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Original Article

Soluble vascular cell adhesion molecule-1 levels and severity of dengue hemorrhagic fever in children

Nolitriani, Rinang Mariko, Mayetti

Abstract

Background The clinical manifestations of dengue infection vary widely, ranging from asymptomatic to severe forms that can cause death. In severe infections, the expression of soluble vascular cell adhesion molecule-1 (sVCAM-1) in endothelial cells is reportedly excessive, causing endothelial cell gaps through VE-cadherin and plasma leakage, which is the basic mechanism for shock in dengue hemorrhagic fever (DHF).

Objective To determine the association between sVCAM-1 levels and severity of dengue hemorrhagic fever in children.

Methods This cross-sectional study was done in children with DHF at Dr. M. Djamil Hospital, Padang, West Sumatera. Subjects were diagnosed according to the 2011 WHO criteria and selected by consecutive sampling. They were grouped as DHF with or without shock. Examination of sVCAM-1 levels was done by ELISA method. Mann-Whitney test with a significance of $P<0.05$ was used for statistical analysis.

Results A total of 66 patients were collected from January 2018 to December 2019, but 2 patients were excluded. The 64 subjects who met the inclusion criteria consisted of 32 (50%) DHF without shock and 32 (50%) DHF with shock. Median sVCAM-1 was significantly higher in the DHF with shock group (840 ng/mL) than in DHF without shock group (598 ng/mL) ($P<0.05$).

Conclusion There was a significant association between higher sVCAM-1 levels and greater severity of dengue hemorrhagic fever in children. **[Paediatr Indones. 2021;61:328-35 ; DOI: 10.14238/pi61.6.2021.328-35**].

Keywords: *pediatric; dengue hemorrhagic fever; sVCAM-1*

Dengue hemorrhagic fever (DHF) is caused by the dengue virus, which consists of four serotypes, namely, DEN-1, DEN-2, DEN-3, and DEN-4.[1] Infection by any one or a combination of the four serotypes causes a clinical spectrum of dengue fever (DD), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).[2] This DHF is transmitted through mosquito bites, especially by *Aedes aegypti* or *Aedes albopictus*.[3]

More than half of the world population is estimated to live in endemic areas. It is estimated that 50 million cases of dengue virus infection occur each year, with a death rate of more than 20,000 people per year in the world.[4] Asia ranks as the first in the world with the highest number of people with dengue infection every year. The *World Health Organization* (WHO) recorded Indonesia as the country with the highest number of cases in Southeast Asia from 1968 to 2009.[1] In 2013, there were 112,511 cases of dengue with 871 deaths, while in 2014 there were 100,347 cases with 907 deaths.[5]

From the Department of Child Health, Universitas Andalas Medical School/ Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia.

Corresponding author: Nolitriani. M. Djamil Hospital. Jl. Perintis Kemerdekaan, Sawahan, Padang, West Sumatra. Phone: +6285272096450. Email: O_iporta@yahoo.com.

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There are many pathogenesis theories that try to explain the variation in clinical appearance of dengue viral infection. Severe cases of DHF and DSS are marked by an increase in blood vessel permeability resulting in plasma leakage.[4] Dengue viral infection can trigger endothelial leakage, causing edema and shock.[6] Endothelial cells line the inner walls of blood vessels and play an important role in maintaining the integrity and permeability of blood vessels. While the pathogenesis of dengue infection is determined by various mechanisms, endothelial cells are likely involved.[7]

Under normal circumstances, the endothelium prevents blood cells from sticking to the walls of blood vessels, but allows leukocytes to pass along the surface and regulates the movement of leukocytes into tissues. The regulation of proliferation, adhesion, and migration of endothelial cells is regulated by special glycoproteins known as cellular adhesion molecules (CAM), which are proteins located on the cellular surface that function in bonds between cells or cells within the extracellular matrix.[8] There are four main families of CAMS, namely, the superfamily of immunoglobulin adhesion molecules (IgCAMs), integrins, cadherins, and selectins. Members of the IgCAMs include vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which contribute to modulating cell-cell interactions.[9]

The ICAM-1 is constantly expressed on resting or non-inflamed endothelial cells.11 While VCAM-1 is only slightly expressed at rest, it rapidly increases when induced by inflammatory conditions.[10] Both VCAM-1 and ICAM-1 are expressed by endothelial cells activated by cytokines.[11] The normal level of sVCAM-1 ranges from 395-714 ng/mL.[12,13] A study revealed that the normal level (cut off point) of sVCAM-1 in healthy or normal people was 631 ng/mL.[14]

