Crimean-Congo Hemorrhagic Fever Virus Infection: Clinical and Laboratory Observations and Predictors of Fatality

Kırım-Kongo Kanamalı Ateş Virüs Enfeksiyonu: Klinik ve Laboratuvar Gözlemleri ve Fatalitenin Öngördürücüleri

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ABSTRACT Objective: In this study we described the predictors of fatality among Crimean-Congo hemorrhagic fever (CCHF) patients who admitted to our hospital based on epidemiological, clinical and laboratory findings between 2005 and 2006. **Material and Methods:** Definitive diagnosis was based on the detection of CCHF virus-specific IgM by ELISA and/or of genomic segments of the CCHF virus by real time polymerase chain reaction. Related data were collected prospectively. **Results:** The study included 63 patients. Thirty-two of the patients (50.8%) were females and 31 of them (49.2%) were males. The overall case fatality rate was 4.8 %. The age and sex were similar between favorable and fatal cases (p>0.05). The mean duration to the onset of symptoms for all patients was 4.7 days and differences in the mean duration to the onset of symptoms between surviving and died patients were not statistically significant (p=0.22). Weakness, myalgia, somnolence, bleeding from multiple sites and presence of petechia/ecchymosis were observed more often in the patients who died (p=0.02, p=0.03, p=0.015, p=0.02, respectively). Significant elevation of LDH levels (p=0.004), prolonged APT (p=0.004), prolonged PT (p=0.04) and thrombocytopenia (p=0.01) were detected in patients who died. Oral ribavirin was prescribed to 46 patients (73%). Prescription of ribavirin was considered to three patients with fatal disease, but this could not be realized because of hematemesis and melena. **Conclusion:** The existence of various clinical and laboratory findings like weakness, myalgia, somnolence, bleeding, elevation of LDH levels, prolonged APT and PT and thrombocytopenia may be considered as risk factors for fatality in CCHF.

Key Words: Hemorrhagic fever virus, crimean-congo; diagnosis; ticks

ÖZET Amaç: Bu çalışmada 2005 ile 2006 arasında hastanemize başvuran Kırım-Kongo kanamalı ateş (KKKA) hastalarındaki fatalite öngördürücüleri epidemiyolojik, klinik ve laboratuvar bulgularına dayanarak tanımladık. **Gereç ve Yöntemler:** Kesin tanı ELISA ile KKKA virus-spesifik IgM'nin saptanması ve/veya gerçek zamanlı polimeraz zincir reaksiyonu ile KKKA virüsün genomik segmentlerinin saptanması ile koyuldu. İlgili veriler ileriye dönük olarak toplandı. **Bulgular:** Çalışma 63 hastayı kapsıyordu. Hastaların 32'si (%50,8) bayan, 31'i (%49,2) erkekti. Tüm olgularda fatalite hızı %4,8 idi. İyi olgularla ölümcül olgular arasında yaş ve cinsiyet benzerdi (p>0,05). Tüm hastalarda belirtiler ortaya çıkana kadar geçen ortalama süre 4,7 gündü ve hayatta kalan ve ölen hastalar arasında belirtiler ortaya çıkana kadar geçen süre bakımından istatistiksel olarak anlamlı fark yoktu (p=0,22). Güçsüzlük, myalji, uyku hali, birden fazla bölgeden kanama ve peteşi/ekimoz varlığı ölen hastalarda daha sık gözlendi (p=0,02, p=0,03, p=0,015, p=0,02). Ölen hastalarda LDH düzeylerinde önemli yükselme (p=0,004), uzamış APT (p=0,004), uzamış PT (p=0,04) ve trombositopeni (p=0,01) saptandı. Kırkaltı hastada (%73) oral ribavirin verildi. Fatal üç hastaya ribavirin verilmesi düşünüldü fakat hematemez ve melena nedeniyle gerçekleştirilemedi. **Sonuç:** Güçsüzlük, myalji, uyku hali, kanama, LDH düzeylerinde yükselme, uzamış APT ve PT, trombositopeni gibi çeşitli klinik ve laboratuvar bulgularının varlığı KKKA'da fatalite için risk faktörleri olarak düşünülebilir.

