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Detection of Hantaan Viral Antigens in Renal Tissues from a Patient with Korean Hemorrhagic Fever in Convalescent Phase†

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=Abstract=**A case of a patient with Korean hemorrhagic fever (KHF) who showed typical clinical manifestations is described for the purpose of reporting the detection of Hantaan viral antigens in renal tissues. The pathophysiologic mechanisms of renal damage are not well known, and several mechanisms including direct renal injury by the virus itself have been proposed to explain the renal lesions and the clinical manifestations. We detected Hantaan viral antigens in renal tissues from a KHF patient in the convalescent phase by the immunohistochemical method using the monoclonal antibodies to Hantaan viral envelope glycoproteins (G1, G2). The immunostainings demonstrated Hantaan viral antigens in the renal tubular epithelial cells, the intraluminal desquamated tubular cells, the infiltrated cells in the interstitium, and the capillary endothelial cells in the interstitium and glomeruli. The presence of viral antigens in renal tissues may support the hypothesis that direct renal injury by the virus is one of the pathophysiologic mechanisms of renal damage in KHF**

Key Words: *Korean hemorrhagic fever, Hantaan virus, Envelope glycoprotein, Immunohistochemistry, Monoclonal antibodies*

INTRODUCTION

Korean hemorrhagic fever (KHF) is an acute febrile viral disease. The etiologic agent, Hantaan virus, was first isolated in 1978 (Lee *et al.* 1978). Thereafter, other causative viruses were identified and many clinical and experimental studies were performed in order to elucidate the pathophysiologic mechanisms of KHF. But the fatality rate of KHF is still high (up to 7%), and the primary shock is the leading cause of death (Lee 1991). So, elucidation of the pathophysiology of KHF is strongly needed to develop new treatment modalities.

The direct renal injury by the virus, renal

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injury secondary to several mediators including kallikrein-kinin (Han *et al.* 1989), and other mechanisms have been proposed. But the exact pathophysiologic mechanisms for renal injury have not so far been well established. A direct renal injury by the virus or viral toxic substances was suggested as one of the possible pathophysiologic mechanisms of the renal damage (Collan *et al.* 1978; Dantas *et al.* 1986; Jokinenm *et al.* 1977; Smorodintsev *et al.* 1963). But the etiologic agents, Hantaan virus or its related viruses, have not been demonstrated in human renal tissues. Recently, we detected the viral envelope antigens in a KHF patient in the convalescent phase whose renal and other biochemical functions were nearly normalized. This finding may support the direct renal injury hypothesis. So for this reason we report this case.

CASE REPORT

A 25 year old Korean man was admitted to Seoul National University Hospital (SNUH) because of fever and oliguria. He had been well upto 4 days earlier, when he began to suffer from mild fever, headache and generalized myalgia. Two days before admission, high fever and chills developed suddenly. One day before admission, he entered another hospital because the fever continued and generalized myalgia was aggravated. On the next day, the fever was resolved, but the systolic blood pressure had fallen to 70mmHg and urine output was decreased markedly. So, he was transferred to the SNUH emergency room for further evaluation and management.

On arrival in the emergency room, his temperature was 35.8° C, the pulse rate 150/min and the respiration rate 32/min. The blood pressure was too low to be measured. The physical examinations revealed that the patient looked severely ill and irritable, but mental state was clear. The limbs were cool and clammy, and cyanosis was present in the lips and the fingers. Multiple petechiae were found on the soft palate and both axillas. The conjunctival suffusion and chemosis were present in both eyes. No lymphadenopathy was found.

The hematocrit was 65.3 percent and hemoglobin was 24.1 gm/dl. The white blood cell count was 38,100/mm^3, and the platelet count 23,000/mm^3. Blood urea nitrogen (BUN) was 21 mg/dl and creatinine 1.4mg/dl. Arterial blood gas analysis was done while the patient was breathing room air, and it disclosed that the partial pressure of oxygen (PaO_2) was 32 mmHg, partial pressure of carbon dioxide ($PaCO_2$) 41 mmHg, pH 7.26 and bicarbonate (HCO_3^-) 18 mmol/L. On arrival, colloid solutions and dopamine were injected intravenously for the management of shock, and blood pressure was raised to the range of 90 to 160 mmHg systolic. He was admitted to an intensive care unit for conservative treatment and careful monitorings.