The endothelium is activated by exposure to inflammatory mediators produced by monocytes infected by dengue virus to express the adhesion molecules VCAM-1 and ICAM-1.[4] Such expression of ICAM and VCAM can cause adhesion and transfer of leukocytes as well as lead to plasma leakage. The expression of VCAM-1 by endothelial cells is excessive in severe infections. All these processes lead to endothelial cell weakness and leakage of fluid into the serous cavity.[15]

To date, only a few studies have assessed for an association between sVCAM-1 and dengue infection. The search for an accurate biomarker directly related to plasma leakage in dengue infection prompted us to evaluate sVCAM-1 in pediatric DHF patients. The general objective of this study was to determine the relationship between sVCAM-1 levels and severity of DHF in children, with the hope of using this laboratory examination modality to predict the occurrence of shock in dengue hemorrhagic fever.

Methods

This cross-sectional, analytic study was done from January 2018 to December 2019 at the Department of Child Health, Andalas University/Dr. M. Djamil Hospital, Padang, West Sumatera. Subjects were included by consecutive sampling. Of 66 pediatric patients diagnosed with DHF by a consultant in pediatric infections and tropical diseases, 64 patients fulfilled the inclusion criteria of DHF according to the 2011 WHO critieria. The WHO 2011 clinical criteria included clinical symptoms of thrombocytopenia, hemoconcentration, as well as confirmed by positive anti-dengue IgM and IgG serology tests, or positive anti-dengue.[15] Subjects' parents provided written informed consent. Exclusion criteria were patients with comorbidities (diabetes mellitus, cardiovascular disease, malignancy, or sepsis).

The level of soluble vascular cell adhesion molecule 1 (sVCAM-1) was defined in the critical phase, namely, the period of plasma infiltration, the transition from fever to afebrile phase, and/or the 4th to 6th sick days. Dengue hemorrhagic fever severity was divided into two groups based on WHO 2011 criteria, with and without shock. DHF without shock was defined as fever duration of 2-7 days that appeared suddenly, and was high, persistent (continuously), accompanied by two or more symptoms such as: spontaneous bleeding manifestations (petechiae, purpura, ecchymosis, epistaxis bleeding gums, hematemesis and/or melena); a positive tourniquet test; headache, myalgia, arthralgia, or retroorbital pain; presence of DHF cases in the school environment, home, or around the house; hepatomegaly; plasma leakage such as increased hematocrit value >20% from baseline or from population data according to age, pleural effusion, ascites, hypoalbuminemia,

hypoproteinemia; thrombocytopenia <100,000/mm^3 and proven by positive anti-dengue IgM and IgG, or positive anti-dengue IgM. DHF with shock was defined as DHF with signs of circulatory failure. Subjects underwent data collection (age, sex, nutritional status, clinical symptoms, routine laboratory and diagnosis) at admission, and blood sampling for complete blood count, anti-dengue IgM and IgG, and sVCAM-1 examinations in critical phase, the period of plasma leakage on 4th to 6th day of illness with soluble human VCAM-1 ELISA kit by BT Lab.

Data were grouped into numerical and categorical variables. Numerical variables were displayed as mean (standard deviation) or median (minimum-maximum); categorical variables were displayed as frequencies and percentages. Categorical variables were analyzed by Chi-square test. Normality for numerical variables (sVCAM-1 level results, age, platelet and hematocrit level) were analyzed by Kolmogorov-Smirnov test. Normally distributed numerical data were analyzed by independent sample T-test. Non-normally distributed numerical data were analyzed by Mann-Whitney test. Statistical analyses were performed with *SPSS version 20.0* software. This study was approved by the Medical Ethics Committee of Universitas Andalas/Dr. M. Djamil Hospital, Padang.

