Tropical Medicine, 25 (3), 155–164, September, 1983

Virological and Epidemiological Studies on Encephalitis in Chiang Mai Area, Thailand, in the Year of 1982

IV. Serological examination on hospitalized patients

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Abstract: One-hundred and eight paired sera including 40 encephalitis and 54 dengue hemorrhagic fever(DHF), and 71 single sera including 15 encephalitis and 25 DHF cases, were examined by the hemagglutination-inhibition (HI) against Japanese encephalitis (JE) and dengue antigens, as well as by the enzyme-linked immunosorbent assay(ELISA) against JE antigen. By the HI test, 10 encephalitis and 3 DHF cases showed primary type antibody response, while there were 7 encephalitis and 28 DHF with secondary responses. Only 7 encephalitis and 10 DHF cases with paired sera showed monospecific HI antibody reponse to JE and dengue antigens, respectively, and there were 7 encephalitis cases with monospecific HI antibody response to dengue antigens. IgM-ELISA against JE antigen appeared to be a more specific indicator of recent infection by JE virus, and could detect 27 positive cases. Twenty two of them were the cases with paired sera and 16 of them did not give positive and monospecific HI antibody response to JE antigen. Remaining 5 case were with single serum specimen. Serological tests suggested that at least 4 encephalitis case were due to dengue virus infections.

Key words: Encephalitis, Thailand, Serology, Chiang Mai.

Received for publication, September 26, 1983.
Contribution No.1375 from the Institute for Tropical Medicine, Nagasaki University.

INTRODUCTION

Since 1969, many encephalitis cases have been reported from Chiang Mai Area, Northern Thailand, and most of the cases were observed during rainy season of June, July, and August (Grossman *et al.*, 1973; Statistics of the Ministry of Public Health of Thailand). Although some of the patient's sera were tested by the HI, only one-third of them were confirmed as infection by JE virus, mainly because of the cross-reactions between JE and dengue viruses. During our study period from July 19 to August 17, 1982, we examined hospitalized patients of encephalitis, DHF, and some other diseases (Igarashi *et al.*, 1983a; Uzuka *et al.*, 1983). Serum specimens from these patients were examined by the HI and ELISA and the results are presented in this paper.

MATERIALS AND METHODS

Serum specimens and the HI test: Sampling and collection of patient's sera were described in the preceeding paper (Uzuka *et al.*, 1983). Sera were treated with kaolin to remove nonspecific inhibitors, followed by absorption with goose red blood cells. The procedure of Clarke and Casals (1958) was followed with modification to microtiter system using goose red blood cells. Sucrose-acetone extracted antigen of JE virus, JaGAr-01 strain, was kindly supplied by Chemoserotherapeutic Institute, Kumamoto, Japan. The antigens of dengue virus, type 1 (D1), Mochizuki and Hawaiian strains; type 2 (D2), TR1751 strain; type 3 (D3), H87 strain; and type 4 (D4), H241 strain; were prepared from infected suckling mouse brains by sucrose-acetone extractions.

ELISA procedures: Indirect micromethod of Voller *et al.* (1976) was used with modifications as described before (Igarashi *et al.*, 1981; Bundo *et al.*, 1981), except that the incubations were performed at room temperature (25-30°C). Peroxidase-conjugated anti-human IgG (heavy and light chains) and anti-human IgM (μ-chain specific) goat IgG were obtained from Cappel Laboratories, Pa. USA. Formalin-inactivated and purified JE vaccine concentrate (Takaku *et al.*, 1968) was kindly supplied by the Research Foundation for Microbial Diseases of Osaka University and was used as ELISA antigen.