On the following day (the 4th day of illness, counted from the day of fever onset), blood pressure ranged from 110 to 150 mmHg systolic although dopamine was discontinued. On examination, the patient was less irritable than the previous day, but the face looked more puffy. Urine volume was 200 ml for the 24 hours. The urinalysis revealed that the albumin was 4(+), and blood 3(+); the sediments contained many red blood cells, 5 to 7 white blood cells, and many bacteria per high power field. Hematocrit was 60.2 percent, hemoglobin 19.0gm/dl, white blood cell count was 54,400/mm^3; 69 percent neutrophils, 4 percent band forms, 19 percent lymphocytes, 2 percent monocytes, 2 percent eosinophils, and 4 percent atypical cells. The platelet count was 9,000/mm^3 and the erythrocyte sedimentation rate was 1 mm/hr. The prothrombin time was 1.57 in International Normalized Ratio and activated partial thromboplastin time was 82 seconds. Fibrinogen was 350 mg/dl and a test for fibrin/fibrinogen degradation products was more than 40 μg/ml. BUN was 82 mg/dl, creatinine 4.7 mg/dl, total bilirubin 1.2 mg/dl, uric acid 10.0 mg/dl, cholesterol 82 mg/dl, HDL-cholesterol 8 mg/dl, calcium 6.8 mg/dl, phosphorus 3.2 mg/dl, and total protein 5.1 gm/dl (albumin 2.9 gm/dl). Sodium was 129

mmol/L, potassium 3.9 mmol/L, chloride 101 mmol/L, and total carbon dioxide 10 mmol/L. Serum aspartate aminotransferase(AST) was 331 U/L, serum alanine aminotransferase (ALT) 81 U/L, and lactic dehydrogenase 1616 U/L. Total hemolytic complement (CH_{50}) was 11.0 u/ml, C_3 27 mg/dl, and C_4 30 mg/dl. A test for Hantaan virus antibody in serum by indirect immunofluorescent technique was positive in a titer of 1:80. An electrocardiogram and an X-ray film of the chest were normal.

During his hospitalization, the oliguria continued till the 12th hospital day (the 14th day of illness), after that day diuresis developed, and he received hemodialysis on 9 different occasions during the oliguric period. The serum creatinine concentration increased up to 16.8 mg/dl on the 13th hospital day (the 15th day of illness), and then decreased and normalized with diuresis. A follow-up test for Hantaan virus antibody by indirect immunofluorescent technique was positive in a titer of 1:640. Magnetic resonance images of the kidney and sella, performed on the 17th hospital day (the 19th day of illness, the diuretic phase), revealed low signal intensity in the renal medulla, and a hemorrhagic lesion in the anterior pituitary gland (Fig. 1). The cocktail test, performed on the 30th hospital day (the 32th day of illness, the convalescent phase) showed flat response compatible with panhypopituitarism. During the convalescent phase, when the serum creatinine concentration was normalized, the polyuria persisted and daily urine volume reached 13,310ml on the 39th day of his illness, serum sodium was 143 mmol/L, serum osmolarity 289 mOsm/kg, urine sodium 44 mmol/L, and urine osmolarity 149 mOsm/kg. On the 38th hospital day (the 40th day of illness), the water deprivation and pitressin test was performed and the result was compatible with central diabetes insipidus. With dDAVP nasal sufflation, the urine output was decreased to within normal range. A kidney needle biopsy was done on the 30th day of illness, when the serum creatinine concentration was 2.6 mg/dl. Microscopically, the glomeruli were normal in size, shape

