ORIGINAL ARTICLE

Immunoglobulin M and G in Virologically Confirmed Dengue Hemorrhagic Fever

by

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Abstract

Starting from September 1987, a one year prospective study on IgM and IgG in dengue hemorrhagic fever, was carried out at the Department of Pediatrics, Sumber Waras Hospital West Jakarta. This report describes the preliminary finding of the study from September 1987 through June 1988.

Virus isolation and serologic analysis (HI, IgG and IgM capture ELISA) for DHF were done by NAMRU 2 in Jakarta. The subjects were 151 virologically confirmed DHF patients consisting of 82 boys and 69 girls of 6 months - 15 years old.

Serum samples were collected: (I) on the day of admission; (II) on the day of discharge and (III) 2 weeks after the first samples. Serum samples I, II and III were collected from respectively 151, 131 and 64 subjects on respectively 3.5 ± 1.7; 8.8 ± 2.7 and 16.8 ± 2.1 days of illness. Positive IgM titer from acute sera was observed in 20% of subjects. A positive correlation between HI - IgM and HI - IgG was also observed.

The percentage of positive IgM titers rose with the increase of HI titer, the percentage of positive IgG titer was lower than that of IgM but a sudden increase exceeding that of IgM was observed at the HI titer of 320 and more.

This study revealed that HI titer of 640 and 1280 were indicators for the primary and secondary dengue infection respectively and IgM capture ELISA can be used as a reliable predictor for DHF even more in fatal cases where only single serum is available.

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Introduction

Dengue hemorrhagic fever (DHF) in Indonesia was first recognized in Jakarta [1] and Surabaya [2] in 1968 and the number of reported cases since then has increased sharply. At present the disease is endemic with cases occurring throughout the year and has become one of the major health problems in Indonesia.

Clinical manifestations of dengue infections are not generally so distinctive as to permit diagnosis by clinical criteria alone [3] and hence reliable diagnosis of dengue requires the application of a rapid, sensitive, specific and economic diagnostic laboratory test to confirm dengue infections. Currently available serologic test for the confirmation of dengue infections is the hemagglutination inhibition (HI) test. The test is of diagnostic importance if a seroconversion in titers of fourfold or greater rise between the acute and convalescence sera can be demonstrated [3].

In our clinical experience it is very difficult to collect well spaced paired sera especially after hospital discharge. That was the reason why in our series well spaced sera could only be obtained from less than 50% of DHF patients.

Anti dengue IgM is produced transiently during both primary and secondary dengue infections and detection of it in any single serum indicated an acute or recent infections.

The present study was designed to elucidate the nature of IgM and IgG in virologically confirmed DHF patients.

Materials and methods

The subjects consisted of virologically confirmed DHF patients admitted to the Department of Pediatrics, Sumber Waras Hospital-Tarumanegara University in West Jakarta during the period of September 1987 - June 1988.

Virus isolations and serologic analysis for hemagglutination inhibition (HI) test, IgM and IgG capture ELISA were done by NAMRU 2 Jakarta detachment.

Blood samples were taken from each patients admitted with the clinical diagnosis of DHF based on the criteria outlined by WHO (1986): (I) on the day of admission, (II) on the day of discharge and (III) 2 weeks after the first.

Virus isolations were attempted from the acute sera and were inocculated in Toxorhynchite splendens adults and TRA - 284 (T. amboinensis) cells.

All sera were tested for dengue antibody by the HI test according to Clarke and Cassals (1958) [1], IgG and IgM capture ELISA. HI interpretation was based on the WHO criteria (1986) [3] and IgM and IgG was considered positive if the titer was 0.100 od (optical density) or more.

Statistical analysis was done using Chi-squared test and t-test.

Results

Of 1021 patients with clinical diagnosis of DHF admitted from September 1987 through June 1988 virus isolations were obtained from 151 (14.7%) subjects although culturing has then not been completed yet. The subjects consisted of 82 boys and 69 girls of 6 months to 15 years old. It was evident that statistically no significant difference could be observed in the percentage of positive IgM and IgG by age.

