

CORRELATION BETWEEN DENGUE VIRUS SEROTYPE AND HUMORAL IMMUNE RESPONSE IN PEDIATRIC DENGUE HEMORRHAGIC FEVER

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ABSTRACT

Dengue hemorrhagic fever (DHF) is an endemic disease caused by dengue virus. The pathogenesis of dengue hemorrhagic fever is still unclear, while virus virulence and heterologous antibody still play important roles in the pathogenesis of dengue haemorrhagic fever. The aim of this study was to find the correlation between dengue virus serotype distribution and humoral immune response on dengue haemorrhagic fever in children. This infection pattern was based on the antibody profile as a humoral immune response in dengue virus infection. Dengue virus serotype examination was done by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), while humoral immune response was done by IgM-IgG Captured ELISA. The result of this study showed that from 35 dengue haemorrhagic fever cases, majority (74.4%) were secondary infection with mild gradation, grade I (48.6%) and grade II (34.4%). Den-3 and Den-4 serotypes were found, Den-3 have mild clinical manifestation, whereas Den-4 has a fatal manifestation as Dengue Shock Syndrome.

Keywords: Humoral immune response, DHF, DSS, Den

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is an endemic disease caused by dengue virus with distribution in tropical and subtropical countries, particularly in urban areas. The growth of DHF as a global problem becomes stronger, as it becomes an endemic disease in more than 100 countries, affecting about 2.5 billion individuals. World Health Organization (WHO) estimated that there were 50-100 million cases of dengue virus infection in the whole world annually, from which 250-500 thousands patients had DHF cases and 24 thousands of who died (Shu 2004). DHF incidence in Indonesia during 1991-1992 was 11.56 and 0.45 per 100,000. DHF cases increased significantly in 1998 and reduced in 1999. The majority of DHF cases occurred in children less than 15 years old or between 4-14 years old, and there was increased proportion of the cases in adults until recently (Soemarno 2004; Suroso 1999). The cause of fatality in DHF is the condition designated as Dengue Shock Syndrome (DSS). The pathogenesis of DHF remains, however, unclear, but it is suspected that viral virulence and heterologous antibody play a role in this phenomenon.

Recently, there are two theories representing the elaboration of DHF and DSS pathogenesis. The first theory indicates that DHF and DSS result from

secondary infection of heterologous dengue virus, which induces subneutralizing Antibody-Dependent Enhancement, while the second theory mentions that DHF and DSS occurs because the virus has genetic variations in its genome, which in phenotypic expression the genetic changes may present as capability in replication and viremia induction, the disease severity, virulence, and potentiality to induce epidemics (Gubler 1998). Based on the replication of human-infecting dengue virus serotype, dengue virus infection can be divided into two, the primary and secondary infection. Lifelong immunity against homologous serotype emerges after primary infection. Epidemiological study in Southeast Asia indicated that DHF/DSS occurs mainly during secondary infection by virus serotype different from that causing primary infection. Clinical manifestation of secondary dengue virus infection is more severe than that of primary infection. Several literatures indicate that primary infection only causes a condition designated as febrile self-limiting disease, while secondary infection may cause severe complications. Therefore, the better prognosis of DHF/DSS should also be based on the necessity to differentiate primary or secondary dengue infection, not only differentiating positive or negative result of the dengue infection (Suroso 2004). As has been mentioned above, DHF pathogenesis has not been widely disclosed. The correlation between the role of

dengue virus and antibody in DHF pathogenesis referring the degree of DHF severity becomes important.

MATERIAL AND METHODS

This was a molecular virological and serological study, with analytical laboratory study was the type of the study. The population in this study comprised pediatric patients with clinical DHF according to WHO criteria 1997 who were treated at Intensive Care Unit and Inpatient Wards, Department of Pediatrics, Dr Soetomo Hospital, Surabaya, in August 2005. The blood of DHF patients, whose diagnosis had been established according to WHO criteria 1997, was taken to obtain the serum, and subjected to serological examination using Capture ELISA method to find the presence of antidengue IgM and IgG antibody, and the type of infection was subsequently determined. Furthermore, using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) the virus serotype was determined. To find the serotype of DHF virus by involving 10 samples who were taken using random selection based on DHF grades. Three samples from grade 1, 3 samples grade 2, 2 samples grade 3, and 2 samples grade 4, by also taking into account the type of infection.

