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CORRELATION OF DENGUE VIRUS SEROTYPE AND DVI SEVERITY IN ADULT PATIENTS

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ABSTRACT

The clinical manifestation of dengue virus infection is often not clear, varies widely from mild to severe. Exposure of dengue virus which serotype is different from a previous infection is a risk factor for the severe manifestation of dengue virus infection. Dengue hemorrhagic fever is classified into four degrees of severity based on clinical manifestations and laboratory results. Real-time RT-PCR Dengue can detect dengue virus serotype in early dengue virus infection. The aimed of this study was to prove the correlation between dengue virus serotype and degree of severity in adult patients. This study was a cross-sectional observational design done in February until July 2016. Subjects consisted of 100 dengue virus infection patients. Serum of the patients was examined using Real-time RT-PCR Dengue (Simplexa™ Dengue). It was shown that from 46 patients with DENV-3 serotype was 63%, DENV-2 serotype 17.4%, DENV-1 serotypes 17.4% and mixed infection of DENV-1 and DENV-3 serotype 2.2%. There was not any DENV-4 serotype. Dengue Hemorrhagic Fever (DHF) stage I was 47.8%, DHF stage II was 30.4%, DHF stage III was 10.9% and Dengue Fever was 10.9%. There was not any DHF stage IV. There was not enough evidence that DENV-3 correlated with the degree of severity (p= 0.510). Based on this research, DENV-3 serotype was the dominant serotype prevalent at the Dr. Soetomo Hospital. There was no correlation between viral dengue serotype and severity in dengue adult patients in this study.

Keywords: Dengue, Real-time RT-PCR dengue virus, serotype, degree of severity

INTRODUCTION

In Indonesia, the prevalence of dengue hemorrhagic fever (DHF) in 2015, according to the Ministry of Health, was 1817 cases. In 2014, the prevalence of Dengue Hemorrhagic Fever (DHF) in the East Java Province even was considered as an extraordinary incidence due to an increase in its prevelence as much as 980 (46%).[1]

Dengue virus is a genus of *flavivirus* of the *flaviviridae* family spread by Aedes aegypti and Aedes albopictus mosquitoes transmitted through vectors. Clinical manifestations of Dengue Virus Infection (DVI) associated with its severity degrees are often known to be not typical, varying from asymptomatic to symptomatic ones.[2] The symptomatic manifestations of Dengue Virus Infection (DVI) consist of undifferentiated fever, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS), Expanded dengue syndrome and various organ abnormalities. Further complications of DVI even can lead to death.[2]

Therefore, clinical and laboratory manifestations, according to the WHO in 2011, must be examined to determine and establish the diagnosis of dengue virus infection.[2] Patients who have been exposed to different dengue virus serotypes will have risk factors for severe DVI manifestations or DSS.[3,4] Serological test is usually performed to detect antigens in the form of NSI3, while anti-dengue IgM and IgG tests are conducted to detect IgM and IgG antibodies.[4,5] The method commonly used in those examinations is immunochromatography. Nevertheless, although the serological test is useful for the detection of DVI from day 2-3 to 7 after the fever, it is difficult to detect specifically at the onset of fever (≤ five days).[6]

Consequently, Polymerase Chain Reaction (PCR) test is also carried out to detect dengue virus infection.[6] Polymerase chain reaction is a gold standard test to detect dengue virus serotype, also considered as a diagnostic parameter an epidemiologic study within the first five days of fever.[7,8,11] Polymerase chain reaction with biomolecular method even is considered as a more sensitive, specific, faster and better method to detect dengue virus than viral culture method.[7,9,11] In general, PCR as a dengue screening method consists of two types, namely conventional PCR Dengue and Real-time RT-PCR Dengue assays.[10] Compared to conventional PCR Dengue, Real-time PCR Dengue is not only more sensitive, accurate and objective, but also capable of detecting various dengue virus serotypes.[11-13]

As a result, this research aimed to examine the correlation of dengue virus serotypes and DVI severity in adults using Real-time RT-PCR dengue assay.[12-14] Results of this research then are expected to be used as a reference for determining diagnosis and therapy of DVI.[15]

METHODS

This research was an observational analytical study with a cross-sectional design. This research was conducted from February to July 2016. Screening of NS1, anti-dengue IgM and anti-dengue IgG was performed with the immunochromatographic rapid test.[13] The screening was conducted at Clinical Pathology Laboratory of the Dr.Soetomo Hospital in Surabaya. Meanwhile, Dengue virus RNA was examined with Real-time PCR Dengue assay at Dengue Laboratory of Eijkman Institute for Molecular Biology in Jakarta.

Moreover, subjects of this research were DVI patients treated at the Emergency Unit and Tropical Infection Division of the Internal Medicine Department, Dr. Soetomo Hospital, Surabaya. Those subjects were selected since they were patients aged over 14 years and diagnosed with DVI by an internist due to clinical and laboratory examinations' results as determined by WHO (2011) without other accompanying diseases, such as autoimmune and HIV/AIDS. Those patients also had signed the informed consent for participating in this research.