Serum samples I, II and III were collected from respectively 151 (100%), 131 (86.7%) and 64 (42.4%) subjects, on respectively 3.5 ± 1.7; 8.8 ± 2.7 and 16.8 ± 2.1 days of illness. The comparison of the percentage of positive HI and IgM titers is presented in Figure 1. It was evident that positive IgM from acute sera (I) was ob-served in 20% of subjects (Table 1).

The percentage of positive HI and IgM titer from paired sera I - II on admission and discharge was respectively 64.7% and 81.7% while that from well-spaced sera (I - III) was observed in respectively 95.3% and 87.5% (Figure 1).

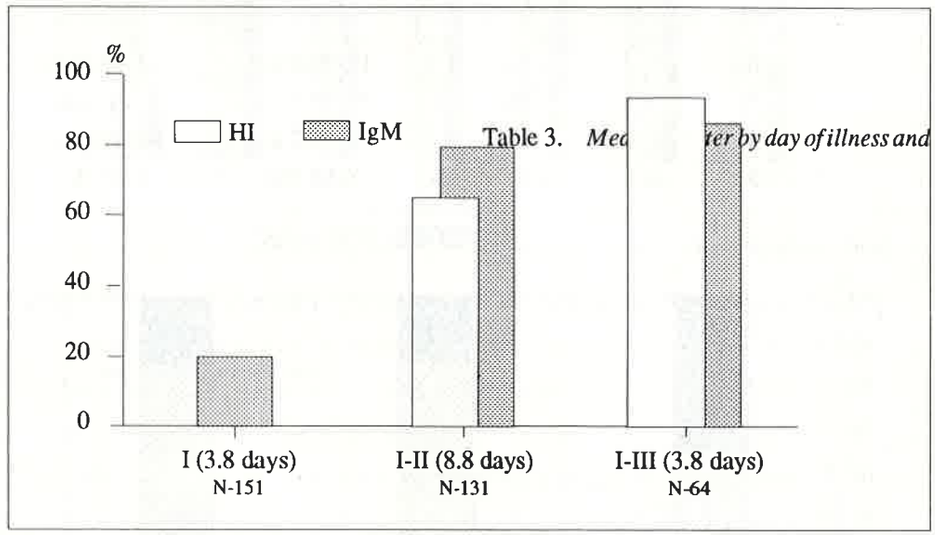

Table 3. *Mea ter by day of illness and*

Figure 1. *Percentage of positive IgM and HI among virologically confirmed DHF Cases*

HI test and IgM capture ELISA could confirm 62.9% and 77.5% subjects respectively and the combination of both (HI and IgM) could confirm 86.8% subjects (Figure 2).

The percentage of positive IgM and IgG is presented in figure 3. In the HI titer of less than 1 : 320 the percentage of positive IgM was higher than that of IgG whereas in the HI titer of 320 or higher the percentage of IgG was higher than that of IgM.

Positive correlation was observed between HI - IgM and HI - IgG. The equation for the correlation of HI-IgM and HI-IgG was respectively log Y = 1.7056 + 0.1271 log X and log Y = 1.2289 + 0.3665 log X (Figure 4 and Table 2).

Mean IgM and IgG titer by day of illness and HI titer is presented in figure 5. It appears that mean IgM titer was not different in any

HI titer. The mean IgG titer of HI 1280 was above the mean IgM titer and the difference with that of HI ≥ 2560 was statistically not significant, while that of HI ≤ 640 lay below the IgM and the difference with that of HI ≥ 1280 was statistically significant (Table 3).

The mean IgM titer of HI ≤ 640 and HI ≥ 1280 is presented in Table 4. The differences were statistically not significant.