RESULTS

Data on the results of clinical examination in DHF patients and the grading of disease severity indicated that the majority of the patients had DHF grade 1 (48.6%) and degree 2 (34.3%), while a minority of them had grade 3 (11.4%) and grade 4 (5.7%). The results of anti-dengue IgM and IgG examination in DHF-diagnosed patients is presented in Figure 1.

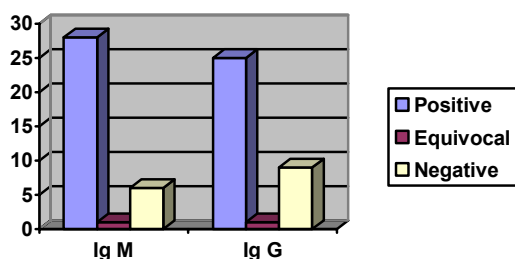


Figure 1. IgM and IgG profiles.

Figure 1 indicated that from 35 patients with DHF clinical diagnosis, 6 patients (17.1%) had negative IgM, 1 patient (2.9%) was equivocal, and 28 patients (80%) had positive IgM. This indicated that the percentage of positive IgM was higher than the percentage of negative

IgM. The figure also indicates that from 35 patients with DHF diagnosis, 9 patients (25.7%) had negative IgG, 25 patients (71.4%) had positive IgG, and 1 patient (2.9%) had equivocal IgG. The infection status of patients diagnosed with dengue virus infection is presented in Figure 2.

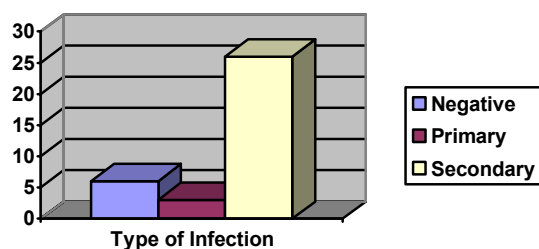


Figure 2. Dengue virus infection pattern

Figure 2 shows that from 35 DHF patients who had been serologically examined, 6 patients (17.1%) were negative, 3 patients (8.5%) had primary infection, and 26 patients (74.4%) had secondary infection. The result of serological examination above indicated that the highest percentage of DHF patients had secondary infection. Dengue virus serotype examination with RT-PCR was conducted to 10 samples. The result of dengue virus serotype examination is presented in Figure 3. Electrophoresized PCR results revealed three positive patients. Two (66.7%) had serotype Den-3 and one (33.3%) showed serotype Den-4.

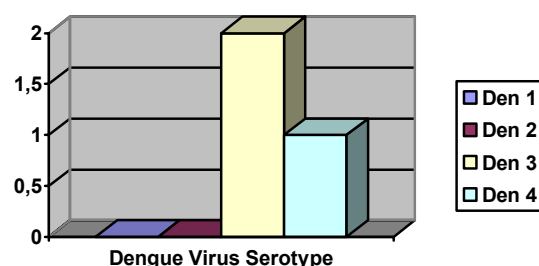


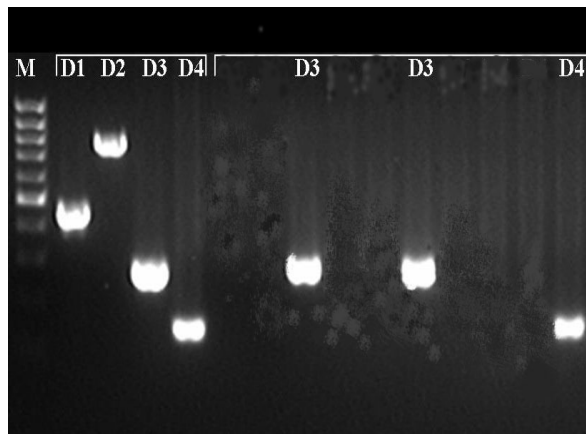
Figure 3. The number of dengue virus serotype

Correlation between the grade of DHF severity and the result of serological examination can be seen in Table 1. Table 1 shows that negative antibody response was found in 6 patients with grade 1. Primary infection was found in one patient with grade 1, and two patients with grade 2. Secondary infection was found in four grades

of DHF disease, grade 1 in 10 patients, grade 2 in 10 patients, grade 3 in 4 patients, and grade 4 in 2 patients.

