Correlation of the Presence of Non Structural-1 (NS1) Antigen Dengue Virus with Severity of Dengue Infection

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ABSTRACT

Dengue is a major public health threat worldwide, affecting approximately 3 billion people. More than 100 countries in the world located in tropical and subtropical areas, there are at least 100 to 400 million people infected with the dengue virus which causes dengue hemorrhagic fever (DHF). Soluble Non Structural Protein (sNS1) DENV is a soluble NS1 protein that is secreted and found in the serum of patients during acute infection. Because of its presence early in infection, sNS1 is used as a diagnostic indicator of acute dengue infection. NS1 can directly activate platelets through TLR4 and can further increase platelet aggregation, endothelial cell adhesion, and phagocytosis by macrophages that can cause thrombocytopenia so that high sNS1 levels are associated with disease severity. From the results of the study showed p <0.05. This indicates that there is a correlation between the presence of NS1 and the severity of dengue infection.

Key words: Dengue Virus, NS1 antigen, Thrombocytopenia.

INTRODUCTION

The dengue virus (DENV) is a major public health threat worldwide, affecting approximately 3 billion people. More than 100 countries in the world in tropical and subtropical regions, at least 100 to 400 million people are infected with DENV which causes DHF and causes 20,000 people to die every year.[1] The incidence of dengue fever has increased more than eight times in the last 20 years. Weak mosquito control, reforestation, climate change and global warming are some of the reasons for the increasing number of dengue cases. DENV infection can cause abdominal pain, vomiting, nosebleeds, hematemesis, melena, malaise, anxiety and even organ failure.[2]

DENV is a positive single-stranded RNA virus and is a member of the Flaviviridae family that causes dengue hemorrhagic fever (DHF). This virus is transmitted through the bite of the *Aedes aegypti* mosquito.[3] There are 4 different dengue virus serotypes that have been found worldwide. This serotype can cause mild self-limiting infection or severe dengue fever, namely Dengue Shock Syndrome (DSS).[3,17-19]

The dengue virus genome consists of 5' UTR, ORF, and 3' UTR. The polyproteins that code for ORF are three structural proteins, namely capsid (C), premembrane (PrM) and envelope (E) proteins and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The ORF adjacent to the UTR encodes a polyprotein that is a protein maturation precursor. Processing of this polyprotein contributes to both the post-translation of three structural proteins and seven non-structural proteins. As the name implies, the protein structure of the capsid (C), envelope (E),

premembrane (prM) is a structural component of viral particles involved in viral RNA encapsulation, nucleocapsid formation, membrane formation, virion maturation and encapsulation. The non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 have diverse enzymatic activities. It has been studied that non-structural proteins have multiple roles in the infection cycle.[4,5] To highlight some of the roles of NS proteins: NS5 is a viral RNA-dependent RNA polymerase It is (RdRp) S-adenosylmethionine methyltransferase (MTase). NS3 has nucleoside RNA triphosphatase (RTP) Triphosphatase (NTPase) and plays an important role in NS5 co-replication. NS2B is a viral serine protease. Proteins NS1, NS2A, NS4, and NS4B have roles in viral replication, assembly, and release of new viruses.[4,5]

The NS1 DENV protein is a 48kD glycosylated protein that has a role in viral replication and evasion of the immune system.[6,7] NS1 is initially translated as a glycosylated monomer in the endoplasmic reticulum, but rapidly forms a dimer that has a role in viral replication on the surface of the endoplasmic reticulum membrane, plasma membrane association *via* glycophosphatidylnocytol linkage on infected cells, or the binding of hexameric NS1 to the surface of uninfected cells *via* interactions. glycosaminoglycans.[8]

Secretion Soluble NS1 (sNS1) DENV is a soluble NS1 that is secreted and found in the serum of patients during acute infection and is used as a diagnostic indicator of acute dengue infection. High levels of sNS1 are associated with disease severity although it is unclear whether this measure has functional significance or is simply a marker of higher viremia in severe cases. sNS1 can bind to components of host complement directly and inhibit complement activation on the cell surface.[9] Antibodies to NS1

can cross-react with platelets, endothelial cells, and thrombin which are thought to play a role in the pathogenesis of dengue infection.[10] Thrombocytopenia is a decrease in the number of platelets which is a common feature in mild and severe dengue infection and correlates with the severity of the disease. NS1 can directly activate platelets through TLR4 and can further increase platelet aggregation, endothelial cell adhesion, and phagocytosis by macrophages that can cause thrombocytopenia.[11] Therefore, it is necessary to study whether there is a relationship between the presence of NS1 and the severity of an infection by looking at the presence of thrombocytopenia.

