

# Upregulation of SCUBE1 in Dengue Virus Infection

Hirowati Ali<sup>1,2\*</sup>, Coyza Prana<sup>2</sup>, Ellyza Nasrul<sup>2,3</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, Indonesia; <sup>2</sup>Graduate School of Biomedical Sciences, Andalas University, Padang, Indonesia; <sup>3</sup>Department of Clinical Pathology, Faculty of Medicine, Andalas University, Padang, Indonesia

## Abstract

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**\*Correspondence:** Hirowati Ali, Department of Biochemistry, Faculty of Medicine, Andalas University, Padang, Indonesia. E-mail: [hirowatiali@med.unand.ac.id](mailto:hirowatiali@med.unand.ac.id)

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**BACKGROUND:** Dengue is a major communicable disease in tropical areas, with an increasing prevalence every year. Thrombocytopenia is one of the commonly used laboratory parameters for predicting the severity of the disease. It is detected on day 6 or day 7 after the febrile stage, and its presence indicates that the disease has become potentially fatal. Therefore, it is necessary to identify a marker for the early recognition of dengue virus infection during the febrile stage before the detection of thrombocytopenia on day 6 to prevent severe disease outcomes. Signal peptide-CUB- (complement C1r/C1s)-EGF (epidermal growth factor)-like domain-containing protein 1 (SCUBE1) is secreted in activated platelets under inflammatory conditions and enhances platelet-platelet adhesion and agglutination. This gene was first identified in human vascular endothelium, but its biological role in platelets remains unknown.

**AIM:** This study aims to identify SCUBE1 expression during the febrile stage of dengue virus infection and examine the correlation of its expression with thrombocytopenia occurrence on day 6.

**MATERIAL AND METHODS:** Blood samples were collected from 17 patients infected with dengue virus on day-3 fever and from 16 healthy controls who met the inclusion and exclusion criteria for dengue virus infection according to the World Health Organization (WHO) classification for dengue virus infection. All samples were subjected to SCUBE1 gene analysis using real-time reverse transcription quantitative PCR (RT-PCR).

**RESULTS:** The results showed that upregulation of SCUBE1 gene in infected patients ( $8.9 \pm 3.1$ -fold) compared to that in healthy controls, indicating SCUBE1 involvement in dengue virus infection. Furthermore, we analysed the laboratory parameters of infected patients on day 3 and day 6, when thrombocytopenia is usually detected. Platelet count was found to be significantly decreased from day 3 until day 6 in the infected patients. Unfortunately, our results showed no correlation between SCUBE1 expression in the febrile stage and the occurrence of thrombocytopenia on day 6.

**CONCLUSION:** The conclusion of this study is SCUBE1 might play a role in dengue virus infection but does not correlate with thrombocytopenia on day-6 fever.

## Introduction

Dengue virus infection is an acute mosquito-borne viral infection and is still one of the major health problems in Indonesia, where its incidence has been rapidly increasing in recent years [1]. Due to the broad spectrum of clinical manifestations of this infection, ranging from asymptomatic, mild-to-severe symptoms, there is a need for new biomarkers for diagnosis, proper treatment, and prognosis [2], [3]. The clinical onset of dengue virus infection is observed as an initial febrile phase on days 1-3, a critical phase where plasma leak is apparent on days 4-7, and a spontaneous recovery phase on days 8-10. Laboratory parameters may vary from mild to

moderate thrombocytopenia and leukopenia. In a small proportion of infected patients, systemic vascular leak progresses into impending deterioration symptoms that require clinician awareness [3], [4], [5]. Thrombocytopenia is one of the warning signs for the severity of dengue virus infection. A low platelet count detected in dengue infection might indicate an association with platelet dysfunction. Further decrease in platelet count suggests additional complications. Therefore, monitoring the warning signs of the critical phase of dengue infection is essential to prevent the severity of this infection [6], [7].

