Serodiagnosis by the ELISA on Encephalitis in Chiang Mai, Thailand, 1983

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Abstract: One hundred forty two serum specimens from 114 encephalitis cases in Chiang Mai, Thailand collected during epidemic period in 1983, were measured for their antibody titers against Japanese encephalitis (JE) and dengue virus type 2 antigens by the ELISA. Seventeen cases were diagnosed as JE, while 12 were considered as possible dengue encephalopathy by IgM-ELISA. Age distribution of JE cases showed its peak in the group of 10-14 years old, while possible dengue encephalopathy distributed from 10 up to 49 years old. Indirect IgG-ELISA using JE antigen detected 26 cases of secondary flavivirus infections, with highest incidence in 10-14 years old.

Key words: encephalitis, ELISA, Chiang Mai, Thailand

INTRODUCTION

Serological tests on viral infection are very important for serodiagnosis to confirm clinical diagnosis and for epidemiological survey to know the spread of the virus infections in human environment. In the case of arbovirus infections, such as Japanese encephalitis (JE) or dengue, hemagglutination-inhibition (HI) test has most commonly been used (Clarke and Casals, 1958). Since JE and dengue viruses belong to the same genus of flavivirus in family Togaviridae, sharing common antigens, the HI test could not often give clear-cut answer whether the patient was infected by JE or dengue virus, especially

when the specimens were taken from areas where both JE and dengue virus infections coexist. For example in Chiang Mai Area, Northern Thailand, Both JE and dengue hemorrhagic fever (DHF) have been reported to be prevalent (Yamada *et al.*, 1971; Grossman *et al.*, 1973; Igarashi *et al.*, 1983a). Since IgM-antibodies were reported to be more type-specific and appear earlier than IgG-antibodies in flavivirus infections (Westaway, 1968a, b), assay on IgM-class of antibodies was used in the serodiagnosis on JE in Thailand either by fractionation of the sera by sucrose gradient sedimentation followed by the HI test (Edelman and Pariyanonda, 1973), or more recently by using IgM-capture immunoassays on patients' sera or cerebrospinal fluid (Burke and Nisalak, 1982; Burke *et al.*, 1982). Bundo and her colleagues have been trying to apply the ELISA in the serodiagnosis and seroepidemiological studies on JE (Bundo *et al.*, 1981, 1982a, b, 1983a, b) and also on DHF cases (Bundo and Igarashi, 1983), developing their own system to differentiate JE and dengue by their IgM-capture mathod (Bundo and Igarashi, 1984). In the IgG-ELISA, Bundo and Igarashi (1983) showed that antibody levels in the secondary DHF cases were significantly higher than those in the primary cases of DHF. In this report, we describe the result of serodiagnosis by the ELISA on sera taken from encephalitis cases in Chiang Mai observed during the epidemic season in the year of 1983.

MATERIALS AND METHODS

Serum specimens: One hundred and forty two serum specimens were taken from 114 encephalitis cases admitted to the Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Northern Thailand, from July to October in 1983. The sera were taken as 90 single specimens, 23 pairs of acute and convalescent, and each one of the triplicate and quadruple specimens. The sera were dried on bleeding filter paper strips with calibrated absorbing capacity of 0.1ml volume (Type I, Toyo Kagaku Sangyo, Japan) and were sent to Japan by Airmail. One-fifth of each filter paper was cut and eluted with 2ml of phosphate buffered saline containing 0.05% Tween 20 and 0.01% of NaN_3 (PBS-T) to make 1 : 100 dilution of test serum.