Results

Table 1 shows that the majority of subjects were female in both the DHF without shock and DHF with

Table 1. Characteristics of DHF subjects with and without shock

Characteristics	Dengue hemorrhagic fever		P value
	Without shock (n=32)	With shock (n=32)	
Demographics			
Gender, n (%)			0.418[a]
Male	12 (37.5)	8 (25.0)	
Female	20 (62.5)	24 (75.0)	
Mean age (SD), years	8.34 (3.99)	7.22 (3.55)	0.239[b]
Nutritional status, n (%)			0.030[a*]
Malnourished	17 (53.1)	6 (18.8)	
Well-nourished	12 (37.5)	18 (56.3)	
Overweight	2 (6.3)	4 (12.5)	
Obese	1 (3.1)	4 (12.5)	
Clinical circumstances			
Median duration of fever at admission (range), days	4.5 (3-7)	4.5 (3-6)	
Bleeding manifestation, n (%)			
Spontaneous	23 (71.9)	21 (65.6)	
Not spontaneous	9 (28.1)	11 (34.4)	
Abdominal pain, n (%)			
Yes	7 (21.9)	16 (50.0)	
No	25 (78.1)	16 (50.0)	
Vomiting			
Yes	2 (6.3)	12 (37.5)	
No	30 (93.8)	20 (62.5)	
Hepatomegaly			
Yes	5 (15.6)	17 (53.1)	
No	27 (84.4)	15 (46.9)	
Laboratory findings			
Median platelet (range), /mm^3	60,000 (9,000–92,000)	26,000 (8,000–84,000)	<0.001[c*]
Median hematocrit (range), vol%	41 (21–53)	45.50 (27-57)	0.071[c]
Type of dengue infection, n (%)	12 (37.5)	4 (12.5)	0.043[a*]
Primary infection, positive IgM	20 (62.5)	28 (87.5)	
Secondary infection, positive IgG & IgM			

Note: [a]Chi-square test; [b]independent sample T-test ; [c]Mann-Whitney test; [*]P<0.05 significant

shock groups. The mean age of subjects was 8.34 (SD 3.99) years in the DHF without shock group and 7.22 (SD 3.55) years in the DHF with shock group. Neither sex nor age were significantly associated with DHF severity (P>0.05 for both). Most subjects were well-nourished in the DHF with shock group, but malnourished in the DHF without shock group, which was a statistically significant relationship (P<0.05).

The mean duration of fever at the time sVCAM-1 levels were examined was 4.5 days. Spontaneous bleeding manifestations were found in more than half of the subjects. Complaints of abdominal pain in the DHF without shock group (21.9%) were lower than in those with shock (50.0%). Complaints of vomiting in the DHF without shock group (6.3%) were lower than in those with shock (37.5%). Hepatomegaly in the DHF without shock group (18.8%) was lower than in the DHF with shock group (50.0%).

The platelet count was significantly lower in the DHF with shock group than in the without shock group. The median hematocrit value in the critical phase was not significantly different between groups (41% *vs.* 45.5%, respectively; P>0.05). Secondary infection was significantly lower in the DHF without shock group (62.5%) than in DHF with shock group (87.5%).

Figure 1 shows that the sVCAM-1 levels were not normally distributed, with a range of 399-2,184 ng/mL in the without shock group and 398-2,365 ng/mL in the DHF with shock group. The P value of Kolmogorov-Smirnov normality test was <0.05, so that the analysis was continued using non-parametric statistics.

Table 2 shows that there is a significant relationship between sVCAM-1 levels and the severity of DHF in children. The median sVCAM-1 levels were 598 ng/mL in subjects without shock and 840 ng/mL in those with shock.

