

## Original Article

# Detection of Japanese Encephalitis (JE) Virus-Specific IgM in Cerebrospinal Fluid and Serum Samples from JE Patients

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**SUMMARY:** Detection of Japanese encephalitis virus (JEV)-specific IgM by IgM-capture enzymed-linked immunosorbent assay (IgM-capture ELISA) has been accepted as the standard for serological diagnosis. In the present study, we analyzed the time course of the positive rate of JEV-specific IgM in serum and cerebrospinal fluid (CSF) specimens from confirmed JE patients. Serum and CSF samples were obtained from 155 JE cases for diagnostic purposes at hospitals in Thailand from 2002 to 2004. The levels of specific IgM were assessed by IgM-capture ELISA in the 171 serum and 156 CSF samples. Anti-JEV IgM was detected in 26 of 44 serum samples collected on days 1-4 of the disease period, in 31 of 44 samples collected on days 5-8, in 23 of 26 samples collected on days 9-12, and in all the samples collected on day 13 or later. Specific IgM was detected in 60 of 66 CSF samples collected on days 1-4 of illness, and in all the CSF samples but one collected on day 7 or later. The results indicate that the detection of JEV-specific IgM in CSF by IgM-capture ELISA is a reliable laboratory diagnostic method for confirmation of JE throughout the disease period, while the detection of IgM in serum samples is a reliable method on day 9 or later.

## INTRODUCTION

Japanese encephalitis virus (JEV) is the most common cause of viral encephalitis in Southeast Asia. JEV is a member of the family *Flaviviridae*, genus *Flavivirus*, and is transmitted to humans through a pig-*Culex* mosquito-pig cycle. Humans are an incidental host. The clinical features are manifested as a febrile headache syndrome, aseptic meningitis, or encephalitis (1,2).

JEV can not usually be isolated from clinical specimens because of the low levels of viremia and the rapid development of neutralizing antibodies (3). The detection of JEV-specific IgM by IgM-capture enzymed-linked immunosorbent assay (IgM-capture ELISA) has been accepted as the standard for serological diagnosis. This assay distinguishes between JE and dengue virus, which are serologically cross-reactive (4). Presence of JEV-specific IgM in cerebrospinal fluid (CSF) is considered to be a sign of JEV infection of the central nervous system. Serum samples are also used for detection of specific IgM, because serum can be obtained from patients more easily than CSF. In the present study, we analyzed the time course of the detection of JEV-specific IgM in serum and CSF specimens from confirmed JE patients.

## MATERIALS AND METHODS

**Patients:** Patients with fever ( $\geq 39^{\circ}\text{C}$ ) and showing at least two of six symptoms – headache, changes of consciousness, convulsions, abnormal movements, neck rigidity and pres-

ence of Kernig's sign – were clinically diagnosed with viral encephalitis if no bacteriological causative agent was found.

**Specimens:** Serum and CSF specimens were obtained for diagnostic purposes from 1,453 patients with clinically suspected viral encephalitis in hospitals in Thailand from 2002 to 2004. These specimens were sent to the Arbovirus Section, National Institute of Health, Thailand for serological tests. Of these 1,453 patients, 155 patients were confirmed to have JE based on the serological tests, in which positivity for the disease was taken as an anti-JE IgM titer of 40 units or more in a CSF specimen collected at any time point of illness. In the present study, disease day 1 was defined as the day of the onset of symptoms.

**IgM-capture ELISA:** All the specimens were examined for anti-JEV IgM by IgM-capture ELISA as reported by Innis et al. using mouse brain-derived, acetone-extracted JE antigen and a tetravalent of dengue antigen (5). Briefly, 96-well microtiter plates (Nunc Maxisorp; Nunc, Roskilde, Denmark) were coated with rabbit anti-human IgM antibody (ICN/Cappel; ICN Biomedicals Inc., Aurora, Ohio, USA). Fifty microliters of serum diluted at 1:100 in PBS or CSF diluted at 1:10 in PBS was added to the wells and incubated overnight at  $4^{\circ}\text{C}$ . The plates were then washed 6 times with PBS plus 0.05% Tween 20, and 50  $\mu\text{l}$  of either JE antigen or tetravalent dengue antigen diluted in PBS containing 20% acetone-extracted normal human serum prepared as described by Burke et al. (6) was added to the wells. The dengue antigen was a mixture of 16 hemagglutination units each of dengue serotypes 1-3 and 8 hemagglutination units of dengue serotype 4 in 50  $\mu\text{l}$ . The JE antigen was diluted to 50 hemagglutination units in 50  $\mu\text{l}$ . After 2 h of incubation at room temperature, the plate was washed as described above and 25  $\mu\text{l}$  of optimal diluted HRP-conjugated anti-flavivirus IgG, prepared from pooled convalescent sera of patients with dengue hemorrhagic fever with secondary antibody responses

