RESEARCH

ANALYSIS OF DENGUE SPECIFIC IMMUNE RESPONSE BASED ON SEROTYPE, TYPE AND SEVERITY OF DENGUE INFECTION

(Analisis Respons Imun Spesifik Dengue terhadap Serotipe, Jenis dan Derajat Infeksi Virus Dengue)

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ABSTRAK

Infeksi Virus Dengue (IVD) menimbulkan derajat klinis beragam dari DD hingga DBD/SSD. Respons imun spesifik dengue berupa IgM dan IgG anti dengue masih merupakan perdebatan untuk patogenesis DBD di samping faktor virulensi virus dan jenis infeksi. Penelitian ini bertujuan untuk menganalisis respons imun spesifik dengue terhadap serotipe, jenis dan derajat IVD di Surabaya. Subjek adalah pasien IVD yang dirawat di Ruang Tropik Infeksi Penyakit Dalam RSUD Dr. Soetomo dengan hasil penyaringan uji cepat NS1 (SD Bioline Dengue Duo) dan/atau PCR (Simplexa Dengue) positif. Pemeriksaan IgM dan IgG anti dengue kuantitatif dengan metode ELISA (Panbio Dengue Duo IgM and IgG Capture). Penelitian dilakukan Maret–Agustus 2016 dan didapatkan 61 pasien dengan hasil NSI dan/atau PCR dengue positif. Identifikasi serotipe didominasi DEN-3, namun serotipe yang lebih virulen ditunjukkan DEN-1 yaitu semua pasien bermanifestasi sebagai infeksi sekunder dan DBD. Jenis infeksi primer sebanyak 19 (31,1%) dan infeksi sekunder 42 (68,9%). Derajat IVD meliputi DD 10 (16,4%), DBD 47 (77%) dan SSD 4 (6,56%). Nilai indeks rerata IgM dan IgG anti dengue di kelompok infeksi serotipe DEN-1 (5,140 dan 5,774), DEN-2 (2,971 dan 2,222), DEN-3 (1,863 dan 2,792); kelompok jenis infeksi primer (1,478 dan 0,746), sekunder (4,028 dan 4,864) dan kelompok derajat DD (1,170 dan 1,492), DBD I (3,370 dan 3,651), DBD II (3,924 dan 4,439) dan DBD III (4,164 dan 4,243). Sebagai simpulan respons imun spesifik dengue didapatkan lebih tinggi bermakna di kelompok infeksi serotipe DEN-1, kelompok jenis infeksi sekunder dan kelompok DBD/SSD.

Kata kunci: IgM, IgG, serotipe, infeksi sekunder, DBD/SSD

ABSTRACT

Dengue infection causes a clinical severity from DF to DHF/DSS. Dengue specific immune response such as anti-dengue IgM and IgG is still controversial for the DHF pathogenesis besides virus virulence and type of infection. The study aim was to analyze dengue specific immune response to serotype, type and severity of dengue infection in Surabaya. Subjects were dengue infection patients hospitalized in the Tropical Infectious Disease Ward, Dr. Soetomo Hospital with positive results for NS1(SD Bioline Dengue Duo) and/or PCR (Simplexa Dengue). Examination of quantitative IgM and IgG was performed by Panbio Dengue Duo IgM and IgG Capture ELISA. This study was done in March–August 2016 and comprised 61 patients with positive results for NSI and/or PCR. Serotype identification was dominated by DEN-3, but DEN-1 seems to be more virulent as all patients manifested as secondary infection and DHF. Primary infection consisted of 19 (31.1%) and secondary infection of 42(68.9%) patients. Clinical manifestation were DF 10 (16.4%), DHF 47 (77%) and DSS 4 (6.56%). Mean index value of anti-dengue IgM and IgG in DEN-1 was (5.140 and 5.774), DEN-2 (2.971 and 2.222), DEN-3 (1.863 and 2.792); primary infection (1.478 and 0.746), secondary infection (4.028 and 4.864) and severity group of DF (1.170 and 1.492), DHF I (3.370 and 3.651), DHF II (3.924 and 4.439) and DHF III (4.164 and 4.243). In conclusion, dengue specific immune response was significantly higher in DEN-1 serotype infection group, secondary infection group and DHF/DSS group.