RESULTS

The numbers of patients with paired serum specimens were 40 encephalitis, 54 DHF, 6 fever of unknown origin (FUO), 4 meningitis and myelitis, and 4 other disease or without clinical diagnosis. While, the numbers of patients with single serum specimens were 15 encephalitis, 25 DHF, 6 FUO, 4 meningitis, and 21 other disease or without clinical diagnosis. These specimens were tested by the HI against JE and dengue

antigens, as well as by the ELISA against JE antigen. Some patterns of these reactions for encephalitis patients were shown in Table 1, and those for DHF in Table 2, respectively. Patients were classified according to their patterns of HI antibody responses using the guide line of World Health Organization (1983) as shown in Table 3. There were 10 primary and 7 secondary infections of encephalitis, with 1 presumptive secondary and 6 definite infections. On the other hand, there were 3 primary and 28 secondary infections of DHF, with 9 presumptive secondary and 2 definite infections. The age distribution of the primary and secondary infections of encephalitis and DHF cases were summarized in Table 4. The primary infection was observed in younger age groups compared with secondary infection, however, one encephalitis with primary response was observed at the age of 35, and 2 primary DHF cases were at the age of 15 and 16 years old.

Table 1. Serological resonse of some encephalitis patients with paired sera, in Chiang Mai, Thailand, 1982

Patient code	age sex	Date of onset	Specimen	days after onset	HI JE	HI D1	HI D2	HI D3	HI D4	JE-ELISA IgG	JE-ELISA IgM	Virus isolated (from)
P-2	56M	Jul 16	A	5d	40	40	160	80	160	8000	100	
			C	7d	640	320	160	80	160	4000	50	
P-10	7M	Jul 18	A	3d	10	<20	<10	<10	<10	250	50	
			C	12d	80	<20	<10	<10	<10	1000	200	
P-19	5M	Jul 18	A	4d	20	<20	<10	<10	<10	500	50	JE
			C	8d	80	<20	<10	<10	<10	1000	400	(brain)
P-22	12F	Jul 17	A	3d	<5	<20	<10	<10	<10	500	50	
			C	9d	<5	<20	10	10	10	2000	1600	
P-28	2M	Jul 22	A	3d	<5	<20	<10	<10	<10	500	50	
			C	12d	160	160	80	40	80	2000	1600	
P-39	14M	Jul 16	A	4d	5	<20	<10	<10	<10	250	50	
			C	11d	<5	<20	<10	<10	<10	8000	50	
P-90	26M	?	A	?	<20	40	20	10	20	4000	50	
			C	?	>2560	>2560	>2560	320	>5120	>8000	100	
P-95	13F	?	A	?	80	160	160	20	40	8000	50	
			C	+13d	160	320	320	160	320	8000	50	
P-97	14M	?	A	?	80	10	10	10	20	2000	1600	
			C	+7d	80	<10	40	20	40	8000	800	
P-103	2M	Jul 30	A	6d	<10	<20	<10	<10	<10	500	50	
			C	?	10	80	20	10	10	500	50	
P-125	30M	Jul 28	A	9d	20	80	40	20	20	4000	50	
			C	12d	80	80	40	320	160	4000	50	
P-127	10M	Aug 6	A	5d	<10	<10	<10	<10	<10	500	50	
			C	19d	20	10	10	10	10	500	50	
PP-1	18M	Jul 3	A	6d	40	20	nT*	nT	nT	4000	400	
			C	13d	80	40	nT	nT	nT	4000	800	
PP-2	35F	Jul 3	A	6d	<20	10	nT	nT	nT	2000	<100	
			C	13d	40	40	nT	nT	nT	8000	<100	
B-8	67F	Jul 12	A	7d	20	10	20	80	160	8000	<100	D1
			C	21d	40	40	10	80	80	4000	<100	(C)
B-17	12F	Jul 5	A	3d	20	40	<10	20	20	1000	50	
			C	15d	5120	1280	2560	5120	20480	8000	200	

* nT : not tested

Table 2. Serological response of some DHF patients with paired sera, in Chiang Mai, 1982