Next, venous blood samples were taken from the cubital vein in sterile and lege artist condition as much as 10 milliliters and then put into vacutainer tubes without anticoagulants. Afterward, those tubes were labeled (name of patient and date of sampling). Those samples then were centrifugated at a rate of 3,000 rpm for 5 minutes to obtain serum, and stored at -80^{0}C until examined.then were centrifugated at a rate of 3,000 rpm for 5 minutes to obtain serum and stored at -80^{0}C until examined.

RESULTS AND DISCUSSIONS

The study was conducted from February to July 2016. The number of samples selected was 100 subjects. After those samples had positive screening results, Real-time RT-PCR Dengue test was conducted for detecting dengue virus serotypes.

Table 1. Results of the RT-PCR dengue test and the characteristics of subjects

RT-PCR dengue assay	Total (n = 100)	Percentage (%)
Positive	46	46
Negative	54	54
Total	100	100
Characteristic of samples	**Total (n=46)**	**Percentage (n=%)**
Sex		
Male	27	58.7
Female	19	41.7
Total	46	100
Day of fever		
3	10	21.7
4	16	34.8
5	10	21.7
6	5	10.9
7	5	10.9
Total	46	100
Dengue virus serotypes		
DENV-1	8	17.4
DENV-2	8	17.4
DENV-3	29	63.0
Mixed DENV-1 and	1	2.2
DENV-3	0	0
DENV-4	46	100
Total		
The degree of DVI severity		
DF	5	10.9
DVI type I	22	47.8
DVI type II	14	30.4
DVI type III	5	10.9
DVI type IV	0	0
Total	46	100
Hematology leukocytes		
Decreasing	15	32.6
Normal	31	67.4
Increasing	0	0
Total	46	100
Platelets ≤ 100	39	84.9
>100	1	2.2
Total	46	100

Results of the Real-time RT-PCR Dengue test showed that there were 46 (46%) subjects with positive dengue virus as seen in Table 1.

Those forty-six subjects consisted of 27 (58.7%) males and 19 (41.7%) females aged between 14-61 years-old with an average age of 24-25 years. Those DVI subjects went to the hospital on day 3 to 7 after the fever. The 4th day sampling indicated the highest positivity (34.8%) followed with the 3rd day, the 5th day, the 6th day and the 7th day of sampling. In other words, the positivity of the Real-time RT-PCR Dengue test' results decreased after day 5. Thus, many patients mostly come on day 3 to 5.

After those one-hundred samples suspected with DVI showed positive screening results, they were examined with Real-time RT-PCR Dengue assay. Results of the Real-time RT-PCR Dengue assay showed that there were 46 (46%) subjects with positive dengue virus as seen in Table 2.

In Table 1, the dengue virus serotype of the research subjects was dominated by DENV-3, followed by DENV-2, DENV-1 and mixed DENV-1 and DENV-3. DENV-4 was not found in the dengue virus serotype of the research subjects.

In addition, the degree of DVI severity in the research subjects was dominated by DVI type I as many as 22 subjects (47.8%), followed by DVI type II 14 subjects (30.4%), DVI type III 5 subjects (10.5%), was 1,700/mm^3 found in DVI type II with DENV-2. and DF 5 subjects (10.5%). None of the research subjects suffered from DVI type IV and DSS.

Plasma leakage is a sign of pathognomonic DVI detected by hematocrit, albumin, pleural effusion and ascites as its parameters. Besides, it is also indicated with other organ abnormalities as well as unusual expanded dengue syndrome or isolated organopathy manifestations that can be identified with pleural effusions and ascites.[2,4,15] The parameters are usually also used to distinguish DF from DVI.[2,15] In this research, an increase in the amount of hematocrit and a decrease in the amount of albumin were used as parameters.

Moreover, the laboratory results showed that fifteen research subjects (32.6%) had decreased leuko cytes/leukopenia, while thirty-one research subjects (67.4%) had normal leukocytes. In other words, none of the research subjects had increased leukocytes/ leukocytosis. The lowest leukocyte count Table 3, besides, thrombocytopenia was known to be mostly found in DVI type I (56.4%), followed with DVI type II (30.8%), DVI type III (7.7%) and DF (5.1%). Thrombocytopenia was not found in DVI type I.

Also, forty-three research subjects had hematocrit examination result data, while three research subjects did not have any result data. The increased hematocrit count (≥20%) is considered as a sign of the most prominent hemoconcentration. In Table 5, there were six research subjects who had plasma leakage, three of them (50%) with DVI type I, two of them

Table 3. The frequency of thrombocyte percentage towards DVI severity degree

Thrombocyte percentage	DVI severity degree					Total n (%)
	DF	DVI type I	DVI type II	DVI type III	DVI type IV	
≤ 100	2 (5.1)	22 (56.4)	12 (30.8)	3 (7.7)	0 (0)	39 (100)
101 – 150	3 (42.9)	2 (28.6)	2 (28.6)	0 (0)	0 (0)	7 (100)
Total	5 (10.9)	24 (52.2)	14 (30.4)	3 (6.5)	0 (0)	46 (100)

