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Coagulopathy Parameters in Patients With Crimean-Congo Hemorrhagic Fever and Its Relation With Mortality

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Background: Crimean-Congo hemorrhagic fever (CCHF) is an acute illness affecting multiple organ systems and characterized by ecchymosis, visceral bleeding, and hepatic dysfunction. In this study, we aimed to investigate the profile of coagulopathy markers (platelet count, activated partial tromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR), fibrinogen, protein C, protein S, antithrombin III, activated protein C resistance (APCR), and D-dimer) and their clinical significance in 83 CCHF-infected patients. *Subjects and methods*: We studied 83 CCHF patients who were admitted to Ankara Numune Education and Research Hospital during the spring and summer of 2007. We compared the coagulopathy markers of fatal CCHF patients ($n = 9$) with nonfatal cases ($n = 74$). *Results*: Platelet count, PT, aPTT, INR, and fibrinogen were prognostic factors associated with mortality for CCHF. Especially, platelet count $< 20 \times 10^9$ cells/l and aPTT>60 sec were important. Protein C, protein S, APCR, and antithrombin III levels were not associated with mortality. *Conclusion*: Laboratory tests including classical parameters (platelet count, PT, aPTT, INR, and fibrinogen) of coagulopathy seem to be enough for the followup of CCHF. Protein S, protein C, APCR, and D-dimer levels were not associated with mortality. J. Clin. Lab. Anal. 24:163–166, 2010.

Key words: Crimean-Congo hemorrhagic fever; protein C; protein S; antithrombin III; activated protein C resistance

INTRODUCTION

Infection by the Crimean-Congo hemorrhagic fever (CCHF) virus is transmitted through the bite of Hyalomma or by contact with blood or tissues of infected livestock. In addition to zoonotic transmission, CCHF virus can be spread from person to person. Since 2002, in endemic season an increase in the number of CCHF cases has been observed in Turkey. CCHF can present a mild, self-limited, or a severe sickness, which may endanger the life of a patient, characterized by ecchymosis, visceral bleeding, and hepatic dysfunction (1,2). The virus belongs to the genus Nairovirus in the *Bunyaviridae* family (3,4). The reported fatality rate ranges 5–50% (2,3). Increased white blood cell count (WBC), decreased platelet count, elevated alanine transferase (ALT), aspartate transferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) levels, prolonged activated partial tromboplastin time (aPTT) and prothrombin time (PT), decreased fibrinogen, neopterin levels, viral load ecchymosis, melena, hematemesis, and somnolence, have been described as risk factors for fatal CCHF in different studies (2,5–7). The pathogenesis of this disease is not completely understood. Infection of the endothelium has an important role in CCHF pathogenesis (8). Bleeding in infectious disease is most likely a multifactorial process resulting from a combination of thrombocytopenia, consumption of local clotting factors, hyperfibrinolysis, and vascular damage or leakage. In addition, immunologically mediated vasculitis may contribute to bleeding in specific infections (9). Direct and indirect endothelial damage contributes to haemostatic failure by stimulating

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platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade (6,7,10). Limited data suggest that activation of coagulation plays a role in pathogenesis of CCHF infection. However, the pathogenetic mechanism underlying bleeding complications and multiorgan failure has not yet elucidated. Indeed, fatal CCHF cases had grossly abnormal indicators of coagulation system function from an early stage of illness, and disseminated intravascular coagulation (DIC) is noted as an early and prominent feature of the disease process. DIC was more prominent in patients with fatal CCHF, although it could be seen in various degrees in patients with nonfatal CCHF (11).

Protein C is a major physiological anticoagulant and protein S is its cofactor. It is activated by thrombin into activated protein C (APC). The activated form degrades Factor Va and Factor VIIIa (12). Activated protein C resistance (APCR) is a hemostatic disorder characterized by a poor anticoagulant response to APC. Antithrombin III is a glycoprotein produced by the liver. It is a serine protease inhibitor that inactivates several enzymes of the coagulation system with the physiological targets in both intrinsic and extrinsic pathways (13). Protein C, protein S, antithrombin III, and APCR have not been studied in CCHF previously.

In this study, we aimed to investigate a wide range of coagulopathy parameters, including protein C, protein S, antithrombin III, and APCR in CCHF patients, and their clinical significance as a predictor factor of mortality.

