CASE REPORT

HANTAVIRUS INFECTION IN THAILAND: FIRST CLINICAL CASE REPORT

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Abstract. This study involved 115 cases of fever of unknown origin (FUO) patients who were admitted to the Department of Medicine, Siriraj Hospital from May 1999 to November 2000. Among the patient sera screened by ELISA for IgG Hantavirus, five were positive for IgG Hantavirus-reacting antibodies and eight tested positive for IgM Hantavirus-reacting antibodies. One serum had both IgG and IgM antibodies. The patient exhibited acute encephalitic febrile illness, thrombocytopenia, high AST and ALT levels, and prolonged coagulation time. It appears that a form of the Hantaan virus is circulating in Thailand, which can infect humans and be pathogenic in some instances.

INTRODUCTION

Hantaviruses are negative-stranded RNA viruses of the Bunyaviridae family (Schmaljohn *et al*, 1985). Their role as a causative agent of hemorrhagic fever with renal syndrome (HFRS) in the Old World and Acute Respiratory Syndrome in the Americas is well established (Ksiazek *et al*, 1995). Hantavirus infection is a zoonosis associated with a rodent reservoir species of the Muroidae family. Clinical human cases have been described in Korea, China, Japan (Haantan-type viruses); Scandinavia, Holland, the United Kingdom, France, Belgium (Puumala-type viruses); the Balkans (Puumala, Belgrade, and Porogia virus types); and North and South America (Sin Nombre virus types) (Schmaljohn and Hjelle, 1997). Human infection generally occurs via inhalation of an aerosolized virus excreted in rodent feces, urine or saliva (Nuzum *et al*, 1988). Different Hantavirus types have been associated with specific rodents (genotypes and serotypes) worldwide.

Clinical manifestations are characterized by a febrile phase accompanied by headache, abdominal and lumbar pain, facial flush, and petechiae. After three to five days, the febrile phase is followed by a hypotensive phase during which shock can occur, often in association with low cardiac output and increased systemic vascular resistance. The oliguric phase transpires subsequently, with a common photocytosis. Metabolic acidosis with a decreased bicarbonate level and lactic acidemia has also been witnessed in severe cases. The fibrinogen level is generally normal. Mild to moderate proteinnuria is frequently present, but frank renal failure is not a feature in most cases; mild elevation in the creatinine level (usually <2.5 mg/dl) occurs only in severe cases (Butler and Peters,1994).

Serological survey, both in rodents and humans, has shown that Hantavirus infection can also circulate in Thailand, although clinical cases have never been documented (Elwell *et al*, 1985; Sawasdikosol *et al*, 1989; Nitatpattana *et al*, 2000, 2002). This paper reports the first clinical case of Hantavirus infection in Thailand.

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MATERIALS AND METHODS

Hantavirus biological tests were used as a diagnostic to exclude patients with infectious clinical syndromes.

Human cases

This study involved 115 cases of fever of unknown origin (FUO) patients who were admitted to the Department of Medicine, Siriraj Hospital from May 1999 to November 2000. The surveillance criteria of FUO admissions were: fever (≥38ºC) for at least three days and no signs of gastrointestinal infection or upper respiratory infection.

Enzyme-linked immunosorbent assays (ELISA) for Hantavirus serology IgG

ELISA plates (MaxiSorp; Nunc, Roksile Denmark) were coated with a 1:1,000 diluted Hantavirus antigen (Hantaan ROK 84/105 SMRV, kindly provided by Professor Ho Wang Lee), in sterile PBS (pH 7.2) and a negative uninfected cell culture (1:100), in sterile PBS (pH 7.2). These were dispensed at 100 μl per well, with a positive and a negative antigen colon overnight at 4ºC. The plates were then washed with 0.5% PBS/tween; 100 μl of human serum (1:100 dilution in PBS/tween/3% skim milk) was added to the plate wells and incubated for one hour at 37ºC after washing three times with 0.5% PBS/tween; 100 μl of HRP/anti-human IgG (HRP conjugate goat anti-human IgG, ZYMED) was added and the plate wells were incubated for one hour at 37ºC. The plates were washed three times with 0.5% PBS/tween, and 100 μl OPD (O-Phenylenediamine). A substrate was added and incubated for 30 minutes at room temperature in the dark. The reaction was stopped with 50 μl 4M H_2SO_4, and the plates were read in a Metertech Σ490 spectrophotometer at a wavelength of 490 nm. The cut-off value for the presence of antibodies was defined as three times the negative control absorbance. The sample of positive sera was checked against the Seoul and Puumala antigens and did not react (Centre Nationale de Reference, Institute Pasteur, Paris, France; Dr Hervé Zeller).

