DAILY OBSERVATION OF ANTIBODY LEVELS AMONG DENGUE PATIENTS DETECTED BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Abstract: Serial serum specimens from forty eight dengue patients admitted to the hospital on day 2, day 3, or day 4 post onset were examined sequencially by enzyme-linked immunosorbent assay for laboratory diagnosis according to the criteria set by Innis et al.(3) Cumulative ELISA positive rates among forty secondary dengue infection patients were 95 % and 100 % at day 6 and day 7 post onset, respectively while the ELISA positive rates at day 3, day 4, and day 5 were 17.5 %, 37.5 %, and 75 %. Cumulative ELISA positive rates among eight primary dengue patients were 87.5 % and 100 % at day 6 and day 7 post onset, while the rates at day 4, and day 5 were 12.5 % and 50 %. Thus, in order to achieve better diagnostic efficiency according to the criteria, convalescent sera should be taken after the 6th day from the onset of the disease. Four out of 74 secondary infection patients, corresponding to 5 % of the patients, showed poor response of dengue-specific IgM antibody (less than 10 units) even when discharged, indicating that both IgG and IgM examinations are necessary in secondary dengue infection.

INTRODUCTION

Dengue/dengue hemorrhagic fever is epidemic in most of the countries of southeast Asia and is an important problem in public health.(2) Because most of the clinical symptoms of dengue infection are not specific to dengue, serological diagnosis is important for confirmation of the etiological agents.

Enzyme-linked immunosorbent assay (ELISA) for dengue was developed by several institutions, and measurement of anti-dengue IgM antibody has been proved to be useful as a rapid and sensitive diagnostic method for acute dengue infection.(1, 3, 4)

In Thailand, an ELISA system produced by Innis et al.(3) has been widely introduced and applied by HIN of Thailand for laboratory diagnosis. Although the system is very useful for rapid serological examination than and easier to use than hemaglutination inhibition test (HI),

some of the test specimens did not show elevation of anti-dengue IgM and IgG antibodies greater than the cut -off level. We supposed that in such cases the convalescent sera were collected too early to demonstrate antibody elevation. For this paper, we collected serum and plasma specimens from dengue patients every day and observed the antibody levels in order to determine what day after the onset of the disease was most suitable for collecting the test sera for laboratory diagnosis by the ELISA.

MATERIALS AND METHODS

Serum specimen.

Sera were collected from patients at admission to and discharge from the Department of Pediatrics in Nakhornphanom Provincial Hospital, Thailand, in July 1992. Plasma fractions in hematocrit tubes were col-

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Table 1 ELISA positives according to the criteria by Innis *et al.*

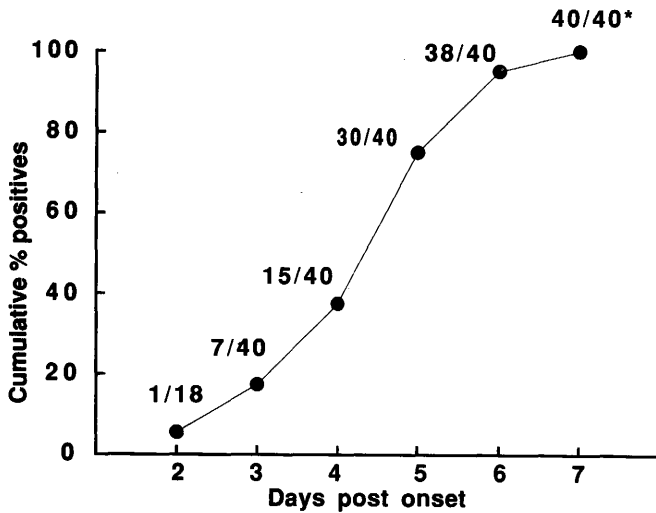|	Day admitted post onset of the disease					
	2	3	4	5	6	Total
No. of Cases	18	22	31	10	1	82
No. of Positives	1	6	11	5	1	24
% Positives	5.5	27	35	50	100	29

Figure 1 Cumulative ELISA positive rates among forty secondary dengue patients who were admitted on day 2 and day 3 post onset.
*: number of positive patient(s)/Total number of patents.
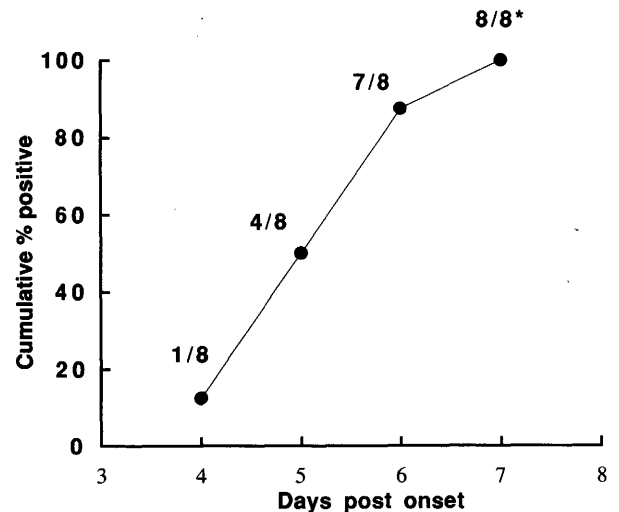

Figure 2 Cumulative ELISA positive rates among eight primary dengue patients.
*: number of positive patient(s)/Total number of patents.

lected every day after routine hematocrit examination. All serum and plasma specimens were kept at $-20°C$ until use.