Table 1. *Positive IgM and IgG in virologically confirmed dengue hemorrhagic fever (RSSW, 1987 - 1988)*

Age (year)	Number	IgM	IgG
< 4	21	17 (80.9%)	14 (66.7%)
5 - 9	75	58 (77.3%)	51 (68.0%)
10 - 14	47	37 (78.7%)	34 (72.3%)
≥ 15	8	5 (62.5%)	4 (50 %)

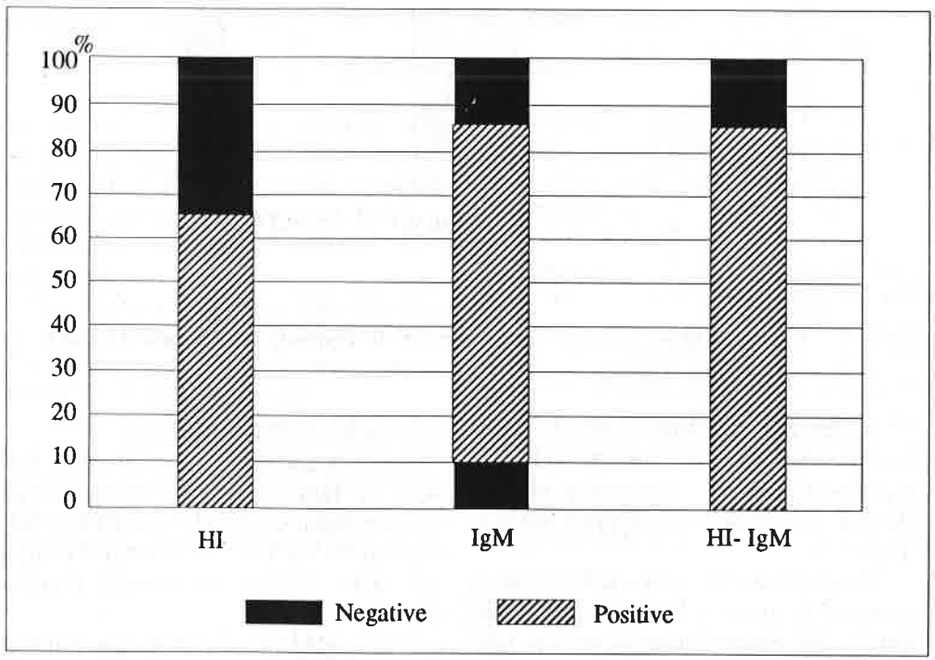

Figure 2. *Percentage of positive HI, IgM and HI-Igm in virologically confirmed DHF*

