

Short Communications

Dengue Hemorrhagic Fever in a Japanese Traveler with Pre-existing Japanese Encephalitis Virus Antibody

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Abstract: An adult Japanese man who had just returned from Thailand developed dengue hemorrhagic fever (DHF). A primary infection of dengue virus (DENV) was confirmed, specifically DENV serotype 2 (DENV-2), on the basis of the detection of the virus genome, a significant increase in the neutralizing antibody and the isolation of DENV-2. DHF is often observed following a secondary infection from another serotype of dengue virus, particularly in children, but this case was a primary infection of DENV. Japan is a non-endemic country for dengue disease. In fact, only Japanese encephalitis (JE) is known to be a member of the endemic flavivirus family. In this study, IgG antibody against Japanese encephalitis virus (JEV) was detected. JEV belongs to the family of dengue virus and prevails in Japan, particularly Kyushu. Among many risk factors for the occurrence of DHF, a plausible candidate could be a cross-reactive antibody-dependent enhancement (ADE) mechanism caused by JEV antibody. This indicates that most Japanese travelers who living in dengue non-endemic areas, particularly Kyushu, should be aware of the occurrence of DHF.

Key words: dengue hemorrhagic fever, Japanese encephalitis virus antibody, cross-reactive antibody, imported infection, petechiae, thrombocytopenia, antibody-dependent enhancement (ADE)

INTRODUCTION

Dengue virus (DENV) is a mosquito-borne virus common in tropical and subtropical areas. The prevalence of dengue disease has widely expanded geographically in recent decades [1]. Imported DENV infection is increasing in Japan [2]. DENV infection can result in a sub-clinical infection, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Although most cases are self-limiting febrile illnesses, DHF can be fatal if its plasma leakage is not treated early. There are four different antigenetic serotypes of DENV (DENV-1, DENV-2, DENV-3, and DENV-4). A primary infection with a single serotype leads to an antibody production that cross-reacts with all serotypes. Despite the cross-reactivity, the produced antibody dose not protect against infection from other serotypes [3]. Epidemiological studies suggest that pre-existing cross-reactive antibodies may enhance the severity of the disease following secondary infection with a differ-

ent DENV serotype. Antibody-dependent enhancement (ADE) has been cited as an explanation for the mechanism underlying DHF/DSS. DENV also serologically cross-reacts with Japanese encephalitis virus (JEV), and both belong to the virus family *Flaviviridae*. Although the clinical implications of JEV/DEV cross-reactivity remain undefined, some evidence suggests that infection with pre-existing JEV antibody may be linked to the severity of a subsequent DENV infection [3, 4]. Herein we report the case of a primary DENV-2 infection presenting with DHF with a pre-existing JEV antibody.

CASE REPORT

A 64-year-old male Japanese patient complained of diarrhea, fever (38.2°C) and rash. He was admitted to the Asakura Medical Association Hospital six days after the onset of disease. The patient remembered that he had suffered many mosquito bites nine days before the onset of

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symptoms during a visit to Bangkok, Thailand. The patient presented with a minimal degree of thrombocytopenia (Fig. 1) and a petechial rash on the body, with the exception of his face. A tourniquet test was positive. He also showed liver dysfunction (AST: 72 IU/l, ALT 44 IU/l) and an increase in atypical lymphocyte count (Fig. 1). Hematocrit values were 48% on admission and 40.6% on the third day of admission. Rapid tests for antibodies (SD BIOLINE Dengue IgG/IgM kit, Standard Diagnostics Ltd., Yongin, Korea) and antigens (NS1 Ag Strip, Bio-Rad, Hercules, CA) to DENV were both positive. The patient's physiological parameters on admission were as follows: blood pressure 105/61 mmHg, respiration rate 15/min, and PaO₂ 40.4 mmHg. Neither leukocytosis (white blood cells, 4,800/ μ l) nor inflammation (C-reactive protein, 0.4 mg/dl) was observed. A treatment of intravenous rehydration and rest was prescribed. A significant increase in the white blood cell count was observed on days eight to nine after

