

Evaluation of Immunoglobulin A-capture Enzyme-linked Immunosorbent Assay for Serodiagnosis of Dengue Virus Infection

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Abstract

In order to determine the usefulness of anti-dengue IgA-capture enzyme-linked immunosorbent assay (IgA-capture ELISA), specific IgA was examined in the serum samples from 87 patients with symptoms of dengue in Kaohsiung city, Taiwan. Of these 87 patients, 34 cases who were confirmed to be dengue by reverse transcription-PCR (RT-PCR), and 39 cases who were RT-PCR-negative but IgM-positive, were separately analysed. Dengue virus-specific IgA antibody was first detected on disease day 5 and continued to be detected until day 39. The results indicate that the duration of dengue virus-specific IgA is short, and suggest that the detection of specific IgA in the serum is a useful diagnostic measure, especially when only a single serum sample is available from patients.

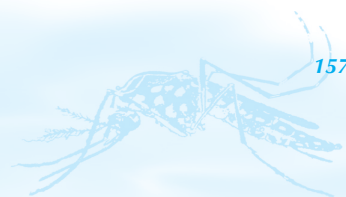
Keywords: Dengue, serodiagnosis, IgA antibody, ELISA.

Introduction

Dengue is one of the most serious viral infections affecting tropical and subtropical countries in the world. Dengue has a great relevance for us as a re-emerging disease spread to a large extent by air-travel of humans who are incubating the virus after getting infected in dengue-endemic areas. At present, dengue virus infection is confirmed by RT-PCR detecting viral RNA in acute-phase serum sample, or testing IgM antibody in acute- and convalescent-

phase serum samples. In Taiwan, dengue epidemics have occurred since 1980s.^[1,2] Additionally, it has been reported that a number of epidemics were associated with imported cases, primarily with travellers who come from neighbouring countries where dengue is endemic.^[3] It has been reported that specific IgA is induced by dengue virus infections, but persists for a shorter period of time than specific IgM.^[4] In order to test the usefulness of dengue virus-specific IgA for confirmation of dengue virus infections, we examined the specific IgA responses in dengue patients in Taiwan.

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Materials and methods

A total of 151 serum samples were obtained for diagnostic purpose from 87 patients manifesting symptoms of dengue virus infection at clinics and hospitals in Kaohsiung city in 2005. Samples were sent to the Center for Disease Control, Department of Health, Kaohsiung City Government in Taiwan. The presence of dengue viral RNA was examined by RT-PCR with acute-phase serum samples, and the presence of anti-dengue IgM antibody was examined by IgM-capture ELISA. The presence of anti-dengue IgA antibody was examined by IgA-ELISA in Japan, as previously reported by Nawa et al.^[5] In the study, disease day 1* was defined as the day when symptoms were first recognized. Primary or secondary infection was not determined for many of these cases.

Results

Of the 87 cases, 34 cases were RT-PCR positive and defined as dengue confirmed cases in the study (Figure 1A). Of the remaining 53 cases who were RT-PCR-negative or not tested by RT-PCR, 39 were specific IgM-positive and defined as dengue cases (Figure 1B). A total of 123 serum samples obtained from these 73 cases on various days were tested by IgA-capture ELISA.

Anti-dengue IgA antibody responses were examined in 56 serum samples from 34 dengue-confirmed cases (Figure 1A). Anti-dengue IgA antibody was detected in one of 13 samples on disease days 1–3, in 4 of 13 samples on days 4–6, and in all the 13 samples on days 12–18. Specific IgA was not detected in 2 samples collected on days 31 and 32. As a total, 18 of the 34 confirmed cases were IgA-positive and the remaining 16 cases were IgA-negative.

*Some authors define day 1 as day 0 when the symptoms are first recognized – **Editor**

Next, we examined samples from 39 dengue cases, who were diagnosed as dengue only by IgM antibody responses (Figure 1B). A total of 67 serum samples were collected from these 39 cases on disease days 1–62. Specific IgA was first detected on day 5 and last detected on day 39. Specific IgA was not detected in serum samples collected on day 40 and after. The results shown in Figures 1A and 1B suggest that specific IgA start to be detected at several days after the onset of illness, but are not detected after day 40.

The levels of anti-dengue IgA in 119 sequential serum samples from 55 cases are shown in Figure 2. Serum samples were sequentially collected at various intervals during disease days 1–60. The levels of IgA appeared to reach the peak on disease days 10–20 and decline thereafter.

Discussion

The dengue virus infection is confirmed by viral isolation or viral RNA detection in acute-phase serum samples, or by detecting virus-specific IgM in serum samples. Detection of IgM in the single serum sample does not necessarily confirm dengue virus infection of a febrile patient in dengue-endemic countries. It has been reported that IgM can persist for longer than eight months after dengue virus infections.^[6] It has also been reported that serum IgA responds simultaneously with IgM in dengue patients but persists for a shorter period.^[4,5] In order to confirm the usefulness of specific IgA detection for the diagnosis of dengue, we examined specific IgA antibody in the 87 patients who were diagnosed as dengue in Taiwan where dengue is not endemic. A total of 151 serum samples from 87 dengue patients with signs and clinical manifestations consisted of single- and paired-sera from primary and secondary dengue virus infections.



