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Original Article

The value of IgG to IgM ratio in predicting secondary dengue infection

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ABSTRACT

Background The determination of primary or secondary dengue infection using hemagglutination inhibition (HI) test is time-consuming. The IgG to IgM ratio which can be obtained earlier was used by several studies to differentiate secondary from primary infection, but they still reported various cut-off points.

Objective To find the diagnostic value and best cut off point of IgG to IgM ratio for predicting secondary dengue infection.

Methods This was a prospective study carried out between July 2003 and June 2004. Children with suspected dengue hemorrhagic fever (DHF) were tested for HI during acute and convalescent phase. The IgG and IgM titer were examined during the acute phase using ELISA method.

Results Sixty-two children were recruited, 48 with secondary infection and 14 with primary infection. The prevalence of secondary infection was 77%. The best cut off point of the IgG to IgM ratio to predict secondary infection was ≥1.1 with sensitivity of 87.5%, specificity 92.9%, likelihood ratio 12.3, and post test probability 97.7%.

Conclusion The IgG to IgM ratio of ≥1.1 is a good predictor for secondary infection **[Paediatr Indones 2006;46:113-117].**

Keywords: *dengue hemorrhagic fever (DHF), primary and secondary infection, hemagglutination inhibition (HI) test, ratio of IgG:IgM*

Dengue hemorrhagic fever (DHF) is one of the top ten causes of morbidity and mortality in South East and West Pacific with mortality rate of 1-30%.[1-3] In Indonesia, DHF is endemic with incidence of 35.2 per 100 000 people (the second rank after Thailand) in 1998.[4]

Based on the antibody response, dengue infection is classified into two types, the primary and secondary.[5] Primary infection is usually mild and often self-limited.[3,6] Secondary infection often results in severe clinical manifestations especially in South East and West Pacific.[7,8] Almost 100% of patients with dengue shock syndrome (DSS) have secondary infection, but less percentage is found in paatients with DHF grade I or II.[8]

At present, WHO recommended hemagglutination inhibition (HI) test as the gold standard to determine primary or secondary infection. This test is relatively easy, sensitive, reliable, and can be done with local reagents.[9] Unfortunately, it takes longer time for interpretation as it requires two samples, at the acute and convalescence periods (2-3 weeks after the first specimen collection).[10] Several studies had been performed to find the association between immune response and the type of infection using the IgG to IgM ratio at the acute phase, but they found different ratios.[9-11] This might be due to different settings and seroepidemiologics.[9,10]

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The aim of this study was to assess the diagnostic value and find the best cut off point of IgG to IgM ratio for differentiating secondary from primary dengue infection.

Methods

This prospective study was performed from July 2003 until June 2004 at the Division of Tropical Infection, Department of Child Health, Udayana University, Sanglah Hospital. Children hospitalized with suspected DHF, aged >1 year old, and had IgM titer of ≥1.0 unit/ml were included with parental consent. Patients suffering from diabetes mellitus or sickle cell anemia, or receiving immunosuppressive treatment were excluded. Consecutive sampling was done until the minimal sample of 62 was obtained.

All subjects (or their parents) were interviewed using a questionnaire, underwent physical examination, and had their clinical, demographic, and laboratory data recorded. Treatment was based on the standard procedure of treatment in Sanglah Hospital. Blood samples for routine hematology were taken each day. On follow up after discharged, routine hematology examination was also done on patients who did not have a rise in hematocrite during the acute phase. Hemoconcentration was determined by hematocrite elevation by 20% during the acute phase or between the highest level during the acute phase with that of the convalescence phase.