Signal peptide-CUB-(complement C1r/C1s)-EGF (epidermal growth factor)-like domain-containing protein 1 (SCUBE1) is a newly identified secreted and

membrane-anchored protein consisting of nine copies of EGF-like repeats and one CUB domain. The expression of SCUBE1 has been reported in the vascular endothelium and activated platelets under inflammatory conditions [6], [7], [8]. A recent report showed that SCUBE1 expression was elevated in patients with Crimean–Congo hemorrhagic fever (CCHF), another viral disease that exhibits mild-to-severe clinical features similar to those of dengue fever. Upregulation of SCUBE1 levels in patients with CCHF may predict a fatal case where thrombocytopenia can cause massive hemorrhage [9]. Considering that SCUBE1 is detected in endothelial dysfunction and activated platelets, we assumed that this gene might play a role in dengue virus infection, which could impact the vascular endothelium and platelets. This study was conducted to assess whether SCUBE1 could be a promising predictive marker for dengue virus infection.

## Material and Methods

### Collection of blood samples

Blood samples were collected from 17 patients infected with dengue virus with a sudden onset of fever for at least 3 days. The inclusion criteria were fulfilling the World Health Organization (WHO) criteria for dengue virus infection, being anti-NS1-positive, and agreeing to sign the informed consent form. The exclusion criteria were patients confirmed as having no other infectious diseases such as influenza A, influenza B, and HCV infections, and not undergoing any drug treatment. Blood samples were collected from patients confirmed as infected with the dengue virus on day 3 and day 6 after the onset of illness. SCUBE1 expression was measured on day 3 of the febrile stage. Healthy volunteers as controls were randomly recruited from individuals who had no clinical symptoms of infectious diseases. Basic laboratory data included the results of white blood cells, platelet counts, and hematocrit.

### RNA extraction and real-time quantitative PCR (qRT-PCR)

Total RNA was isolated using Trizol reagent according to the manufacturer's instruction (Invitrogen, USA). The total RNA was dissolved in 20  $\mu$ L of RNase-free water and stored at  $-80^{\circ}\text{C}$  until further use. Reverse transcription was performed to obtain cDNA using iSCRIPT cDNA synthesis kit (Bio-Rad, USA). SCUBE1 expression was confirmed using SsoFast Evagreen Supermix (Bio-Rad, USA) with Bio-Rad CFX96TM Real-Time PCR System. The primer for SCUBE1 was forward: GTGCCCTATGTCACCTACGAT and reverse:

GAACATCTCCTTGGATTCCTGG and that for the reference gene GAPDH was forward: ATG GGT GTG AAC CAT GAG AAG TA and reverse: GGC AGT GAT GGC ATG GAC.

### Research Ethics

This study was approved by the Faculty of Medicine, Andalas University Ethics Committee for Health Research.

### Data analysis

The relative expression of SCUBE1 was calculated by the  $2^{-\Delta\Delta\text{Ct}}$  formula. Data were expressed as mean  $\pm$  SEM, with statistical significance being evaluated using Student's t-test for normally distributed data or Mann – Whitney test for non-normally distributed data. Pearson correlation and significance analysis were conducted using SPSS software.  $P < 0.05$  was considered as statistically significant.

## Results

In this study, we analysed SCUBE1 expression in 17 patients with dengue virus infection in the febrile phase and healthy donors. We observed an increased SCUBE1 expression ( $8.89 \pm 3.1$ -fold) compared to that in controls (Figure 1).

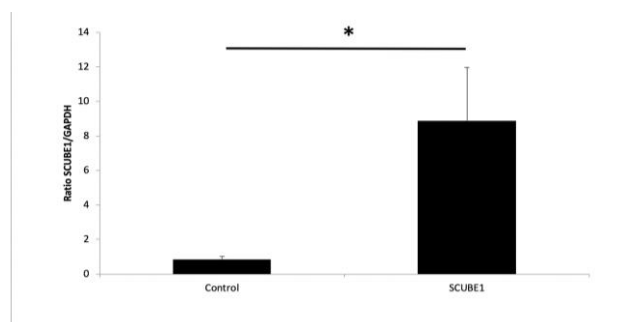


Figure 1: mRNA expression of SCUBE1. An upregulation of SCUBE1 expression was detected in 17 patients infected with dengue virus on day 3 compared with healthy controls

Laboratory parameters of the infected patients revealed a lower platelet count on day 6 than that on day 3 (Table 1).