Patient code	grade	age sex	Date of onset	Specimen days after onset	HI JE	HI D1	HI D2	HI D3	HI D4	JE-ELISA IgG	JE-ELISA IgM	Virus isolated (from)
P-13	Ⅱ	1 F	Jul 19	A 2d	<5	<20	<10	<10	<10	500	50	D1
				C 4d	<5	<20	<10	<10	<10	500	50	(A)
P-15	Ⅲ	13 F	Jul 18	A 1d	10	<20	<10	<10	<10	1000	50	D3
				C 4d	20	20	20	10	20	2000	50	(A)
P-31	Ⅱ	22 F	Jul 17	A 7d	320	320	40	40	80	4000	50	
				C 10d	320	>2560	5120	2560	2560	8000	50	
P-58	Ⅰ	15M	?	A	<10	<20	<10	<10	<10	500	100	
				C+14d	40	80	20	10	20	1000	100	
P-68	Ⅰ	40M	Jul 27	A 3d	320	320	320	160	320	8000	100	D1
				C 6d	>2560	>1280	>10240	2560	>5120	8000	50	(A)
P-76	Ⅰ	5M	Jul 27	A 7d	<20	<20	<10	<10	<10	250	50	D1
				C 15d	40	80	40	10	40	2000	100	(A)
P-100	Ⅰ	10 F	Jul 31	A 4d	1280	2560	1280	640	640	8000	50	
				C 13d	2560	2560	1280	2560	640	8000	50	
P-112	Ⅱ	23 F	Jul 28	A 4d	80	160	160	40	40	1000	50	
				C 13d	80	320	80	80	40	8000	100	

Table 3. Patterns of HI response and clinical diagnosis

	Pattern of HI response	Clinical diagnosis* Enc	DHF	FUO	Men	Other	Total
Paired sera	Primary	10	3	1		2	17
	Secondary	7	28			1	37
	Presumptive secondary	1	3	1			5
	Definite infection	6	2				7
	Uninterpretable	8	17	3			28
	Negative	8	1	1	4	1	14
	Total	40	54	6	4	4	108
Single serum	Presumptive secondary		6				6
	Uninterpretable	15	19	6	4	21	65
	Total	15	25	6	4	21	71
Sum of paired and single serum		55	79	12	8	25	179

* Enc: encephalitis;
DHF: dengue hemorrhagic fever
FUO: fever of unknown origin
Men: meningitis and myelitis
Other: Other diseases and without clinical diagnosis

One-hundred and eight cases with paired sera were classified into 13 groups according to their patterns of serological responses with combinations of positives and negatives in the HI and ELISA as shown in Table 5. The upper quarter of the Table (groups A, B, and C) contained patients showing positive HI responses both to JE and dengue antigens, and they are hard to differentiate by the HI whether they were infected with JE or dengue viruses. The specimens in group A showed positive IgG and IgM-ELISA to JE antigen, and there were 8 encephalitis, like P-28 and B-17 in Table 1, and

Table 4. Distribution of encephalitis and DHF patients by age and pattern of HI antibody response

Age group	Encephalitis		DHF	
	Primary	Secondary	Primary	Secondary
1– 4	2			2
5– 9	3	2	1	4
10–14	4	1		3
15–19		1	2	4
20–29		3		9
30–	1			5
Total	10	7	3	27
Mean age	10.4	15.7	12	20.1

Table 5. Relationship between clinical diagnosis and serodiagnosis paired serum specimens

Group	HI		JE-ELISA		Clinical Diagnosis*					Total	
	JE	DEN	IgG	IgM	Enc	DHF	FUO	Men	Other		
A	+	+	+	+	8				1	9	
B	+	+	+	–	5	27				32	45
C	+	+	–	–	1	2			1	4	
D	+	–	+	+	3					3	
E	+	–	–	+	3					3	9
F	+	–	–	–	1		1		1	3	
G	–	+	+	+	3		1			4	
H	–	+	+	–	3	6	1			10	20
I	–	+	–	–	1	4	1			6	
J	–	–	+	+	4			1		5	
K	–	–	+	–	1	8	2			11	34
L	–	–	–	+	1					1	
M	–	–	–	–	6	7		3	1	17	
Total					40	54	6	4	4	108	

* Enc: encephalitis; DHF: dengue hemorrhagic fever;
FUO: fever of unknown origin; Men: meningitis and myelitis:
Other: other disease or without clinical diagnosis
HI(+): 4-fold or more titer rise or the titer $\geqq$ 2560
ELISA(+): 4-fold or more titer rise or IgG –ELISA $\geqq$ 8000
IgM–ELISA $\geqq$ 400