Indirect ELISA to measure IgG antibodies: The method of Voller *et al.* (1976) was modified as described before (Igarashi *et al.*, 1981; Bundo *et al.*, 1981). U-shaped 96-well microplate (Immulon, Greiner Labortechnik, West Germany) was coated with purified JE vaccine concentrate (Takaku *et al.*, 1968) kindly supplied by Research Foundation for Microbial Diseases of Osaka University. After washing with PBS-T, the plate was reacted with test serum at 1 : 1000 dilution or standard serum with serial dilution in 2-fold steps starting from 1 : 1000 dilution. Plate was washed again with PBS-T and reacted with peroxidase conjugated anti-human IgG (heavy and light chain) goat IgG, followed by washing with PBS-T and subsequent peroxidase reaction. Optical density (OD) of the colored product was measured at 490 nm using Micro ELISA Autoreader (Dynatech, USA). The titer of the test specimen was calculated by comparing the OD for each test

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specimen with those developed by serial dilution of the standard positive serum of known endpoint titer (Igarashi *et al.*, 1981; Morita *et al.*, 1982).

Preparation of peroxidase-conjugated anti-flavivirus human IgG: The method is described elsewhere (Bundo and Igarashi, 1984). Hightitered DHF patients' sera were kindly supplied by Dr. K. Chatiyanonda, Virus Research Institute, Bangkok, Thailand. The sera were pooled and dialysed against 0.005 M phosphate buffer, pH 8.0, and then applied to the column of DEAE Sephacel (Pharmacia, Sweden). Fractions of 5 ml volume was collected by eluting with the above buffer, and OD_{280} was measured. IgG in high OD fractions were pooled and concentrated by negative-pressure dialysis. The concentrated IgG was conjugated with horseradish peroxidase (Sigma, Type VI) by Nakane and Kawaoi's method (1974).

IgM-capture ELISA: The method of Duermeyer *et al.* (1979) was modified to measure antibody titers against JE and dengue antigens (Bundo and Igarashi, 1984). The ELISA microplate was coated with antihuman IgM (μ-chain specific) goat IgG. After washing with PBS-T, test sera at 1:100 dilution and standard sera diluted in serial 2-fold steps were distributed into the wells on the plate. After incubation and washing with PBS-T, the plate was reacted with virus antigen, using purified JE vaccine concentrate for JE and infected culture fluid from *Aedes albopictus* clone C6/36 cells (Igarashi, 1978) for dengue type 2 antigens, respectively. The plate was incubated and washed with PBS-T again, and then reacted with peroxidase-conjugated anti-flavivirus human IgG, followed by washing with PBS-T and peroxidase reaction. Recording of the ELISA-OD and calculation of the ELISA titer for test speciemns were as described above.

Cell cultures and preparation of infected culture fluid: Origins and cultivation of *Ae. albopictus* clone C6/36 cells were described before (Igarashi, 1978). The cells were inoculated with seed virus and virus adsorption was carried out for 2 hours. The cells were incubated for 7 days under maintenance medium and infected fluid was collected. After low speed centrifugation at 2500 rpm for 15 min., the supernatant was used as virus antigen.

Virus strains and reagents: The JE vaccine concentrate used as ELISA antigen was prepared by the Nakayama strain. New Guinea B strain of dengue virus type 2 was used to prepare infected culture fluid of C6/36 cells. Anti-human IgM (μ-chain specific) goat IgG and peroxidase-conjugated anti-human IgG (heavy and light chain) goat IgG were the products of Cappell Laboratories, USA.

RESULTS

Fig. 1 shows age and sex distribution of 114 encephalitis cases in Chiang Mai, in 1983. Male cases were more numerous than females and the highest numbers were observed in the age group of 15−19, followed by the equal numbers in 5 − 9 and 10−14 years old.