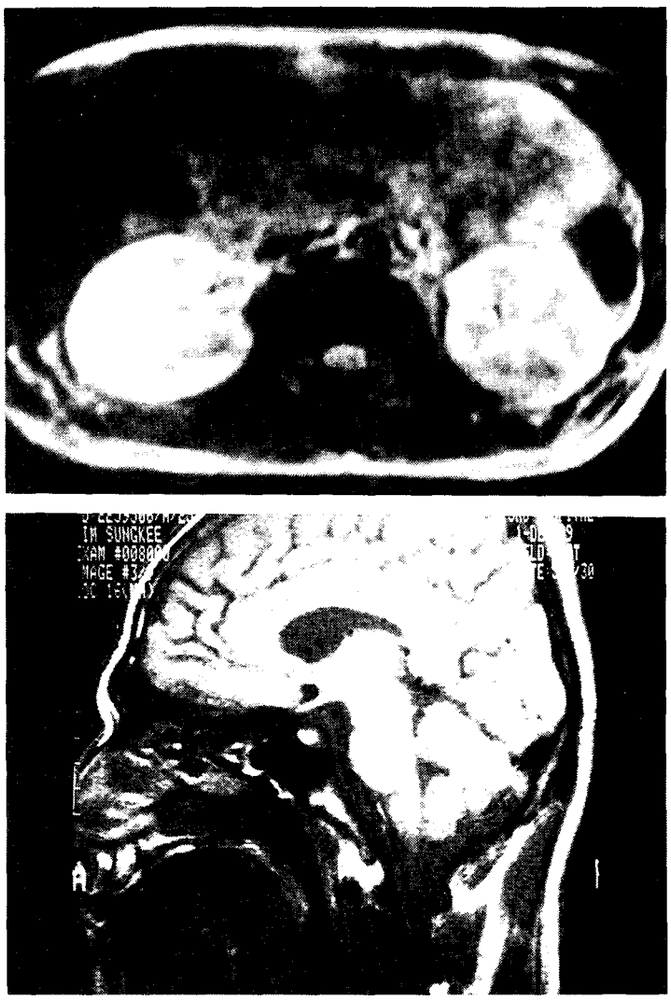

Fig. 1. The kidney (A) and sellar (B) MRI findings. These figures show low signal intensity in the renal medulla, and high signal intensity suggesting hemorrhage in the anterior pituitary gland.

and approximation except for a slight increase of mesangial matrix. Renal tubules showed mild dilatation with denudation of epithelial cells. There was mild interstitial fibrosis and edema with patchy round cells infiltration (Fig. 2).

For the detection of Hantaan viral antigens, the 6 monoclonal antibodies to the G1 or G2 envelope glycoproteins of Hantaan virus (HCO2, EBO6, 11E10, 3D7, 8E10, JDO4 ; supplied by the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, U.S.A.), which were prepared from hybridomas between SP 2/0-Ag 14 myeloma cells and the spleen cells from mice immunized with a suspension of immune-precipi-