Table 1: Laboratory parameters of dengue virus-infected patients on day 3 and day 6

| Parameters                        | Day 3             | Day 6             | p*    |
|-----------------------------------|-------------------|-------------------|-------|
| Leukocytes                        | 4769 $\pm$ 454.18 | 4605 $\pm$ 402.02 | 0.79  |
| PLT ( $\times 10^3/\text{mm}^3$ ) | 168 $\pm$ 10.8    | 137 $\pm$ 9.2     | 0.04* |
| HB (mg/dL)                        | 14.3 $\pm$ 0.4    | 14.5 $\pm$ 0.4    | 0.61  |
| HT (%)                            | 41.5 $\pm$ 0.9    | 41.3 $\pm$ 0.9    | 0.90  |

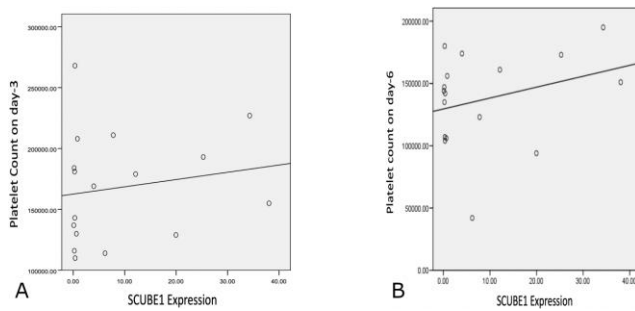


Figure 2: A) SCUBE1 expression and platelet count on day-3 fever showed no statistically significant correlation; B) Extended observation of the platelet count on day 6 showed a decreased count, but no significant correlation was observed with increased SCUBE1 expression

Furthermore, we determined whether there was any correlation between increased SCUBE1 expression on day 3 and platelet counts on day 3 and day 6 among the infected patients. However, we did not observe any significant correlation between SCUBE1 expression and decreased platelet count on day 3 ( $r = 0.170$ ,  $p = 0.51$ ). Similarly, no significant correlation was observed between SCUBE1 expression and platelet count on day 6 ( $r = 0.292$ ,  $p = 0.26$ ) (Figure 2).

## Discussion

The mechanism of dengue virus infection and its complexity remain ambiguous. Dengue virus infection exhibits a wide variety of clinical manifestations ranging from asymptomatic to mild-to-severe illness [4], [5]. Awareness during the critical phase is essential to prevent the development of vascular leakage that could lead to dengue shock syndrome [5]. Numerous identification or assay methods have been developed for the detection of dengue virus infection, but they remain suboptimal [3], [4]. Therefore, it is necessary to develop a predictive marker and identify warning signs for close observation in dengue virus infection to reduce mortality due to the severity of illness [5], [6], [7].

SCUBE1 has been documented to be expressed in the vascular endothelium and platelets both at the mRNA and protein levels. Therefore, SCUBE1 may also be involved in the pathogenesis or the progression of atherosclerotic thrombus by promoting platelet adhesion and agglutination [8], [9]. Increased SCUBE1 levels have also been detected in acute coronary syndrome and ischemic stroke, which impact the vascular endothelium [10]. Moreover, a recent study reported increased SCUBE1 levels in patients with CCHF, which predicted severe cases of CCHF [11].

In this study, SCUBE1 expression was

observed on day 3 of the febrile stage for early recognition. The expression of SCUBE1 was significantly higher in infected patients than that in the healthy controls ( $p < 0.05$ ). We followed up these infected patients until day 6 of fever and checked their basic laboratory parameters. We observed that the infected patients showed significantly lower platelet counts from day 3 until day 6, implicating a role for SCUBE1 expression in thrombocytopenia occurrence. Furthermore, we analysed whether increased SCUBE1 expression in the febrile stage correlates with platelet count on both day 3 and day 6. However, no significant correlation was observed between increased SCUBE1 expression in dengue virus-infected patients and lower platelet count on either day 3 or day 6. Among the infected patients, some platelet count may decrease from day 3 until day 6, but the count was still in the normal range.

This study demonstrated an increase in the novel secreted protein SCUBE1 in the acute febrile phase of dengue virus infection. Moreover, the platelet count was found to be decreased from day 3 to day 6, but no significant correlation was observed between SCUBE1 expression and platelet count. The major limitation of our study is the small number of patients and controls. Nevertheless, the novel finding of increased SCUBE1 expression in the acute febrile stage of dengue virus infection can be considered as pioneering research for identifying a predictive marker for dengue virus infection. This result can also encourage further comprehensive studies for elucidating the pathogenic mechanisms of this mosquito-borne viral infection.

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