

# The Difference of sVE-Cadherin Levels between Dengue Hemorrhagic Fever Patients with Shock and without Shock

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## Abstract

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**BACKGROUND:** Dengue virus infection is an infectious disease caused by the dengue virus and transmitted by the *Aedes aegypti* mosquito. Dengue virus (DEN-V) consists of 4 serotypes, namely DEN-1, DEN-2, DEN-3, and DEN-4. The most feared result of DHF is death. Death in children is caused by hypovolemic shock due to plasma leakage from intravascular to extravascular space due to endothelial dysfunction.

**AIM:** This study aims to analyse difference in sVE-Cadherin levels in Dengue Hemorrhagic Fever (DHF) with and without shock.

**MATERIAL AND METHODS:** The method of taking samples is consecutive sampling, namely the research subjects obtained based on the order of entry in the hospital with a comparative cross-sectional design. From the results of the calculation using the sample formula, the sample size for each group is set at 32 people. So that the total sample size used for both groups is 64 people. The serum sVE-Cadherin levels using the ELISA method. The statistical test used is the independent t-test. The value of  $p < 0.05$  was said to be statistically significant.

**RESULTS:** The result showed that there was no difference in mean sVE-Cadherin levels between DHF patients with shock and without shock ( $p > 0.05$ ).

**CONCLUSION:** This study concluded that there was no difference in mean of sVE-Cadherin level in DHF patients with shock and without shock.

## Introduction

Dengue virus infection is an infectious disease caused by the dengue virus and is transmitted by the mosquito *Aedes aegypti* [1]. In dengue infection after the virus enters the body, the virus will infect Langerhans, dendrites, macrophages and B lymphocytes [2], [3], [4]. These infections produce various mediators that have an impact on endothelial cell function [5]. Langerhans, dendrites, macrophages and B lymphocytes that are infected will experience activation, securing mediators TNF- $\alpha$ , IL-8, IL-10, IL-15, IL-18, RANTES, MCP-1 $\alpha$ , MCP-1 $\beta$ , monokine, histamine and vascular endothelial growth factor (VEGF) [6], [7], [8].

Furthermore, MHC class II presents the

dengue virus to T lymphocytes and T lymphocytes will stimulate macrophages to kill viruses that have been previously deposited. Infected B lymphocytes, after binding to T lymphocytes, will transform into plasma cells and then produce antibodies. Furthermore, antibodies will bind and neutralise circulating viruses, activate the complement system and cross-react with platelets, endothelial cells and hepatocytes (transient autoimmune) [9]. Antibodies that cannot neutralize the virus will bind the dengue virus and function as opsonin. The antibody-virus bond then binds to the Fc receptor on the surface of the macrophage to cause signals into the cell and activate macrophages [2].

Proinflammatory cytokines, VEGF, complement and antibodies released by the immune system including macrophages result in endothelial cells contracting actin filaments in the capillary endothelial cell cytoplasm. The contraction will pull in

the link protein between cells, JAMs and sVE-Cadherin that enter the cells resulting in widening of the gap between endothelial cells resulting in plasma leakage. Severe and prolonged plasma leakage can cause hypovolemic shock and even death of the patient [10].

Dengue research using endothelial tissue culture in patients with dengue infection showed endocytosis of sVE-Cadherin in endothelial cells that were activated. Endocytosis decreases levels of sVE-Cadherin, in endothelial cells which are directly proportional to the severity of plasma leakage. This shows that sVE-Cadherin plays an important role in maintaining the integrity of the link between endothelial cells and its level can be used as a parameter of plasma leakage [11].

This study aims to analyse difference in sVE-Cadherin levels in Dengue Hemorrhagic Fever (DHF) with and without shock.

## Material and Methods

This study was an observational study with a comparative cross-sectional design. The sVE-Cadherin examination was carried out in the Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang.

### Study Population

The study population was patients with dengue virus infection (DHF and DSS) who were hospitalised at Dr M. Djamil Central General Hospital according to WHO 2011 criteria [12]. Subjects were part of the population that met the inclusion and exclusion criteria. The inclusion criteria were patients with dengue hemorrhagic fever who had received informed consent from parents to participate in the study with the age of 1-15 years. Exclusion criteria were patients suffering from other viral or bacterial infections based on clinical and laboratory examinations, receiving corticosteroid therapy, malnutrition and obesity.

### Examination of sVE-Cadherin Levels

Blood samples  $\pm$  2-3 cc (which is checked in the critical phase) that were inserted into the serum tube were sent to the Biomedical Laboratory, Faculty of Medicine, Andalas University using media transport at 4°C. After that, prepare the microplate well as needed. Then, add 100  $\mu$ L Diluent RD1-78 Assay into each well and add 50  $\mu$ L of serum or standard or control into each well, cover with adhesive strip then incubate at room temperature and above the

horizontal orbital microplate shaker set at 500 rpm + 50 rpm. The aspirations of each well and washing, do 3 times from a total of 4 washing times. Washing is done by entering 400  $\mu$ L wash buffer. After that, add 200  $\mu$ L conjugate sVE-Cadherin to each well. Then cover with a new adhesive strip and incubate for 2 hours. Perform the washing process again as in point 5. After that, add 200  $\mu$ L Substrate Solution to each well and incubate for 30 minutes at room temperature and on benchtop avoid light and then add 50  $\mu$ L Stop Solution to each well to stop the reaction. The colour inside the well must change from yellowish blue. Read using a microplate reader with a wavelength of 450 nm and a correction wavelength of 540 nm or 570 nm. Plot the standard curve and estimate the concentration of the sample against the curve.