Key words: Anti dengue IgM and IgG, serotype, secondary infection, DHF/DSS

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INTRODUCTION

Dengue Viral Infection (DVI) is an infectious disease caused by dengue virus (DEN) and transmitted to humans by infective female mosquitoes of *Aedes sp,* especially *Aedes aegypty* or *Aedes albopictus* mosquito. Clinical manifestations of dengue infections are highly variable and not typical, ranging from no symptoms (asymptomatic), non-specific mild-fever, Dengue Fever (DF), or more severe forms i.e Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[1]

Based on the frequency and serotypes of the infecting virus, dengue infection is classified into two types, primary and secondary infection. Secondary infection needs to be distinguished from primary infection because it often results in severe clinical manifestations. Based on the literature, primary infection only causes a condition called febrile self-limiting disease whereas secondary infections can cause more a severe complication form of DHF or DSS.[2]

Humoral immune response in primary infection is marked by the emergence of anti- dengue IgM 3-5 days of fever, the titers will increase in the next 1-3 weeks and can be detected up to 3-8 months. Immunoglobulin G is produced about 2 weeks after infection and can last a lifetime. Humoral immune response in a secondary infection occurs more rapidly due to anamnestic reaction of antibody formation, especially of the IgG class, where on day 2 IgG increased rapidly. Immunoglobulin M if it is found tends to be very low and usually does not exceed IgG, even in some cases it is not detected.[2,3]

Dengue virus is a single-stranded RNA virus member of Flaviviridae family and flavivirus genus. Antigenically there are four serotypes of dengue, namely DEN-1, DEN-2, DEN-3 and DEN-4. Antigen structure of all four serotypes is very similar to one another, but antibodies against each serotype are not be able to provide cross protection.[4]

Two hypothesis of pathogenesis DHF in many cases are viral virulence theory and immunopathogenesis based theory. The viral virulence theory suggested that the virulent dengue strains causes DHF, while the non-virulent dengue strain causes DF. This theory is based on the fact that although dengue fever occurs more frequently in secondary infection than in primary infection, but DHF may also occur in primary infection. This suggests that the virulence of the virus contributes to the development of DHF.[5] Immunopathogenesis theory is based on the serotype cross-reactive antibodies generated from previous infections. Cross-reactive antibodies that lack neutralizing activity are induced in the primary infection. In secondary infection, dengue virus and non-neutralizing antibodies form virus-antibody complexes bound to Fcγ receptors on target cells and result in enhancement of dengue virus infection. This phenomenon is called Antibody Dependent Enhancement (ADE).[5,6]

As mentioned above the dengue pathogenesis is not yet fully understood. The relationship between dengue virus and antibodies against the severity of DVI becomes important. This study aimed to analyze the humoral immune response against specific dengue serotypes, types and severity of dengue infection in Surabaya.

METHODS

This study was an analytical observational cross-sectional design done at The Tropical Infectious Diseases Ward Dr. Soetomo Hospital for the selection and sampling; The Clinical Pathology Laboratory of Dr. Soetomo Hospital for serum separation and examination of NS1 rapid test; Laboratory of Tropical Infectious Diseases Hospital Surabaya for anti-dengue IgM and IgG ELISA methods and Eijkman Biology and Molecular Institute Jakarta for RNA extraction and PCR dengue.

The inclusion criteria were samples of patients hospitalized at the Tropical Infectious Diseases Ward Dr Soetomo Hospital with 2–7 days of fever accompanied by symptoms and signs of dengue infection (clinical manifestations DF or DHF) according to WHO criteria 2011; with positive results of NS1 and/or dengue PCR and willing to participate in the study by signing an informed consent. Criteria for sample rejection was dengue infection patients with concomitant diseases such as autoimmune diseases, HIV/AIDS, other immunodeficiency diseases and malignancy that were obtained from medical records lysis, jaundice and lipemic sera.

Samples were included in this study by consecutive sampling and specimens used were sera. Screening of NS1 rapid test using the SD Bioline Dengue Duo kit, positive or negative results was determined from color lines appearing in the test area and the result was invalid if it appeared the color line of the control area. RNA extraction was done automatically using Magna Pure LC Total Nuclei Acid Isolation (Roche) kit and the Magna Pure LC 2.0 instrument. Amplification and identification of dengue virus serotypes used Simplexa Dengue Real Time RT-PCR (Focus Diagnostics) kit and the 3M Integrated Cycler instrument. Interpretation of positive results if each serotype detected by the cycle threshold (Ct) was ≤40.0 and ≠0.

Examination of anti-dengue IgM and IgG quantitatively was performed using Panbio Dengue Duo IgM and IgG Capture ELISA kit. The positive or negative value was determined based on the specifications of the kit. Primary or secondary infection type was determined based on the results of the anti-dengue IgM and IgG ELISA method. Samples with positive IgG anti dengue positive in the acute phase (2-7 days) were considered as secondary infections.

RESULT AND DISCUSSION

The study was done from March to August 2016 and comprised 61 samples with positive results of NS1 and/or dengue PCR (Table 1). One of the confirmed criteria of dengue infection was through virus isolation or detection of viral antigen or viral RNA in the serum.[1,7] It was expected that antibodies formed and analyzed in the subjects of this study were an antibody specific response against dengue.

Table 1. Results of screening NS1 immunochromatography and dengue PCR

Screening results	n (%)
NS1(+), PCR(+)	34 (55.7%)
NS1(+), PCR(−)	15 (24.6%)
PCR(+), NS1(−)	10 (16.4%)
NS1(+), PCR not done	2 (3.3%)
Total	**61**

Identification of 44 positive dengue PCR results were dominantly DEN-3 serotype (63.6%), followed by DEN-1 (18.2%) and DEN-2 (18.2%), while DENV-4 serotype was not detected in this study (Table 2).