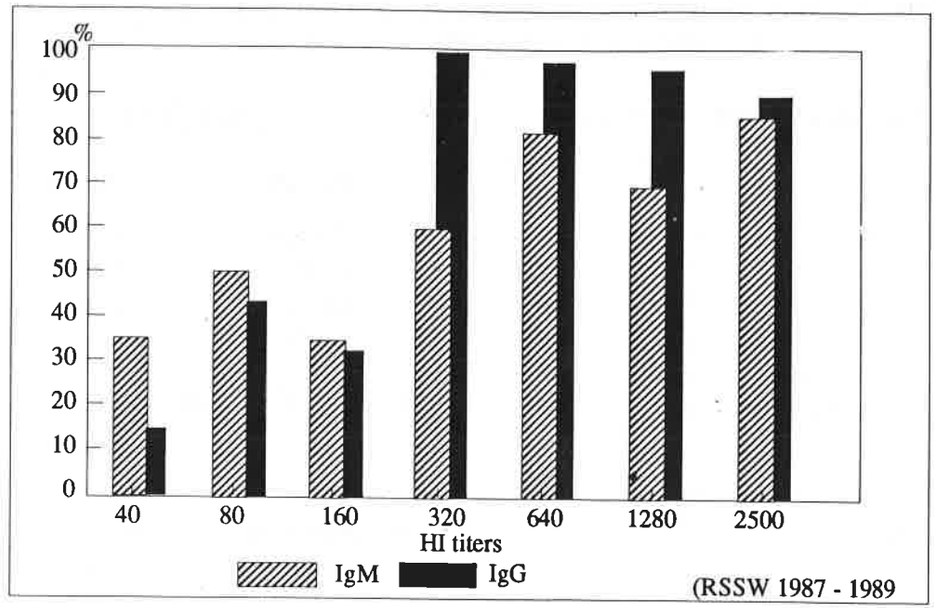

Figure 3. *Percentage of positive IgM and IgG by HI in virologically confirmed DHF*

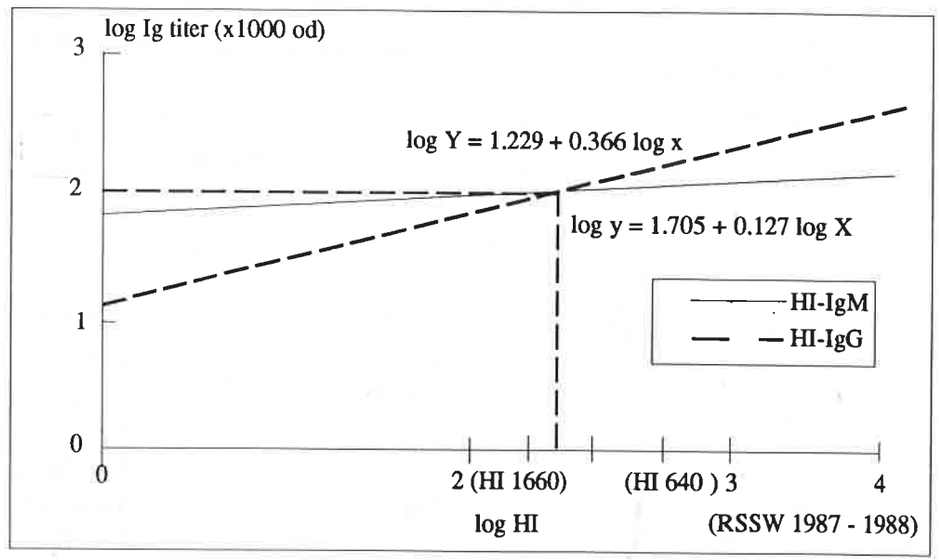

Figure 4. *HI - IgM and HI - IgG correlation among virologically confirmed DHF*

Table 2. *The correlation between HI-IgM and HI-IgG in virologically confirmed dengue hemorrhagic fever (RSSW, 1987 - 1988)*

Correlation equation Y = a + x —> log Y = log a + b log x		
	HI - IgM	HI - IgG
X = HI	Y = IgM	Y = IgG
log a	1.7056	1.2289
b	0.2171	0.3665
Coefficient of correlation (r)	0.29 —>	0.77 —>
t test	5.56 Null Hypothesis rejected	21.94 Null hypothesis rejected
N = 335	df = 332	t 0.995 = 2.58

Table 3. *Mean IgG titer by day of illness and HI titer in virologically confirmed DHF (RSSW, 1987 - 1988)*

Days of Illness		I gG Titer		
		HI ≤ 640	<—	HI ≥ 1280
1 - 4	Mean	0.050	0.026	t = 2.32
	SD	0.061	0.118	df = 108
	N	66	44	t < 2.62
5 - 8	Mean	0.091	0.281	t = 13.827
	SD	0.078	0.151	df = 94
	N	59	37	t > 2.62
≥ 9	Mean	0.159	0.326	t = 23.267
	SD	0.116	0.122	df = 119
	N	57	64	t > 2.62

Table 4. *Mean IgM titer by day of illness and HI titer in virologically confirmed DHF (RSSW, 1987 - 1988)*