Enzyme-linked immunosorbent assays (ELISA) for Hantavirus serology IgM

ELISA plates (MaxiSorp; Nunc, Roksile Denmark) were coated with a goat anti-human IgM gamma chain specific antibody (KPL, USA) at a concentration of 1 μg/ml in a carbonate buffer. The serum samples and the positive/negative controls (0.1 ml) were diluted at 100 in 0.05%/tween 20 with PBS containing 3% non-fat dried milk and were added to the two wells. After one hour of incubation at 37ºC, the plates were washed with 0.05% PBS/tween and then reacted with the HTN-antigen (Hantaan ROK 84/105 SMRV, kindly provided by Professor Ho Wang Lee) or the uninfected control fluid. After being left over night at 4ºC, the plates were washed and given an additional anti-HTN incubation of one hour (at 37ºC), washed again and applied with mouse monoclonal anti-HTN. Subsequently a goat anti-mouse IgG conjugated antibody (Zymed, USA) and a 100 μl OPD (O-Phenylenediamine) substrate was added and incubated for 30 minutes at room temperature in the dark. The reaction was stopped with 50 μl 4M H_2SO_4, and the plates were read in a Metertech Σ490 spectrophotometer at a wavelength of 490 nm. The cut-off value for the presence of antibodies was defined over three standard deviations from the mean of eight negative controls' absorbance.

Indirect immunofluorescent test (IFA)

The IFA was conducted using Hantavirus (Hantaan-infected cells) on spot slides ordered from the PROGEN Biotechnik GmbH D-69123 Heidelberg/Germany (Cat. No./Kat.-Nr.: PR77065). The inactivated antigen slides were fixed with cold acetone for 10 minutes and dried at room temperature. Ten microliters of PBS-diluted human sera (1: 16, 1: 32 and 1: 64) were then added to the spot slides. The serum-positive control was supplied by PROGEN Biotechnik. After 30 minutes of incubation in a moisture chamber at room temperature, the slides were washed in the pH 7.4 PBS for 10 minutes. They were then stained with 10 microliters of rabbit anti-human conjugated with Fluorescein-6-isothiocyanate (FITC) then incubated in a moisture chamber at room temperature for 30 minutes. After washing, the slides were mounted with buffer glycerol and examined by fluorescent microscope.

RESULTS

Among the 115 FUO-selected patient sera screened by ELISA for IgG Hantavirus, five were

Table 1
FUO patient sera tested for *Hantaan* virus reacting antibodies (ELISA) and IFA test, May 1999 -November 2000, Thailand.

No.	Test	IgG	IgM	IgG+IgM
1	ELISA	5/115	8/115	1/115
2	IFA	0/5	0/8	1/1

Table 2
FUO patient sera testing positive for *Hantaan* virus by ELISA (IgG/IgM) and IFA test.

Sera sample	Day of onset	ELISA		IFA
		IgG	IgM	
Acute	5	Neg	>12,800	1:512
Convalescent	14	1:12,800	1:1,600	1:512

ELISA cut-off positive ≥OD

positive for the IgG Hantavirus-reacting antibody and eight tested positive for the IgM Hantavirus-reacting antibody. One serum had both IgG and IgM antibodies (Table 1). The sera were confirmed by IFA test titration and paired sera of the patients were tested (Table 2).

