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RESEARCH ARTICLE

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Diagnosis of Dengue Hemorrhagic Fever Based on Laboratory Test of Serology and PCR from Suspected Patients in Hospital

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Background: DHF (Dengue Hemorrhagic Fever) still become one of the health problems in Indonesia. Incidence rate of DHF was higher than 20 per 100.000 population. The clinical symptoms were not specific anymore. The aim of this study was to compare the sensitivity and specificity of DHF diagnosis between serology and PCR test. *Method*: This study was a descriptive observational using cross sectional approach. Twenty four patients of suspect DHF in a hospital in Semarang were recruited. Diagnosis was based on a rapid test which usually done in Hospital, and then the results were compared to PCR technique. Data were analyzed descriptively. *Result*: The result showed that all of the subjects (24 patients) had thrombocytopenia ≤150,000 cells/mm^3. The number of males and females are equal 50%, the mean of age were 35.4 year old, and 36.4% of them were unemployed. This study found that IgM positivity were 50% (primer infection), and IgG were 37,5% (secondary infection). Based on PCR tests to the subjects, 35% positivity were found and 42,9% Den-3 strain were detected. One patient had mixed infection of Den-2 and Den-4. *Conclusion*: There was a difference in diagnosis between clinical symptoms and laboratory tests.

Keywords: Dengue Hemorrhagic Fever (DHF), IgM, Serology, PCR.

1. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is a vector-borne disease which still be the big public health problem in Indonesia. National reports showed that dengue cases tended to increase from year to year. Incidence rate of DHF in Central Java province in 2015 was 47.90/100,000 population but the incidence rate of dengue in Semarang city reached 99.46/100,000, while the target of National DHF Program was 50/100,000 population. Case Fatality Rate in Central Java Province was 1.56%.[1] DHF often cause an outbreak with high mortality.[2] Mortality Rate of Dengue Shock Syndrome (DSS) at PICU of Kariadi Government Hospital was 5–7% (2007–2009).[3]

Based on WHO guideline, diagnosis of DHF was based on clinical criteria or clinical symptoms supported by laboratory tests. Laboratory criteria are thrombocytopenia (100,000/ul or less), hemo-concentration signed by the increase of 20% or more in hematocrit. The recent growing problem is the less specific or highly diverse symptoms of clinical DHF. Symptoms that appear almost identical to other infectious diseases, such as influenza, chikungunya, typhoid, etc., which made the diagnosis assessment is very difficult. It should be supported by laboratory tests.[4]

This disease is caused by dengue virus derived from Arthropod-Borne Virus (Arbovirus), which has four serotype of dengue viruses, i.e.,: DEN1, DEN2, DEN3, and DEN4. All of the four serotypes have been found in various regions in Indonesia. A study in Padang, Indonesia showed that DEN3 virus was the dominant virus serotypes that cause severe cases.[5]

Given the high mortality of DHF cases, a rapid test is very helpful to medical personnel. IgG/IgM Dengue is a quick test that appears earlier than the Dengue NS1 Ag; IgG/IgM test detects the presence of antibodies while NS1 detects antigen against dengue virus. Immunoglobulin G (IgG) and immunoglobulin M (IgM), appear as the body's response to the dengue virus that infect the patient's body. Immunoglobulin G will appear on the 4th day after the initial infection and will last up to six months after infection. The presence of antibodies indicates that a person ever had dengue virus infection, at least in the last six months.

Other laboratory method to confirm diagnosis of DHF is dengue virus isolation from patient serum, identification of dengue virus by antigen ELISA, immunohistochemistry, and immunofluo-rescence as well as the identification of the virus genome sequence by PCR.[6] Detection of antibodies has some weaknesses, such as: it can't be used to detect infection in early days since the body needs time to form antibodies after being

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infected by the virus. Antigen detection for dengue infection is necessary to anticipate the occurrence misdiagnosed or cross reaction with other diseases. Antigen detection can be performed by molecular and immunological technique.

In general, molecular detection showed some advantages such as quick as well as high sensitivity and specificity. However, this technique has a relatively high level of difficulty and requires special skills. This study aimed to determine the sensitivity and specificity of dengue immunological technique in compared to PCR.

2. METHOD

This study used observational cross-sectional design. The study was conducted at hospitals in Central Java Province. Subjects were suspected dengue patients at Private and the Government hospitals. Suspected dengue patients were tested for serology by taking 1 cc of whole blood to confirm the DHF diagnosis. During the three months period, we was found 24 patients with suspected dengue.

This study measured the characteristic variables of suspected DHF and the results of serological and virological examination. Serological examination was based on IgG and IgM antibody response and virological examination used PCR method. Positive IgG and IgM indicates that the patients have ever been infected by dengue virus. Data on characteristics of the suspected patients were collected by interviews. Serological examination performed in a hospital, while the virological examination conducted at Gadjah Mada University laboratory of Molecular Biological in Yogyakarta Province.