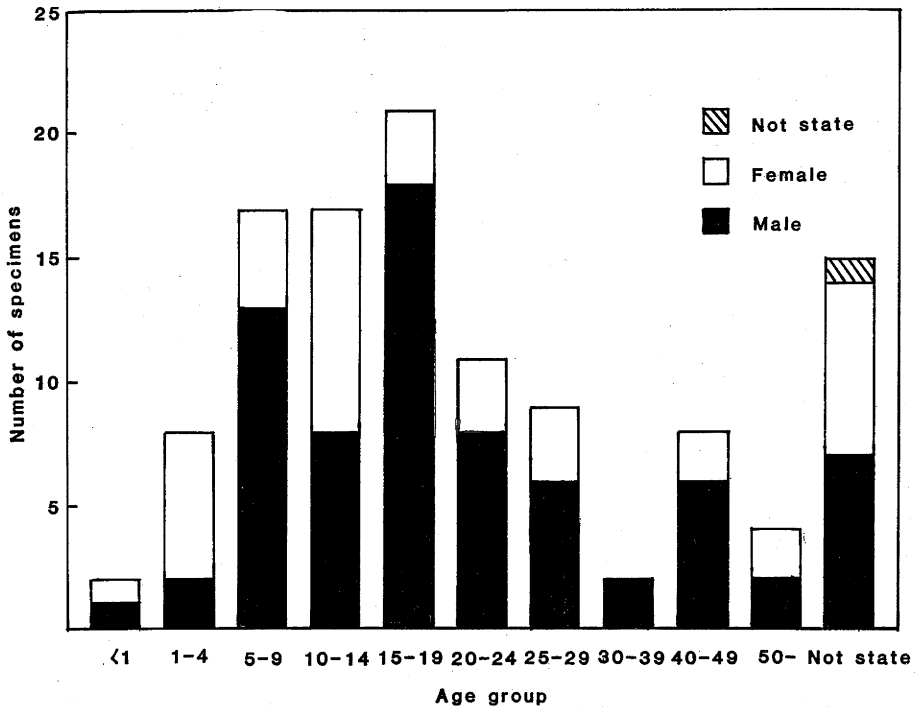The encephalitis patients in each age group were categorized into JE, dengue,

Fig. 1. Age distribution of male and female encephalitis patients
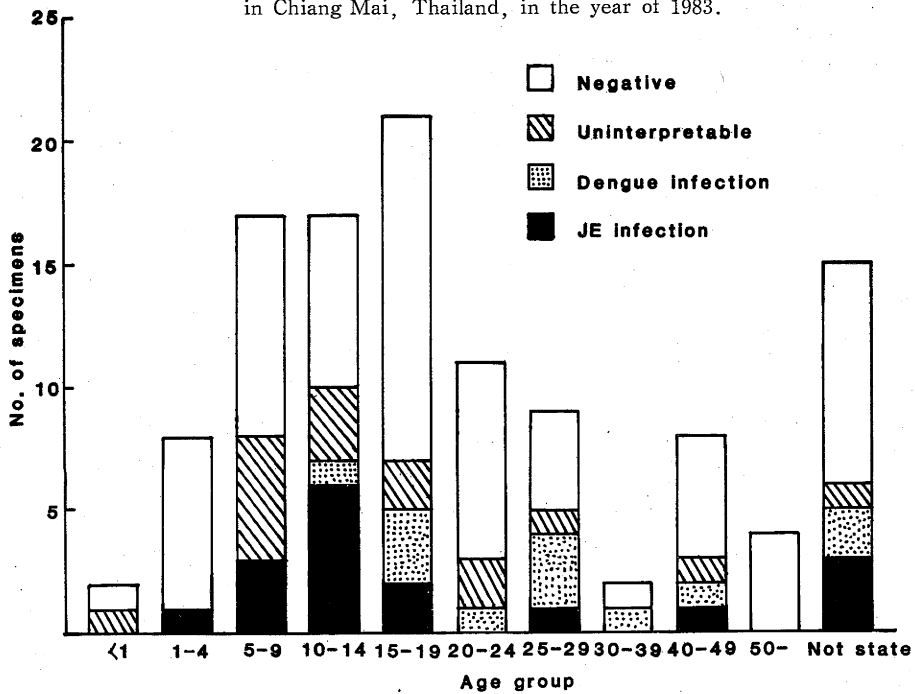in Chiang Mai, Thailand, in the year of 1983.