Anahtar Kelimeler: Hemorajik ateş virüsü, kırım-kongo; tanı; keneler

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the arbovirus Crimean-Congo hemorrhagic fever virus, which is a member of the Nairovirus genus (family Bunyaviridae).[1] This potentially lethal disease was detected in about 30 countries worldwide. CCHF virus spreads through tick bite, or direct exposure to infected animal or human blood, body fluids or other infected tissues.[2] Therefore, it cannot be considered only as a zoonotic tick-borne disease, but also a nosocomial infection, with high inter-personal transmission rates.[3] The most common clinical signs of CCHF are fever, nausea, headache, diarrhea, myalgia, petechial rash, and bleeding. For humans, infection with tickborne CCHF virus often results in a serious illness followed by death (with a fatality rate of up to 80%, most deaths occurring five to 14 days after the onset of illness).[4,5] Treatment options for CCHF are limited. Immunotherapy and ribavirin have been tried with varying degrees of success during sporadic outbreaks of disease, but no case-controlled trials have been conducted.[1]

In this study, we determined the predictors of fatality and some outcome features in patients with CCHF, based on epidemiological, clinical and laboratory findings.

MATERIAL AND METHODS

Between January 2005 and December 2006, we carried out a prospective study on patients with acute febrile syndrome characterized by malaise, bleeding, leukopenia and thrombocytopenia. The patients were admitted to the Infectious Diseases and Clinical Microbiology Department of Ataturk University Research Hospital, a 1200-bed tertiary hospital. Patients with positive IgM antibody and/or viral RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) in blood or tissue were included in this study. Acute and convalescent sera from all of the acute cases were sent to the Refik Saydam Hygiene Center of Ankara, Turkey for enzyme-linked immunoassay (ELISA) and RT-PCR tests. Specimens were tested by ELISA IgG/IgM using CCHF native antigen grown on VeroE6 cells, and RT-PCR on the S segment of the viral genome. Biochemical and hematological laboratory parameters which were measured on daily basis after admission to the hospital were recorded prospectively on individual forms. Erythrocytes, platelets, total blood preparations and fresh frozen plasma were given to patients depending on their homeostatic state. Oral ribavirin therapy was administered before laboratory evidence of CCHV infection at the dose recommended by the WHO (4 g four times daily for 4 days; 2.4 g four times daily for 6 days) because the results of diagnostic tests were not available for up to four weeks.[6]

STATISTICAL ANALYSIS

Due to the small number of cases, bivariate comparisons of the numeric variables were done with the Mann Whitney U test. Chi-square Fisher's exact tests were used for categorical variables. A P value <0.05 was considered as statistically significant. Power of the study was based on "Days from symptoms to admission" as the main outcome, using independent samples t test. Taking the standard deviation as 2, significance level as 0.05, effect of interest as two days, and the number of cases in the study and control arms as 3 and 60 respectively, we would have a power of 38.3%. A statistical package (SPSS 10.0) was used for statistical analysis.

LIMITATIONS

One limitation of our study was its low power. However, due to the seasonality of the disease, it is very difficult to find enough cases in the study group. The results may be re-calculated using a longer time span.

RESULTS

The study included 63 patients. Thirty-two of the patients (50.8%) were females and 31 of them (49.2%) were males. Anti-CCHF IgM was positive in 52 patients and RT-PCR was positive in 13 patients (Table 1).

The overall case fatality rate was 4.8%. The age and sex were similar between favorable and fatal cases ($p>0.05$). The mean duration to the onset of symptoms for all patients was 4.7±2.5

TABLE 1: Serology and PCR results for 63 patients with CCHF

Variable	Favorable cases n= 60 (%)	Fatal cases n=3 (%)
Ig M	51 (85 %)	1 (33.3%)
RT-PCR	11 (18.3%)	2 (66.7%)

PCR: polymerase chain reaction, CCHF: Crimean-Congo hemorrhagic fever.

days, and the differences in the mean duration to the onset of symptoms between surviving and died patients were not statistically significant (p=0.22). Weakness, myalgia, somnolence, bleeding from multiple sites and presence of petechia/ecchymosis were observed more often in the patients who died (p=0.02, p=0.03, p=0.015, p=0.02, respectively) (Table 2).

