First Cases of Hemorrhagic Fever with Renal Syndrome from the Middle Anatolia Region of Turkey and the First Case of Hantavirus and Crimean-Congo Hemorrhagic Fever Virus Co-Infection in a Patient

Türkiye'nin Orta Anadolu Bölgesi'nden Renal Sendromlu İlk Kanamalı Ateş Olguları ve Hanta Virüs ile Kırım Kongo Kanamalı Ateş Virüsü Koenfeksiyonu Olan İlk Olgu

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ABSTRACT The first laboratory confirmed cases of hemorrhagic fever with renal syndrome caused by hantavirus infection in Turkey were published in 2009. We reported the first cases of hantavirus infection from the Middle Anatolia Region of Turkey as well as the first case of co-infection caused by hantavirus and Crimean-Congo hemorrhagic fever (CCHF) virus in a patient. The co-infected patient was tested for hantavirus infection because the duration of the illness lasted longer than expected for CCHF and renal function was impaired. Diagnosis of CCHF was confirmed with serological and molecular methods; in addition, IgM and IgG antibodies for hantavirus were positive. All three patients were discharged after they received supportive therapy.

Key Words: Hantavirus; hemorrhagic fever with renal syndrome; hemorrhagic fever virus, crimean-congo

ÖZET Hantavirüsün neden olduğu, renal sendrom ile seyreden ve laboratuvar tanıları doğrulanmış ilk kanamalı ateş olguları Türkiye'de 2009 yılında yayımlanmıştır. Bu makalede, Türkiye'nin Orta Anadolu bölgesinden ilk hantavirüs enfeksiyonu olguları ve hantavirüs ve Kırım Kongo kanamalı ateş (KKKA) virüsünün birlikte neden olduğu ilk koenfeksiyon olgusu bildirilmiştir. Koenfeksiyonu olan hasta, hastalık süresinin KKKA için beklenenden daha uzun olması ve böbrek fonksiyonunun bozulması nedeniyle hantavirüs enfeksiyonu açısından serolojik olarak incelenmiştir. KKKA tanısı serolojik ve moleküler yöntemlerle doğrulanmıştır; ayrıca hastada, hantavirüs IgM ve IgG antikorları da pozitif bulunmuştur. Üç hasta da uygun destek tedavinin uygulanmasının ardından taburcu edilmiştir.

Anahtar Kelimeler: Hantavirüs; böbrek sendromu ile birlikte kanamalı ateş; hemorajik ateş virüsü, kırım-kongo

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Crimean-Congo hemorrhagic fever (CCHF) virus and hantavirus belong to the bunyaviridae family and cause severe diseases in humans.[1,2] CCHF virus has wide geographic distribution in the Central and South-Eastern Europe, the Middle East, Asia and Africa. Humans become infected with CCHF virus by tick bites, direct contact with the blood or tissue of infected humans or viraemic livestock.[3-6] Clinical features of CCHF usually include liver dysfunction, ecchymosis, extensive bleeding

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and disseminated intravascular coagulation.[7] In contrast to other bunyaviridae, hantavirus does not have any relation with an arthropod vector; each hantavirus is carried by a specific rodent species (subfamilies Murinae, Arvicolinae, Sigmodontinae) or insectivore species. Hantavirus is the causative agent of hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS).[2] It is transmitted to humans via inhalation of excretions of the infected animals such as saliva, urine and feces. To date, about 4400 cases have been reported between 2002 and 2009 according to the report of the Turkish Ministry of Health. The first laboratory confirmed cases of HFRS caused by hantavirus in Turkey were puplished in 2009.[8] We reported three case of HFRS from the Middle Anatolia Region of Turkey as well as the first case of co-infection with hantavirus and CCHF virus.

CASE REPORTS

CASE 1

On June 5, 2010, a previously healthy 50-year-old man presented to the Cumhuriyet University Hospital with a history of fever, chills, headache, nausea, vomiting, fatigue, weakness and generalized myalgia for the last 5 days. He had no history of unconsciousness or bleeding. On admission, his body temperature was 39°C, the pulse rate was 84/min and the blood pressure was 100/60 mmHg. Physical examination was unremarkable except for macular lesions on the upper trunk. The other physical examination was unremarkable. Complete blood count (CBC) and blood biochemistry results including liver and renal functions were shown in Table 1. The baseline CBC revealed pancytopenia and serum biochemistry showed increased aminotranspherases [aspartate aminotransferase (AST) 336 IU/L, alanine aminotransferase (ALT) 74 IU/L), lactate dehydrogenase (LDH) 1862 IU/L, and creatinin phosphokinase (CPK) 1907 IU/L], serum creatinin (1.3 mg/dL), and activated partial thromboplastin time (aPTT) (77s).