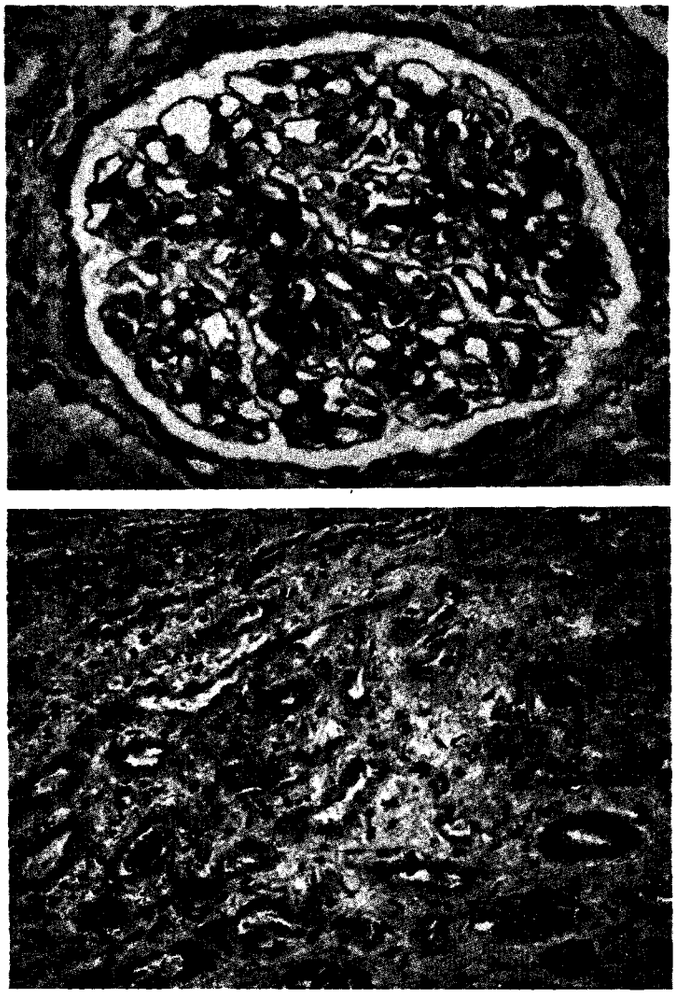

Fig. 2. The kidney biopsy findings (light microscopy). The glomeruli are normal in size, shape and approximation except a slight increase in the mesangial matrix (A). The renal tubules are mildly dilatated with denudation of epithelial cells. There is mild interstitial fibrosis and edema with patchy round cell infiltration (B).

tated viral envelope glycoproteins purified by binding to the Protein-A Sepharose beads, were reacted with the previously prepared biopsy specimen and stained by the indirect immunoperoxidase technique. These procedures were performed by following the standard immunohistochemical method. The fluorescent reactions to the Hantaan viral envelope glycoproteins were positive in the tubular epithelial cells, the intraluminal desquamated tubular cells, the infiltrated cells in the interstitium, and in the capillary endothelial cells of the interstitium and glomeruli (Fig. 3). The antigens were

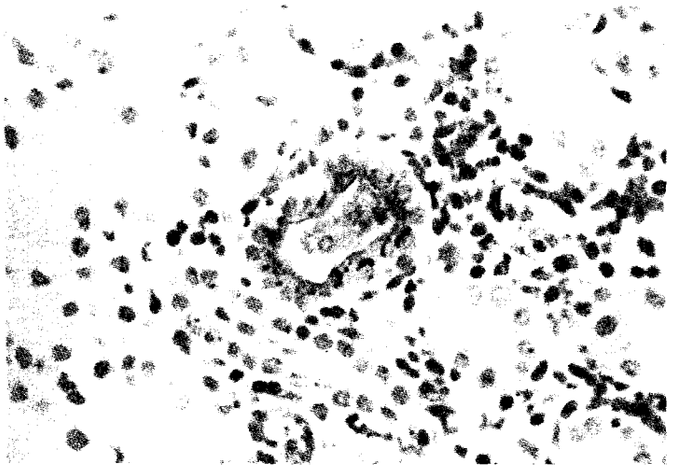

Fig. 3. The immunohistochemical stainings. The fluorescent reactions are positive in the tubular epithelial cells, the intraluminal desquamated tubular cells, the infiltrated cells in the interstitium, and in the capillary endothelial cells of the interstitium and glomeruli.

located mainly in the cytoplasm of the renal tubular epithelial cells and the capillary endothelial cells.

DISCUSSION

Korean hemorrhagic fever (KHF) is a disease characterized by an acute onset with fever, circulatory collapse, hemorrhagic tendency and acute renal failure. Most patients recover after such a course (Lee 1991). The most prominent pathologic features in KHF are vascular changes in capillaries, which are characterized by diffuse arteriolar dilatation, congestion, extravasation of the red blood cells and plasma, and focal disruptions of the capillary wall integrity that finally lead to diffuse capillary endothelial cell damage (Hullinghorst and Steer 1953). The kidney is one of the 3 major organs in which the hemorrhagic phenomena are most prominent (Lukes 1954). The microscopic findings in the kidney vary greatly depending on the site of biopsy, on the stage, and on the severity of the disease (Oliver and McDowell 1957), but are mainly tubulointerstitial changes ; tubular dilatation, tubular cell atropy, interstitial edema, occasionally medullary

hyperemia or hemorrhages, and cellular infiltrations composed of lymphocytes and, less frequently, plasma cells and polymorphonuclear cells (Hullinghorst and Steer 1953; Smorodintsev *et al.* 1963; Steer 1955).