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as previously described (7), was added. After 1 h of incubation at 37°C, 100 µl of o-phenylene diamine (OPD) substrate (Sigma-Aldrich Co., St. Louis, Mo., USA) was added to develop the color. The reaction was stopped by the addition 50 µl of 4M sulfuric acid. On all test plates, 1:100 dilutions of negative control (NC), weak positive control (WPC), and strong positive control (SPC) serum samples were run simultaneously with duplicate test samples. The plate was read for absorbance at 490 nm by an ELISA reader (Elx800; Bio-Tex Instruments, Inc., Winooski, Vt., USA). The ELISA titer was calculated by this formula: units =  $100 \times (A_{490} \text{ test sample} - A_{490} \text{ NC}) / (A_{490} \text{ WPC} - A_{490} \text{ NC})$ . The cutoff point for IgM was 40 units and corresponded to a mean test absorbance of 0.175 (4,5,8). A ratio of anti-JE IgM to anti-dengue IgM higher than 1.0 was defined as JEV infection. It was necessary to measure the level of antibodies against both viruses because dengue and JEV co-circulate in Thailand.

**Statistical analysis:** The correlation between IgM titers in CSF and serum from the same patients was analyzed. The correlation was considered to be significant when the *P* value was less than 0.05.

## RESULTS

One-hundred and seventy-one serum samples and 156 CSF samples were obtained from 155 patients with confirmed JE. Figures 1 and 2 show the numbers of JEV IgM-positive or -negative serum and CSF samples according to the day of disease. Anti-JEV IgM was detected in 26 of 44 serum samples collected on days 1-4 of disease, in 31 of 44 samples collected on days 5-8, in 23 of 26 samples collected on days 9-12, and in all the samples collected on day 13 or later (Figure 1). Specific IgM was detected in 60 of 66 CSF samples collected on days 1-4, and in all the CSF samples but one collected on day 7 or later (Figure 2). The percentages of anti-JEV IgM-positive samples were higher in CSF samples (diluted at 1:10) than in serum samples (diluted at 1:100) on disease days 1-12 (Figures 1 and 2). Anti-JEV IgM was detected in all the CSF and serum samples collected on day 13 or later.

The levels of anti-JEV IgM were compared in pairs of serum and CSF samples collected from the same patients on the same day (Figure 3). There was a significant correlation between the IgM titers in CSF and those in serum samples ( $R^2 = 0.575$ ,  $P < 0.001$ ).

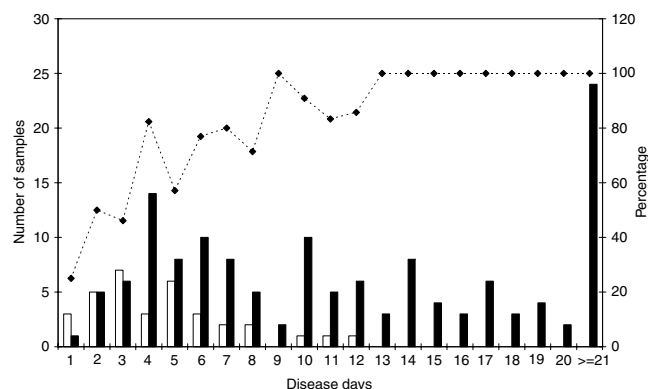


Fig. 1. Detection of anti-JEV IgM and percentage of positive samples in serum specimens according to the day of disease. Open bar, negative; black bar, positive; diamond and dotted line, percentage of positive samples.

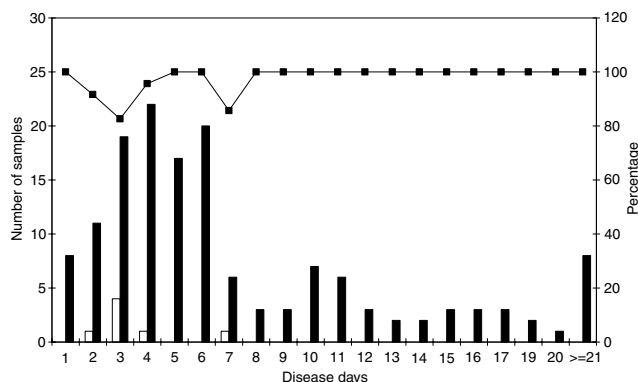


Fig. 2. Detection of anti-JEV IgM and percentage of positive samples in CSF specimens according to the day of disease. Open bar, negative; black bar, positive; rectangle and solid line, percentage of positive samples.