Fig. 2. Serodiagnosis on encephalitis patients in each age group
according to the diagnostic criteria by IgM–capture ELISA
worked out by Bundo and Igarashi (1984).

uninterpretable and negative cases, according to their IgM-ELISA titers using the diagnostic criteria which have been worked out by Bundo and Igarashi (1984). The age-distribution of these categorized pateints in Fig. 2 shows that JE cases had the peak in the group of 10-14 years old followed by 5-9 and then 15-19 years old. On the other hand, dengue infection distributed in older age groups and the largest numbers were recorded in the age groups of 15-19, and 25-29 years old. There were also quite a bit of cases which could not be interpreted either as JE or dengue, because their sera showed
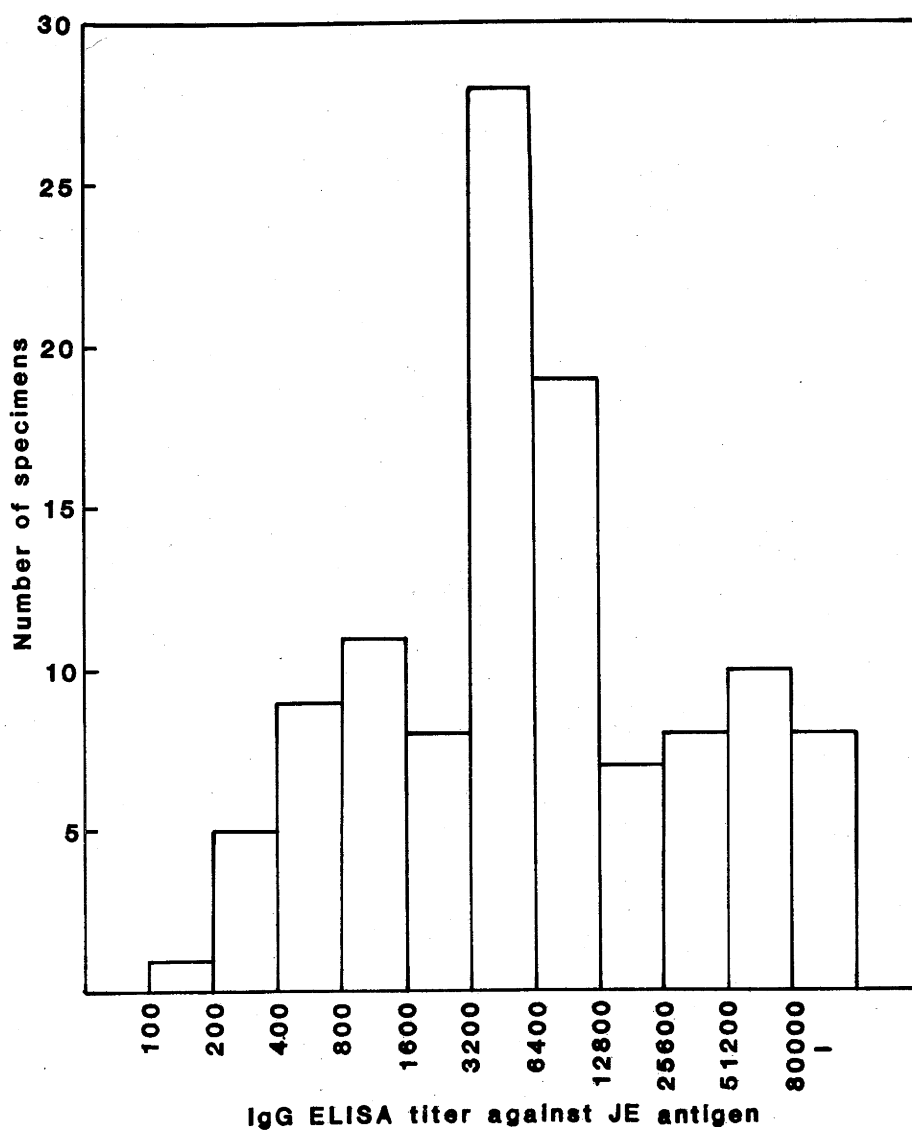

Fig. 3. Frequency distribution of IgG-ELISA titers of encephalitis patients in Chiang Mai, Thailand, 1983.

significant levels of IgM-ELISA antibody titers to JE and dengue antigens without showing more than 4-fold difference between the titers against these 2 antigens. Such cases were most numerous in the age group of 5-9 years old. Also more than half of the encephalitis cases were considered as negative by the IgM-ELISA using JE and dengue virus type 2 antigens. These negative cases were most numerous in the age group of 15-19 years old.

