PRESENCE OF MANNOSE BINDING LECTIN ON DENGUE INFECTION

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Abstract– Dengue Hemorrhagic Fever (DHF) is endemic in Indonesia. DHF is caused by Dengue virus (DENV) as a result of the bite of the mosquito Aedes aegypti. Responses innate immune are activated in DENV infection. Mannose binding lectin (MBL) protein plays a role in the activation of the lectin pathway of complement so that the protein acts as a Pattern Recognition Receptor for dengue virus envelop. In this study we wish to understand the relationship between the concentration of MBL with clinical degree of dengue infection. The study was conducted with a cross-sectional method. In all 64 dengue patients in hospital Cipto Mangunkusomo and Cijantung, Jakarta in May-October 2014 were studied. The results of this study showed degree of DHF has a weak relationship for MBL. Calculations with multivariate regression analysis on degree of DHF with mannose binding lectin protein, gender, age, platelets, hematocrit, and infection of primary/secondary obtained a very strong correlation. The results of this study demonstrate that MBL protein has a weak relationship for degree of DHF and MBL levels in pediatric patients infected with dengue acute phase more than the adult group. We assume MBL binds to the platelet cell membrane thereby activating the lectin pathway of complement system that cause thrombocytopenia.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by the dengue virus (DENV) through the intermediary of Aedes aegypti. Dengue hemorrhagic fever is endemic in Indonesia. In Indonesia based on data from the Ministry of Health of DHF patients decreased in 2010 as many as 75 thousand cases and in 2011 to 50 thousand cases. With the decline in deaths from 0.87% in 2010 to 0.80% in 2011. The decline rate is still positioned Indonesia as the first country with DHF infection in ASEAN. When the body is infected with dengue virus (DENV) resulted activation of the various components of non-specific immune system (innate immune system) as the lectin pathway of complement system activation and increased activity of macrophages. Activation of the lectin pathway of the complement system using mannose binding lectin (MBL) protein contained in the blood plasma (Abbas et al., 2012).

Mannose Binding Lectin belongs to a class of molecules Pattern Recognition Receptor (PRR) soluble proteins that can recognize protein envelop DENV (Avirutnan et al., 2011). Mannose binding lectin protein has a C-terminal lectin structure that function to bind carbohydrate ligand N-linked glycosylation group (N-Glycan) contained in the envelop and NS1 protein DENV. So that can activate the lectin pathway of the complement system (Hidari and Takashi, 2011). The bond MBL protein with envelop protein (E) and NS1 proteins can activate the complement system to lyses DENV (Alen and Dominique, 2012).

Macrophages also play a role in opsoninfagositosis DENV particles. Macrophages that have fagocyt DENV will experience the intervention of DENV so macrophages will perform a variety of responses such as activation of receptors B7-1, ICAM-1, MHC molecules to interact with Th1 lymphocytes, secrete cytokines IFN type 1 as a form
of communication between cells and paracrine interactions between macrophages with Th1 lymphocytes cells will trigger Th1 lymphocytes producing IFN-γ which would trigger macrophages to secrete proinflammatory cytokines such as IL-1, TNFα, and IL-6 (Abbas et al. 2012). Secretion of IL-1 and IL-6 triggers the production of prostaglandins that interfere with thermoregulation center, causing sudden high fever. Other impacts resulting from the activity of proinflammatory cytokines headache, joint pain, muscle pain, nausea, vomiting and decreased appetite (Dantzer, 2010).

MBL protein levels in the blood plasma in the acute phase of DENV infection is expected to affect the degree of clinical manifestations of dengue fever (DB) into DHF and DSS. Assuming that the presence of MBL protein increases the activity of the lectin pathway of the complement system as a response of the innate immune system that can be linked to the degree of clinical manifestation of dengue fever. Complement activation process between MBL protein with envelope DENV until the formation of the membrane attack complex produces protein C5a, C4a and C3a which is anafilatoxin as C5a molecule can induce degranulation of mast cells and basophils to release histamine which causes the symptoms of itching and other mediators which causes vasodilation, redness, swelling and contraction of smooth muscle (Abbas et al., 2012).

Endothelial damage also cause thrombocytopenia. This is due to platelet dysfunction due to immune complex binding to DENV-IgM. Immune complexes with DENVantigen-IgM- platelet membrane (immune-mediated clearance of platelets) caused the destruction of platelets by the reticuloendothelial system in the liver and spleen. In addition, the presence of specific autoantibodies that bind to platelets and platelet DENV antigen complex can form bonds directly with platelet stimulating proinflammatory cytokine induction. Platelets contain many proinflammatory cytokines such as IL-1 which modulate the immune response resulting in inflammation (Li et al., 2012).

Levels of MBL protein has a high variation so that there is no normal limits on the levels of MBL protein. MBL function as molecules that activate the complement lectin pathway is also thought to contribute to the clinical symptoms of dengue fever which can cause an increase in dengue higher degree. We assume MBL levels affect the degree of DHF. In this study, the control factor is also taken gender, age, value platelets, hematocrit values, and antibody IgM / IgG.