Table 4. The frequency of hematocrit percentage towards dengue virus serotypes

Hematocrit percentage	Dengue virus serotypes					Total n (%)
	DENV-1 n (%)	DENV-2 n (%)	DENV-3 n (%)	Mixed DENV-1 and DENV-3 n (%)	DENV-4 n (%)	
³ 20%	1 (16.7)	1 (16.7)	4 (66.6)	0 (0)	0 (0)	6 (100)
< 20%	6 (16.2)	8 (21.6)	22 (59.5)	1 (2.7)	0(0)	37 (100)
No data available	-	-	-	-	-	3 (100)
Total	7 (16.3)	9 (20.9)	26 (60.5)	1 (2.3)	0 0)	43 (100)

(33.3%) with DVI type II and another (16.7%) with DVI type III. Plasma leakage, however, ware not found in DVI type IV and DF since none of the research subjects suffered from DVI type IV and DF.

Plasma leakage is difficult to detect if the patient is admitted to hospital and treated with fluid-therapy due to unobserved laboratory examination results. Thus, albumin is used as a parameter to determine plasma leakage. A decrease in albumin level as much as > 0.5 g/dL from baseline or <3.5 g% is considered as an indirect indicator of plasma leakage.

In this research, there were twenty-six research subjects without albumin examination result data, while the other twenty research subjects had data. In Table 6, six of those twenty subjects (43.7%) had decreased albumin level. The decreased albumin level was not found in the subjects with DENV-1, mixed DENV-1 and DENV-3 and DENV-4. It is difficult to determine plasma leakage by using this parameter since not all subjects had checked

In Table 7, the decreased albumin level was found in four research subjects with DVI type I, two research subjects with DVI type II and one research subject with DF. The decreased albumin level ware not found in DVI type III and DVI type IV. There were plasma leakage signs, such as pleural effusion, ascites and edema on the gallbladder wall. Chest X-Ray photo (with Right Lateral Decubitus = RLD position) and abdominal ultrasonography, thus, is usually used to detect plasma leakage. Nevertheless, there was no data from such examinations in this research.

Next, Fischer's exact test was performed to analyze the correlation of dengue virus serotypes and DVI severity degree. Results of the statistical test revealed that there was no correlation between dengue virus serotypes and DVI severity degree with a p-value of 0.510. The dominance of dengue virus severity was DVI type I (22 cases), followed with DVI type I1 (14 cases), DF (5 cases) and DVI type III (5 cases). Undifferentiated fever, DVI type IV and expanded dengue syndrome were not found in this research.

CONCLUSION AND SUGGESTION

Finally, it can be concluded that the dengue virus serotype of the research subjects was dominated by DENV-3, followed by DENV-2, DENV-1 and mixed DENV-1 and DENV-3. DENV-4 was not found in the dengue virus serotype of the research subjects. Meanwhile, the degree of DVI severity in the research subjects was dominated by DVI type I, followed by DVI type II, DVI type III and DF.

Table 5. The frequency of hematocrit percentage towards DVI severity degree

Hematocrit percentage	DVI severity degree					Total (n %)
	DF (n %)	DVI type I (n %)	DVI type II (n %)	DVI type III (n %)	DVI type IV (n %)	
³ 20%	0 (0)	3 (50)	2 (33.3)	1 (16.7)	0 (0)	6 (100)
< 20%	6 16.2)	16 (43.2)	10 27.1)	513.5)	0 (0)	37 (100)
No data available	-	-	-	-	-	3 (100)
Total	6 (14)	19 (44.1)	12(27.9)	6 (14)	0(0)	43(100)

Table 6. The frequency of albumin percentage towards dengue virus serotypes

Albumin	Dengue virus serotypes					Total n (%)
	DENV-1 n (%)	DENV-2 n (%)	DENV-3 n (%)	Mixed DENV-1 and DENV-3 n (%)	DENV-4 n (%)	
< 3.5	0(0)	3(6.5)	3(6.5)	0 (0)	0	6 (15.2)
3.5 – 5.0	2(4.4)	0(0)	10(21.7)	1(2.2)	0	13 (28.3)
No data available	-	-	-	-	-	26(56.5)
Total	2(4.4)	3(6.5)	13 (28.3)	1(2.2)	0	46(100)

Table 7. The frequency of albumin percentage towards DVI severity degree

Albumin (g/%)	DVI severity degree					Total n (%)
	DF (n %)	DVI type I (n %)	DVI type II (n %)	DVI type III (n %)	DVI type IV (n %)	
< 3.5	1(14.3)	4 (57.1)	2 (28.6)	0(0)	0(0)	7(100)
3.5 – 5.0	1(7.7)	8 (61.5)	2(15.4)	2(15.4)	0(0)	13(100)
No data available	-	-	-	-	-	26(100)
Total	2(4.3)	12(21.7)	4(8.7)	2(4.3)	0(0)	46(100)

None of the research subjects suffered from DVI type IV and DSS. Also, there was no correlation between dengue virus serotypes and DVI severity degree in patients. PCR test, as a result, is recommended for detecting dengue virus infection early to prevent its severity. Nevertheless, further researches are still needed to determine dengue virus serotypes in Indonesia.

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