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Crucial parameter of the outcome in Crimean Congo hemorrhagic fever: Viral load

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ABSTRACT

Background: Crimean Congo hemorrhagic fever (CCHF) is a fatal disease with a mortality rate of 5–30%. CCHF can be asymptomatic or it may progress with bleeding and cause mortality.
Objectives: To evaluate relation of viral load with mortality, clinical and laboratory findings in CCHF.
Study design: A total of 126 CCHF patients were included. Serum samples obtained from all patients on admission for measurement of viral load.
Results: In our study, mortality rate was 11.1%. The most important prognostic factor was viral load. Mean viral load was 8.3×10^7 copy/ml and 4.6×10^9 copy/ml in survived and dead patients, respectively ($p < 0.005$). Probability of survival is found to be significantly reduced where AST >1130 U/l, ALT >490 U/l, CPK >505 U/l, LDH >980 U/l, platelet count $<23 \times 10^3$/l, creatinine >1.4 mg/dl, INR >1.3, D-dimer >7100 ng/dl, and viral load $>1.03 \times 10^8$ copy/ml. Patients with 10^8 copy/ml or higher viral load had diarrhea, headache, unconsciousness, bleeding, and seizure significantly more frequently ($p < 0.05$). WBC, hemoglobin, platelet counts were significantly lower whereas AST, ALT, CPK, LDH, creatinine levels, PT and aPTT time, D-dimer levels, and INR were found to be significantly higher in these group.
Conclusions: There are several severity criteria for prognosis of CCHF. In addition to these parameters, we introduce creatinine as a predictive factor for prognosis. Our study, which has the largest number of patients among studies that evaluate viral load on CCHF shows that viral load is the most effective parameter on mortality.

1. Background

Crimean Congo hemorrhagic fever (CCHF) is a zoonotic viral infection which is transmitted primarily through contact with ticks. It is endemic in the Middle East, southeastern Europe, Asia, and Africa. In Turkey, it is seen in a wide region encompassing south of the Black Sea coastal line and middle and eastern Anatolia [1]. The disease is caused by a RNA virus in the Bunyaviridae family and *Nairovirus* genus. Signs and symptoms of the disease include high fever, malaise, headache, diffuse myalgia, nausea, vomiting, diarrhea, and bleeding through the skin and mucosal surfaces. Important laboratory findings are thrombocytopenia, leukopenia, elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) levels, and prolonged activated

Abbreviations: CCHF, Crimean Congo hemorrhagic fever; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatine phosphokinase; LDH, lactate dehydrogenase; aPTT, activated partial thromboplastin time; PT, prothrombin time; RT-PCR, reverse transcriptase polymerase chain reaction; ELISA, enzyme linked immunosorbent assay; WBC, white blood cells; Plt, platelet; CI, confidence interval; uNGAL, urine neutrophil gelatinase-associated lipocalin.

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partial thromboplastin time (aPTT) and prothrombin time (PT). Although it varies by region, the mortality rate is 3–30% [1,2].

Diagnosis of CCHF is based on immunological or molecular assays. Detection of viral nucleic acid in serum samples using reverse transcriptase polymerase chain reaction (RT-PCR) or virus specific IgM by enzyme linked immunosorbent assay (ELISA) provide a rapid and reliable method for early diagnosis. In addition, real-time RT-PCR can be used to determine viral load, which is found to be a prognostic parameter on survival in a few recent studies [3,4].

2. Objectives

In our study we aimed to evaluate mortality related risk factors in CCHF and relation of viral load with mortality and clinical and laboratory findings.

3. Study design

A total of 126 adult patients that were treated in Infectious Diseases and Clinical Microbiology Department in Ankara Ataturk Education and Research Hospital with diagnosis of CCHF between June 2008 and September 2013 were included in this prospective study.

Clinical and laboratory features of patients are obtained from medical records and follow up charts. While white blood cells (WBC) and platelet (Plt) counts, hemoglobin, fibrinogen, and cholesterol levels were based on lowest levels in the patient's follow up; AST, ALT, CPK, LDH, creatinine, PT, aPTT, INR, and D-dimer were based on highest levels observed. At the presence of ecchymosis, hematoma, petechia, melena, epistaxis, hematuria, or vaginal bleeding; parameter of bleeding was considered as positive.

Serum samples obtained from all patients on admission were stored at −80 °C before measurement of viral load. Laboratory analysis for viral load was carried out at Public Health Institute of Turkey, Microbiology Reference Laboratories Department, National Arbovirus and Viral Zoonoses Reference and Research Laboratory. Viral RNA extraction assay was performed with EZ1 Virus Mini Kit (QIAGEN) and measurement of viral load was performed with Altona RealStar® CCHFV RT-PCR Kit 1.0 in accordance with the manufacturer's instructions. Analysis of real time PCR were done with Applied Biosystems, LightCycler® 480 Instrument II (Roche), Rotor-Gene™ 3000/6000 (Corbett Research).