MATERIALS AND METHODS

This research has graduated from the Research Ethics Committee of the Faculty of Medicine Indonesia University-RSCM No. 674 / UN2.F1 / ETHICS / 2014. This type of research is observational analytic research. The study population was all children with Dengue Fever (DD), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (SSD) which met the inclusion criteria sample derived from Cipto Mangunkusumo Hospital and Hospital Cijantung, Jakarta. Inclusion criteria sample of men and women, fever d’ 4 days, suggesting the diagnosis of dengue virus infection symptoms in the acute phase, the clinical examination and laboratory, according to the WHO in 1997, showed symptoms of DD or DHF or SSD, and ethnic Malay. Criteria for sample rejection doubt the existence of clinical and laboratory examination. The study was conducted in the month of May to October 2014. The sample study 64 samples of plasma acute phase of dengue patients which were divided into two groups: children and adult groups. The level of MBL protein was measured using ELISA technique. Mannose Binding Lectin ELISA kit used in this study is the MBL Oligomer ELISA Kit BioPORTO Thermo Scientific. Detection of primary and secondary infections in patients with DHF then used rapid detection of IgM antibody test / IgG antidengue NOVA TEST.

Statistical Analysis

Research data such as age, sex, degree of DHF, the value of platelets, hematocrit value, IgM/IgG, and protein levels of MBL were tested for normality of data distribution. Normal distribution of data to test the correlation and regression at $\alpha = 0.05$.

RESULTS

Characteristics of Study Population

The number of samples researched 64 serum samples of patients DBD. These patients were classified by the WHO 1997 guideline with details of 9 samples DHF grade 1, 52 samples of DHF grade 2, 3 samples had DHF grade 3. Sample 35 men and 29 women. Distribution of data normality test results
showed all study variables with normal distribution. Data MBL protein levels in samples of normal distribution, with an average value in the group of children $4215.17 \pm 5042.39$ ng / mL and adult groups $3158.66 \pm 1642.21$ ng / mL. With a minimum value of 50 ng / mL and maximum 23112.9 ng / mL. Data variables such as degree of DHF research, age, platelets value, hematocrit value, IgM / IgG, and protein levels of MBL are described in Table 1.

**Relationship of MBL Protein With a Degree of DHF Infection**

Correlation and regression test results obtained by the correlation between MBL protein concentration with a degree DHF in children group $R = 0.023$ and the adult group has a correlation value of $R = 0.087$. If both of groups combined, the correlation between the levels of MBL protein for DHF degree of $R = 0.043$. Correlation test results showed a very weak correlation value as it approaches the value 0. A positive value indicates which demonstrated a positive correlation between the levels of MBL protein and the degree of DHF. Data results of correlation and regression between MBL protein levels with the degree DHF are described in Table 2.

**Relationship of Degree of DHF Infection With Platelets and Hematocrit Value**

Results of correlation and regression test a value of relationships degrees DHF with platelets and hematocrit values in the group of children obtained value of $R = 0.639$ and the adult group rate $R = 0.737$. Value relationship a degrees DHF with platelets and hematocrit value of the two groups is $R = 0.685$. This means a strong relationship between a degree of DHF with platelet and hematocrit values. Regression test result data of the relationship between platelet and hematocrit values with degrees DHF are described in Table 3.

**Table 1. Descriptive analysis of samples**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of DHF</td>
<td>DHF1</td>
</tr>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td>IgM/IgG</td>
<td>IgM (+)</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Age</td>
<td>23 ± 13 year</td>
</tr>
<tr>
<td>Value platelets (cell/ mL)</td>
<td>88,000± 33,549</td>
</tr>
<tr>
<td>Value Hematocrit</td>
<td>39.477±5.45 %</td>
</tr>
<tr>
<td>Protein MBL (ng/mL)</td>
<td>3455.81±3002.74</td>
</tr>
<tr>
<td>Protein MBL (ng/mL) on control samples</td>
<td>2393.3±908.86</td>
</tr>
</tbody>
</table>

**Table 2. Correlation and Regression test results between MBL protein concentration with a degree of DHF**

<table>
<thead>
<tr>
<th>Test</th>
<th>Samples of DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children Group</td>
</tr>
<tr>
<td>Correlation Sig. value</td>
<td>0.929 &gt; 0.05</td>
</tr>
<tr>
<td>Regression R value</td>
<td>0.023</td>
</tr>
</tbody>
</table>
Value relationship of MBL protein with hematocrit value both of group was $R = 0.078$. This means a weak correlation between the levels of MBL protein with hematocrit values in patients with DHF. Regression test result data of the relationship between levels of MBL protein with platelet and hematocrit values are described in Table 4.

**DISCUSSION**

The complement system is an innate immune system that is automatically activated if there is an infection or the entry of foreign molecules into body. Activation of the complement starts with molecular recognition.