MATERIALS AND METHODS

We studied 83 CCHF patients who were admitted to Ankara Numune Education and Research Hospital during the spring and summer of 2007. Suspected patients for CCHF were defined as the cases with clinical symptoms and signs of CCHF such as fever, myalgia, malaise, and bleeding, and also the history of tick bite or inhabit endemic region. Diagnosis of all patients in the study group was confirmed with elevated IgM antibodies and/or viral RNA by RT-PCR. Blood samples of suspected cases were collected on admission to hospital for IgM antibodies and RT-PCR test of CCHF virus. The IgM antibodies were detected by using ELISA. TaqMan-based one-step RT-PCR assay was used for the detection of CCHF virus RNA (14). The assay was performed in a Perkin-Elmer 7700 Sequence Detection System by using the combination of reverse-transcriptase (MBI Fermentas, Vilnius, Lithuania) and hot start Taq DNA polymerase (Birion GmBH, Munchen, Germany) enzymes. All of the manipulations were performed in a biosafety class II cabinet.

The venous blood samples from patients were taken into plastic tubes containing 3.8% trisodium citrate (4.5 ml blood sample and 0.5 ml citrate). After centrifugation by $3000 \times g$ for 20 min, the plasma samples were kept at -70°C until the time of analysis. The functional level of antithrombin III (Berichrom, Dade Behring, Marburg, Germany), protein C (Dade Behring), protein S (Dade Behring), and APCR (proC Global, Dade Behring) were assessed using chromogenic assays, performed on the coagulation analyzer (Dade Behring's BCS). The normal reference ranges of various tests were protein C: 50–150% of normal; protein S: 50–150% of normal; AT: 80–120% of normal; APCR: 0.8–1.2.

Age, sex, duration of symptoms, presence of fever, bleeding, tick bite, hepatomegali, splenomegali, consciousness, cattle dealing, platelet count, WBC, ALT, AST, LDH, CK, PT, aPTT, international normalized ratio (INR), fibrinogen, D-dimer, antithrombin-III, protein S, protein C, and APCR levels were recorded for all patients. Protein C, protein S, antithrombin-III, and APCR levels were measured in the first 24 hr of hospitalization. All of the other laboratory parameters were measured on a daily basis after admission to hospital.

Patients with fatal and nonfatal CCHF were compared by χ^2 tests and Mann–Whitney U test. Statistical analysis was performed by using the SPSS 11.5 Statistical Package Program for Windows (SPSS Inc., Chicago, IL). Differences were considered significant at $P < 0.05$.

RESULTS

Nine of all CCHF patients cases died (fatal CCHF group), and 74 cases were survived (nonfatal CCHF group). The overall fatality rate was 10.1% and overall bleeding in hospitalized patients of CCHF in this study was 30.1%.

Of all CCHF patients, antithrombin III, protein S, protein C, and APCR were less than the normal range in 16.9, 67.5, 41.0, and 15%, respectively. D-dimer was higher than the normal range in 73.8% of all patients.

For fatal and nonfatal CCHF patients, the parameters of coagulopathy are presented in Table 1. Low platelet count, longer PT and aPTT, higher INR and fibrinogen level, platelet count $< 20 \times 10^9$ cells/l, and aPTT > 60 sec were significantly associated with mortality ($P < 0.05$). There was no statistically significant difference between fatal and nonfatal groups for antithrombin III, protein S, protein C, APCR, and D-dimer ($P > 0.05$).

DISCUSSION

CCHF infection is a serious clinical condition that presents with bleeding in some patients. Bleeding may present as petechiae, ecchymosis, hemoptysis, epistaxis, hematuria, hematemesis, melena, vaginal, and gingival bleeding in CCHF disease, and is a significant mortality

TABLE 1. Parameters of Coagulopathy for Fatal and Nonfatal CCHF Patients

	Patients with nonfatal CCHF ($n = 74$)	Patients with fatal CCHF ($n = 9$)	P
Lowest platelet count ($\times 10^9$ cells/l)	45.8 ± 28.6	13.6 ± 3.2	0.001
PT[a] (sec)	124.0 ± 18.6	147.5 ± 42.7	0.026
Longest PT during hosp. (sec)	12.8 ± 1.7	15.9 ± 3.2	0.0001
Aptt[a] (sec)	371.9 ± 103.7	524.4 ± 221.0	0.003
INR[a]	0.9 ± 1.3	1.1 ± 1.7	0.008
Highest INR during hosp.	1.0 ± 0.1	1.4 ± 0.2	0.0001
D-dimer[a] (ng/ml)	704.7 ± 1335.9	788.4 ± 667.2	0.371
Fibrinogen[a] (mg/dl)	280.2 ± 75.3	224.0 ± 63.1	0.041
Antithrombin III (%)	97.7 ± 19.3	83.6 ± 9.8	0.056
Protein S[a] (%)	51.0 ± 11.3	53.2 ± 12.5	0.468
Protein C[a] (%)	82.7 ± 24.8	80.0 ± 9.2	0.977
APCR[a]	0.9 ± 0.2	1.0 ± 0.1	0.708