All patients had leukopenia, thrombocytopenia, elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK). None of the patients had leukocytosis. Significant elevation of LDH levels (p=0.004), prolonged APT (p=0.004), prolonged PT (p=0.04), and thrombocytopenia (p=0.01) were detected in patients who died (Table 3). All patients received intensive clinical support, including infusions of platelets, fresh frozen plasma, and packed erythrocyte when indicated. Oral ribavirin was prescribed to 46 (73%) patients; 43 of them survived and three of them died.

DISCUSSION

Serological evidence suggests that CCHF virus existed in Turkey in the previous years, however there were no identified cases among humans.[7] Although epidemics have been reported from neighboring countries since the 1970s, patients with CCHF were first reported in Turkey in 2002.[8] CCHF virus causes a fatal hemorrhagic syndrome, which has been endemic in the central, Northern, and Eastern regions of Turkey in the recent years.[9-12] The typical course of CCHF infection has four dis-

TABLE 2: Demographic and clinical characteristics of favourable and fatal cases with CCHF.

Characteristics		Favorable cases n=60 (%)	Fatal cases n=3 (%)	p value
Number of females		28 (46.7%)	3 (100%)	0.261
Mean age (SD), years		45.8 ± 17.1	50.3 ± 10.4	0.809
Days from symptoms to admission (Mean±SD)		4.6 ± 2.6	5.7±1.2	0.226
Symptoms	Nausea	37 (61.7%)	1 (33.3%)	0.328
	Vomiting	27 (45%)	3 (100%)	0.063
	Myalgia	38 (63.3%)	-	0.029
	Fever	51 (85%)	2 (66.7%)	0.396
	Headache	25 (41.7%)	1 (33.3%)	0.775
	Weakness	58 (96.7%)	2 (66.7%)	0.017
Signs	Fever	51 (85%)	2 (66.7%)	0.396
	Bleeding	20 (33.3%)	3 (100%)	0.021
	Maculopapular rash	6 (10%)	-	0.565
	Conjunctival infection	1 (1.7%)	-	0.822
	Jaundice	1 (1.7%)	-	0.822
	Diarrhea	11 (18.3%)	1 (33.3%)	0.518
	Hepatomegaly	12 (20%)	-	0.389
	Splenomegaly	2 (3.3%)	-	0.748
	LAP	1 (1.7%)	-	0.822
	Neck stiffness	1 (1.7%)	-	0.822
	Peritonal irritation	7 (11.7%)	-	0.530
	Somnolence	3 (5%)	2 (66.6%)	0.015

CCHF: Crimean-Congo hemorrhagic fever, LAP: Lymphadenopathy.

TABLE 3: Laboratory results for favourable cases and fatal cases with CCHF.

	Favorable cases n=60	Fatal cases n=3	p value
Lowest WBC count(WBC X 109)	2716.5±1923.7	1833.3±404.1	0.272
Lowest platelet count (platelets X 109)	83290±48163.7	14000±13000	0.011
Highest AST level, U/l	289.8±314.3	943±737	0.10
Highest ALT level, U/l	138.6±132.3	292±224	0.093
Highest LDH level, U/l	1215.7±809	6366.7±2667	0.004
Highest CPK level, U/l	1052.3±1491 ± 9	463±337.2	0.675
Longest PT, s	13.4±1.7	16.6±3.3	0.044
Longest APT, s	35.9±7.2	75.1±14.2	0.004

WBC: White blood cells; ALT: Alanin aminotransferase; AST: Aspartate aminotransfaerase; LDH: Lactate dehydrogenase; CPK: Creatin phosphokinase; PT: Prothrombine time; APT: Activated partial thromboplastin time.