Dengue fever was defined as clinical manifestation of dengue infection (confirmed by HI test) without hemoconcentration, while those showing hemoconcentration was classifed as suffering from DHF. For grading of DHF, we used the WHO criteria. Grade I was defined if a child had fever, unspecific symptoms, and bleeding manifestation confined only to positive Rumple Leede test. A child with positive grade I criteria plus spontaneous bleeding was classified as suffering from DHF grade II, while those with circulation failure, characterized by soft and rapid pulse, blood pressure narrowing (≤20 mmHg), hypotension with cold clammy skin, and iritability were defined as having grade III. Grade IV was defined if there were shock, unmeassured blood pressure, and unpalpable pulse. Dengue shock syndrome (DSS) comprised DHF grade III and IV.

All subjects were examined for IgG and IgM titers at the acute phase (the 5^{th}-7^{th}day of fever) using ELISA and the HI test as the gold standard. Four ml of venous blood was taken at day 5 or 6 of fever using sterile syringe and centrifuged at 10 000 rpm for ten minutes. The serum was divided into two labeled (identity and date), 2.5 ml syringes, and directly sent in a cool-box to the laboratory for ELISA and HI examination or stored in the refrigerator. The second blood sample for the HI test was collected at convalescence phase (2-3 weeks after the first measurement).

The HI test was interpreted using the WHO criteria. Primary infection was defined as first infection with one serotype of dengue virus, characterized by the increase of antibody titer by ≥4 times, or a titer of ≥1:1280 at the convalescence phase. Secondary infection was defined as second, third, or fourth infection with different serotypes of dengue virus, characterized by antibody titer increase of ≥4 times, or a titer of ≥1:2560 at the convalescence phase.

Based on the HI test results, the subjects were grouped as having secondary or primary infection. The mean age, body weight, white blood cell count, platelet count, IgM level, IgG level, and the IgG to IgM ratio were calculated for each group. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and cut-off point for IgG to IgM ratio were counted using 2x2 tables. The best cut-off point was determined using Receiver Operator Characteristic (ROC) curve. Data were analyzed using SPSS 11.0 for Windows software.

Results

During one year of study period, 191 children were diagnosed with suspected DHF. Sixty-seven from 71 hospitalized children met the elibility criteria. From 67 children, 5 were excluded (8%), 3 patients did not come at the convalescence phase for the second HI test, 1 had insufficient blood sample, and 1 died. The rest 62 subjects showed positive HI test confirming dengue infection. Forty-eight (78%) had secondary infection and 14 (22%) with primary infection. Clinical manifestations of secondary infection were categorized as dengue fever in 17 subjects, DHF in 23, and DSS in 8, while all patients with the primary infection had dengue fever.

From **Table 1,** we can see that the mean IgM level are higher in primary infection (n=14) compared to secondary, while the mean IgG level and IgG to IgM ratio were higher in secondary infection (n=48).

TABLE 1. CHARACTERISTICS OF SUBJECTS WITH SECONDARY (n=48) AND PRIMARY INFECTION (n=14)

Demographic, clinical, and laboratory characteristics	Secondary infection n=14	Primary infection n=48
Sex, n(%)		
Male	27 (56)	6 (43)
Female	21 (44)	8 (57)
Age (month), mean (SD)	70.8 (29.57)	61.4 (38.81)
Body weight (kg), mean (SD)	20.8 (7.64)	19.5 (8.89)
Weight for age, %, mean (SD)*	99.6 (15.58)	100.4 (18.88)
IgM (µ/ml), mean (SD)	41.2 (26.11)	66.7 (39.09)
IgG (µ/ml), mean (SD)	102.4 (62.34)	15.4 (14.24)
IgG to IgM ratio, mean (SD)	4.8 (6.19)	0.4 (0.31)
WBC (K/µl), mean (SD)	4.5 (4.41)	3.9 (1.01)
Platelet (K/µl),mean (SD)	56.2 (22.35)	65.4 (19.10)
Clinical manifestations, n(%)		
Dengue Fever	17 (36)	14 (100.0)
DHF	23 (48)	0 (0.0)
DSS	8 (17)	0 (0.0)

Note: *Standard of body weight based on Ministry of Health, Republic of Indonesia, 1988

We found that IgG to IgM ratio of ≥1.1 confirmed secondary infection with sensitivity of 87.5%, specificity 92.9%, positive predictive value (PPV) 97.7%, negative predictive value (NPV) 68.4%, accuracy level 88.7%, and likelihood ratio of (LR) 12.3 (**Table 2**). The pre-test probability was the same with natural prevalence rate of suspected DHF (77.4%). For determining sensitivity and specificity of each cut off point (**Table 2**), we used ROC.