1 patient without clinical diagnosis in this group. Group B contained 5 encephalitis and 27 DHF cases, and they showed positive IgG-ELISA with negative IgM-ELISA to JE antigen, like PP-2 and P-90 in Table 1, and P-58, P-68, P-76, and P-100 in Table 2. One encephalitis, 2 DHF, and 1 patient without clinical diagnosis in group C showed negative IgG- and IgM-ELISA against JE. (P-125 in Table 1). The second quarter of Table 5 (groups D, E, and F) contained specimens showing positive HI response by JE antigen without showing significant HI responses to dengue antigens, and they could be diagnosed as JE infection by the HI. Three encephalitis, like P-10 in Table 1, in group D showed positive IgG- and IgM-ELISA to JE antigen, while 3 encephalitis in groupE, like P-19 in Table 1, with positive IgM-ELISA only. One encephalitis (P-127 in Table 1), 1 FUO, and 1 patient without clinical diagnosis in group F did not show positive ELISA. The third quarter (groups G, H, and I) in Table 5 contained specimens showing significant HI antibody response to any of the 4 types of dengue antigens without showing positive HI antibody response to JE antigen, and they could be diagnosed as dengue infection by the HI test. Three encephalitis, like P-97 in Table 1, and 1 FUO patients showed positive IgG- and IgM-ELISA and were classified into group G. Group H consisted of 3 encephalitis, like P-95 in Table 1, 6 DHF, like P-31 in Table 2, and 1 FUO patients, and they showed positive IgG- and negative IgM-ELISA against JE antigen. Group I consisted of 1 encephalitis (P-103 in Table 1), 4 DHF, like P-15 in Table 2, and 1 FUO patients, and they did not show positive ELISA. The bottom quarter of Table 5 (groups J, K, L, and M) contained specimens which did not show significant HI antibody response either to JE or dengue antigens. There were 4 ence-

Table 6. Relationship between clinical diagnosis and serodiagnosis single serum specimens

Group	HI		JE-ELISA		Clinical Diagnosis*					Total	
	JE	DEN	IgG	IgM	Enc	DHF	FUO	Men	Other		
B	+	+	+	−		1				1	1
H	−	+	+	−		5			1	6	7
I	−	+	−	−					1	1	
J	−	−	+	+	4					4	63
K	−	−	+	−	1	3	2	2	5	13	
L	−	−	−	+	1					1	
M	−	−	−	−	9	16	3	3	14	45	
Total					15	25	5	5	21	71	

* Enc: encephalitis; DHF: dengue hemorrhagic fever;
FUO: fever of unknown origin; Men: meningitis and myelitis;
Other: other disease or without clinical diagnosis
HI(+): HI titer $\geq$ 2560
ELISA(+): IgG−ELISA $\geq$ 8000, or IgM−ELISA $\geq$ 400

phalitis, like P-22 in Table 1, and 1 meningitis patients in group J, which showed positive IgG- and IgM-ELISA. Group K consisted of 1 encephalitis (P-39 in Table 1), 8 DHF, like P-112 in Table 2, and 2 FUO patients, which showed positive IgG- and negative IgM-ELISA. Only one encephalitis patient, PP-1 in Table 1, belonged to group L, which showed positive IgM- with negative IgG-ELISA. Finally, group M consisted of 6 encephalitis, 7 DHF, 3 meningitis, and 1 patient without clinical diagnosis, and they did not show any significant responses by any of the serological tests used in this study. However, one of the DHF patients in this group, P-13 in Table 2, was found to be infected with dengue virus type 1 as shown by virus isolation (Igarashi *et al.*, 1983b). The results in Table 5 showed that none of the DHF cases showed positive IgM-ELISA against JE antigen, thus it appears that positive IgM-ELISA is a more reliable indicator of recent infection by JE virus. Seventy one patients with single serum specimens were classified according to the same grouping used in Table 5, and the results were summarized in Table 6. Again none of the DHF patients showed significant levels of IgM-ELISA against JE antigen. Thus, the patients with positive JE-IgM-ELISA appears to have been infected with JE virus recently, like 4 encephalitis in group J and 1 encephalitis in group L. So that total number of encephalitis patients with positive IgM-ELISA is 27 (22 paired serum and 5 single serum specimens, respectively). On the other hand, 3 encephalitis in group H and 1 encephalitis in group I in Table 5 could probably have been infected with dengue virus as shown by their HI antibody response and negative IgM-ELISA against JE antigen. The age distribution of these patients is shown in Table 7.