3. RESULTS

The study subjects were 24 patients with the characteristic explained in Table I. Table I showed that the sex ratio of the treated patient was equal 1:1. Most of the respondent were unemployed (36,4% out of 24 respondents). A high mobility employee like civil servant was 27,3%. The youngest Patient was 3 year old and the oldest was aged 80 years, with the mean age of 35,4 years.

Serology test showed that 87.5% of 24 patients suspected DHF had positive IgM. Among the 21 patients with positive IgM, 42,9% had positive IgG. Positive IgM status indicated that there were infected. If both IgM and IgG are positive, it means that the patient had primary and secondary infection of dengue virus.

Table I. Respondents' characteristics.

Respondent characteristic	*F*	%
Gender ($n = 24$)		
Male	12	50.0
Female	12	50.0
Occupations ($n = 22$)		
Unemployed	8	36.4
Civil servant employees	6	27.3
Retired	1	4.5
Farmer	2	9.1
Enterpreneur	4	18.2
Employee	1	4.5
Age (years)		
Mean	35.4	

Table II. Serologic test results.

	IgG					
Serology	+	(%)	–	(%)	Total	(%)
IgM						
+	9	42.9	12	57.1	21	100.0
–	0	0.0	3	100.0	3	100.0
Total	9	37.5	15	62.5	24	100.0

Table III. Diagnosis of DHF based on serological and molecular test.

	Metode PCR					
Serological diagnosis of DHF	+	%	–	%	Total	%
+	7	41.2	10	58.8	17	0.85
–	0	0.0	3	100.0	3	0.15
Total	7	35.0	13	65.0	20	100.0

Based on the comparison between the result of serological diagnostic tests and PCR, it was found that serological rapid diagnostic test can determine DHF precisely with 7/7 (100%) sensitivity. Meanwhile, the ability of rapid diagnostic test to diagnose non-DHF precisely was 3/13 (23%) and the ability to precisely predict DHF patient or called *positive predictive value* (PPV) was 7/17 (41.2%). However, the ability to predict non-DHF patient precisely or called *negative predictive value* (NPV) was 3/3 (100%), as can be seen in Table III.

From the 24 patients, PCR tests were only conducted on 20 patients. PCR results showed that about 35% patients' serum were infected by DHF. From 7 patients with positive DHF, 3 of them (42.9%) was infected by DEN3 virus. All variant strain of dengue virus were found inside the treated patients and one patient (14,3%) had mixed infections of virus strains DEN1 and DEN4.

4. DISCUSSION

In this study, serologic diagnostic of DHF showed the higher sensitivity (100%) compared to PCR diagnostic while the specificity was less than 50%. This study used clinical sample which was trombositopenia of DHF patients (<100.000/ul) and increased of hemoconcentration (>20%) (WHO, 2000). Those numbers supported the primary infection of this case (87,5% IgM positive). Therefore, the sensitivity number of DHF diagnose was higher than its specificity number. The examination using IgG/IgM anti-dengue was quite good to diagnose DHF, however it was weak to diagnose early DHF. This is likely due to the antibody respond towards dengue appear in four days after infection. While NS1 was able to detect the antigen from the virus protein of the patients, at one to four days after infected. The appearance of virus titer will decrease after antibody response appear. Consequently, the choice of using diagnostic test is due to the period after dengue infection. Positivity percentages of PCR would be decrease with the longer time of clinical fever symptom.[7] Variants of dengue virus serotypes could influence the sensitivity of the test.[8] The differences of sensitivity for each serotype were correlated to the differences of immunology factors against certain serotypes, and also geographical factors.

The clinical symptoms of DHF at the beginning of infection period is generally not specific.[9] DHF occur as a saddle fever for three days decreasing and then will increase on the sixth or seventh day. Different serotypes of virus will cause variation among clinical manifestations. In this study, we found 41.2% of

the cases caused by DEN3 virus. A study in Jakarta identified that DEN3 was dominant.[10] DEN3 serotype was commonly dominant and often correlated with severity cases of DHF.[11] There was no single diagnostic test accurately to detect DHF along the infection period. In addition, diagnosis of dengue virus infection based on clinical syndrome was unreliable and must be confirmed by laboratory tests.[12] In summary, the selection test for dengue fever diagnosis should consider the duration of the infection.

5. CONCLUSION

Based on the PCR test, it was found that 35% of patients were positive DHF and most of them were DEN3 strain (42.9%). In this study, IgM and IgG Serologic test were quite sensitive but less specific compared to PCR tests.

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