### Statistical analysis

The data obtained were analysed using computer systems in the form of tables and graphs. Bivariate analysis was performed to see the difference in mean sVE-Cadherin in DHF patients with shock and without shock. First, the data are analyzed using normality test to determine the normality of the data using the Shapiro Wilk test ( $n < 50$ ), then followed by bivariate analysis, if the data is normally distributed then the analysis is done using the dependent test t-test, but if it is known to be not normally distributed Mann-Whitney test was done with confident interval (CI) 95% and  $\alpha = 0.05$ . The conclusion of the test results if the value of  $p \leq 0.05$  then  $H_0$  is rejected, meaning that there is a difference in the mean between the independent variables and the dependent variable.

### Research Ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 175 / KEP / FK / 2016.

## Results

The difference in the results of sVE-Cadherin examination between dengue patients with shock compared to those without shock can be seen as follows.

**Table 1: Difference in the results of the examination of sVE-Cadherin between DHF patients with shock and without shock**

Variable	DHF		p-value
	DSS (n = 62) mean $\pm$ SD	DHF (n = 48) mean $\pm$ SD	
sVE-Cadherin (ng/ml)	5.93 $\pm$ 4.87	5.86 $\pm$ 4.811	0.956

Table 1 showed that the average sVE-Cadherin level in DHF patients with shock was  $5.93 \pm 4.87$  ng/ml, while in DHF patients without shock  $5.86 \pm 4.811$  ng/ml. From the results of statistical tests, there was no difference in mean sVE-Cadherin levels between DHF patients with shock and without shock ( $p > 0.05$ ).

**The cut-off point for sVE-Cadherin levels as a predictor of dengue patients with shock**

The cut-off point of sVE-Chaderin levels as a predictor of dengue patients with shock is shown in Figure 1.

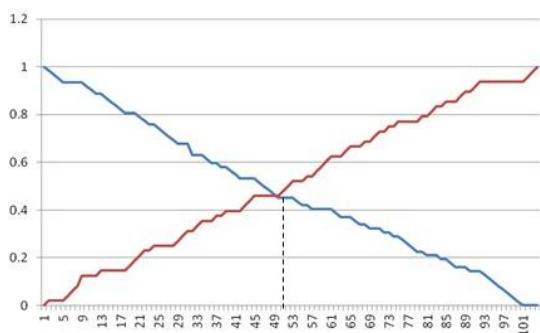


Figure 1: Cut-off of sVE-Cadherin levels as predictors of DHF patients with shock with A); Blue (sensitivity); B) Red (specificity)

Figure 1 shows that the optimal cut-off point on the intersection of sensitivity and specificity lines to determine the cut-off point of sVE-Cadherin levels as a predictor of DHF patients with shock is between point 50. Cut off points of sVE-Cadherin levels as predictors of DHF patients with shock can be explained as follows. Namely, subjects experiencing DSS, if the sVE-Cadherin level is  $\geq 4.04$  ng/ml and the subject has DHF if the sVE-Cadherin level is  $< 4.04$  ng/ml

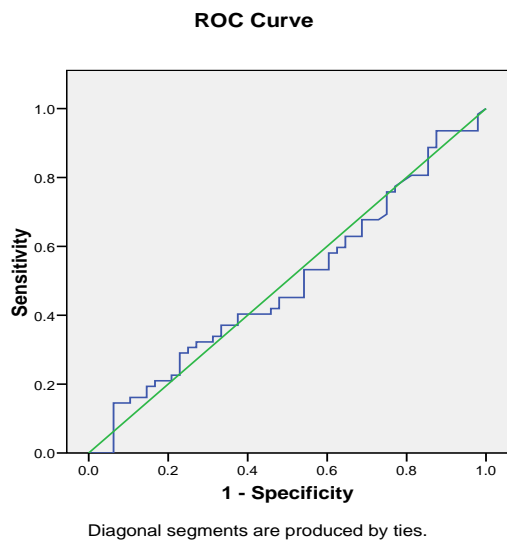


Figure 2: Accuracy of Cut-off point sVE-Cadherin levels as predictors of DHF patients with shock

The cut-off point of this sVE-Cadherin sensitivity was 45.1%, and specificity was 45.8%. The accuracy of the cut-off point of sVE-Cadherin levels as a predictor of DHF patients with shock is shown in Figure 2.

Figure 2 is known based on the receiver operating curve (ROC) analysis that the area under curve (AUC) value of 49.5% means that the cut-off point of sVE-Cadherin level of  $\geq 4.04$  ng/ml has poor accuracy in predicting DSS events.