Table 1. Correlation between DHF severity in children and infection pattern based on the results of serological examination in 2005.

Grade of disease severity	Type of infection		
	Negative	Primary	Secondary
Grade 1	6 (17.1)	1(2.9)	10(28.6)
Grade 2	0	2(5.7)	10(28.6)
Grade 3	0	0	4(11.4)
Grade 4	0	0	2(5.7)
Total	6(17.1)	3(8.6)	26(74.3)



Notes:
Lane 1 : Marker of DNA Molecular Weight Marker VIII (0.019-1.11 kbp) from pUC BM 21 DNA Hpa II digested (cat. No. 1 336 045)
Lane 2, 3, 4, 5 : Positive control Den-1 (453 bp), Den-2 (809 bp), Den-3 (290 bp), and Den-4 (139 bp)
Lane 6 – 15 : DHF patients sample (lane 8 and 11 are Den-3, lane 15 is Den-4)

Figure 4. Electrophoresis of RT-PCR results

From three PCR positive results, it was found that there are two types of dengue serotype, i.e. Den-3 and Den-4. Den-3 presents in the patients with grade 1 and 2, while Den-4 presents in patient with grade 4. Correlation between serological examination and Dengue virus serotype can be seen in Table 2.

Table 2. Dengue virus infection pattern and its serotype in 2005

Type of infection	Den-1	Den-2	Den-3	Den-4	Total
Negative	0	0	1	0	1
Primary	0	0	0	0	0
Secondary	0	0	1	1	2
Total	0	0	2	1	3

Table 2 shows that Den-3 serotype is found in patients with negative IgM and IgG in patients with secondary infection, while Den-4 is found in patients with secondary infection. This study had not been able to disclose whether there was correlation between dengue virus serotype and humoral immune response with the severity of DHF disease in children. This was because the results of positive PCR were less representative for correlational statistical test.

DISCUSSION

This study investigated dengue hemorrhagic fever using samples obtained from the serum of 35 patients who had been diagnosed with DHF according to WHO Criteria 1997 and whose grade of the disease severity had been determined. In general, most of the patients had grade 1 and 2, who, according to WHO, could be classified into one group of DHF. Those with grade 3 and 4 were classified into DSS. DHF patients in this study comprised 29 individuals from total sample of 35 individuals or about 84%, while DSS patients, who were more severe and might be fatal, were only 16% from total sample. It can be observed that, in general, DHF patients in Surabaya had a wide spectrum of DHF, which was proved by the finding of the four grades of DHF severity in this study, starting from the mildest to the most severe one. However, the spectrum of DHF disease is not an exclusive and fixed division due to the development of the disease pathogenesis) and the disease grade may change anytime from mild grade to more severe grade. These changes at times are difficult to find and to predict. It can be identified only through clinical changes and the alteration of the results of laboratory examination. Until recently, there has been no significant parameter as a predictor of change to more severe grade. Studies are only aimed to find the risk factors of those changes.

In this study, we also found patients with SSD, although in a small number (16%), which was indicating that there would be fatal severe condition if DHF patients are not appropriately treated. It has generally been known that the death of DHF patient is resulting from DSS. The death is caused by shock or its resulted bleeding or complications. The death can be prevented if the patient is appropriately treated as early as possible. Delayed treatment and misdiagnosis may be fatal to the patient. A precise early diagnosis is highly necessary.

The result of serological test indicated that the highest percentage of DHF patients had secondary infection. The results of serological examination on anti-dengue IgM and IgG in DHF patients may describe the status or the type of infection. If the result of IgG examination in

the patient's serum is positive, whatever result of IgM examination indicates that the patient had secondary infection of dengue virus. If the results of IgM and IgG are negative, the clinically diagnosed DHF patients may not be infected with dengue virus. Furthermore, if the results of IgM and IgG examination were equivocal, the result of examination should be re-examined. In this study, equivocal IgM and IgG was regarded as negative and not subjected to re-examination a week thereafter.