Figure 1A: Detection of dengue virus-specific IgA antibody in serum samples from 34 dengue infection-confirmed cases

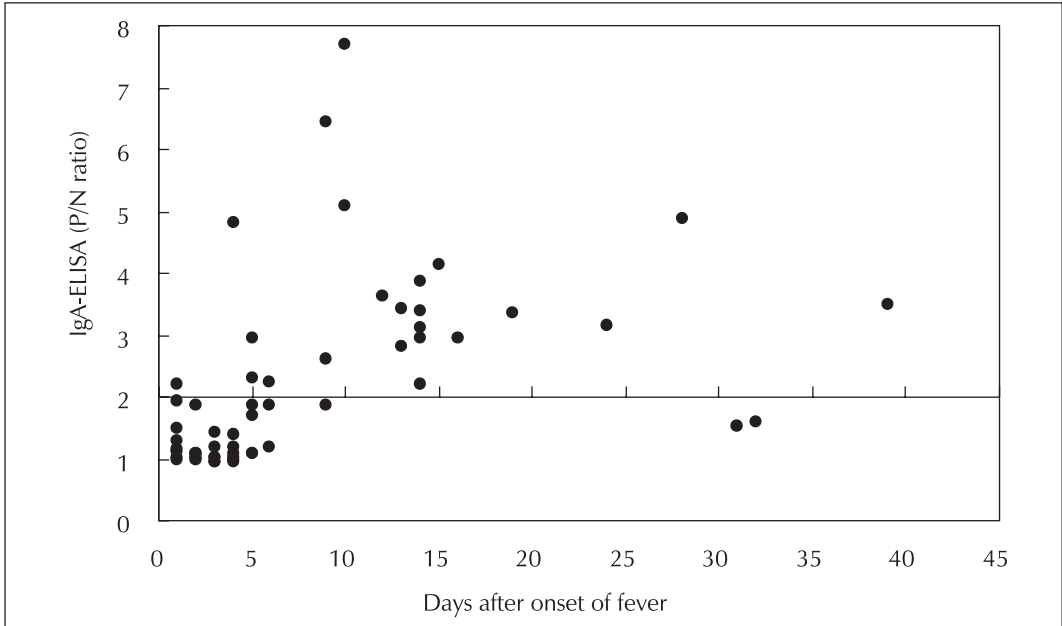
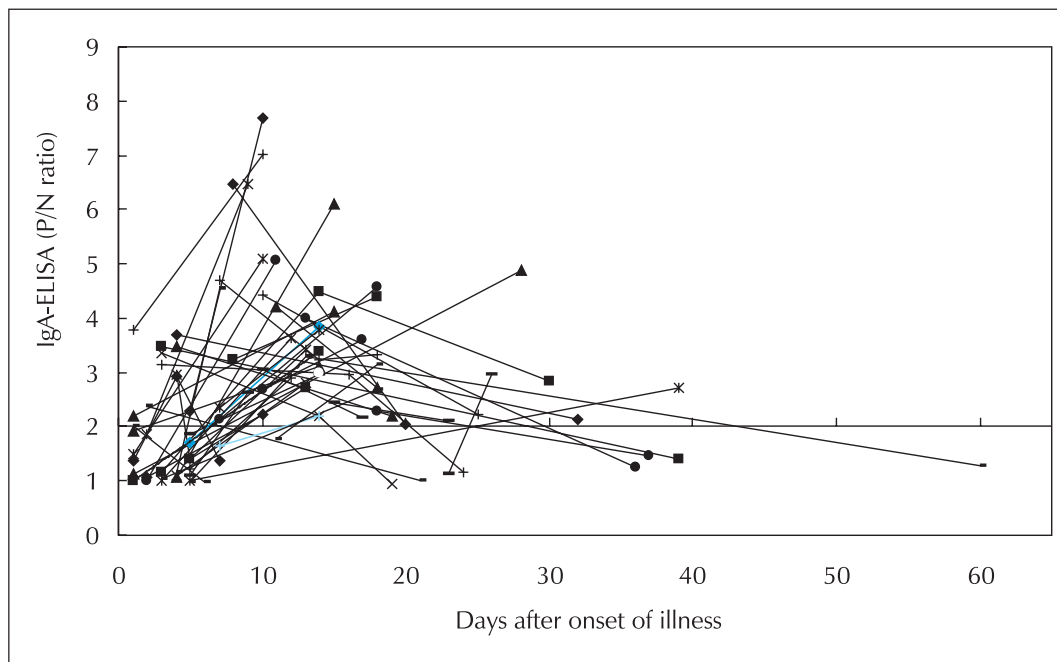


Figure 2: Anti-dengue virus-specific IgA antibody responses in 55 sequential serum samples after the onset of illness



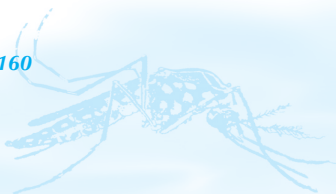
Of the 123 serum samples from 73 cases confirmed by RT-PCR or IgM-capture ELISA, dengue virus-specific IgM was detected in the serum samples from 63 of the 87 cases, and specific IgA was detected in 57 of 63 IgM-positive cases. The 6 discordant cases (IgA-negative and IgM-positive) provided only single serum samples on disease days 3–7. It is expected that IgM is produced prior to IgA in primary infections, and these 6 samples were actually from patients with primary infection. It has been reported that IgA antibody responses in serum were significantly higher in secondary dengue virus infections than in primary infections.^[7]

The present study established the usefulness of IgA-ELISA in dengue diagnosis.

The results suggest that IgA is a useful serological marker for the diagnosis of dengue virus infection, especially when only a single serum sample is available from patients. In the present study, primary or secondary infection was not determined for many of the patients. Dengue-specific IgA responses in primary and secondary infections will be analysed in the next study.

Acknowledgements

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