CCHF, Crimean-Congo hemorrhagic fever; PT, prothrombin time); Aptt, activated partial thromboplastin time; INR, international normalized ratio; APCR, activated protein C resistance; N, normal range; hosp., hospitalization.
[a]On the first day of admission to the hospital, Data are mean ± SD of values.

factor in this disease. Bleeding is significantly frequent (~90%) in fatal CCHF patients compared with nonfatal patients (23%) ($P < 0.05$). Pulmonary and gastrointestinal hemorrhages can be life threatening and carry a very high risk for mortality. Consequently, the analysis of factors contributing to bleeding is important in CCHF. In this study, we investigated the characteristics of markers of coagulopathy in CCHF-infected patients and their clinical significance as a predictor factor of mortality.

Platelet count was generally low in hospitalized patients of CCHF disease. In our study group, overall platelet count was approximately $62 \pm 43 \times 10^9$ cells/l. In fatal CCHF disease, platelet count was significantly lower than that of surviving patients. In the previous studies, lower platelet count was found as a poor prognostic factor in those patients (5,7).

In CCHF disease, PT and aPTT were frequently longer in hospitalized patients. During hospitalization, most of patients had a longer PT with a higher prevalence in fatal patients. INR, a measure of extrinsic pathway of coagulation, was high in fatal cases similarly to PT. Similar to the previous studies, during hospitalization determination of aPTT > 60 sec was a significant prognostic parameter in CCHF (5). More than half of fatal patients had aPTT > 60 sec, whereas it was only 2.7% in nonfatal patients in this study.

In this study, the levels of protein C and protein S were lower than normal in a significant part of CCHF patients. However, we did not found any differences for protein C and S between fatal and nonfatal cases. Antithrombin III was in normal range in most of CCHF patients. It was low in ~16% of all patients. Serum protein C, protein S, and antithrombin III have been investigated in some studies with hemorrhagic fevers including Dengue fever (DF) (9,10,15) and Ebola hemorrhagic fever (16). However, those parameters have not been studied in CCHF disease previously. Gorp et al. studied protein C and protein S concentrations in 50 patients of DF grade III and IV. They found lower protein C and protein S concentrations in fatal DF sera compared with those survivors (9). Nguyen et al. also found lower levels of serum protein C and protein S concentrations in acute and convalescent-phase samples compared with nonshock DF and dengue shock syndrome in infants controls (15). However, decreases in the levels of protein C and S were not correlated with the severity of DF or with gastrointestinal bleeding.

D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. Although a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential etiologies. D-dimer testing was originally developed in the diagnosis of DIC (16). A wide range of D-dimer levels was determined in CCHF disease. Overall ~73% of CCHF patients had high levels of D-dimer. However, it was not a predictor of mortality. Only eight (~10%) of all CCHF patients had lower fibrinogen. However, three of them were in fatal group. Definitive diagnosis of DIC depends on the result of thrombocytopenia, prolonged PT and aPTT, low fibrinogen concentration, and increased levels of fibrin degradation products (17). High levels of D-dimer with thrombocytopenia, prolonged PT and aPTT may be an indicator of a DIC presentation in CCHF.

CONCLUSION

Bleeding was an important problem in CCHF. We analyzed an expanded group of parameters related with coagulopathy, including serum protein C, S, antithrombin III, and APCR, in addition to classical parameters.

Parameters of coagulopathy, including protein S, protein C, antithrombin III, APCR, and D-dimer level, were not associated with mortality. Platelet count, PT, aPTT, INR, and fibrinogen were prognostic factors associated with mortality for CCHF. Especially, platelet count $< 20 \times 10^9$ cells/l and aPTT > 60 sec were important. In conclusion, laboratory tests including classical parameters of coagulopathy seem to be enough for the follow up of CCHF, the others were not contributory. Thrombocytopenia, prolonged PT and aPTT, increased levels of D-dimer, and low fibrinogen concentration may show DIC presentation at least in some of CCHF patients.

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