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Differences in CD95L Levels and Blood Test Results in Primary and Secondary Dengue Infection Patients

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Abstract

Dengue is a disease caused by dengue virus (DENV) that is transmitted mainly by the female Aedes aegypti mosquito. There are four serotypes of DEN, leading to a possibility that a person may be infected four times by this virus, albeit with different serotypes. Recovery from infection with one viral serotype provides lifelong immunity to the same serotype but not to the other serotypes. Secondary infection by other serotypes increases the risk of developing severe dengue. The pathogenesis of severe dengue involves apoptosis of microvascular endothelial cells that leads to plasma leakage. In addition, there is usually a decrease in platelets and leukocytes and an increase in hematocrit. This study aimed to compare the results of the CD95L examination involved in the apoptotic process and the results of blood tests in primary and secondary dengue patients. This was a cross-sectional study performed in a four months period (September–December 2019) involving several clinics and doctor's private practices in Medan, Indonesia. Subjects were eighty-four dengue patients, consisting of 18 (21%) patients with primary infection and 66 (79%) with secondary infection. Data collected were tested with the Mann Whitney test with p-value of <0.05 considered significant. A significant difference (p value=0.007) was observed in the lymphocyte counts between primary and secondary dengue patients, but no differences were seen in CDL95 level, platelet count, leukocyte count, and hematocrit. In conclusion, except for the lymphocyte count, there is no difference in CD95L level and blood test results between primary and secondary dengue patients.

Keywords: Blood test, dengue, primary infection, secondary infection

Introduction

Dengue is a disease found mainly in areas with tropical and subtropical climates. The incidence of dengue has increased dramatically worldwide in recent decades. Most cases are asymptomatic or mild and can be managed independently, many are misdiagnosed as other febrile illnesses, so the actual number of dengue cases is not known with certainty. The number of cases and deaths from dengue appears to be declining during 2020 and 2021. However, the reality is that the data are incomplete, and the COVID-19 pandemic may also hinder case reporting in some countries.

Dengue is caused by the dengue virus (DENV); there are four different serotypes of the virus, namely DENV-1, DENV-2, DENV-3, and DENV-4. Recovery from infection with one viral serotype is believed to confer lifelong immunity against that serotype. However, cross-immunity to other serotypes after recovery is only partial and transient. Another serotype subsequent infection (secondary infection) increases the risk of developing severe dengue. [1]

Dengue with severe symptoms (severe dengue) has a higher risk of death if not appropriately managed. There is no specific treatment for severe dengue. Early detection of disease progression and access to appropriate medical care reduces the death rate from severe dengue to below 1%.[1] The mechanism of severity of the disease in secondary infection is due to pre-existing heterologous antibodies forming antigen-antibody complexes, but they are not neutralized so that they are free to replicate in macrophages.[2] Furthermore, the antigen-antibody complexes activate the complement system, which causes an increase in the permeability of the blood vessel walls and leakage of plasma from intravascular to

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extravascular. The antigen-antibody complex also causes platelet aggregation and activation of the coagulation system.[3]

The immune response in primary infection is the production of IgM antibodies, and secondary infection is the production of IgG antibodies and IgM antibodies. Epidemiological studies in Southeast Asia show that dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) cases are more common during secondary infection with a different serotype of the virus than the virus causing the primary infection.[4]

Apoptosis plays a role in the pathogenesis of several viral infectious diseases.[5] DENV induces apoptosis of various cells involved in the pathogenesis of dengue. Autopsy examination in patients with severe dengue showed apoptosis in the liver, brain, small intestine, and lung cells. Microvascular endothelial cell apoptosis causes plasma leakage.[6] There are two main pathways of apoptosis interconnected, namely the extrinsic pathway and the intrinsic pathway. The extrinsic pathway occurs when the death receptor binds to its ligand. One such death receptor is CD95. This receptor is activated when it binds to its ligand (CD95L) and forms a death-inducing signaling complex (DISC) which activates caspases 8 and 10 to initiate the execution phase of apoptosis.[5] In a previous study, it was found that CD95L levels were higher in DHF patients compared to dengue fever (DF) patients.[7] In addition, the significant hematological parameters in dengue cases are the number of platelets, leukocytes, and hematocrit so that the CD95L levels and hematological parameters can be used as indicators of the severity of dengue disease.[8]

This study aims to compare and analyze CD95L levels and the results of blood tests in patients with primary and secondary dengue infections.