   Frequency distribution of IgG-ELISA titers against JE antigen, which were measured for 114 cases of encephalitis, was shown in Fig. 3, with its mode between 3200-6400 IgG-ELISA titers. Using the results of Bundo and Igarashi (1983), the cases showing IgG-ELISA titer over 16000 could be considered as secondary infection with flaviviruses. Each of the JE cases was then categorized into secondary JE, secondary non-JE, not secondary JE, and not secondary non-JE, cases respectively, as shown in Fig. 4, in each age group. Both secondary JE and not secondary JE cases showed their peak in the age group of 10-14 years old, so was the secondary non JE cases, corresponding to the peak of total JE cases in Fig. 2. Similarly, each of the dengue cases was categorized into secondary dengue, secondary not dengue, not secondary dengue, and not secondary not dengue cases as shown in Fig. 5 according to their age groups. There was only a single secondary dengue case in the age group of 20-24 years old, and the largest num-
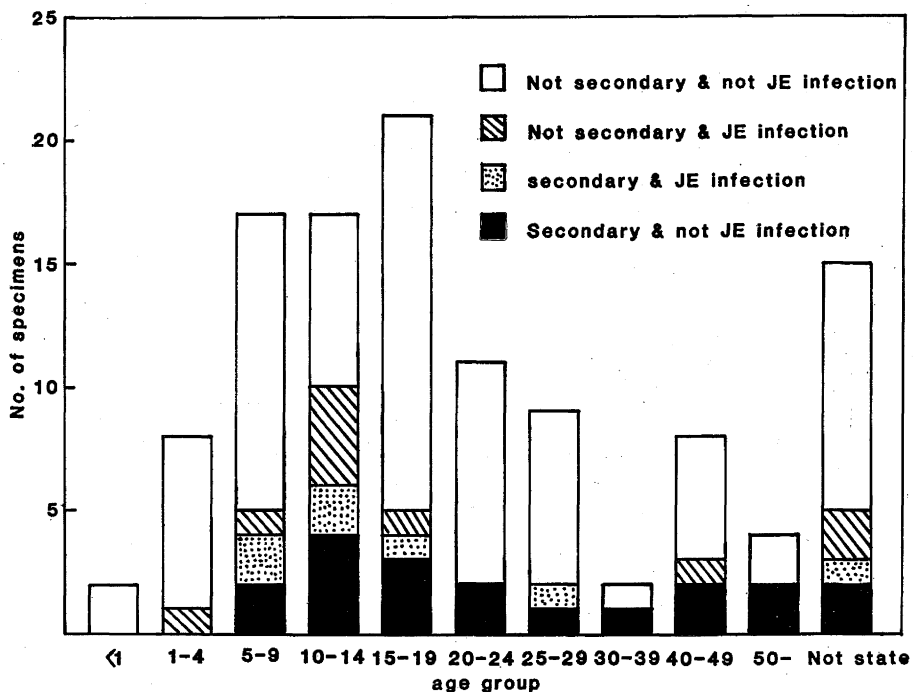

