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Original article

The world first two cases of severe fever with thrombocytopenia syndrome: An epidemiological study in Nagasaki, Japan

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) caused by the SFTS virus (SFTSV), a novel phlebovirus belonging to the family Bunyaviridae, was reported in China for the first time in 2009. We observed two cases where the SFTSV was isolated for the first time in Nagasaki, Japan, in 2005. Two males in their 60s, a farmer and a hunter, respectively, living in Nagasaki developed SFTS during the same period. The patients developed similar clinical symptoms and signs, such as fever, loss of consciousness, and multiple organ dysfunction. The farmer died and the hunter survived. A retrospective diagnosis of SFTS was made in 2013, and genetic analysis revealed that the patients were infected with different SFTSV strains. Retrospective analysis of cytokine production in non-fatal case revealed interleukin (IL)-6, IL-8 and interferon-γ level of acute phase was low and could be potential prognostic factors. As there are no epidemiological studies of positive rate of SFTSV antibody in people living in endemic areas in Japan, a field study was performed. Volunteers at high risk for tick bites, such as hunters, farmers, and soldiers, were recruited in 6 regions, including the areas where the SFTS cases occurred. Three hundred and twenty six volunteers in Nagasaki prefecture were examined and none of these tested positive for the SFTSV antibody. Our data indicates that the risk for SFTSV infection is not high in Nagasaki prefecture. Further collection of blood samples from endemic areas is warranted for the prevention of SFTSV infection.

1. Introduction

An outbreak of 171 cases of severe fever with thrombocytopenia syndrome (SFTS) caused by the SFTS virus (SFTSV), a novel member of the genus phlebovirus in the family Bunyaviridae, was first reported in China in 2009 [1]. A total of 2047 SFTS cases have been reported in China up to the end of 2012 [2]. SFTS cases were also reported in North Korea [3], South Korea [4], and Japan, including our cases [5] in 2009, 2012, and 2013, respectively. A recent phylogenetic study demonstrated that SFTSV isolated from patients in Far East Asia is divided into two clades, which may have evolved separately over time, except for the rare occasion of overseas transmission [6]. This result also suggests that SFTS may have existed without being diagnosed for a long time. We report

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evidence comprising 2 cases of SFTS that occurred in Nagasaki, Japan, in 2005 and were diagnosed retrospectively in 2013. The two patients had no history of travel abroad, and the isolated SFTSVs were the types that predominantly occur in Japan according to the genetic analysis [5]. To the best of our knowledge, these represent the first cases of SFTS infection worldwide and precede the cases reported in China. We present these earliest cases of SFTS worldwide, which occurred in same area of the SFTSV infection with analysis of cytokine production during the course.

The crude mortality rate of SFTS syndrome ranges from 10% to 30% and the pathogenesis of this syndrome has been reported. A total of 170 laboratory-confirmed SFTS cases were reported in Western Japan up to 24 February 2016 and, of these, 46 patients are dead (the mortality rate is 27.1%). *Haemaphysalis longicornis* and *Rhipicephalus microplus* are the most likely vectors for the transmission SFTSV in China [1,7]; however, there are no studies regarding the prevalence of SFTSV infection among these ticks in Japan.

Zeng et al. investigated SFTSV sero- and viral prevalence among Chinese blood donors. Only 0.27%–0.54% were positive for the SFTSV antibody, and asymptomatic SFTSV viremia was found in 0.02% of 17,208 samples [8]. The background and past medical history of SFTS among sero-positive cases are not described in the study. Nine SFTS cases have been reported in Nagasaki, Japan, to date. Currently there are no epidemiological studies on the prevalence of the SFTSV antibody in endemic areas in Japan; therefore, we conducted a field study.

2. Material and methods

2.1. Cytokine measurement in case

Serum samples from two patients with SFTS were stored in a freezer at a temperature of −20 °C from November 2005. Diagnosis was confirmed by SFTSV antibody detection and virus isolation using common methods that have been previously described [5]. Cytokines including interleukin (IL)-6, interferon (IFN)-γ, IL-1 receptor antagonist (RA), macrophage inflammatory protein (MIP)-1α, MIP-1β, and IL-8 in stored serum samples acquired in different timing in Case 2, were measured by standard ELISA method. Due to low volume of serum sample, cytokine production was not measured in Case 1.