Table 7. Age distribution of encephalitis patients with positive JE-IgM ELISA and those with possible infection by dengue viruses

Age group	(+) JE IgM−ELISA	Possible dengue
1− 4	4	2
5− 9	7	
10−14	9	1
15−19	4	
20−29	1	
30−	2	1
Total	27	4

DISCUSSION

By the HI test, only 7 of the 40 encephalitis patients with paired sera were shown to have monospecific antibody response to JE antigen, while only 10 out of the 54 DHF cases showed monospecific HI antibody response to dengue antigens. Thus, serodiagnosis by the HI test was quite inefficient to differentiate JE from dengue infections, mainly because of the cross-reactivity between these viruses as shown in groups A, B, and C in Tables 5 and 6. Although typical DHF symptoms are quite different from those of encephalitis, sometimes encephalopathy due to dengue virus infections have been reported (Sumarmo *et al.*, 1978), and the etiological agent is not always easy to guess

from clinical pictures, especially for encephalitis.

The result of IgM-ELISA appears to help in the differentiation between dengue and JE as shown in this paper. Edelman and Pariyanonda (1973) showed that assay of IgM antibody by the HI test combined with sucrose gradient sedimentation helped to detect recent infection with JE virus even when secondary type of HI antibody response was observed. Recently, Burke and Nisalak (1982), and Burke *et al.* (1982) showed that IgM-assay is diagnomostic for JE by using antibody capture radioimmunoassay on patient's sera or cerebrospinal fluids. Bundo *et al.* (1981; 1982) showed that IgM-ELISA is a good indication of recent JE virus infections both in apparent patients and inapparent infections among healthy inhabitants in Japan, The criterion of positive IgM-ELISA used in this study was quite stringent compared with that in Japan, using 4-fold higher titer as the positive limit. This is because some DHF cases also showed certain levels of IgM-ELISA to JE antigen. We have not determined IgM-ELISA against dengue antigens in this study, however, Burke *et al.* showed that the ratio of dengue IgM to JE-IgM in the ELISA could give clear-cut differentiation between JE and dengue infections (Burke personal communication). More critical works should be performed in the assay of IgM-ELISA using all the 4 types of dengue antigens in order to set up critical diagnostic criteria on JE and dengue infections.

As suggested in this paper and also by the virus isolation in the preceeding paper (Igarashi *et al.*, 1983b), some of the encephalitis patients appeared to have been infected with dengue virus. These possible "dengue encephalopathy" cases (Sumarmo *et al.*, 1978) should further be analyzed more carefully including the findings of cerebrospinal fluids.

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1982年タイ国チェンマイ地区における脳炎のウィルス学的疫学的調査. Ⅳ. 入院患者の血清学的検査

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40名の脳炎と54名のデング出血熱（DHF）を含む108名の患者の対血清と，15名の脳炎と25名のDHFを含む71名の患者の単一血清について，日本脳炎（JE）とデング抗原に対する血球凝集抑制反応（HI）とJE抗原に対する免疫酵素測定法（ELISA）を実施した。HIの結果，10名の脳炎と3名のDHFは初感染の抗体反応を示したが，7名の脳炎と28名のDHFは二次感染

の反応を示した。 HI で JE 抗原にのみ抗体反応を示した脳炎は7例であり， デング抗原にのみ抗体反応を示したものは脳炎で7例，DHF で10例存在した． JE 抗体に対する IgM-ELISA は JE ウイルスの新鮮感染を示す良い指標である事がわかり， この方法で27名の脳炎患者が陽性と判定された． このうち22名は対血清の得られたもので，そのうち16名は JE 抗原のみに陽性の HI 反応を示していなかった。残る5例は単一血清しか得られなかった患者である。 血清学的検査の結果，脳炎患者のうち少なくとも4名はデング感染によると推察された。

熱帯医学 第25巻 第3号，155－164頁，1983年9月