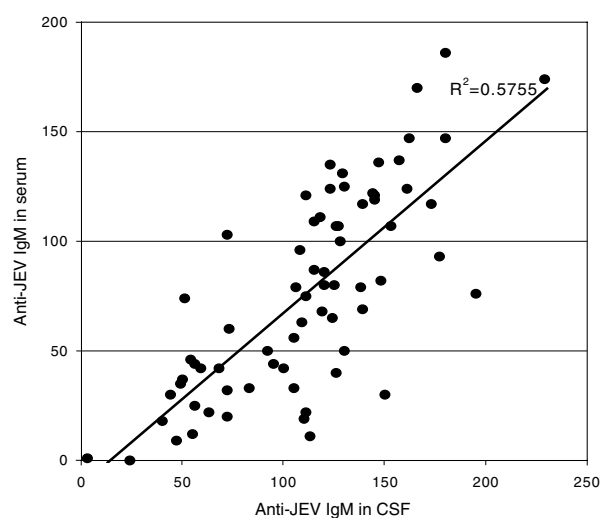


Fig. 3. The correlation between anti-JEV IgM in serum and that in CSF specimens. Each dot represents a pair of serum and CSF samples collected from the same patient on the same day.

## DISCUSSION

From 1993 to 1998, between 500 and 700 cases of viral encephalitis were reported each year in Thailand, and about 34 cases per year were confirmed by serological diagnosis (9). In the present study, we analyzed anti-JEV IgM levels in both serum and CSF using specimens from 155 JE cases that were obtained for diagnostic purposes at hospitals throughout Thailand from 2002 to 2004. These cases constituted approximately 10% of the patients who were clinically diagnosed with viral encephalitis. The levels of JEV-specific IgM were assessed by IgM-capture ELISA in the 171 serum and 156 CSF samples.

The anti-JEV IgM positive-rate was higher in CSF samples than in serum samples until day 13 of the disease period, with day 1 defined as the day of onset of symptoms. Specific IgM was detected in 60 of 66 CSF samples collected on days 1-4, and in all the CSF samples but one collected on day 7 or later. Thus, in most of the JE cases, specific IgM was detectable in CSF as early as day 1. On the other hand, in most cases, specific IgM was not detected in serum samples until day 9 or later. Therefore, for confirming JE, an assay for IgM in CSF is likely to be more effective than an assay for IgM in serum samples in the early days (days 1-8) of the illness.

Detectable levels of anti-JEV IgM were present in all the CSF samples on disease day 8 or later. This result is in good agreement with a report by Burke et al. in which specific IgM was detectable in all the CSF samples on day 7 (10).

The results indicate that the detection of specific IgM in CSF by IgM-capture ELISA is a reliable laboratory diagnostic method for confirmation of JE throughout the disease period, while the detection of IgM in serum samples is a reliable method for the later part of the disease period.

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#### REFERENCES

1. Monath, T. P. and Heinz, F. X. (1996): Flaviviruses. p. 961-1034. *In* Fields, B. N., Knipe, D. M. and Howley, P. M., *Fields Virology*. Lippincott-Raven Publishers, Philadelphia, USA.
2. Solomon, T. (1997): Viral encephalitis in Southeast Asia. *Neurol. Infect. Epidemiol.*, 2, 191-199.
3. Buescher, E. L. and Scherer, W. F. (1959): Immunological studies of Japanese encephalitis virus in man. I. Antibody responses following overt infection of man. *J. Immunol.*, 83, 582-593.
4. Solomon, T., Thao, L. T. T., Dung, N. M., Kneen, R., Hung, N. T., Nisalak, A., Vaughn, D. W., Farrar, J., Hien, T. T., White, N. J. and Cardoso, M. J. (1998): Rapid diagnosis of Japanese encephalitis by using an immunoglobulin M dot enzyme immunoassay. *J. Clin. Microbiol.*, 36, 2030-2034.
5. Innis, B. L., Nisalak, A., Nimmanitya, S., Kusalerdchariya, S., Chongswasdi, V. V., Suntayakorn, S., Puttisri, P. and Hoke, C. H. (1989): An enzyme-linked immunosorbent assay to characterize dengue infection where dengue and Japanese encephalitis co-circulate. *Am. J. Trop. Med. Hyg.*, 40, 418-427.
6. Burke, D. S., Nisalak, A. and Ussery, M. A. (1982): Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. *J. Clin. Microbiol.*, 16, 1034-1042.
7. Bundo, K. and Igarashi, A. (1985): Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *J. Virol. Methods*, 11, 15-22.
8. Strickman, D., Sithiprasana, R., Kittayapong, P. and Innis, B. L. (2000): Distribution of dengue and Japanese encephalitis among children in rural and suburban Thai villages. *Am. J. Trop. Med. Hyg.*, 63, 27-35.
9. Buranathai, C., Sa-ngasang, A., Nuang-plee, S., Punyosri, N., Anantapreecha, S. and Warachit, P. (2000): Laboratory surveillance on Japanese encephalitis in Thailand, 1993-1998. *J. Health. Sci.*, 9, 342-348 (in Thai).
10. Burke, D. S., Nisalak, A., Ussery, M. A., Laorakpongse, T. and Chantavibul, S. (1985): Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. *J. Infect. Dis.*, 151, 1093-1099.