

Anti Dengue IgG/IgM Ratio For Secondary Adult Dengue Infection In Surabaya

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RESEARCH

**ANTI DENGUE IGG/IGM RATIO FOR SECONDARY ADULT DENGUE
INFECTION IN SURABAYA**

(Rasio IgG/IgM Anti Dengue untuk Infeksi Dengue Sekunder Dewasa di Surabaya)

Aryati¹, Puspa Wardhani¹, Ade Rochaeni², Jeine Stela Akualing², Usman Hadi³

ABSTRAK

Infeksi Virus Dengue (IVD) dibedakan menjadi infeksi primer dan sekunder berdasarkan respons antibodi yang dihasilkan. Infeksi sekunder perlu dibedakan dari infeksi primer karena umumnya menimbulkan manifestasi klinis yang berat. Uji hemaglutinasi inhibisi sebagai baku emas untuk menentukan infeksi primer atau sekunder dirasa tidak praktis karena membutuhkan sepasang sera dengan selang waktu yang cukup lama. Penelitian ini bertujuan mengetahui cut-off rasio IgG/IgM anti dengue untuk infeksi dengue sekunder dewasa di Surabaya. Subjek adalah pasien IVD dengan hasil NS1 dan/atau PCR dengue positif. Rasio IgG/IgM anti-dengue diperoleh dari pembagian nilai indeks IgG dan IgM metode ELISA. Nilai cut-off rasio ditentukan berdasarkan kurva ROC. Berdasarkan pola reaktivitas IgM dan IgG ELISA, 19 (31,1%) pasien dikelompokkan sebagai infeksi primer dan 42 (68,9%) infeksi sekunder. Hasil PCR didominasi DEN-3. Nilai cut-off optimal rasio IgG/IgM $\geq 0,927$ sebagai peramal infeksi sekunder memiliki kepekaan 66,7% dan kekhasan 63,2%. Dianalisis pula nilai cut-off optimal IgM dan IgG anti dengue, yaitu IgM $\geq 1,515$ dan IgG $\geq 2,034$ sebagai peramal infeksi sekunder memiliki kepekaan dan kekhasan masing-masing 85,7% dan 84,2%; 100% dan 100%. Disimpulkan bahwa rasio IgG/IgM $\geq 0,927$ tidak dapat digunakan sebagai tolok ukur tunggal peramal infeksi sekunder sedangkan cut-off IgG $\geq 2,034$ dapat dipertimbangkan sebagai peramal infeksi sekunder.

Kata kunci: Dengue, infeksi sekunder, rasio, IgG, IgM

ABSTRACT

Dengue infection is classified into primary and secondary infection based on the antibody response. Secondary infection needs to be distinguished from primary infection because it often results in severe clinical manifestations. Hemagglutination inhibition test as the gold standard for determining primary or secondary infection is not practical because it requires a pair of sera collected at sufficient time intervals. The study aim was to determine the cut-off ratio of anti-dengue IgG/IgM for secondary adult dengue infections in Surabaya. Subjects were dengue infection patients with positive results for NS1 and/or dengue PCR. Anti-dengue IgG/IgM ratio obtained by dividing the IgG to IgM index value by ELISA method. Cut off point for IgG/IgM ratio was determined by ROC curve. Based on the reactivity of IgM and IgG ELISA, 19 (31.1%) were primary infection and 42 (68.9%) were secondary infection. PCR result was dominated by DEN-3 serotype. Optimal cut off ratio of IgG/IgM ≥ 0.927 as a predictor for secondary infection showed a sensitivity of 66.7% and specificity of 63.2%. The optimal cut-off values of anti-dengue IgM and IgG as a predictor of secondary infection dengue were also analyzed. Results were IgM ≥ 1.515 and IgG ≥ 2.034 with a sensitivity and specificity of 85.7% and 84.2%; 100% and 100%, respectively. In conclusion, the ratio of IgG/IgM ≥ 0.927 could not be used as a single predictor of secondary infection while cut off IgG ≥ 2.034 could be considered as a predictor of secondary infection.

Key words: Dengue, secondary infection, ratio, IgG, IgM

INTRODUCTION

Dengue virus infection is an infectious disease caused by dengue virus (DEN), which consists of four serotypes namely DEN-1, DEN-2, DEN-3, DEN-4.