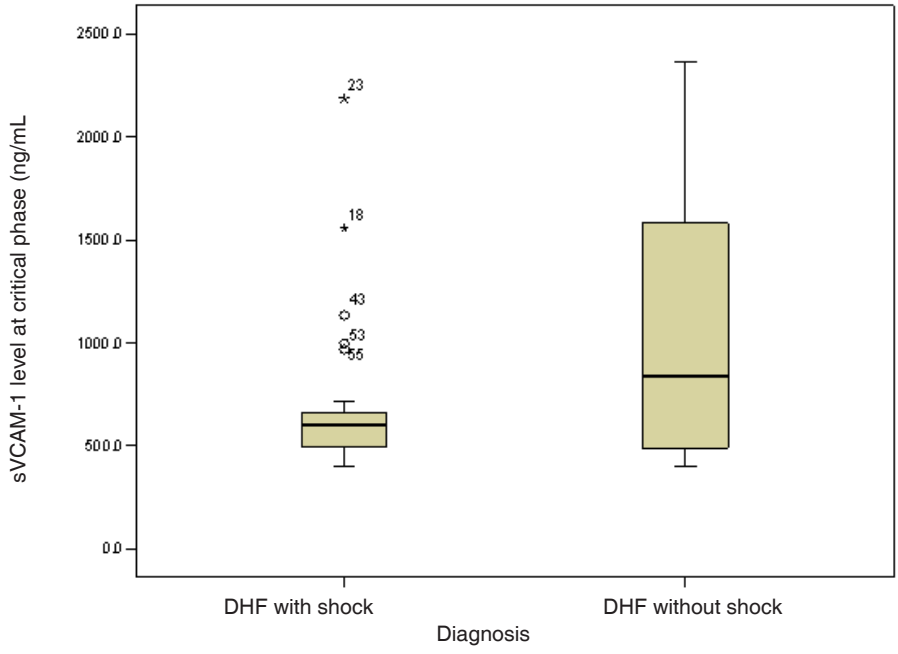

Figure 1. Levels of sVCAM-1 in DHF patients with and without shock

Table 2. Analysis of sVCAM-1 level and DHF severity

DHF classification	n	Median sVCAM-1 level (range), ng/mL	P value
Without shock	32	598 (399-2184)	0.045*
With shock	32	840 (398-2365)	

*Mann-Whitney test

Discussion

National data in 2016 showed that the dengue infection case distribution among age groups was 2.6% in <1-year-olds, 12.2% in 1 to 4-year-olds, 39.9% in 5-14-year-olds, 36.1% in 15-44 year-olds, and 9.13% in >44-year-olds. The report also showed that the incidence of dengue infection in Indonesia was increased in children, adolescents, and young adults.[16] A previous study found that age >6 years was most commonly affected by dengue infection and shock. The risk factors for dengue infection in older children are increased exposure to mosquito bites and secondary dengue infection.[17] Similarly, a mean age of 7 to 8 years was the most common age of dengue infections in our subjects, but age was not significantly different between the groups with and without shock.

More females than males suffered dengue infection, especially DHF with shock in our study. The risk of shock and death are greater in females because their immune response may be more sensitive to the secretion of cytokines, which affect the degree of plasma leakage.[18] However, in our study, gender was not significantly different between groups. Likewise, other studies found that gender was not a risk factor for more severe dengue infection.[18,19]

The number of patients with well-nourished nutritional status was higher in DHF with shock than DHF without shock in our study. Similarly, a previous study reported that the majority of dengue cases had normal nutritional status. Nutritional status is believed to influence disease severity based on the theory that good nutrition increases the antibody response. As such, excessive antigen and antibody reactions lead to more severe dengue infection.[19] A meta-analysis found that obesity was a risk factor for shock in DHF.[20] Obese children are more prone to complications and even death because their immune systems trigger a more severe inflammatory reaction than malnourished children. Obese children have increased adipose tissue which produces various metabolites, hormones, and cytokines such as TNFα, plasminogen activator inhibitor type-1, interleukin-6, and C3 complement. Adiponectin is a hormone that has important biological activity in glucose and fat metabolism and influences insulin resistance. Adiponectin levels are inversely proportional to the mass of intra-abdominal fat.[21] Adipose tissue provides a negative feedback signal causing a decrease in adiponectin levels. Adiponectin itself functions as an anti-inflammatory agent which can inhibit the secretion of IL-6, IL-8, and TNF-α. Hence, if adiponectin hormone levels decrease, as in the case of obese children, capillary permeability will increase. In addition, reduction of adiponectin levels also leads to increased adhesion molecule expression. Dengue virus invasion of endothelial cells causes local inflammation, which combined with increased capillary permeability due to lack of adiponectin hormone, results in vascular leakage and shock.[22] This theory may explain why 4 out of 5 obese patients and 4 out of 6 overweight patients experienced shock in our study.

More of our subjects without shock had malnutrition. A study explained that malnutrition can suppress cell-mediated immune responses, thereby reducing the risk of dengue virus infection severity.[23] Malnutrition can affect the response to infection through decreased cellular immunity as indicated by a decrease in the number of CD4+ T-helper cells, low CD4/CD8+ ratio, decrease of secretory IgA antibody production, various complement (C3, C4 and factor B) factors, and phagocytosis disorders. Thus, secondary infection of dengue virus tends to be less severe in patients with malnutrition.[24]

The median duration of fever when sVCAM-1 levels were measured was 4 to 5 days. There was no significant relationship between duration of fever and DHF severity. Fever can be found in almost 100% of DHF cases either with or without shock.[25] WHO guidelines show that the critical phase of dengue infection generally occurs from day 4 to day 6 when plasma leakage begins.[1] Children with dengue infection with signs of worsening or severe dengue experienced a fever ranging from 3 to 8 days, with a mean fever of 5 days, but there was no significant relationship between fever duration and severity of dengue infection in children.[26]