CCHF was suspected since the patient was referred from an endemic region for CCHF. He was isolated and barrier nursing was started. The patient had no history of tick bite, but had a history of tick removal from livestock without gloves. The first blood sample of the patient was sent to the national reference virology laboratory, Communicable Diseases Research Center, Refik Saydam National Public Health Agency (RSNPHA), Ankara, Turkey. Laboratory results of real time polymerase chain reaction (PCR) (CCHFV real time PCR, Astra, Germany) and specific anti-CCHFV immunoglobulin (Ig) M by sandwich enzyme immunoassay (kindly supported by CDC, Atlanta, US) confirmed the diagnosis of CCHF. On the second day of hospitalization, the patient developed gross hematuria, buccal mucosal and gingival bleeding. Leucopenia, thrombocytopenia and anemia progressed during follow-up (1400 cells/μL, 6000 cells/μL, and 7.9 mg/dL, respectively) and was handled with blood transfusion, platelet and fresh frozen plasma replacement. The patient developed disorientation, incorporation and severe hepatitis (ALT 1852 IU/L, AST 8961 IU/L) on day 5 of hospitalization. He was clinically diagnosed with hepatic precoma due to agitation, meaningless speech followed by unconsciousness on day 10 of hospitalization. Serological tests for differential diagnosis ruled out Lyme disease, leptospirosis, brucellosis, and viral hepatitis. The patient was also tested for hantavirus because the findings were not consistent with CCHF on day 10 of hospitalization. In addition, blood was drawn for bacteriologic cultures and empirical broad-spectrum antibiotic treatment with cefoperazone-sulbactam 2 g q 12 hours, IV was initiated. The patient also received supportive therapy including hydration, parenteral feeding, fresh frozen plasma and platelet solution during the course of the disease. The patient clinically improved on day 18 of hospitalization and was orientated and cooperative on day 20. IgM and IgG antibodies for hantavirus infection were positive. Immunoflourescence tests (Hantavirus profil 1, Euroimmun Medinizische Labordiagnostika AG, Germany) (IFA) carried out at the national reference virology laboratory, Communicable Diseases Research Center, RSNPHA, Ankara, Turkey revealed Dobrava virus. Biochemical and hematological parameters improved dramatically with

TABLE 1: Laboratory data for Case 1 on admission and on the following days.

Date	Hb(g/dL)	WBC/µL	PLT/µL	aPTT (s)	AST (U/L)	ALT (U/L)	LDH (U/L)	Creatinin (mg/dl)
June 5	9.8	2.3x103	17x103	77.2	336	74	1862	1.3
9	8.2	2.3x103	14x103	101.0	8961	1852	12607	
15	9.1	3.9x103	19x103	34.1	1095	444	2886	2.2
18	10.7	3.4x103	18x103	30	648	198	1070	1.5
21	10.3	3.6x103	21x103	29.9	177	132	760	0.9
30	10.4	4.3x103	70x103	33.1	76	48	334	0.9
July 11	9.6	12.6x103	301x103	26	114	102	198	1.3
21	10.1	10.8x103	402x103	25	74	73	170	0.9

Hb: Hemoglobin; WBC: White blood cell; PLT: Platelet; aPTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase.

supportive treatment. The patient was discharged on day 45 of hospitalization.

CASE 2

A 74-year-old man presented to our hospital with fever, weakness, nausea, vomiting, and generalized myalgia for the last 4 days in June 2010. He had no history of tick bite and bleeding. On admission, his body temperature, pulse rate and blood pressure were within normal range. He had no conjunctival hyperemia. Laboratory tests at baseline revealed leucocytosis (15.600 cells/µL), thrombocytopenia (20.000 cells/ µL), increased liver and muscle enzymes (AST 79 IU/L, ALT 28 IU/L, LDH 425 IU/L, CPK 469 IU/L), and high blood urea nitrogen (66 mg/dl) and serum creatinin (3.2 mg/dL) levels. The patient was oliguric (250 mL urine output/24 hours). Urine analysis disclosed proteinuria and microscopic hematuria. Serological tests for hantavirus IgM and IgG antibodies were positive. CCHFV real time PCR, and specific anti-CCHFV immunoglobulin (Ig)M were negative and CCHF was ruled out. The patient developed anuria and acute renal failure on the third day of admission. He underwent hemodialysis for two consecutive days. His renal function recovered gradually and he was discharged on day 10 of hospitalization.

CASE 3

A 55-year-old man presented to our hospital with fever, weakness, nausea, vomiting, diarrhea, headache and generalized myalgia for the last 7 days. He had no history of tick bite and bleeding. On admission, his physical examination revealed that the patient was febrile (38.8 °C), and his pulse rate was 112/min and the blood pressure was 90/50 mmHg. Otherwise, physical examination findings were unremarkable. Hemoglobin and thrombocyte levels were 10.8 g/L and 14,000 cells/µL, respectively. Serum biochemistry revealed AST: 55 IU/L, ALT: 26 IU/L, LDH: 266 IU/L, CPK: 38 IU/L, blood urea nitrogen 100 mg/dL, and serum creatinine 3.7 mg/dL. Urine output was under 400 ml/24 hours and urine analysis disclosed proteinuria. He was treated with ciprofloxacin 200 mg q 12 hours, IV for presumed salmonella infection (tipho-paratific fever). However, blood, stool and urine culture were negative for salmonella. Hantavirus IgM and IgG antibodies were positive. CCHFV real time PCR, and specific anti-CCHFV IgM were negative, which ruled out CCHF. The patient did not need dialysis and developed poliuria on day 4 of hospitalization. Fever, weakness, generalized miyalgia continued for two weeks; body temperature dropped at the end of second week and clinical status of the patient improved at the third week of hospitalization. His renal function recovered gradually and he was discharged on day 21 of hospitalization.