The disease remains a significant health problem in Indonesia because it is endemic and arises throughout the year.¹ Vaccine or effective antiviral agents for dengue virus infection have not been available yet.^{2,3} Dengue infection causes varying clinical manifestation

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1 from an undifferentiated fever (Dengue Fever, DF) to the more severe forms Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).⁴

Based on the antibody response, dengue infection is classified into primary and secondary infection. A primary infection (the first infection by dengue virus or another flavivirus) is characterized by slow and low titre antibody production and IgM anti-dengue is the first class of antibody produced. Anti-dengue IgG is produced 2 weeks after infection. In contrast, during secondary infection (previously infected by dengue virus another flavivirus) IgG titer rises rapidly from 2 days after the onset of fever while the anti-dengue IgM may take a long time to be detected or may be undetectable.^{1,5}

Clinical manifestation in secondary infection is more severe than in primary infection. Some literature stated that primary infection only caused a condition called febrile self limiting disease, while secondary infections could cause severe complications in the form of DHF/DSS. Based on these reasons it is very necessary to distinguish secondary infection from primary infection to determine a better prognosis and not only detecting a positive or negative result of dengue virus infection.¹

5 Hemagglutination Inhibition (HI) test as the gold standard for determining primary or secondary infection is not practical because it requires a pair of sera collected (acute phase and convalescent phase) at appropriate intervals (7–10 days).¹ In dengue-endemic areas, IgM lasting in the circulation can lead to high false-positive results. Those positive results of IgM in such individuals may be caused by IgM formed in 8 months earlier infection. Several studies were conducted to find the relationship between immune response and type of infection using ratio. Research at several different places showed the different cut-off ratio of anti-dengue IgG/IgM for secondary infection.^{6,7} The aim of this study was to determine the cut-off ratio of anti-dengue IgG/IgM for secondary adult dengue infections in Surabaya.

41 METHODS

A total of 61 selected patients were collected from the Tropical Disease Ward Dr. Soetomo Hospital. Patients having fever (2–7 days), signs and symptoms of dengue infection (grading and severity infection according to the 2011 WHO criteria) with a positive result of NS1 rapid test and/or PCR dengue were enrolled in this study conducted in March–August 2016. Patients having concomitant diseases

such as autoimmune diseases, HIV/AIDS, other immunodeficiency diseases and malignancy that were obtained from medical records and lysis, jaundice or lipemic sera were rejected. Blood samples were collected from patients, aseptically, processed for serum separation and immediately transferred to a -80°C deep freezer until further processed. Ethical clearances were obtained from the Medical Research Ethics Committees of Airlangga University Surabaya.

Dengue NS1 antigen qualitative detection was performed using SD Bioline Dengue Duo rapid test according to the manufacture's protocol. The presence of only one color line ("C") indicated a negative result and the presence of two color lines ("T" band and "C" line) indicated a positive result. No "C" line in result indicated invalid and should be re-tested.⁸

RNA virus was extracted automatically using Magna Pure LC Total Nucleic Acid Isolation (Roche) kit and the Magna Pure LC 2.0 instrument. The isolation principle worked by magnetic-beads technology. Samples were lysed through an incubation process using a special buffer containing chaotropic salt and proteinase K. Magnetic glass particles (MGPs) were added so the total viral nucleic acid in the sample were bound to the surface of MGPs. The substance not bound was discharged through multiple washing steps and purified total viral nucleic acid was eluted using a low salt-buffer.⁹

RNA virus detection and serotyping were performed by Simplexa™ Dengue kit (Focus Diagnostics) with 3M Integrated Cycler Instrument. The assay is a real-time reverse transcription Polymerase Chain Reaction (RT-PCR) that discriminate serotypes 1 and 4 in one reaction (well) and serotypes 2 and 3 in another reaction (well). Amplification and detection used bi-functional fluorescent probe-primers and reverse primers. The assay amplified 4 serotypes specific region: dengue 1 (NS5 gene), dengue 2 (NS3 gene), dengue 3 (NS5 gene) and dengue 4 (capsid gene). RNA control is used to monitor the extraction process and to detect RT-PCR inhibitor.¹⁰

Dengue RNA extraction and RT-PCR were performed at the Eijkman Molecular Biology Institute Jakarta.

15 Anti-dengue IgM and IgG were performed by Panbio Dengue Duo IgM and IgG Capture ELISA in the Laboratory of Tropical and Infectious Disease Hospital. In the Panbio Dengue Duo IgM Capture and IgG capture ELISA, IgM and IgG were determined in separate wells of the assay plate using a common assay method. The result was an index value and classified as positive, negative and equivocal according to the manufacturer's protocol.^{11,12} Reactivity of anti-dengue

IgM and IgG ELISA were also used to determine the infection status (primary or secondary infection). Samples with positive IgG antibody results obtained in acute phase was considered as a secondary infection. The ratio of anti-dengue IgG/IgM was calculated from IgG index value dividing by the IgM index value. The cut off point for IgG/IgM ratio was determined by the Receiver Operator Characteristic (ROC) curve.