Days of Illness		I gM Titer		
		HI ≤ 640	HI ≥ 1280	
1 - 4	Mean	0.050	0.059	t = o.543
	SD	0.046	0.048	df = 107
	N	63	46	- 2.58,t,2.58
5 - 8	Mean	0.157	0.172	t = 1.784
	SD	0.107	0.115	df = 89
	N	58	33	- 2.62,t,2.62
≥ 9	Mean	0.223	0.205	t = 2.439
	SD	0.107	0.087	df = 118
	N	57	63	- 2.62,t,2.62

Discussion

The standard serologic test for the confirmation of DHF is HI test [3] but a well spaced paired sera is needed for the conclusive interpretations. IgM is an acute phase reaction of host against primary as well as secondary infections [4,5,6] and the detection of it could confirm the clinical diagnosis.

In the present study a positive correlation was observed between HI and IgM but the coefficient correlation of (r) was only 0.29. On the other hand a good positive correlation (r = 0.77) was observed between HI an IgG. These findings can be explained by the observations that the higher the HI titer the higher was the IgG curves beyond the first week of illness (Figure 5) and the significant difference in the mean IgG titer by day of illness and HI titer (Table 3) while for IgM the difference was statistically not significant (Table 4).

The rise in IgM titer was observed beyond the first 6 days of illness and this finding could explain the observation that viremia in DHF occur during the first 4 - 5 days of illness [7].

The rise in IgM titer in primary and secondary response were similar (Table 4 and Figure 5) and the possibility for a positive IgM titer in DHF would be greater if the sera were collected at the beginning of the second week of illness.

In our study sera collected on discharge (mean 8.8 days of illness) could confirm 82 % subjects. It is obvious that the percentage of positive IgM titer would be less if the sera were collected during the first 4 days of illness.

A field sero-epidemiologic survey done in healthy school children in Jakarta in 1986 [8] revealed that the mean + 2 SD HI titer was less than 640 and they assumed that a HI

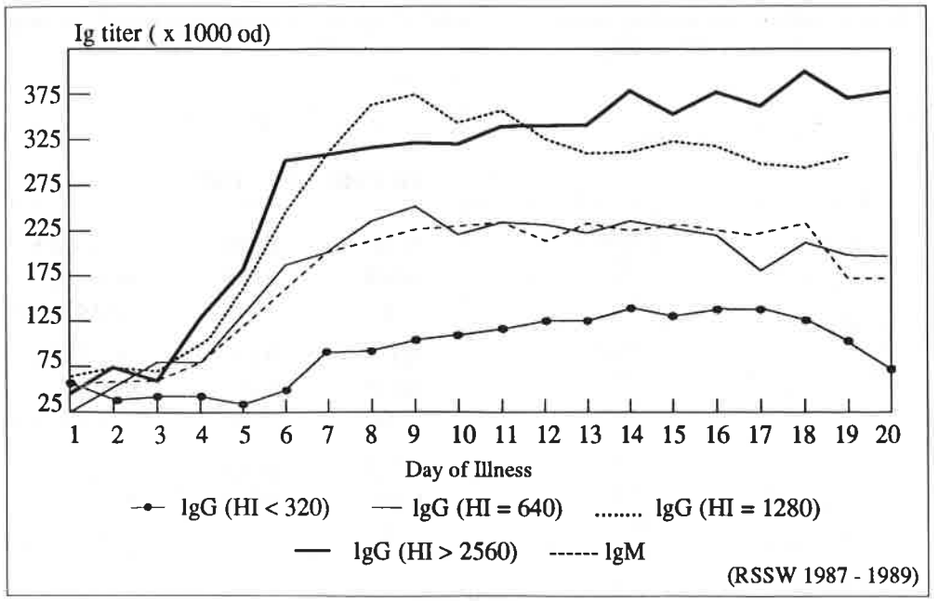

Figure 5. *Mean IgM and IgG titer by day of illness in virologically confirmed DHF*