DISCUSSION

Infections transmitted to humans with tick bites such as Rickettsiosis, Lyme disease and CCHF have been reported from Turkey in previous years.[9-12] In addition, tularemia epidemics transmitted to hu-

mans with tick bite or contact with infected animals such as rhodents or contaminated water were reported from different regions of Turkey between 1988 and 2005.[13] Finally, the first laboratory confirmed case of hantavirus infection in Turkey was reported in the Black Sea Region of Turkey (Zonguldak and Bartın provinces) in 2009.[8] In the present study, we reported the first cases of hantavirus infection from the Middle Anatolia Region of Turkey.

The risk of hantavirus infection increases in persons who are active in forest, fields, farms, and nearby waters.[8,14] Although our patients had no history of rodent bites, they were farmers and they were living in rural areas. The most common route of transmission of hantavirus to humans is the inhalation of aerosolized saliva or excreta of rodents in the field, by ingestion of contaminated food or water, or direct rodent bite.[8]

The incubation period of HFRS varies from 2 to 4 weeks. Typically, the course of HFRS has been divided into febrile, hypotensive, oliguric, diüretic, and convalescent phases, but these phases are not always clinically evident.[14] Case 1 who was co-infected with CCHF virus was shown to be infected with Dobrova virus. Symptoms of Dobrova infections are commonly more severe than the manifestatiosn of Puumela infection. Hemorrhagic complications, disseminated intravascular coagulopathy, severe thrombocytopenia, shock, oliguric renal failure requiring dialysis, pleural and abdominal effusion, and gastrointestinal and cardiac rhythm disorders are the most common. Fluid balance of the body and its replacement are of critical importance for the treatment of hantavirus infections.[14] Only one of three patients (case 2) in this report required dialysis treatment, and none received ribavirin treatment.

The first case of CCHF virus infection was reported in Turkey in 2002. The six provinces in Kelkit valley-Tokat, Sivas, Gümüşhane, Amasya, Yozgat, and Çorum provinces-in northeastern Turkey, were the most strongly affected regions.[1] In our hospital, nearly 200-300 laboratory confirmed CCHF patients are annually diagnosed. Case 1 was referred to our hospital from the CCHF endemic region from where the majority of Turkish cases have been reported in previous years. Therefore, case 1 was hospitalized with a presumptive diagnosis of CCHF and only supportive treatment with fresh frozen plasma, platelet solution and blood products were given.

CCHF is categorized in four distinct phases: incubation, pre-hemorrhagic, hemorrhagic and convalescence. The incubation period varies according to the route of transmission and viral inoculum such as 1-3 days after tick bite, 5 days after contact with livestock blood or tissue, and 5-6 days after contact with human blood. Pre-hemorrhagic period lasts 3-6 days and fever, nausea, vomiting, myalgia, and severe headache are common. The hemorrhagic period develops after 3-6 days following the onset of illness and lasts for 2-3 days. Hemorrhagic manifestations range from petechia, ecchymoses to bleeding at various sites including mucous membranes, gastrointestinal tract (hematemesis or melena), genitourinary tract, respiratory tract (nose, hemophtysis), and brain. Cerebral and severe gastrointestinal hemorrhages are associated with poor prognosis. In patients who survive, the convalescence period begins 15-20 days after the onset of illness and is characterized by prolonged and pronounced weakness, weak pulse and occasional hair loss, polyneuritis, sweating, headache, dizziness, nausea and poor appetite.[1] In case 1, after the prehemorrhagic period, hemorrhagic findings (buccal mucosa bleeding, hematuria and melena) developed on the second day of hospitalization (on day 6 after the onset of illness); a progressive increase was detected in ALT, AST and total biluribin levels. The patient developed hepatic precoma-hepatic coma on day 15 after the onset of disease. Case 1 was screened for hantavirus co-infection due to the atypical clinical course of CCHF and the presence of oliguria and hantavirus infection was confirmed by IFA since real time PCR for hantavirus infection was not available. In the first case the results were interpreted as a cross-reaction between CCHF virus and hantavirus because they belong to the same virus family (bunyaviridaea). However, case 1 had an atypical clinical presentation for CCHF,

and hantavirus infection was also positive in the other two patients (case 2 and case 3) living in the same region at the same time. Thus, the final decision was that case 1 was co-infected with hantavirus and CCHF virus. The presence of hantavirus in Sivas, Giresun, and Tokat provinces is not surprising because it is circulating in neighboring cities.[8,15]

In this study, we reported the first hantavirus infection cases in the Middle Anatolia Region of Turkey. We also presented the first case of hantavirus and CCHF virus co-infection in a patient. In conclusion, hantavirus infection should be considered for the differential diagnosis of febrile disease with atypical and serious life-threatening presentations in Turkey.

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