3.1. Statistical analysis

Results were analyzed with SAS JMP® 11 statistical software package. Bivariate correlations among all variables were calculated in the multivariate analysis. Variables that were correlated with survival and viral load were included in further analysis. Comparisons between groups for continuous variables were performed with Student's *t*-test if they were distributed normally and with Kruskal–Wallis test if they were not distributed normally. Normality was tested with Shapiro–Wilk W. Nominal variables were compared with Pearson χ^2 and Fisher's Exact test. For the calculation of viral load cut-off values, logistic regression and ROC curve analysis were used.

4. Results

Out of 126 patients, 66 patients (49%) were male. Mean age of the patients was 49 (between 16–80) years. Seventy-nine patients (62.6%) had history of tick bite. Time to hospitalization after the onset of symptoms and mean length of stay were 3.7 (1–10) and 7.3 (1–45) days respectively. Fourteen patients died during the follow up and mortality rate was 11.1%.

Clinical features of fatal and non-fatal patients are given in Table 1. We found statistically significant difference between fatal and non-fatal cases in terms of all types of bleeding, diarrhea, loss of consciousness, seizure, and need for hemodialysis ($p < 0.05$).

Significant differences were found in hemoglobin and platelet count, PT and aPTT time, INR ratio, D-dimer, AST, ALT, CPK, LDH, creatinine, triglycerides, and HDL levels across patients who died and survived ($p < 0.05$) (Table 2). The most important prognostic factor was found to be viral load with a mean of 8.3×10^7 copy/ml (CI: 3.09×10^7–1.3×10^8 copy/ml) in patients who survived and 4.6×10^9 copy/ml (CI: 10^8–10^{10} copy/ml) in patients who died. None of the patients who had viral load greater than 2×10^9 copy/ml survived. Fig. 1 illustrates the difference between viral loads of fatal and non-fatal patients. As viral load increases PT and aPTT times significantly extend; INR, AST, ALT, CPK, LDH, and creatinine levels rise and the probability of bleeding

Table 1
Comparison of fatal and non-fatal cases in terms of clinical features.

	Non-fatal CCHF, *n* (%)		Fatal CCHF, *n* (%)		*p* value
	No	Yes	No	Yes	
Fever	13 (%10.3)	99 (%78.5)	0 (%0)	14 (%11.1)	0.1783
Bleeding	81 (%64.8)	31 (%24.8)	2 (%1.6)	11 (%8.8)	**<0.0001**
Myalgia	6 (%4.7)	106(%84.1)	1 (%0.7)	13 (%10.3)	0.7833
Diarrhea	87 (%69)	25 (%19)	7 (%5.56)	7 (%5.56)	**0.0249**
Nausea/vomiting	41 (%32.5)	71 (%56.3)	3 (%2.3)	11 (%8.7)	0.2614
Abdominal pain	83 (%65.8)	29 (%23)	10 (%7.9)	4 (%3.1)	0.8298
Headache	71 (%56.3)	41 (%32.5)	8 (%6.3)	6 (%4.7)	0.6485
Loss of consciousness	102 (%80.9)	10 (%7.9)	10 (%7.9)	4 (%3.1)	**0.0275**
Rash	99 (%78.5)	13 (%10.3)	12 (%9.5)	2 (%1.5)	0.7705
Ecchymosis	96 (%76.1)	16 (%12.7)	9 (%7.1)	5 (%3.9)	**0.0425**
Hemotoma	104 (%83.2)	8 (%6.4)	12 (%9.6)	1 (%0.8)	0.9422
Petechia	100 (%79.3)	12 (%9.5)	8 (%6.3)	6 (%4.7)	**0.0012**
Melena	105 (%84)	6 (%4.8)	6 (%4.8)	8 (%6.4)	**<0.0001**
Epistaxis	97 (%76.9)	15 (%11.9)	7 (%5.5)	7 (%5.5)	**0.0007**
Hematuria	68 (%54.4)	43 (%34.4)	2 (%1.6)	12 (%9.6)	**0.0008**
Pneumonia	104 (%82.5)	8 (%6.3)	8 (%6.3)	6 (%4.7)	**0.0001**
Vaginal bleeding	103 (%81.7)	9 (%7.1)	13 (%10.3)	1 (%0.7)	0.9072
Cholecystitis	109 (%86.5)	3 (%2.3)	13 (%10.3)	1 (%0.7)	0.3690
ICU stay	111 (%88.1)	1 (%0.7)	13 (%10.3)	1 (%0.7)	**0.0777**
Seizure	110 (%87.3)	2 (%1.5)	11 (%8.7)	3 (%2.3)	**0.0004**
Need for hemodialysis	112 (%88.89)	0 (%0.00)	11 (%8.73)	3 (%2.38)	**0.0003**

Bold values are statistically significant ($p < 0.05$).