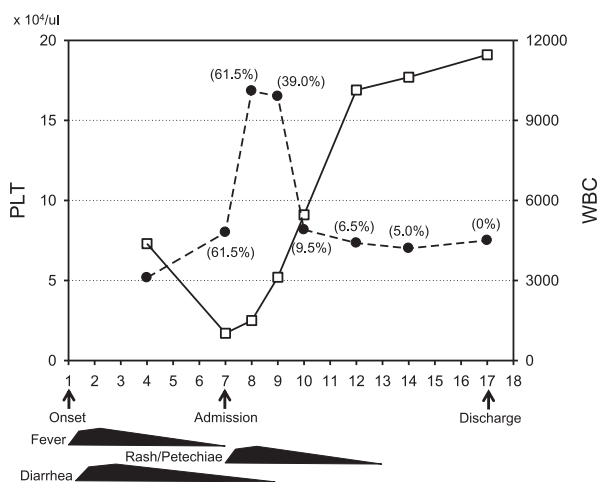


Fig. 1. Clinical course of dengue hemorrhagic fever. Left axis: platelet counts $\times 10^4/\mu\text{l}$ (square); Right axis: white blood cells/ μl (closed circle). % value in the parenthesis shows atypical lymphocytes. Level changes of fever, rash/petechiae, and diarrhea are shown by illustration using distorted chevron.

the onset of disease (Fig. 1). Four days after admission, the leukocytosis had disappeared. The patient's platelet count gradually recovered to a normal level, and he was discharged on day ten after the initial hospitalization. We established a diagnosis of DHF, grade 1.

Since the DENV genome was undetectable using a conventional single RT-PCR [5], we designed new specific primer sets and developed a nested RT-PCR [6]. Total RNA was extracted using whole blood samples from day seven after the onset of disease and subjected to RT reaction using a mixture of three primers, that is, (DNGRT_3048: CTYTCTATCCARTAVCCCAT, DNGRT_2943: ADCCATATRTTGGTHGTGAA, and DNGRT_2657: TCDKWKGHTATTTGYTTCCACA). The RT products were applied to the first PCR using DNGRT_2657 and a FW primer mixture (see below). The second PCR was performed using each of the specific primer pairs, that is, (DNGFW_1: CATCTGGGAGACACTGCATGGGA and DNGRV_1: TGGAATTTATATTGCTCTGTCCA (for DENV-1), DNGFW_2: CATTGGGGCGACACAGCCTGGGA and DNGRV_2: TGGAACTTGTATTGTTCTGTCCA (for DENV-2), DNGFW_3: CATCTGGGAGACACAGCCTGGGA and DNGRV_3: TGAAATTTGTATTGCTCTGTCCA (for DENV-3), DNGFW_4: CATCTAGGTGAAACAGCTTGGGA and DNGRV_4: TGAAATTTGTACTGTTCTGTCCA (for DENV-4)). The cycle conditions were as follows: 45°C for 1 hr (RT step); 40 cycles of 92°C for 1 min, 53°C for 1 min, and 72°C for 1 min (first and second PCR steps). The DENV-2 was identified by amplicon (351 bp) sequencing.

The ELISA test to detect IgG for DENV (Dengue IgG indirect ELISA, Panbio Ltd, Sinnamon Park, Queensland, Australia) was negative on day four but positive on day seven after the onset of disease (Table 1). We isolated dengue virus type 2 from a whole blood sample, which was collected on day four after the onset of disease. Using a plaque reduction assay in C6/36 mosquito cells, we found the neutralization antibody titer to be significantly increased (four-fold, Table 2) against DENV-2 antigen but

Table 1. Virus detection and IgG titers.

Disease days	Virus isolation (DENV)	Real-time PCR*	RT-PCR using newly designed primers	ELISA IgG (DENV) Index**	ELISA IgG (JE) P/N ratio***
4	+(type 2)	+	nt	-(0.63)	+(7.16)
7	nt	nt	+(type 2)	+(2.11)	nt
8	nt	nt	nt	+(2.35)	nt
10	nt	nt	nt	+(2.45)	nt
49	nt	nt	nt	+(2.93)	+(13.7)

* RNA was extracted from isolated dengue virus type 2 [11]. ** Index value more than 1.1 means existence of IgG antibody against dengue virus. *** P/N ratio not less than 2.0 means existence of IgG antibody against Japanese encephalitis virus. nt: not tested.