MATERIALS AND METHOD

Study design

Materials were obtained from EDTA plasma from 80 patients, then examined several parameters such as NS1, and platelet counts. The patient who was examined had a fever on day 3-10, with clinical manifestations of DHF. The results were processed using SPSS with a chi-square test to see whether there was a relationship between the presence of the NS1 antigen and the severity of a DHF infection characterized by the occurrence of thrombocytopenia.

Sample collection

A total of 3 ml blood sample was collected from patient who had a fever on day 3-10, with clinical manifestations of DHF.

NS1 antigen detection

Qualitative and quantitative examination of NS1 using the iChroma II tool. This tool can detect sNS1 secreted by dengue virus-infected cells. The principle of this tool is sandwich immunofluorescence (IFA) where the detector antibody in the cassette will bind to the antigen in the sample to form an antigen-antibody complex. The complex migrates to the nitrocellulose matrix to be captured by the immobilized antibody on the test strip. The more antigens in the sample, more immune complexes will form which leads to a stronger fluorescence signal intensity on the detector antigen. The procedure for examining NS1 with this tool is to enter 75 ul of serum or plasma into 150 ul of diluent then homogenize 10-20 times and incubate. Pipette 75 ul of the master mix and insert it into the sample well on the surface of the cassette, and the instrument will read the results within 12 minutes. The results are expressed in cut-off index (COI) units where results <0.9 are negative for NS1 antigen, >0.9 and <1.1 are indeterminate. This result needs to be retested, if the test results show negative or indeterminate after repeated, the sample is considered negative. Results >1.1 are positive.

Count platelet count

Counting the number of platelets is done using the Sysmex XN-540 tool, where the principle of this tool is fluorescent flowcytometry. Cells that pass through a slit are then irradiated by a laser beam. There are

3 types of light, forward scattered light for detecting cell size, side scattered light for detecting intracellular matrix, and side fluorescent light for type and amount of nucleic acid and cell organelles. Platelet results are expressed in units of cells/mm3. Based on the agreement of the International Committee for Standardization in Hematology, the normal number of platelets is 150,000-400,000 cells/mm3.

Statistical analysis

All statistical calculations were performed using SPSS statistical software. The results were processed using SPSS with a chi-square test to see whether there was a relationship between the presence of the NS1 antigen and the severity of a DHF infection characterized by the occurrence of thrombocytopenia.

RESULT

From table 1 above, it can be seen that from 80 samples of suspected DHF patients, 60 (75%) of them were positive for NS1 antigen and from 60 samples that were positive for NS1, 38 (63%) patients experienced thrombocytopenia.

Table 1 above shows that the statistical test results obtained a positive correlation and p<0.05, which means that there is a correlation between the presence of NS1 and the severity of DENV infection which is characterized by the occurrence of thrombocytopenia. In accordance with the WHO criteria for the manifestation of DENV infection, thrombocytopenia was found as a marker of plasma leakage**.**

DISCUSSION

Until now, DHF is still a global health problem in the world, especially in Indonesia. In this study, 80 samples with suspected DHF were collected. Each sample was examined hematologically to see the number of platelets, examination of NS1 antigen by the IFA method.

Of the 60 samples positive for NS1 antigen, 63% had thrombocytopenia. Meanwhile, 37% showed that the platelet count was still within the normal range, which was around 150,000 – 400,000 cells/mm3. The difference in platelet profiles in NS1 antigen positive patients is thought to be a DENV infection with different serotypes. According to reports, DENV-4 infection showed mild clinical symptoms compared to DENV-2 and DENV-3.

High levels of sNS1 are associated with disease severity although it is unclear whether this measure has functional significance or is simply a marker of higher viremia in severe cases. sNS1 can bind to components of host complement directly and inhibit complement activation on the cell surface.[9,20-22]

Thrombocytopenia is a common feature of mild and severe dengue and correlates with the severity of the disease. There are two mechanisms that can cause thrombocytopenia in DENV infection. One of them is the increased destruction and removal of platelets from the peripheral blood and decreased production of platelets in the bone marrow.[12] Inhibition of megakaryocyte development in the bone marrow has been suggested as the main mechanism causing thrombocytopenia in DENV infection.[13,26-28]

Recently, an increase number of studies have shown that NS1 plays an important role in the pathogenesis of dengue disease both *in vitro* and *in vivo*, including promoting DENV replication/infection and directly inducing vascular leakage, including the induction of cytokine release in immune cells.[14] Furthermore, previous studies have shown that relative levels of NS1 antigen in the serum of dengue patients are negatively correlated with platelet counts. Recently, Modilan *et al.* demonstrated that Toll-like receptor 4 (TLR4), a known receptor for lipopolysaccharide (LPS), functions as a receptor for NS1 in immune

Table 1: Results of NS1 and platelets in patients with suspected DHF.