The subject was a 16-year old student living in the Bangkok Metropolitan Area. She was admitted to Siriraj Hospital in July 1999 with high fever, headache, nausea, vomiting at one day of onset and an alteration of consciousness which started before admission. She had no other constitutional symptoms and no relevant medical history. She did not abuse drugs, keep any pets, or work on a farm; she had not travelled within the past two months to forested or other rural areas. Physical examination revealed a body temperature of 40ºC, a respiration rate of 24/minute, a pulse rate of 120/minute, and blood pressure of 100/60 mmHg. She was confused, agitated, and mildly tachypnic. The rest of the physical examination was unremarkable. A complete blood count revealed pack cell volume of 42.8%, WBC of 2,610/mm^3, and a platelets count of 21,000/mm^3. Urinalysis showed heavy proteinuria (4+) with microscopic hematuria. The serum creatinine was 1.2 mg/dl; the blood urea nitrogen was 13 mg/dl; the total bilirubin level was 0.8 mg/dl; the AST level was 402 IU/l; the ALT level was 331 IU/l; and the alkaline phosphatase level was 89 IU/l. The prothrombin time was 19.6 seconds (normal is 10 to 15 seconds) and the activated partial thromboplastin time was 46.7 seconds (normal is 24 to 38 seconds). The initial diagnosis was acute viral encephalitis. However, the cerebrospinal fluid analysis was completely normal. All cultures obtained from blood and cerebrospinal fluid were negative. Therefore, she received oral doxycycline (200 mg/day) for the treatment of scrub typhus for five days. She had no bleeding complications and no oliguria. Her level of consciousness improved and she became afebrile on day 5 after admission. Thrombocytopenia disappeared on day 4 and she was discharged from the hospital on day 6. All laboratory abnormalities returned to normal two weeks after discharge.

The serological tests performed for leptospirosis, dengue fever, Japanese B encephalitis virus, hepatitis A and B virus, and scrub typhus were negative.

DISCUSSION

For serological considerations of the patients suspected of having Hantavirus infection, the IFA was used as a confirmatory test; among the sera with IgG HTV-reacting antibodies, only one tested positive from the IFA.

Although the serological testing used only the Hantaan virus antigen, some sera showed Hantaan positive-reacting antibodies by ELISA but tested negative against Sin Nombre and Puumala virus antigens.

Also the antibody-reacting Hantaan virus antigen could be related to another closely related type of Hantaan-like virus yet to be discovered.

IgG-reacting sera confirm the correlation of a form of the Hantaan virus in the human population which has been suspected for several years. IgG or reacting sera testing only positive for IgM are suspected of being a more specific reaction and often associated with a leptospirosis infection (Bernadette Murgue, personal com-

munication, IRD, 2003). A cross-reaction of antibodies against the Hantaan virus detected by ELISA with other microorganisms such as *Leptospira interrogans* and *Orientia tsutsugamushi*, was observed in this study. A previous study also showed an antibody of hemorrhagic fever with renal syndrome virus to be related to the B-1 virus (Sawasdikosol *et al*, 1989) in 4/174 human sera. Therefore, the IFA test was used as the confirmation test in this study.

The patient exhibited acute encephalitic febrile illness, thrombocytopenia, high AST and ALT levels, and prolonged coagulation time. A different Hantavirus type was suspected, because her clinical signs overlapped between the Puumala serotype, in which thrombocytopenia is common, and the Seoul subtype, in which hepatitis is common. However acute encephalitis is not a major symptom in both subtypes. A prospective study to detect and isolate the virus from human Hantavirus infection in Thailand is ongoing.

In Thailand the Hantavirus antibody was found in *Bandicola indica*, *Rattus rattus*, and *R. norvegicus* in various regions. This indicates widespread Hantavirus infection in the country. This report confirms that Hantavirus infection is a worldwide problem, and most likely to be underdiagnosed in Thailand and other countries where serological diagnosis is not widely available. In conclusion, a Hantaan virus form appears to be circulating in Thailand, which can infect humans and be pathogenic in some instances.

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