Enzyme linked immuno sorbent assay (ELISA).

ELISA was performed as described previously. (3) Titered standard positive sera of IgG and IgM were kindly provided by Dr. Innis, AFRIMs in Bangkok.

Criteria of ELISA.
  1) Criteria 1.
    Daily continuous increase of anti-dengue IgG or IgM antibody.
  2) Criteria 2. (Advocated by Innis *et al.*)
    The cut off titer for IgG was changed from 40 units to 100 units by Dr. Innis recently. (personal communication)
    Negative: Titer of IgM < 40 units, and IgG < 100 units
    Positive: Others
      IgM ≧ 40 units and IgG < 100 units: Positive
                        (Primary infection)

      IgM < 40 units and IgG ≧ 100 units: Positive
                        (Secondary infection)
      IgM ≧ 40 units and IgG ≧ 100 units: Positive
        IgM/IgG < 1.8   (Secondary infection)
        IgM/IgG ≧ 1.8 (Primary infection)

RESULTS

1. ELISA positive rate of serial specimens.

We followed forty dengue patients diagnosed by criteria 1, who had been hospitalized with secondary dengue on day 2 or day 3 post onset of the disease. Figure 1 shows the cumulative ELISA positive rates from day 2 to day 7, according to criteria 2. ELISA positive rates were 17.5 %, 37.5 %, 75 %, 95 %, and 100 % at day 3, 4, 5, 6, and 7 post onset, respectively.

Figure 2 shows the cumulative ELISA positive rates among 8 patients diagnosed by criteria 1, who had been admitted to the hospital with primary dengue. The ELISA positive rates according to criteria 2 were 12.5 %, 50 %, 87.5 % and 100 % at day 4, 5, 6, and 7 post

Table 2 Anti dengue IgG and IgM units among low IgM responder

Day	Pt1		Pt2		Pt3		Pt4	
(p.o.)	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
3	5*	14	0	19	0	3	—	—
4	0	17	0	23	0	4	2	3
5	0	126	0	105	6	28	4	7
6	1	146	1	121	6	67	5	32
7	8	161	—	—	6	108	6	72
8	—	—	—	—			9	113

p.o.: post onset, Pt1-4: Patients number 1-4
*: units, —: not tested

onset, respectively. All of these patients were diagnosed by IgM-ELISA rather than IgG ELISA, because of the poor and late responce of anti-dengue IgG in dengue primary infections.

These results showed that in order to get more than 90 % effectiveness using the ELISA criteria 2, serum should be taken from the patients after the 6th day from onset.

2. ELISA positive rate at admission.

A total of 82 dengue patients, 74 secondary and 8 primary dengue, were admitted to the hospital during our project. Table 1 shows the ELISA positive cases and rates by criteria 2 on the day of admission. Those who were admitted to the hospital on day 2, 3, 4, and 5 from onset showed 5.5 %, 27 %, 35 % and 50 % ELISA positive rate on the day of admission respectively. These ELISA positive rates were quite compatible with the data in Figure 1. The over-all ELISA positive rate by criteria 2 at admission was 29 %.

3. Low IgM responder levels observed among secondary infection group.

It has been emphasized by several investigators that IgM ELISA is a very useful rapid diagnostic tool for primary and secondary dengue infection. (1, 3, 4)

However, among the 74 secondary dengue cases we observed in this research, four individuals showed very poor IgM responses. All of them showed no elevation of anti-dengue IgM antibody, less than 10 units, even at their discharge. Their IgG and IgM responses are shown in Table 2. These patients numbered about 5 % of the total.

DISCUSSION

It was demonstrated that at least 6 days were required from the onset of the disease for anti-dengue

antibody level to rise a sufficient level to be diagnosed by the criteria of Innis et al. Therefore, when their ELISA system and criteria are used, serum specimens should be collected after the 6th day from onset in order to achieve better than 90 % diagnostic effectiveness.

The ELISA positive rate at admission that we observed was only 29 %, though Innis et al. reported that the IgM-ELISA sensitivity at admission was 78 % in their study. This discrepancy could be explained by the fact that many of the patients in our research area were hospitalized at an early phase of the infection, as was indicated in Table 1.

Examination on sequential serum specimens as in this paper, could provide a confident conclusion on serodiagnosis when daily increase of anti-dengue antibody is observed. Practically speaking, however, for routine laboratory diagnosis examination of a single serum specimen is important. Therefore, it is useful and important to determine criteria for ELISA serological analysis such as Innis et al. proposed.

Many investigators have emphasized the usefulness of IgM ELISA for dengue sero-examination even among the secondary infection group. (1, 3, 4) However, we found that 5 % of secondary infection group showed almost no dengue specific IgM antibody responce during their time in the hospital, though IgG antibody was markedly elevated. On the other hand, eight out of eight primary dengue patients were diagnosed by IgM ELISA alone, because of the low responce of anti-dengue IgG. These results suggest that both IgG and IgM ELISA are always necessary for the dengue Ig capture ELISA diagosis.

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