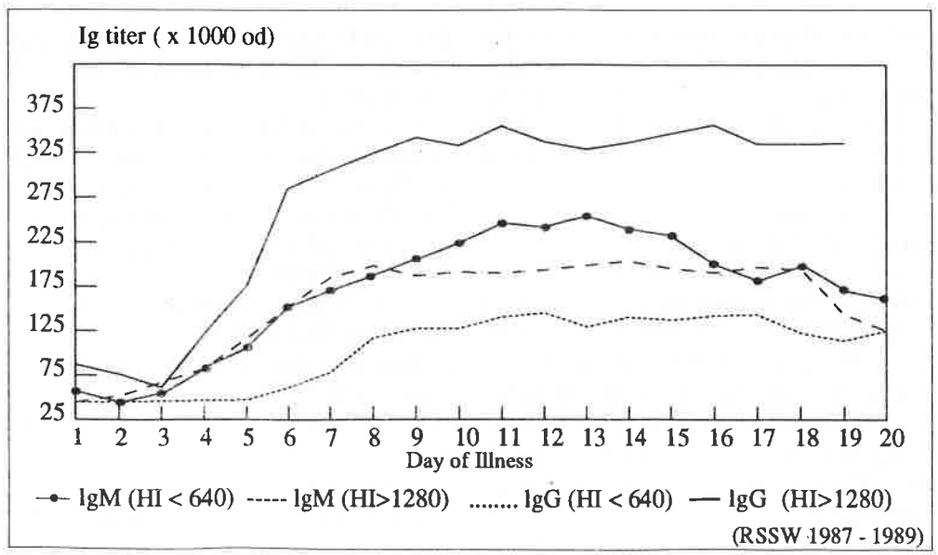

Figure 6. *Mean IgM and IgG titer by day of illness among virologically confirmed DHF*

titer of 640 confirm the diagnosis of DHF in a child with positive clinical signs and symptoms.

The IgG curve of HI titer of 1280 lay above that of IgM and IgG of HI titer of 640 (Figure5) and this observation therefore support the hypothesis that HI titer of 640 is adequate for the serologic confirmation of dengue infections.

This observations could be seen in Figure 6 and by taking this titer (640) as parameter, the IgM and IgG curve for HI of 640 and less and 1280 and over is presented.

This Figure and Table 4 show that no significant difference was observed in the IgM curve of HI titer of ≤ 640 and ≥ 1280. The IgG curve of HI titer of ≤ 640 rose later and but it was still lower than that of IgM and IgG curve of HI titer of ≥ 1280. An early, rapid and higher rise in IgG titer of patients with HI titer of ≥ 1280 is due to the anamnestic reaction occurring in secondary infections and the curve exceeding that of IgM.

In primary infection the rise in IgM titer as an acute phase reaction against infection precedes and exceeds that of the IgG titer [4,5]. Based on the data above we assume that a HI titer of 640 and less corresponds with primary dengue infection and a HI titer of 1280 and more with secondary dengue infections.

This study revealed that IgM capture ELISA could confirm 20% single acute sera and 82% of sera collected on discharge, while HI test requires a well spaced paired sera. The percentage of positive IgM and HI titer in well spaced paired is equal but in our experience the well spaced sera can only be collected from less than 50% patients.

Lam et al. (1987) reported that IgM capture ELISA is more sensitive than HI test [9] and this present study revealed that IgM capture ELISA was as sensitive as HI test and had the advantages of being able to confirm the clinical diagnosis even if only single acute serum was available.

Conclusion

This study revealed that the criteria for the primary and secondary respone of DHF in Jakarta are lower than that outlined by WHO (1986) and HI titer of 640 and 1280 are indicators for the primary and secondary dengue infections respectively.

IgM capture ELISA is as sensitive as HI test and hence IgM capture ELISA could be used as a reliable predictor for DHF even in fatal cases when only single sera are available.

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