tinct phases: incubation, prehemorrhagic, hemorrhagic, and convalescence periods.[13] There is a variety of potential clinical manifestations following infection with this virus, and not all patients develop the classic form of CCHF syndrome.[14] Generally after a short incubation period, CCHF is characterized by a sudden onset of high fever, chills, severe headache, dizziness, as well as back and abdominal pain. Additional symptoms may include nausea, vomiting, diarrhea, neuropsychiatric and cardiovascular changes. In severe cases, hemorrhagic manifestations, ranging from petechia to large areas of ecchymosis can be seen.[1] In this study, the mean duration to the onset of symptoms was 4.7 ± 2.5 days and differences in duration between surviving and died patients were not statistically significant (p=0.27). The mean duration of the disease course before presenting to a hospital was reported as approximately five days in Turkey.[9,15] The clinical presentation of patients with CCHF in this study was similar to those reported previously.[9-12,15] On admission, the most common symptoms were fever, weakness, myalgia, headache and bleeding. Hemorrhagic findings were detected in 36.5% of the cases. Primary laboratory findings in the patients diagnosed with CCHF are thrombocytopenia, leukopenia, and increased levels of AST, ALT, LDH and CPK, like previously reported.[9-12,15] The overall case fatality rate was 4.8% in this study. The mean fatality rate for Turkey is approximately 5%. This rate has not changed over the years and is lower than the rate reported from the other series in the other parts of the world.[16] Various fatality rates for CCHF, up to 80%, have been reported in the literature.[12,15,17] No CCHF virus IgM or IgG antibodies were detected in the serum specimens obtained from two of the fatal cases, but the virus was detected by PCR . The absence or minimal evidence of antibody response in the patients who died was reported previously.[18]

Any of the following clinical pathologic values during the first five days of illness were found to be >90% predictive of fatal outcome in a study: Leukocyte counts <10 x 10^9/L, platelet counts <20 x 10^9/L, AST >200 U/L, ALT >150 U/L, aPTT >60 s, and fibrinogen <110 mg/dL[18] Other case series have showed that elevated AST, ALT, CPK levels and decreased platelet count were significantly higher among severe cases.[9,12,15]

Previously defined severity criteria did not completely fit in the patients in our study (Table 4).[15,18,19] Leukocytosis and renal failure were the fatality criteria described by Swanepoel et al., but we did not observe them.[18] Among the fatal cases, the mean ALT (292 vs. 138) and the mean AST (943 vs. 289.8) levels were higher, but the differences were not statistically significant in our study. In contrast to Swanepol et al. and Ergonul et al., we observed significant elevation of LDH level.[18,19] Somnolence, prolonged APT and PT have previously been reported from Turkey as indicators of poor prognosis, similar to this study.[15,19] General supportive treatment is essential in CCHF.[20] Supportive treatment should be provided with fresh frozen plasma (FFP and erythrocyte) and platelet suspensions.[21] Rib-

TABLE 4: Comparison of predictors of fatality with previous studies.

	Swanepoel et al.[18]	Ergonul et al.[19]	Cevik et al.[15]	Present study	p Value
Increased WBC count (WBC X 109)	Yes	No	No	No	0.272
Decreased platelet count (platelets X 109)	Yes	Yes	Yes	Yes	0.011
Elevated AST level, U/l	Yes	Yes	Yes	No	0.10
Elevated ALT level, U/l	Yes	Yes	Yes	No	0.093
Elevated LDH level, U/l	No	No	Yes	Yes	0.004
Elevated CPK level, U/l	No	No	Yes	No	0.675
Prolonged PT, s	No	Yes	Yes	Yes	0.044
Prolonged APT, s	Yes	Yes	Yes	Yes	0.004
Somnolence	No	Yes	Yes	Yes	0.05

avirin has been shown to inhibit in vitro viral replication.[22] Ribavirin treatment was administered to 46 cases (43 non fatal-three fatal cases). The efficacy of ribavirin in the treatment of CCHF is not clear.[12] Currently, there are no Food and Drug Administration (FDA)- approved antiviral agents for the treatment of CCHF.[1] Studies from our country have reported a decreased mortality rate among severe cases who were given oral ribavirin treatment, while other studies have reported that ribavirin had no effect on mortality.[9,12,23] Multidrug transporter Pglycoprotein 1 and cytochrome P450 isozyme 2D6 genes may play a crucial role in pharmacokinetics, immunological response and drug metabolism in the management of CCHF infection.[24]

In conclusion, the results of our study indicate that the most important poor prognostic factors for CCHF are existence of somnolence, myalgia, bleeding, thrombocytopenia, increased LDH level, prolonged APT and PT. We suggest that such patients should be observed more carefully for intensive supportive therapy.

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