Methods

This study used a cross-sectional method, the sample was taken for 4 months (September–December 2019 period) and obtained from several clinics and doctor's practices in Medan. Sampling was done by the consecutive sampling method. Inclusion criteria were aged ≥18 years old and diagnosed with DENV infection based on WHO 2011 criteria with positive NS1 (Nonstructural antigen 1) and/or anti-dengue IgM and/or anti-dengue IgG. The exclusion criteria were patients suffering from infectious diseases other than dengue. Blood tests were performed when the patient first came and was diagnosed with dengue infection. Patients were included in the primary infection group if the blood tests found anti-dengue IgM or NSI, and patients were included in the secondary infection group if anti-dengue IgG and anti-dengue IgM or NS1 were found together.[9] Blood examination includes the examination of CD95L, hemoglobin, platelets, hematocrit, leukocytes, lymphocytes, monocytes, and granulocytes. Then the data was analyzed using the Kolgomorove-Smirnov test to determine whether the data were normally distributed or not. In this study, the data were not normally distributed, so the Mann-Whitney test was used to determine the difference between the data in primary and secondary dengue infection patients. The significance level was set at $p<0.05$. The study was conducted after obtaining an ethical approval from Health Research Ethics Committee UMSU (293/KEPK/FKUMSU/2019).

Results

The subjects in this study were 84 people, consisting of 18 (21%) primary infection dengue patients and 66 (79%) secondary dengue infection patients. Table 1 describes the characteristics of the research subjects, the average age of primary dengue subjects was 33.72 (±17.00) years, and secondary dengue subjects were 23.69 (±9.05) years.

The sex of the subject was 43 males,

Table 1 Characteristics of Data

Age and Gender	Primary Dengue n=18(%)	Secondary Dengue n=66 (%)
Average age (years)	33.72±17.00	23.69±9.05
Gender		
Man	9	34
Woman	9	32

Table 2 Results of the Examination of CD95L Levels and the Average Results of Blood Tests based on the Results of Serological Examinations

Blood Test	Serological Examination		P-value
	Primary Infection	Secondary Infection	
CD95L (pg/mL)	110.15±29.95	123.40±34.17	0.114
Hemoglobin (g/dL)	12.92±2.25	12.67±1.48	0.194
Hematocrit (%)	39.21±5.84	38.89±4.02	0.371
Platelets (103 per L)	151.33±66.14 131.45±59.94		0.389
Leukocytes (103 per L)	5.20±1.86	4.83±1.96	0.383
Lymphocytes (%)	19.84±10.91	29.61±11.36	0.007
Monocytes (%)	6.82±3.33	5.79±2.93	0.389
Granulocytes (%)	70.60±9.45	64.19±12.92	0.093

Mann Whitney test, P-value <0.05 is significant

consisting of 9 (21%) primary dengue patients and 34 (79%) secondary dengue patients. There were 41 female genders, 9 (22%) primary dengue patients, and 32 (78%) secondary dengue patients. On average, blood sampling for both groups was carried out on the 3rd-5th day of fever.

Table 2 showed that CD95L and lymphocyte levels were higher in secondary infection than in primary infection, while hemoglobin, hematocrit, platelet count, leukocyte, monocyte, and granulocyte in primary infection were higher than in secondary infection.

Discussion

Most of the subjects in this study were secondary dengue patients (79%). This shows the high rate of dengue infection. In this study, the average age of primary dengue patients was higher (33.72±17.00) than the age of secondary dengue patients (23.69±9.05), this is not the same as previous studies which found that secondary infection occurs at a higher average age than primary infection. But the important thing to note is the previous studies concluded that for both primary and secondary infections, the older age group was more likely to develop more severe fever symptoms than the younger age group.[9,10]

Based on gender, male and female subjects were almost the same. In a study conducted in six ASEAN countries (Laos, Philippines, Singapore, Malaysia, Sri Lanka, and Cambodia), it was found that the incidence of dengue by gender found that the incidence of dengue in males was greater than that of females. This difference is associated with different daily activities between men and women.[11]

Blood sampling for both groups was carried out on average on days 3rd-5th of fever. During dengue infection, IgM antibodies are usually produced five days after symptoms appear, and persist in the patient's body for 2–3 months, sometimes even longer. The primary infection had a higher IgM antibody response, while the secondary infection had a higher IgG antibody response. Nonstructural antigen 1 (NS1) plays an essential role in DENV replication in host cells. This antigen is considered an essential biomarker for detecting dengue infection at a stage before the appearance of IgM. NS1 is detectable in the acute phase and persists longer than viremia in the blood. According to the CDC, NS1-based tests show the same results as molecular tests in the first week of infection.[12]

Dengue has a comprehensive spectrum of symptoms, from mild symptoms such as acute flu symptoms to the most severe, in the form of several complications associated with severe bleeding, organ damage, or plasma leakage.[1] The pathogenesis of severe dengue is still unknown. Several hypotheses explain the occurrence of severe dengue, one of which is the antibody-dependent enhancement (ADE) theory. According to this theory, patients who have a secondary infection with a different viral serotype from the first infection are more likely to develop severe dengue.[13]

Host cells respond to viral infection by initiating apoptosis. There is ample evidence that the dengue virus (DEN) can trigger host cells to undergo apoptosis. Dendritic cell apoptosis is induced by viral replication within cells and can induce apoptosis of surrounding uninfected dendritic cells via cytokines or exosomes secreted by infected dendritic cells. Dendritic cell apoptosis can attenuate the host immune response, increasing viral load and cytokine storm observed in severe cases of dengue fever.[6] Induction of apoptosis involves activation of intracellular signaling systems.