RESULTS AND DISCUSSION

A total of 133 adult patients with clinical sign of dengue were initially enrolled in the study conducted from March–August 2016, therefore only of 61 samples were eligible for serological testing with positive result of NS1 and/or PCR dengue.

Both NS1 antigen and RT-PCR are useful for early diagnosis of dengue infection. NS1 antigen is essential for viral replication and can be detected in infected serum samples from the first day up to day 9 to 18 after the onset of fever.¹³ The Specificity of NS1 antigen was as high as the gold standard of dengue viral culture or PCR.¹ In the present study, the highest positivity examination NS1 was on day 5 of fever whereas PCR on day 4 of fever (Figure 1). A previous study, both NS1 and RT-PCR was detectable on day 1 of days post

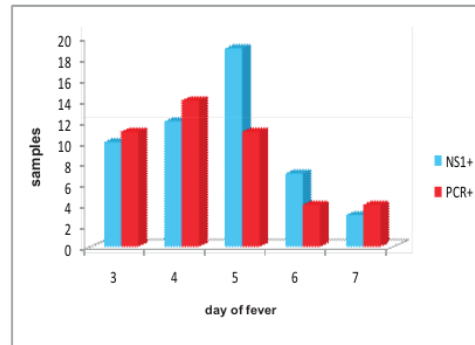


Figure 1. Positivity of NS1 and dengue PCR based on day of fever

onset of illness, while maximum positivity was seen from day 2 to 5 post onset of illness.¹³

Based on the reactivity of anti-dengue IgM and IgG ELISA, 19 (31.1%) patients were classified into primary infection and 42 (68.9%) were secondary infection. Subject characteristics, clinical, laboratory and serological data of patients with primary and secondary infection are shown in Table 1.

The most prevalent severity of dengue primary infection was DHF grade I and DF, while in secondary

Table 1. Subject characteristics

Subject characteristics	Primary infection (n=19)	Secondary infection (n=42)	P
Gender, n (%)			
Male	13 (68.4)	23 (54.8)	0.404 ^a
Female	6 (31.6)	19 (45.2)	
Age, year, mean (SD)	27.9 (12)	22(8.7)	0.064 ^b
Thrombocyte count, mean (SD)	83521.1(46100)	49207.1(33680,9)	0.000 ^b
Day of Fever, n (%)			
3	10 (52.6)	1 (2.4)	
4	4 (21.1)	11 (26.2)	
5	3 (15.8)	18 (42.8)	0.000 ^c
6	2 (10.5)	7 (16.7)	
7	0 (0)	5 (11.9)	
Clinical manifestation, n (%)			
DF	7 (36.8)	3 (7.1)	
DHF I	8 (42.1)	20 (47.7)	0.026 ^c
DHF II	3 (15.8)	16 (38.1)	
DHF III	1 (5.3)	3 (7.1)	
Serotype, n			
DEN-1	0	9	
DEN-2	4	4	
DEN-3	13	15	0.031 ^c
DEN-4	0	0	

^a fisher's exact test, $p < 0.05$

^b independent t test, $p < 0.05$

^c chi-square, $p < 0.05$

infections DHF grade I and DHF grade II. This result showed that DHF could also occur in primary infection, although DHF occurred more frequently in secondary infection. This is supported by a virus virulence theory that stated virulent dengue virus strains caused DHF while avirulent dengue virus strains caused DF.¹⁴

Dengue PCR result was dominated by DEN-3 serotype, similar to the result of Fedik¹⁵ at the Dr Soetomo Hospital.¹⁵ Different results obtained by Ginting¹⁶ with the dominant serotype was DEN-2 and Aryati¹⁷ was DEN-1.^{16,17} Dengue domination serotype in dynamic and domination changing can happen all the time. Dengue serotype domination changing as well as of the presence of multiple infections every year, played an important role in spreading of dengue infection.¹⁷

Index value of anti-dengue IgM ELISA in primary infection varied between 0.325 to 6.578 and in the secondary infection varied between 0.506 to 6.793. The index value of anti-dengue IgG ELISA in primary infection varied between 0.123 to 1.777 and in the secondary infection varied between 2.291 to 6.687. The study found that anti-dengue IgG/IgM ratio ≥ 0.927 as a predictor for secondary infection showed a sensitivity of 66.7% and specificity of 63.2%, Positive Predictive Value (PPV) 80.0%, Negative Predictive Value (NPV) 46.2%, Positive Likelihood Ratio (LR +) of 1.81 and a Negative Likelihood Ratio (LR-) 0.53. (Figure 2 and Table 2).