More virulent serotype seemed to be demonstrated by DEN-1 infection in which all patients with DEN-1 infection manifested as secondary infections and DHF (with or without shock). The mean index value of anti-dengue IgM and IgG on DEN-1 infection group was also obtained significantly higher than those of infection by other serotypes. Dengue virus had different virulence levels in causing clinical manifestations. This was related to the virus ability to infect, causing viremia, replication and stimulating immune response.[4,8]

ELISA serology results obtained mean index value of anti-dengue IgM varying between 0.325 to 6.578 and anti-dengue IgG varies between 0.123 to 1.777. Based on serology reactivity 19 (31.1%) patients were classified as primary infection and 42 (68.9%) patients as secondary infection.

In this study, the mean index value of anti-dengue IgM in secondary infection was higher than in primary infection. These results were not consistent with the theory that stated that during primary infection IgM titers are higher than secondary infection. The exact reason was unknown, possibly because from 19 samples of primary infection there were only 6 samples that had positive results of anti-dengue IgM and 3 (50%) were which obtained at day 3 of fever. Humoral immune response in primary infection is marked by the emergence of anti-dengue IgM, starting on day 3 of fever, but generally it can be detected on day 7 of fever or more and IgM levels will continue to increase in 1-3 weeks.[2]

The mean index value anti dengue IgG in secondary infection was significantly higher than primary infection. Humoral immune response in secondary infection occurred more rapidly due to the anamnestic reaction, especially of the IgG class, as on day 2 IgG had increased significantly.[2]

Patients with secondary antibody response had 1.5 times a possibility to manifest as DHF than patients with primary antibody response (93.9% vs 63.2%, p=0.026). Similar results were obtained by Vaughn, patients with secondary antibody response were 2 times a possibility to manifest as DHF compared to

Table 3. Severity and mean index values of anti-dengue IgM and IgG ELISA based on type of infection

Characteristic	Primary Infection n=19 (31.1)	Secondary infection n=42 (68.9)	p
Severity, n(%)			
DF	7 (36.8)	3 (7.1)	
DHF I	8 (42.1)	20 (47.7)	0.026b
DHF II	3 (15.8)	16 (38,1)	
DHF III	1 (5.3)	3 (7.1)	
IgM, mean	1.478	4.028	0.000c
IgG, mean	0.746	4.864	0.000d

[b] Chi-square test, p < 0.05
[c] Mann-Whitney test, p < 0.05
[d] Independent t test, p < 0.05

primary antibody response. Secondary antibody response proved that circulating enhancing antibody was able to amplify dengue virus by facilitating the entry of the virus into susceptible cells.[8,9] Table 3 showed that not all DHF cases occurred in secondary infection, primary infection could also manifest as DHF (with or without shock). This was supported by the viral virulence theory suggesting that the virulent dengue strains caused DHF.[5]

Analysis of mean index value of anti-dengue IgM and IgG against the severity of dengue infection showed that there were significant differences between DF, DHF grade I, DHF grades II and DHF grade III and its index value tended to increase along the increasing of severity (Table 4).

Table 4. Mean index values anti-dengue IgM and IgG ELISA in various severity of dengue infection

Index value	DF n=10	DHF I n=28	DHF II n=19	DHF III n=4	p
IgM, mean	1.170	3.370	3.924	4.164	0.015a
IgG, mean	1.492	3.651	4.439	4.243	0.005a

[a] one-way ANOVA, $p < 0.05$

Koraka et al.,[10] examined the correlation between the kinetic classes of IgA, IgM a subclass of IgG specific dengue with clinical outcomes and showed that IgM, IgG1 and IgG3 were the dominant immunoglobulins in DF, DHF and DSS. Detection of anti-dengue IgM in serum of dengue patients is more related to the type of primary or secondary infection rather than the severity of dengue infection, while IgA, IgG1, IgG3 and IgG4 were associated with the incidence of DHF and DSS. It was described that the subclass IgG1 and IgG3 were most effectively binding the complement and complement activation that occurred subsequently induced clotting factors and intravascular coagulation which aggravated the degree of disease.[10]

CONCLUSION AND SUGGESTION

The mean index value of anti-dengue IgM and IgG was higher in DEN-1 serotype infection, group, secondary type infection group and DHF/DSS group. More virulent serotype as demonstrated by DEN-1 infection in which all patients with DEN-1 infection

manifested as secondary infections and DHF (with or without shock). Patients with secondary antibody response had a 1.5 times possibility to manifest as DHF than patients with primary antibody response. The mean index value of IgM and IgG anti-dengue was significantly different between DF, DHF grade I, DHF grades II and DHF grade III and its index value tended to be increased along with the increasing of DVI severity.

Examining viremia titer, genotyping, level of Ig, class and sub-class of Ig specific dengue in acute and convalescent phase is needed in order to obtain more specific and clearer humoral immune response analysis of dengue.

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