In regard with DHF grade, negative antibody response was found in six patients with grade 1, primary infection in one patient with grade 1 and two patients with grade 2. Secondary infection was found in four DHF grades, grade 1 in 10 patients, grade 2 in 10 patients, grade 3 in four patients, and grade 4 in four patients. It can be inferred that primary infection is related to mild DHF grade (grades 1 and 2), while secondary infection provided wide clinical manifestations, from grade 1, 2, 3, and 4.

Results obtained in this study confirmed the currently prevailing theories. The theory of Secondary Heterolog Infection, as introduced by Scott B. Halstead (1993), states that primary infection in dengue patients provides protective immunity in the individuals and also provides mild clinical manifestation. If subsequent infection occurs with virus in different serotype, the formed antibody in primary infection will be non-protective and non-cross reactive. The antibody may result in severe manifestation, a phenomenon designated as the Antibody Dependent Enhancement (ADE). Secondary infection may result in severe outcome that leads to shock, because the complement is also activated due to the effect of dengue virus-antibody complex, resulting in anaphylatoxin (C3a, C4a, C5a) that caused vasodilatation and the increase of blood vessel capillary permeability, and ultimately result in plasma leakage.

Results of this study using RT-PCR revealed that from 10 DHF patients, three (30%) showed positive result and seven patients (70%) showed negative result. From positive RT-PCR results, two (66.7%) showed serotype Den-3 and one (33.3%) showed serotype Den-4. The result of RT-PCR is highly determined by the presence of the virus in the patients' serum. This was closely related to the incidence of viremia in dengue virus infection. The incidence of viremia in dengue virus infection occurs until five days after the first day of fever, and the virus will drastically decline until it disappears from circulation. WHO 1997 also provides sampling procedure in this viremic phase, which is designated as S1. The positive result of RT-PCR revealed two types of detected dengue serotype, i.e., Den-3 and Den-4. Den-3 existed in patients grade 1 and 2, while Den-4 existed in those with grade 4.

Serotype Den-3 was found in 1 patient with negative IgM level, negative IgG (negative) and 1 patient with positive IgM, positive IgG (secondary infection), and serotype Den-4 in 1 patient with positive IgM, and positive IgG (secondary infection). This study found two dengue virus serotypes, i.e., Den-3 and Den-4. Den-3 was found in 1 patient with grade 1 and negative serological results (negative IgM and IgG). Subsequently, Den-3 was found in grade 2 patients with secondary infection (positive IgM and positive IgG). Den-4 was found in grade 4 patient with secondary infection (positive IgM and positive IgG).

In patients with Den-3 with grade 1 and negative serology, negative serological result did not exclude the presence of dengue virus infection in the patient. This may occur during the sampling procedure in patients with early viremia during which the antibody has not been formed. This was reflected from negative results, in which the patients were possibly having primary infection, as confirmed by the presence of mild manifestations. Patients with other Den-3 serotypes were with grade 2 and positive serological results revealing secondary infection. This confirmed the Secondary Heterolog Infection theory, even though with mild clinical manifestation.

Patients with Den-4 showed severe clinical manifestation of grade 4 as supported by positive serological result of secondary infection. This phenomenon proved the role of heterologous antibody, again confirming the currently prevailing Secondary Heterolog Infection theory. Den-3 serotype in this study provided mild DHF clinical picture (grade 1 and grade 2), while grade 4 was caused by Den-4. The role of Den-4 in severe dengue infection cannot be generalized. It was apparent that in Surabaya, as a DHF hyperendemic area, DHF was found to have several dengue virus serotypes, among which were Den-3 and Den-4.

CONCLUSION

It can be concluded that the severity of DHF in children was mostly in grade 1 (48.6%) and grade 2 (34.3%), and lesser part of those children had grade 3 (11.4%) and grade 4 (5.7%). The pattern of DHF infection in children was mostly the secondary infection (74.4%), while primary infection was 8.5% and negative 17.1%. Dengue virus serotypes in children were Den-3 of 66.7% and serotype Den-4 of 33.3%. Serotype Den-3 was found in patients with grade 1 and negative IgM and negative IgG (negative) and in grade 2 with secondary infection (positive IgM and positive IgG). Serotype Den-4 was found in patients with grade 4 and secondary

infection (positive IgM and positive IgG). Correlation between Dengue virus serotype and humoral immune response with DHF severity in children has not been proved.

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