Discussion

From 62 subjects enrolled in this study, 48 (77%) were confirmed to have secondary infection. Among them, 17 (36%) had dengue fever, 23 (48%) DHF, and 8 (17%). All fourteen subjects with primary infections have dengue fever. This result was different from that of Halstead study[5] which reported that not all DHF patients have secondary infection. A study in Malaysia also found that 8.6% of DHF patients had primary infection.[8] Those results supported the virulence theory stating that a first or primary infection could result in DHF. The DEN-2 viral serotype has high virulence that can cause severe symptoms.[12] The occurrence of DSS in this study is similar with previous studies, in which all patients had secondary infections.[8]

Several studies asessing the diagnostic value of IgG to IgM ratio for predicting primary or secondary infections revealed various cut-off points. In our study, the best cut off point was ≥1.1 (sensitivity 87.5%, specificity 92.9%, positive predictive value 97.7%, negative predictive value 68,8%, accuracy 88.7%, and likelihood ratio 12.3). A study in Thailand[10] using HI test as the gold standard found that the prevalence of secondary infection was 82% and the best cut off point for the IgG to IgM ratio was ≥1.78, with sensitivity of 92% and specificity 96%. Another study in Malaysia[9] reported that the prevalence of secondary infection was 83.3% and the best cut off point was ≥2.0 (sensitivity 95%, specificity 94%). Similar results between those two studies might be due to close geographical location causing similar seroepidemiology besides the possibility of cross-reactivity with other flaviviruses. A study in China[13] found that 10% of dengue patients showed IgG cross-reactivity with other flaviviruses.

TABLE 2. DIAGNOSTIC VALUE OF VARIOUS CUT-OFF POINT OF THE IGG TO IGM RATIO IN DIAGNOSING SECONDARY DENGUE INFECTION

IgG to IgM ratio	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR
≥0.1	100.0	0.0	77.4	0.0	77.4	1.0
≥0.2	95.8	21.4	80.7	60.0	79.0	1.2
≥0.3	91.7	64.3	89.8	69.2	85.5	2.6
≥1.1*	87.5	92.9	97.7	68.8	88.7	12.3
≥1.8	79.2	100.0	100.0	58.3	67.0	∞
≥2.5	56.3	100.0	100.0	40.0	60.1	∞

* Best cut-off point

Many studies tried to explain the association between immune response and the severity of clinical manifestations. According to the antibody-dependent enhancement (ADE) theory, dengue cross-reactive antibody combining with T-cell is the center of dengue infection pathogenesis. This hypothesis stated that cross-reactive non-neutralized antibody from previous infection facilitated dengue virus to enter the Fc receptor in monocyte or macrophage as the antigen presenting cells (APCs). The increased number of infected APCs activates the cross-reactive memory in the T-cell precursor which is already in a large amount because of previous heterologous dengue infection. This will cause massive release of chemical mediators, which can cause damage of the plasma membrane and plasma leakage resulting in a more severe disease. This mechanism will also cause the plasma cell to produce large amounts of IgG, a heterotype antibody that is ineffective in eliminating infection.[14,15]

In our study, 17 of 48 subjects with secondary infection showed only dengue fever symptoms. This might be explain by the the IgM theory stating that specific IgM at the first infection can protect and eliminate the virus. If the amount of IgM is sufficient, the symptoms is milder, unfortunately how much is considered sufficient still remain unknown.