2.2. SFTSV antibody titer measurement

For the epidemiological antibody study, volunteers at high risk for tick bites, such as hunters, farmers, and soldiers, were recruited in 6 regions where the documented cases of SFTS had occurred. Written informed consent was obtained from each volunteer along with information including sex, age, medical history, history of the tick-bite, development of symptoms after the tick-bite, and work location. Then, 6 mL of blood samples were collected and antibody levels were measured using an indirect IgG ELISA, with the recombinant SFTSV-N protein, as described previously [9]. Briefly, recombinant SFTSV-N protein and recombinant Rift Valley fever virus nucleocapsid (RVFV-N) protein are used as positive and negative antigens, respectively. Serum samples from one case of confirmed SFTS and from a healthy volunteer were used as positive and negative controls, respectively. A serum sample in this ELISA test was considered positive if the adjusted OD value was greater than or equal to the assay cut-off of 0.2. We adhered to the Japanese ethical guidelines for epidemiological studies, and the ethics committees of Nagasaki University Hospital approved the protocol for this study.

3. Results

3.1. Case reports

The serum samples from two patients with SFTS had been stored at −20 °C since November 2005. Diagnosis was confirmed on the basis of SFTSV detection and antibody detection in Spring, 2013.

3.1.1. Case 1

A 62-year-old farmer who lived in the Northern part of Nagasaki city presented with fever, respiratory, and neurological symptoms. He visited the local hospital (hospital A) on the third day after the onset of fever. There was no past medical history of note. On the fourth day, he developed tremor of the extremities and headaches. He became restless and disorientated on the seventh day. He did not complain of gastrointestinal symptoms during the course. He was admitted to the hospital. On admission, physical examination revealed the following: pulse, 94/minute with sinus rhythm; blood pressure, 130/70 mmHg; body temperature, 38.5 °C; oxygen saturation, 93% (at room atmosphere) and diminished consciousness (Glasgow Coma Scale: E4V3M5). There was no history of a tick bite and no obvious eschar although the patient's occupation was associated with an increased risk of tick bites. Chest and abdominal radiographs and magnetic resonance imaging (MRI) of the brain revealed no abnormalities. A spinal fluid test showed no increase in the number of cells, an opening pressure of 120 mm H_2O, and a closing pressure of 70 mm H_2O. Bicytopenia of white blood cells and of platelets, disseminated intravascular coagulation (DIC), liver dysfunction, and renal failure were diagnosed on admission (Table 1). Aseptic encephalomeningitis with multiple organ failure and DIC were suspected, and treatment with electrolyte infusion and nafamostat mesilate were commenced. No antibiotics were administered. The patient developed shock 58 h after admission and died 64 h after admission. (10th day after onset of fever).

3.1.2. Case 2

A 58-year-old hunter with a past history of tick bites, presented with a fever 7 days after being bitten by a tick in the mountains of central Nagasaki prefecture during wild boar hunting. He also complained of nausea, vomiting, and diarrhea on the second day after the onset of fever. He was admitted to a local hospital on the third day after the onset of symptoms. He became disorientated and subsequently developed hallucinations and convulsions. He was admitted to our intensive care unit on the sixth day after the

Table 1
Laboratory findings on admission for Case 1 and Case 2.

	Case 1	Case 2
WBC ($\times 10^3/\mu L$)	1.5	1.7
Hemoglobin (g/dL)	16.1	18.4
PLT ($\times 10^4/\mu L$)	1.6	3.0
PT-INR	4.9	1.4
APTT (s)	34.9	75.9
Cr (mg/dL)	2.3	1.3
ALP (IU/L)	305	433
AST (IU/L)	907	981
ALT (IU/L)	310	434
LDH (IU/L)	1926	2679
CPK (IU/L)	2523	901
CRP (mg/dL)	0.09	0.38

WBC, white blood cell counts; PLT, platelet; PT-INR, prothrombin time-International Normalized Ratio; APTT, activated partial thromboplastin time; Cr, serum creatinine; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; CRP, C-reactive protein.