		Infection severity		Total
		Normal	Trombocytopenia	
NS1 Antigen	Negative	20	0	20
	Positive	22	38	60
Total		42	38	80

Table 2: Relationship of presence of NS1 antigen to severity of dengue infection.

Variable	r	P
NS1 Antigen Infection Severity (platelet count)	0,000	0,000

cells.[15] It is known that LPS induces platelet activation and increases platelet aggregation through TLR4/MyD88 signaling.[16,23-25] Since NS1 and LPS can activate cells *via* TLR4, this study shows that NS1 induces platelet activation and promotes aggregation *via* TLR4, which causes thrombocytopenia and bleeding during dengue infection.

In a study conducted by *et al* showed that platelets activated by NS1 were susceptible to aggregation, adhesion to endothelial cells, and phagocytosis by immune cells. NS1 can directly bind to resting platelets, then platelets will be activated and produce ADP. These active platelets can increase platelet aggregation, endothelial cell adhesion, and phagocytosis by macrophages that can cause thrombocytopenia.[11,29,30] Therefore, NS1 is one of the key factors contributing to thrombocytopenia during DENV infection.

CONCLUSION

The presence of NS1 secretion released by DENV-infected cells in the serum indicates a state of thrombocytopenia as a sign of the severity of DENV infection.

ACKNOWLEDGEMENT

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GRAPHICAL ABSTRACT

Non Structural-1 (Ns1) Antigen Dengue Virus

Severity Dengue Virus
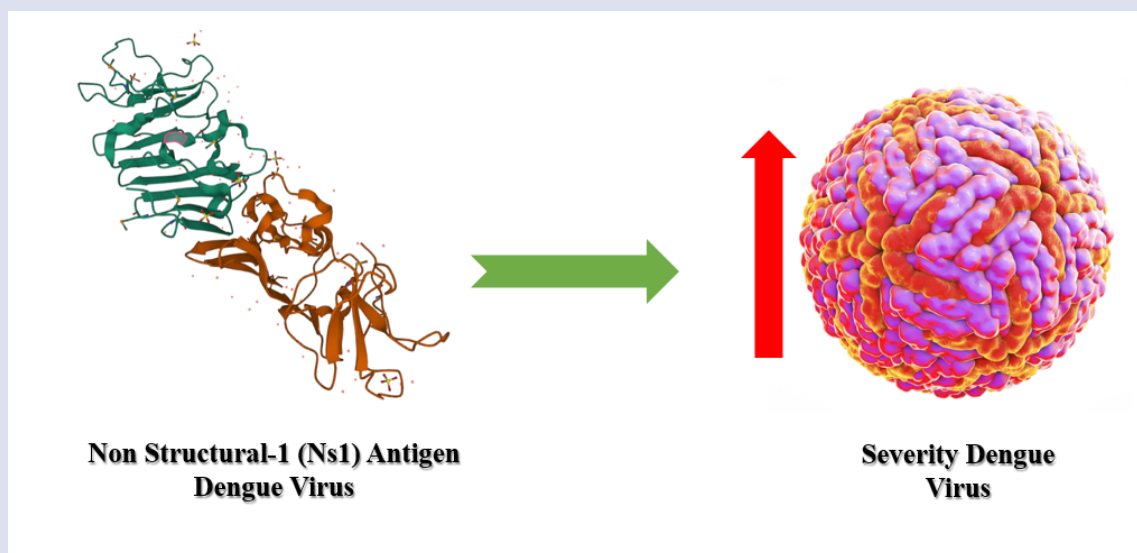
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Rahadian Zainul has completed a Bachelor of Educational Chemistry in IKIP Padang, then continued his studies and obtained a Master of Chemistry at Universitas Andalas, and earned a Doctoral Chemistry degree at Universitas Andalas. He is a researcher on the design and modification of copper oxide for inactivation SARS-CoV-2 by stimulated indoor lights and a researcher on the design and modification of copper oxide by computation approach with DFTB+. He is also the Head of Cambiotics Research Center, Universitas Negeri Padang. The author has published 41 manuscripts in Scopus-indexed journals and also 8 h-index.