Spontaneous bleeding manifested in more than half of our subjects, but it was not higher in DHF patients with shock than those without shock. A study also found that bleeding manifestations in the form of mucosal bleeding were not related to shock in children.[27] Another study explained that bleeding in dengue infection can occur due to platelet dysfunction, vasculopathy, and coagulopathy.[18]

At the peak of plasma leakage, the patient may

go into shock. Persistent vomiting and abdominal pain are early signs of plasma leakage, worsening as the patient goes into shock. These symptoms can persist even after shock. Abdominal pain can be caused by bleeding in the gastrointestinal tract, hepatomegaly, decreased blood flow during shock and shock itself, causing hypoxia in the gastrointestinal tract.[18] A meta-analysis found abdominal pain (14 studies) and vomiting (17 studies) had a significant relationship with the degree of dengue infection in children.[28] A previous study also obtained the same results, namely, that abdominal pain and vomiting were more common in cases of severe dengue infection and had a significant association with the occurrence of shock.[25]

Hematocrit and platelet levels change according to the degree of dengue infection, with more severe dengue infection, hematocrit level increases and platelet level decreases. Thrombocytopenia at the start of treatment, especially in patients with a platelet count of less than 20,000/mm^3, was a significant predictor of shock.[17] This finding suggests that children with progressively decreasing need special attention because they can develop severe dengue infection.[29] However, a study found that sVCAM-1 levels did not correlate with platelet counts. This finding may have been due to thrombocytopenia in DHF occurring through several mechanisms, including the destruction of platelets by the direct effect of the virus. In addition, immune complexes attached to the platelet surface facilitate their destruction by the reticulo-endothelial system in liver and spleen.[13] In our study, DHF patients with shock had much lower platelet levels than those without shock. Low platelets are indicative of platelet destruction due to a strong immune response. As such, patients are at risk for hypovolemic shock if there is heavy bleeding due to thrombocytopenia.

We found that hematocrit levels in DHF patients with shock were higher than those without shock, but the difference was not significant. A previous study showed that hematocrit level cannot be used as an indicator of shock because it is influenced by bleeding and fluid administration. Heavy bleeding can reduce hematocrit levels, while dehydration and plasma leakage can increase hematocrit levels.[22] Increased hematocrit levels indicate plasma leakage and are a risk factor for hypovolemic shock. We attempted to collect the highest hematocrit data available, as a marker of critical phase occurrence, from Dr. M. Djamil Hospital, Padang, or previously-visited health facilities.

Our study found a significantly higher incidence of secondary infection than primary infection with greater severity of DHF. This finding supports the theory of secondary heterologous infection which states that plasma leakage in secondary infection causes more severe clinical manifestations.[30] The greatest risk factor for severe dengue infection is secondary infection by a viral serotype that is different from previous infections. The severity of disease in secondary infection is associated with higher plasma viral titers.[31]

The level of sVCAM-1 in DHF patients with shock was significantly higher than those without shock ($P<0.05$). There have not been many studies on the role of adhesion molecules, such as VCAM-1, in the pathogenesis of dengue infection. Liao et al. also found that sVCAM-1 levels were significantly increased in the severe dengue infection group than in the dengue fever group and was continuously high from the start of the fever to the recovery phase, thus, sVCAM-1 was closely related to the degree of disease severity.[32] Another study showed that sVCAM-1 levels were associated with the severity of dengue infection and showed an increase in sVCAM-1 levels in serial serum blood tests of patients during the acute phase. They also reported that sVCAM-1 levels were higher in DSS patients compared to DHF patients.[33]

A study revealed that the median VCAM-1 level in normal people is 630 ng/mL.[34] Six dengue with shock subjects had normal sVCAM-1 levels in our study. During the plasma leakage phase, the cytokines also cause angiogenic protein activation, such as increased VEGF level and decreased sVEGFR2, as well as decreased angiopoietin-1 and increased angiopoietin-2 levels.[4] These angiogenic protein levels were not measured and compared in our study. In addition, the examination of viral load, dengue virus serotype, and levels of NS1, which are part of the pathogenesis of dengue fever, were also not examined. Such factors may have led to the extreme values in our study.

Conflict of Interest

None declared.

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