Some researches were done to differentiate between primary and secondary infection using the ratio of IgG/IgM resulting various cut off points. A study in Bali (2006) in children hospitalized for suspected dengue infection found an IgG/IgM ratio of ≥ 1.1 as a good predictor of secondary infection (sensitivity 87.5% and specificity 92.9%). Innis et al⁶ in Thailand using HI test as gold standard found that the best cut off points for secondary infection was ≥ 1.78 (sensitivity 92% and specificity 96%). Sai Kit Lam⁶ in Malaysia found that the best cut off point for secondary infection was ≥ 2.0 (sensitivity 95% and specificity 94%). Cucunawangsih⁷ in Banten found that the best cut off point for secondary infection was ≥ 1.14 (sensitivity 80.56% and specificity 91.67 %). These differences in cut off values of ratio can be caused due to the different geographic locations, characteristics of the subjects and seroepidemiology which may affect the antibody response generated and the severity of the disease.^{6,7} It also has been recently demonstrated that the ratios vary depending whether the patients has a serological non-classical or classical dengue infection and the ratios have been redefined taking into consideration the four subgroups of classical infection with dengue.⁴ Vaughn¹⁸ revealed that some important factors that play a role in the pathogenesis of DHF included specific virulent virus genotypes with high replication rate that can lead to an increase of immune response and disease severity; a genetic

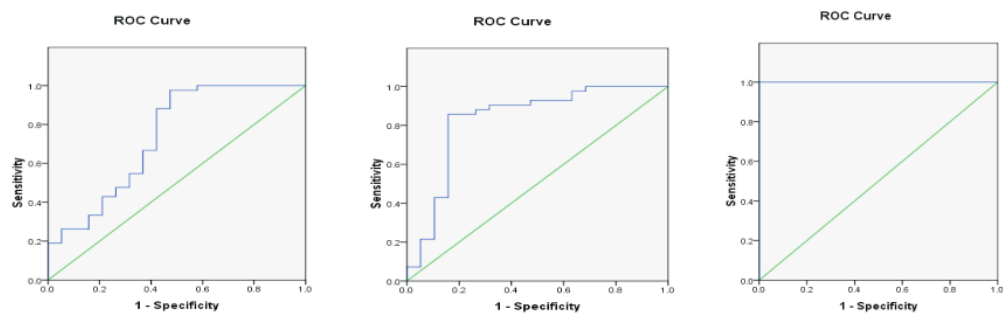


Figure 2. ROC Curve : (a) Cut-off value of anti-dengue IgG/IgM ratio (b) Cut-off value of anti-dengue IgM and (c) Cut-off value of anti-dengue IgG for secondary infection

Table 2. Cut off value of IgG/IgM ratio, IgM and IgG anti-dengue for secondary infection

Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
IgG/IgM ≥ 0.927	66.7%	63.2	80.0	46.2	1.81	0.53
IgM ≥ 1.515	85.7	84.2	92.3	72.7	5.43	0.17
IgG ≥ 2.034	100	100	100	100	NA	NA

NA = not available

predisposition to certain disease and other risk factors such as age, sex and nutrition.¹⁸

The optimal cut-off values of anti-dengue IgM and IgG as a predictor of secondary infection dengue were also analyzed. Results were IgM cut-off ≥ 1.515 and IgG cut-off ≥ 2.034 with a sensitivity and specificity of 85.7% and 84.2%; 100% and 100%, respectively (Figure 2 and Table 2). A study by Sang et al¹⁹ found that the results of anti-dengue IgG capture ELISA (Panbio Dengue Duo) showed an excellent correlation with HI test. Cut-off ratio of IgG anti-dengue ≥ 3.0 can be used to distinguish between primary infection and secondary, where the cut off values classify correctly 88% of primary infection and 98% of secondary infections using the HI test as the gold standard and this method was superior to the use of IgG/IgM ratio reported previously.¹⁹

CONCLUSION AND SUGGESTION

The cut-off ratio of anti-dengue IgG/IgM $\geq 0,927$ with a sensitivity 66.7% and specificity 63.2% could not be used as a single predictor of secondary infection while the cut-off IgG ≥ 2.034 with a sensitivity 100% dan specificity 100% could be considered as a predictor of secondary infection.

A larger sample size is required and anti-dengue IgM and IgG examination performed in the acute and convalescent phase to determine the kinetics (serologic pattern) of anti-dengue IgM and IgG associated with the type and severity degree of dengue infection.

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