One of the intracellular signals detected in the blood is CD95L, which plays a role in stimulating apoptosis. In this study, the levels of CD95L were higher in dengue patients with secondary infection than in primary infection, although not significantly. A previous study found that CD95L levels increased in cases of severe dengue, and increased viral load and cytokine storm were observed in severe cases of dengue fever.[14] Induction of apoptosis involves activation of intracellular signaling systems. One of the intracellular signals detected in the blood is CD95L, which plays a role in stimulating apoptosis. In this study, the levels of CD95L were higher in dengue patients with secondary infection than in primary infection, although not significantly. A previous study found that CD95L levels increased in cases of severe dengue, and increased viral load and cytokine storm were observed in severe cases of dengue fever.[6] Induction of apoptosis involves activation of intracellular signaling systems. One of the intracellular signals detected in the blood is CD95L, which plays a role in stimulating apoptosis.

This study found that the number of lymphocytes in secondary infections was higher than in primary infections; in previous studies, it was concluded that the higher the percentage of lymphocytes, the faster the recovery from dengue fever and the shorter the duration of hospital stay.[15] Atypical lymphocyte counts may be a useful diagnostic tool for infections. dengue and recovery from disease can be assessed when the cell count increases significantly.[16]

In this study, the levels of hemoglobin, hematocrit, number of platelets, leukocytes, monocytes, and granulocytes in primary infections were higher than in secondary infections. The study compared the results of routine blood tests that had previously been carried out, comparing the dengue and non-dengue groups and the dengue fever and dengue hemorrhagic fever (severe dengue) groups. If we assume that secondary dengue is more severe than primary dengue, then the examination results of hemoglobin, hematocrit, platelet, leukocyte, monocyte, and granulocyte counts in this study do not match this assumption. It is possible that most of the patients in this study had secondary infections from the same DENV serotype as the DENV serotype in primary infection. Hematocrit levels are elevated in dengue patients as a result of plasma leakage. In vitro studies revealed the cross-reaction of proinflammatory mediators with surface proteins on endothelial cells leading to apoptosis of these cells and subsequent plasma leakage. Leukopenia usually occurs from the second day of fever and the lowest leukocyte count occurs on the fifth day of fever.[17] Leukopenia in dengue infection is caused by the destruction or inhibition of myeloid progenitor cells. An examination of the bone marrow showed mild hypocellularity in the first seven days of fever, then returned to normal in the recovery phase. [15,17]

Monocytosis was found more frequently in cases of dengue hemorrhagic fever (severe dengue) than in dengue fever. Thus, monocytosis can be used to predict the severity of dengue infection.[18] The increase in monocytes in the first few days of fever is caused because monocytes and macrophages that are part of primary immunity carry out phagocytosis and present antigens to helper T cells. However, several other conditions are associated with monocytosis, so monocytosis is not specific to dengue infection.[19] In a previous study, the percentage of neutrophils increased in the first five days of fever, then in the following days, the percentage of lymphocytes dominated. These results are consistent with previous studies, which showed that lymphocytes predominated on the tenth day of fever.[16]

Platelets of dengue-infected patients have mitochondrial dysfunction, which activates the apoptotic cascade and causes cell death. Prolonged thrombocytopenia is more common in dengue hemorrhagic fever than in dengue fever, so the duration of thrombocytopenia is also considered a predictor of the severity of dengue infection. The parameter to reflect the level of thrombopoiesis is the immature platelet fraction (IPF), which can predict platelet recovery in dengue fever patients on the fourth day of fever,[20] while in this study blood sampling was used out on average on the third to the fifth day of fever. Cytopenia is the main parameter

of peripheral blood count that can differentiate dengue infection from others. A review of bone marrow studies in dengue patients demonstrated a transient suppression of hematopoiesis within 3–4 days after infection, possibly a protective mechanism to limit injury to marrow stem cells during the eradication of infected cells.[20]

Difference in the results of the CD95L examination and other blood tests between primary and secondary dengue patients, except lymphocyte levels, but further research needs to be carried out by distinguishing patients with secondary dengue infection with the same serotype or different serotypes.

The limitation of this study is that this study did not examine whether the secondary infection was of the same DENV serotype or was different from the first infection.

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