Many investigators have studied the etiologic agent and the pathophysiologic mechanisms since the 1930's, and the causative agent, a single-stranded RNA virus named Hantaan virus, was first isolated in 1978 from rodents, *Apodemus agrarius coreae,* and from the affected person (Lee et al. 1978). Possible pathophysiologic mechanisms for the development of renal lesions have also been suggested by some authors ; a direct renal injury by the virus or viral toxic substances (Collan *et al.* 1978; Dantas *et al.* 1986; Jokinenm *et al.* 1977; Smorodintsev *et al.* 1963), immunologic mechanisms mediated by the immune complex, complement system, kinin-kallikrein system or fibrinolysis system (Han *et al.* 1989; Jokinenm *et al.* 1977; Park *et al.* 1986), and hemorrhages induced by thrombocytopenia and/or intravascular coagulopathy (Lee *et al.* 1989). But the exact mechanisms that lead to the endothelial cell damage have so far not been made clear, and the sites of distribution of Hantaan virus in tissues have not been disclosed as yet. So, we tried to demonstrate the Hantaan viral antigens in renal tissues obtained by biopsy, which support the hypothesis that direct injury by the virus may be the pathophysiologic mechanism of the renal damage.

Hantaan virus is the prototype virus of the Hantavirus genus of the Bunyaviridae family (McCormick et *al.* 1982; Schmaljohn *et al.* 1983; White *et al.* 1982). Like other Bunyaviridae, Hantaan virus possesses a three-segmented, single-stranded RNA genome of negative polarity (Schmaljohn and Dalrymple 1983). The large (L), medium (M), and small (S) genome segments have relative molecular masses of approximately 2.7, 1.2, and 0.6 $\times$ 10^6, respectively, as estimated by agarose gel electrophoresis, and are enclosed in three separate nucleocapsid structures which are surrounded by a lipid envelope containing two virus-specified glycoproteins. The S segment encodes the nucleocapsid protein and the M segment encodes two envelope glycoproteins, designated as G1 and G2, and the L segment is presumed to encode a polymerase protein (Elliot *et al.* 1984; Schmaljohn and Dalrymple 1983; Schmaljohn *et al.* 1983; Schmaljohn et al. 1985; Schmaljohn *et al.* 1986; Schmaljohn *et al.* 1987). Hantaan viral envelope proteins possess hemagglutinating activity, and induce pH-dependent cell fusion (Arikawa *et al.* 1985; Okuno *et al.* 1986; Tsai *et al.* 1984). These functions are thought to play important roles in virus attachment to the susceptible cell surfaces and in the uncoating of virions during the initial stages of infection. The viral neutralization sites are known to be located on the G1 and G2 proteins (Dantas *et al.* 1986; Yamanishi *et al.* 1984). With these findings, the M genome segment and the envelope glycoproteins of Hantaan virus have been implicated as containing the major determinant of virulence of the virus (Jansen *et al.* 1986; Shope *et al.* 1981). Therefore, we used the monoclonal antibodies to G1 or G2 envelope glycoproteins of Hantaan virus.

The patient presented to us with the typical clinical manifestations of KHF, and the diagnosis was confirmed by an 8 fold rise in the serum antibody titers for Hantaan virus during the oliguric phase. He was managed with conservative treatment including hemodialysis, and recovered from the illness except for the panhypopituitarism and the central diabetes insipidus. The renal biopsy was performed on the 30th day of illness, when the serum creatinine was 2.6 mg/dl and his general condition was almost recovered.

The biopsy specimens, which were reacted with the monoclonal antibodies to the envelope glycoproteins of Hantaan virus, demonstrated the Hantaan viral antigens in the renal tubular epithelial cells, the intraluminal desquamated tubular cells, the infiltrated cells in the interstitium, and the capillary endothelial cells of the interstitium and glomeruli. The detection of Hantaan viral antigens in the human renal tissues from a KHF patient in convalescent phase is

probably the first in the world as far as the literature concerned. This finding may support initial step of the hypothesis that the renal lesions may be from direct injury of the Hantaan virus. However, the detection of viral antigens in renal tissues cannot be direct evidence of viral replication and its functional participation in the pathophysiologic mechanism of renal injury. Still, the isolation of the virus and detection of viral genome in renal tissue has not been performed. Therefore, our report is insufficient to prove the hypothesis that direct renal injury by the virus is one of the pathophysiologic mechanisms of renal lesions. So, further studies including the isolation of the virus and the detection of viral genome in renal tissue are required to confirm the pathophysiologic mechanism of renal injury in KHF.

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