39	
DHF	35	4	<0.001
DSS	36	5	

*X^2 test

clinical dengue (DF, DHF, DSS) on a sample of the first group (Table 2) was conducted.

Table 2 shows there was a significant relationship between the ratio of albumin/globulin <1.2 and the ratio of albumin/globulin ≥1.2 patients with clinical degrees of DVI (DF, DHF, DSS). In the DF group, none had a ratio of albumin / globulin <1.2. In the DHF group there were 4 of the 39 patients, and in DSS group there were 5 of 41 patients that had the ratio of albumin/globulin ≥1.2.

DISCUSSION

Dengue virus infection is a dangerous viral infection transmitted to humans through mosquito bites. Clinical spectrum of DVI varies from DF to a small portion to life-threatening disorder characterized by hemostasis, endothelial permeability, thrombocytopenia, and coagulation (DHF). Vascular leakage is a characteristic of DHF which indicates the presence of vascular endothelial damage. Changes in vascular permeability can trigger hypovolemic shock known as DSS.

Data on patient characteristics of the study in DVI (DF, DHF, DSS) show that there were more females than males in the age range of 6–10 years (Table 1). A study in Semarang showed more females (14 out of the 26 children, 53.8%) suffered from DHF and DSS (16 out of 23 children, 69.6%).[16] The opposite was found in a study in Brazil; there were more male patients in the group without plasma leakage (33 out of 56 children, 59%), with plasma leakage (31 out of 49 children, 63%), and the group of healthy children (9 out of the 15 children, 60%).[17]

Based on the age of the subjects, we found significant differences in mean age between the DF, DHF, and DSS groups, with a mean age of 7–9 years. Age is one factor that influences the susceptibility to DVI. In Indonesia, Philippines, Thailand, and Malaysia, DHF affects mostly children during the age span of 5–9 years.[18] These results are similar with the results of a study conducted in Jakarta, wherein it was found that the highest percentage of dengue cases was noticed in children in the age group of 5–14 years (36%), followed by those aged below 5 years (31%), those in the age range of 15–44 years (22%), and those aged above 45 years (11%).[19] Several studies were reported that young age is associated with the occurrence of DSS.[20,21] A study in Denpasar reported the mean age of subjects with DSS group 7.5 (*SD* = 2.5) years,[22] and a study in Semarang found a mean age of 8 years (range 6–10 years) for both subjects with DHF and with DSS.[16]

Shock in patients with DHF is more common in children with good nutritional status; it is associated with increased antigen antibody reaction resulting in more severe infection.[18,23] This study found a different result; there was no effect of the nutritional status on DVI. This is due to the characteristic of the study sample. This study shows the frequencies of DF, DHF, and DSS were more common in children with good nutritional status (80%) compared to those with malnutrition status (20%). Studies in Thailand and Vietnam showed a significant association between nutritional status and severity of dengue; the risk for complications of dengue in normal children is 3.2 times higher compared to malnourished children.[24] The same result was reported by two other researchers who showed obesity as a risk factor in the severity of dengue.[25,26] This is in contrast to the studies in Latin America, El Salvador, which did not see the connection between nutritional status and the severity of dengue.[27]

The duration of fever determines the clinical course of DHF and the ratio of albumin/globulin. In the early days of DHF, dengue virus viremia was still going on and the infected macrophages produced cytokines.[28] This study shows a very significant difference of duration of fever between DF (2.56 days), DHF (4.1 days), and DSS (4.41 days) groups. This is similar to a study in Brazil which showed a significantly different duration of fever in patients with plasma leakage compared to patients without lasma leakage in DVI.[17]

Furthermore, the study results show that all patients in the DF group (39 subjects) did not experience leakage of plasma, whereas those in the DHF group (39 subjects) and DSS group (41 subjects) had plasma leakage with one or more markers of plasma leakage according to WHO criteria, 1997. The grouping of patients into DHF and DSS is based on several clinical and laboratory parameters of DHF/DSS according to WHO criteria, 1997. Moreover, there is an increase in hematocrit ≥ 20% and the presence of pleural effusion or RLD photo and/or thickening of the gallbladder/ascites on ultrasound. Hematocrit can be influenced by the presence of anemia, bleeding, hydration status, fluid, and often we do not have the initial laboratory data that can be used as the initial benchmark. Chest X-ray also

has weaknesses. It can only detect the presence of fluid effusion in a relatively large number and it is difficult to do in series (for ethical reasons, no additional information on the diagnosis and treatment of patients was colleted and thus there may have been technical difficulties during diagnosis. The abdominal ultrasound in this study also was not performed on all patients because of the technical difficulties of implementation. The use of the ratio of albumin/globulin, hematocrit, albumin, and the differences in albumin, x-rays, and ultrasound are the parameters of plasma leakage that should be interpreted with the same time, to improve the accuracy of these parameters as a marker of plasma leakage.[29-32]

Moreover, in this study, in the DF group there was no subject that had a ratio of albumin/globulin <1.2, while in the DHF and DSS group we found 4 patients and 5 patients, respectively, who had albumin/globulin ≥1.2. This is possible because the parameter of albumin/globulin can be influenced by the severity of leakage of albumin and globulin plasma, so that both can be out of the intravascular space together and cannot meaningfully reduce the levels of albumin/globulin. Further analysis showed that in 4 patients and 5 patients (in DHF and DSS group, respectively) had a ratio of albumin/globulin ≥1.2 showing albumin level <3.5 g/dl and the changing level of albumin from the begining till the end of study at > 0.5 g/dl. This is a sign of plasma leakage by WHO criteria, 2011. In this case, in evaluating plasma leakage using the ratio of albumin/globulin we should consider the levels of albumin and the changing level of albumin. We should keep in mind also that there are other factors that affect the synthesis of albumin. In this study, it is found out that duration of fever influenced the ratio of albumin/globulin.[30]

Albumin molecule has a smaller size (69 kDa) compared to globulin molecules (90–156 kDa), resulting in the state of increased vascular permeability; the albumin has relatively more leakage than globulin at an early stage; consequently, the ratio of albumin and globulin was reversed. In more severe cases an increasing pore size of the blood vessels can lead to leakage of good albumin and globulin molecules, thus resulting in no reversal in the ratio of albumin and globulin.[14]

In addition, albumin and globulin were affected by vascular permeability, and by the reduction of synthesis, by reduction through the gastrointestinal tract and kidneys, as well as by reducing the lymphatic backflow.[33]

In this study, the ratio of serum albumin/globulin was significantly different between the DF, DHF, and DSS groups. The ratio of serum levels of initial/first albumin/globulin in DHF and DSS groups was significantly lower than the DF group. Dengue shock syndrome group has a lower ratio of the initial serum albumin/globulin than the DHF group but this diference was not significant.

This study also shows that there was a decrease in the ratio of the initial levels of albumin/globulin in all groups at different degrees. According to Wills, a light increase in the capillary permeability results in relatively slow leakage of albumin and gives time to activate the mechanism of regulation of homeostasis compared to the massive leak. If the leak exceeds the capacity of regulation, there will be hemoconcentration followed by cardiovascular disorders. Thus, in DF the capillary leak was light or only small, so hemoconcentration did not occur. Wills also reports that the levels of IgG, transferrin, and albumin in patients with secondary DVI will be reduced in patients who experienced shock than those who are in convalescence. There are significant differences in the levels of albumin in moderate-to-severe shock patients because they had lower albumin levels than patients who experienced only a mild shock.[34,35]

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