Pathogen Associated molecule Pattern (PAMP) that are antigenic contained on dengue virus (DENV). Structural proteins of dengue virus that are most antigenic is protein Envelop (Protein E) (Yustinasari, 2011). Introduction of complement with PAMP molecules can pass through three pathways, namely classical pathway, alternative pathway and lectin pathway. In each of the molecular pathways that act to recognize PAMP. The molecule known as the Pattern Recognition Receptor molecules (PRR). In the classical pathway activation is required antibody capable of binding Protein E, after the plasma protein C1q binds to antibody IgG on CH2 domain or CH3 domain of IgM antibodies antidengue. In the alternative pathway C3 molecules which are capable of recognizing proteins E directly. C3 molecules is more dominant function in bacterial and parasitic infections (Abbas et al., 2012). In the lectin pathway use Mannose Binding Lectin protein for activation of the complement system. Protein Mannose Binding Lectin capable of binding to the N-linked glycosylation group (N-Glycan) contained protein E and NS1 DENV (Hidari and Takashi, 2011).

The results showed a weak correlation between the levels of MBL protein with a degree of DHF. This does not mean MBL does not play a part in during DENV infection because the average value of the MBL levels in patients with DHF higher than normal samples. High levels of MBL protein in patients with DHF indicating immune response has occurred. High levels of MBL protein in patients with DHF may be caused by the induction of IL-1 and IL-6. Rathakrishnan et al., 2012 stated that the levels of IL-1 and IL-6 increased in acute DENV infection. IL-1 and IL-6 induces the production of mannose binding lectin protein in the liver.

Weak correlation between MBL protein levels against a degree of DHF because the classical pathway of the complement system is activated which is characterized by the presence of IgM / IgG antidengue detected in samples. Detection of IgM / IgG conducted to determine the primary infection and secondary infection in patients with DHF. With assessment results IgM (+), IgG (-) is a primary dengue infection and results IgM (-), IgG (+) / IgM (+), IgG (+) is a secondary dengue infection (Li et al., 2012). The presence of IgM / IgG in plasma samples indicates that the adaptive immune response have contributed in inhibiting the replication of DENV toward target cells. Interactions between macrophages with Th1 lymphocytes cells will trigger a Th1 lymphocytes produce interferon (IFN) $\gamma$ and IL-2 (Hatch et al., 2011). Interferon $\gamma$ stimulates macrophages to secrete proinflammatory cytokines such as interleukin 1 (IL-1), tumor necrosis factor $\alpha$ (TNF-$\alpha$), IL-6, chemokines, and lipid mediators such
as prostaglandins, leukotrienes, and platelet activating factor (PAF). IFN-γ, TNF-α, and IL-1 can increase capillary permeability, causing leakage of blood vessel walls, leakage of plasma fluid into tissues of the body that cause DHF / SSD (Rathakrishnan et al., 2012).

Plasma leakage can cause thrombocytopenia due to damage to the vascular endothelium, causing aggregation of platelets to the reticuloendothelial system (Somnuke et al., 2011). Platelet cell surface collagen receptors (α2β1 and GPVI collagen), fibrinogen (GPIb / Ila) and von Willebrand factor (GPIB / IX / V) which helps platelets bind to endothelial cells (Cox et al., 2013). Demonstrated a strong relationship of platelet and hematocrit values in degrees DHF. Decreased platelet count causes increased hematocrit levels in the blood and is associated with the degree of DHF. The results of this study showed higher degrees of DHF then the value of the lower platelet and higher hematocrit levels. One of the factors which cause low platelet values is suspected because of the the bond of platelets with mannose binding lectin proteins that trigger the lectin pathway of complement activation and platelet destruction occurs in the reticuloendothelial system. Although the results of correlation and regression produces a weak correlation between the levels of MBL protein with platelet values. But it did not rule out the influence of MBL protein levels to symptoms of thrombocytopenia, because on the surface of platelet cells have complement receptor gC1qR / P33 and cC1qR proteins which can bind to MBL (Vershoor and Harald, 2013). Although receptor gC1qR / P33 and cC1qR a protein receptor for C1q (Abbas et al., 2012) that play a role in the classical pathway of the complement system but the similarity between the structure of the protein C1q MBL may cause MBL protein can also bind to cell membranes receptor gC1qR / P33 and cC1qR. In the platelet cell surface complement receptors 1 (CR1) and complement receptor 2 (CR2) (Vershoor and Harald, 2013). Hamad et al., (2010) expressed thrombocytopenia in DHF is caused by activation of the complement system. This means that in both the classical pathways of the complement system, the alternative pathway and the lectin pathway of platelet cells are capable of thrombocytopenia.

**CONCLUSION**

We conclude that the relationship between the degree of DHF with mannose binding lectin protein is weak because of the adaptive immune system has been activated. Although the adaptive immune system has been activated, mannose binding lectin protein remained in production and plays a role in the activation of the lectin pathway of the complement system and allegedly associated with symptoms of thrombocytopenia due to mannose binding lectin proteins can bind to the surface of the cell membrane of platelets.

**RECOMENDATIONS**

More research needed to prove that mannose binding lectin proteins can bind to the surface of the cell membrane of platelets.

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