Fig. 4. Classification of encephalitis patients into secondary or not
        secondary, and JE, according to the levels of IgG-ELISA
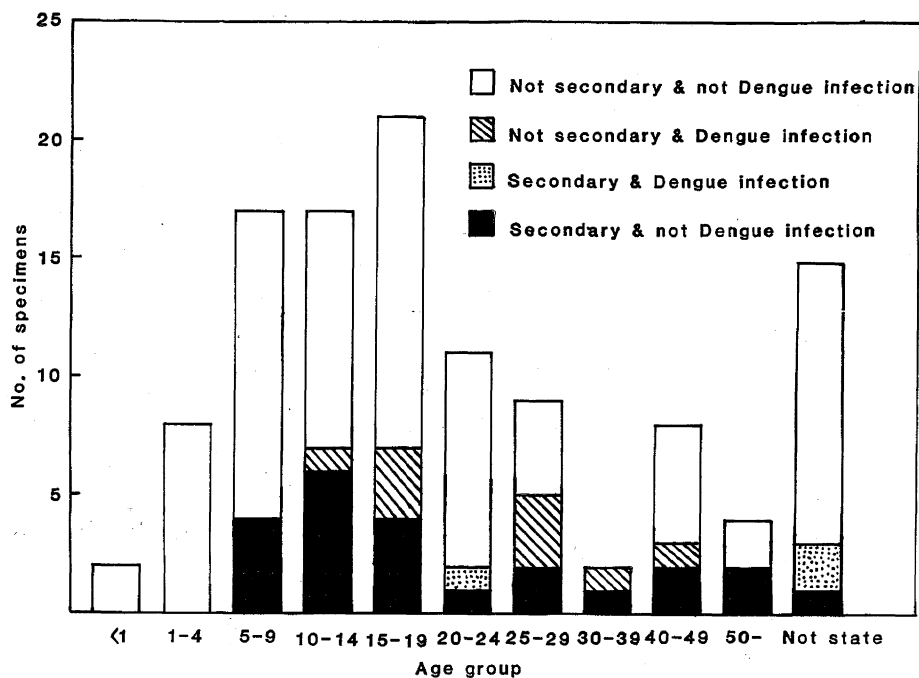   and serodiagnosis by IgM-ELISA.

Fig. 5. Classification of encephalitis patients into secondary or not
secondary, and dengue or not dengue, according to the
levels of IgG–ELISA and serodignosis by IgM–ELISA.

bers of not secondary dengue cases were observed in the age group of 25–29 followed by
15–19 years old. On the other hand, secondary not dengue cases showed their peak in
the age group of 10–14 years old, corresponding to the peak of JE cases.

DISCUSSIONS

The data showed that 17 cases (14.9%) of 114 encephalitis cases in Chiang Mai,
1983, were diagnosed as JE by IgM–ELISA, while 12 cases (10.5 %) were dengue in-
fections. The ratio of positive JE cases was lower and that of dengue cases was higher
than the previous observation in 1982 using indirect IgM–ELISA against JE antigen
(Fujita et al., 1983), although we did not use IgM–ELISA against dengue antigens in the
previous studies. Here again, we found quite a bit of encephalitis cases which were
serologically considered as dengue infections. Possible dengue encephalopathy cases (Sum-
armo et al., 1978) in Chiang Mai were described previously both by virus isolation (Iga-
rashi et al., 1983b) and serology (Fujita et al., 1983). Age distribution of these possible
dengue encephalopathy cases shifted towards older age groups compared with JE cases.
However, secondary non JE cases were not preferentially observed in older age groups.
Therefore, it is rather hard to consider that the cases which showed high titere IgG

antibodies and categorized as secondary infections were diagnosed preferentially as dengue as a result of higher IgM-ELISA antibody titers against dengue antigen compared with JE antigen. In the case of JE patients in Japan, their IgM-ELISA showed virtually monospecific reaction to JE and almost negligib'e reaction to dengue antigens (Bundo and Igarashi, 1984). On the other hand, DHF patients' sera from Rangoon, Burma, did not show cross-reactions to JE antigen (May La Linn, 1984). Relatively high cross-reactivity of sera from encephalitis cases in Chiang Mai in the IgM-ELISA may be due to a kind of anamnestic response either to JE or dengue virus. These problems should be clarified by appropriate animal experiments or by careful observation on selected cases.

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1983年タイ国チェンマイにおける脳炎の ELISA による血清診断

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1983年の流行期にタイ国チェンマイにおいて114例の脳炎患者から採血した142検体の血清について日本脳炎 (JE) とデングウイルス2型抗原に対する抗体価を ELISA によって測定した結果, IgM-ELISA によって17例は JE, 12例は多分デング脳症と診断された. JE の年令分布は10−14才にピークを示したが, デング脳症は10−49才とより高年令側に分布した. JE 坑原に対する間接 IgG-ELISA によって26例はフラビウイルスの2次感染と考えられ, その年令分布は10−14才に最も多く存在した.