There are several limitations in our study, such as the small number of subjects, the presence of external variables which can interfere with the immune response such as age, sex, and nutritional status, and the possibility of cross-reactivity with other flavi viruses.

In conclusion, the IgG to IgM ratio of ≥1.1 during the acute phase is a good predictor of secondary dengue infection.

References

1. Suroso, Ali Imran Umar. Epidemiologi dan penanggulangan penyakit demam berdarah dengue (DBD) di Indonesia saat ini. In: Sri Rezeki, Hindra Irawan Satari, editors. Demam berdarah dengue. Naskah lengkap pelatihan bagi pelatih, dokter spesialis anak, dan dokter spesialis penyakit dalam, dalam tatalaksana DBD. Jakarta: BP FKUI, 2002. p. 14-31.
2. Chairulfalah A, Setiabudi D, Ridad A, Colebunders R. Clinical of dengue haemorrhagic fever in children in Bandung, Indonesia. Ann Soc Belge Med Trop 1995;75:291-5.
3. Darmowandono W. Correlation between humoral immune response and the severity of dengue hemorrhagic fever. Proceeding of the international seminar on dengue fever/dengue hemorrhagic fever in South East Asia. Surabaya: Tropical center disease, 1999. p. 68-75.
4. Suroso T. Pencegahan dan penanggulangan demam berdarah dengue (DBD) di masyarakat. Presented at 11th National Child Health Congress, Jakarta; 1999.
5. Sumarmo. The epidemiology control and prevention of dengue hemorrhagic fever (DHF) in Indonesia. Cermin dunia kedokteran 1994;92:5-10.
6. Pinheiro FP, Chuid R, WHO. Emergence of dengue hemorrhagic fever in the American. Infect Med 1998;15:244-51.
7. Usawattanakul W, Nimmantya S, Sarabenjawarong K, Tharavanij S. Endotoxin and dengue hemorrhagic fever. Southeast Asian J Trop Med 1996;17:8-12.
8. Sutaryo. Perkembangan patogenesis demam berdarah dengue. Dalam: Sri Rezeki, Hindra Irawan Satari, editors. Demam berdarah dengue. Naskah lengkap pelatihan bagi pelatih, dokter spesialis anak, dan dokter spesialis penyakit dalam, dalam tatalaksana DBD. Jakarta: BP FKUI, 2002. p. 32-43.
9. Behrman RE, Kliegman RM, Nelson WE, Vaughan VC. The nervous system. In: Behrman RE, Kliegman RM, editors. Nelson Textbook of Pediatrics. 15th ed. Philadelphia: W.B. Saunders Company, 1997. p. 11-23.
10. Innis BL, Nisalak S, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, *et al*. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 1989;40:418-27.
11. Martinez E, Guzman MG, Valdes M, Soler M, Kouri G. Dengue and dengue hemorrhagic fever in infants with a primary infection. Rev Cubana Med Trop 1993;45:97-101.
12. Sutaryo. Dengue. 1st ed. Yogyakarta: Medika Fakultas Kedokteran Unversitas Gajah Mada, 2004. p. 1-241.
13. Pei YS, Li KC, Shu FC, Yi YY, Ling Chow, Li JC, *et al*. Comparison of capture imunoglobulin M (IgM) and IgG enzym-linked immunoabsorbent assay (ELISA) and nostructural protein NS1 serotype-specific IgG ELISA for diffrentiation of primary and secondary dengue virus infections. J Clin Immunol 2003;10:622-30.

14. Vaughan DW, Green S, Kalanarooj S, Innis BL, Nimmannitya, Suntayakorn, *et al*. Dengue in the early febrile phase: Viremia and antibody responses. J Infect Dis 1997;176:322-36.

15. Koroka P, Suharti C, Setiati E, Mairuhu ATA, Van Gorp E, Hack CE, *et al*. Kinetics of dengue-specific serum immunoglobulin classes and subclasses correlate with clinical outcome infection. J Clin Microbiol 2001;39:4332-8.