Table 2. Titer of neutralization antibodies against four dengue virus serotypes.

serotype	disease day	
	4	49
1	< 10 ×	10 ×
2	< 10 ×	40 ×
3	< 10 ×	10 ×
4	< 10 ×	20 ×

not against DENV-1, 3 or 4 antigens. Thus, we confirmed a primary infection with DENV, particularly DENV-2 for this patient. It was also noted that the patient had pre-existing IgG antibody to JEV (Table 1).

DISCUSSION

Between 1 and 3% of all cases of dengue disease exhibit DHF/DSS, and 95% of DHF/DSS occurs in children [7]. The risk of developing DHF is higher with a secondary DENV infection compared with only a primary DENV infection. The patient in the present study developed DHF but was confirmed to have only a primary DENV infection. Epidemiological data reveal several risk factors for severe dengue, including age, sex, high body-mass index, MHC-I related sequence B, and phospholipase C epsilon 1 [1, 8]. Secondary infection by a different serotype has also been cited as a risk factor. A highly pathogenic dengue virus strain was suggested [9]. Among the above, the induction of a previous heterotypic dengue antibody may cause severe dengue through an antibody-dependent enhancement (ADE) mechanism. This mechanism could work even when the previous antibody is derived from another virus, for example JEV. We similarly detected a pre-existing JEV antibody (Table 1). There is at least one study reporting an unusual case where antibodies of DENV and JEV coexisted and presenting several interesting interpretations [10]. The author suggested that co-invasion could occur in an area that is endemic for both viruses. However, only JE is endemic in Japan. Therefore, the cross-reactivity between DENV and JEV suggested that the pre-existing JEV antibody might have been associated with DHF upon primary DENV infection, for example via an ADE mechanism.

The DENV genome can be detected only during the early days of infection before 0-fever day of DF. The detection rate via RT-PCR is reported to be 33% [11]. The virus titer of a DHF patient is supposed to be lower than that of a DF patient [12]. We tested a whole blood sample on the seventh day of disease, and, using a nested RT-PCR with a new design of primers, we were able to detect the

DENV genome whereas a conventional single RT-PCR could not. This nested RT-PCR is applicable to the detection of DENV genome in DHF patient samples.

Recently, dengue infections have increased in Japan among people traveling to endemic areas [13]. Japanese encephalitis (JE) is almost the only endemic flavivirus disease in Japan, particularly in southern regions [14, 15]. An estimated 2% of the population are believed to have been naturally infected with JEV. Vaccination programs have increased the number of people receiving neutralization antibody against JEV, and people who are less than 50 years of age have been targeted. Therefore, some Japanese travelers may have an increased risk of developing DHF via the ADE mechanism. In the United States, the West Nile fever is endemic and corresponds to JE in Japan [16]. West Nile virus (WNV) is a member of the JE serocomplex of the family *Flaviviridae*, genus *Flavivirus*. WNV and DENV have common epitopes [17]. In the United States, imported dengue fever is also increasing [18]. American travelers may have a latent risk of manifestation of DHF through a mechanism similar to that of the case described in this paper. In fact, an unusual manifestation of WNV infection (hemorrhagic fever) was reported in the United States [19]. In that case, a high titer of neutralization antibody against DENV-2 was found retrospectively. Although the present case was a reverse example showing the relationship between WNV infection and the DENV antibody, the severe symptoms caused by WNV could have been modified by the DENV antibody through, for example, an ADE mechanism.

In conclusion, with increases in the global spread of the dengue virus, the risk of DHF or modified symptoms of indigenous flavivirus infection caused by dengue infection might increase in countries that are non-endemic to dengue. Therefore, people in all countries should take precautions against such a risk.

CONFLICTS OF INTEREST

None.

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