Table 2: Selection of candidate variables in predicting payments in DHF patients

Variables	p-value
Long fever	0.274
Mucosal bleeding	0.001†
Abdominal pain	0.000†
Sedentary vomiting	0.000†
Hepatomegaly	0.000†
Hematocrit	0.005†
Platelets	0.000†
sVE-Cadherin	0.956

† qualify if  $p < 0.25$ .

**Discussion**

**The difference in the results of sVE-Cadherin examination between DHF patients with shock compared to without shock**

Inter-cell links that maintain the paracellular path are tight junction and adhering junction. From the two links the main one is the adhering junction. The large gap between endothelial cells is maintained constant by various proton adhesions in the gap between endothelial cells. Among these adhesion proteins, sVE-Cadherin is the main adhesion protein. sVE-Cadherin is embedded in the actin tissue of the cortex of the endothelial cell and forms a homophilic bond with neighbouring sVE-Cadherin cells. The movement of water and various molecules that dissolve in the blood, mainly through the paracellular pathway, the integrity of the protein sVE-Chaderin adhesion is very necessary [13], [14].

The Pober (2007) study found a statistically significant difference in the levels of sVE-Cadherin among DHF patients with and without shock ( $p < 0.05$ ). Leukocyte interaction with the endothelium during inflammation can change the composition of endothelial permeability. The stimulation of proinflammatory cytokines will result in the emergence of adhesion molecules on the surface of the leukocytes and endothelium. Activated endothelial cells due to cytokine stimulation will express adhesion molecules such as FIK-1 (E-selectin), ICAM-1, VCAM-1, p-selectin and PECAM-1 on the endothelial surface [15], [16].

These adhesion molecules make leukocytes

stick to the endothelial surface and secrete free radicals, proteases and cause local inflammation and endothelial cell damage. Also, leukocytes that bind to ICAM-1, through SRC and Rho GTPase, interfere with sVE-cadherin adherens junction. PECAM-1 which is the most important molecule binds to leukocyte cells in the inter-endothelial gap, attracts and causes leukocyte migration. Endothelial damage that interferes with VE-cadherin adherent junction and migrated leukocytes widens the gap between the endothelium, causing and aggravating plasma leakage [17], [18].

The study of sVE-cadherin in dengue infection has so far only been in the in vitro research stage using endothelial tissue culture. This approach shows that the levels of sVE-cadherin decrease in leaky endothelial tissue (11). The release of proinflammatory cytokines, VEGF, antibodies and complement activation in the infection resulting in disruption of endothelial cell links, widening of the endothelial gap and leakage of plasma from the intravascular space to the extravascular space.

Cardozo *et al.*, (2017) investigating the effect of plasma leakage in patients with severe dengue infection getting vascular endothelial homeostasis plays an important role in plasma leakage, which is influenced by the immune response. Dengue virus affects endothelial cells to produce proinflammatory cytokines and chemokines such as IL-8, RANTES, MMP-2 and VEGF. Dengue infection also suppresses the production of TNF- $\alpha$  which mediates vascular hyperpermeability. PMBCs (peripheral mononuclear blood cells) also play a role in increasing endothelial cell permeability by decreasing the expression of sVE-cadherin. It can be concluded that the decrease in sVE-Cadherin values in individuals with dengue infection indicates an increased risk of becoming more severe infections [19], [11].

In vitro research by Yacoub *et al.*, (2016) and Kanlaya *et al.*, (2009) in the endothelial model found that the dengue virus can bind to EGL, reducing the expression of VE-cadherin and tight junction ZO-1 proteins, causing an increase in plasma permeability [20], [21].

### ***The difference of candidate variables in predicting payments in DHF patients***

Fever, abdominal pain and vomiting are also symptoms that are often found in DHF and are a warning sign in dengue cases. Abdullah *et al.*, (2018) found that there were significant differences between persistent vomiting, fluid accumulation and mucosal bleeding with the severity of dengue infection and had high sensitivity and specificity in predicting the occurrence of severe dengue infection [22]. Nagaram (2017) found 73 cases with complaints of abdominal pain and 115 cases with vomiting. In DHF patients, 32.8% of cases of abdominal pain were obtained, and

60.4% of cases of vomiting in patients with DSS had 96% of cases reduced and 100% of cases of vomiting. Research conducted by researchers also found that there was a relationship between abdominal pain and vomiting with DHF in shock. Although dengue virus is a nonhepatotropic infection, liver injury often occurs, ranging from mild dysfunction to an increase in liver enzymes to those with severe yellow symptoms and even fulminant liver failure [23].

The Nagaram (2017) study obtained 100% hepatomegaly in the DSS case group and 77% in the DHF group [23]. Research by Zhang *et al.*, (2014) found hepatomegaly in children with dengue infection had a 5 times greater risk of death compared to children infected with dengue without the discovery of hepatomegaly. From the above review compared to this study, there was a relationship between mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly with DHF with shock ( $p < 0.05$ ) [24].

This study concluded that there was no difference in mean levels of sVE-Cadherin in DHF patients with shock and without shock.

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