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Original Article

Presepsin Levels of Patients with Crimean-Congo Hemorrhagic Fever

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SUMMARY: Levels of presepsin (a soluble cluster of differentiation subtype 14 [CD14]) are thought to increase in cases of bacterial infection. CD14 has also been found to play a role in the pathogenesis of various viral diseases. Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic arboviral infection. Our study focuses on presepsin levels as a biomarker for CCHF. Serum presepsin levels in a CCHF group (n = 59) and control group (n = 28) were compared. Patients with CCHF were classified according to severity grading score as having mild, moderate, or severe infection and were allocated to corresponding subgroups (groups 1, 2, and 3, respectively). Presepsin levels were measured in serum samples by using a commercial enzyme-linked immunosorbent assay kit. The mean presepsin levels in the CCHF group as a whole and the healthy group were found to be significantly different (1,499.46 ± 411.96 pg/ml and 430.68 ± 61.21 pg/ml, respectively). The mean presepsin levels of the CCHF subgroups (1, 2 and 3) and the healthy group were also found to be significantly different (1,204.53 ± 371.18, 1,464.21 ± 338.37, 2,007.36 ± 82.18, and 430.68 ± 61.21 pg/ml, respectively) ($p < 0.05$). We also found that as the severity of the disease increased, the presepsin level also increased. We postulate that the presepsin levels could be used as a supportive biomarker for diagnosis and follow-up of the disease.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne human disease caused by a negative-stranded RNA virus of the *Nairovirus* genus and *Bunyaviridae* family (1). CCHF is an acute zoonotic viral disease causing high mortality and is seen in many countries in Africa, Asia, Europe, and the Middle East (2). The mortality rate among adults is between 3 and 30% in countries where the disease has been observed (3,4).

The limited information available about the pathogenesis of the disease has been obtained by analyzing changes in liver biopsies and blood test results (5). The most common pathological findings are increase in capillary fragility, endothelial damage, platelet aggregation, and degranulation, and similar hemostatic disorders.

The development of bleeding, which is an important predictive clinical symptom of disease progression, is not necessarily a result of the interaction between virus and cells, but can occur as a result of the effect of proinflammatory cytokines released in response to infection (2,6). Natural immunity, tumor necrosis factor α (TNF-α), type I interferon (type I IFN), interleukins-1, 6, and 10 (IL-1, 6, and 10), and similar cytokines and toll-like receptors (TLRs) play an important role in disease pathogenesis (7–9).

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Cluster of differentiation 14 (CD14) is a receptor with glycoprotein structure that is expressed on the surface of monocytes and macrophages. CD14 activates TLR4 causing release of some proinflammatory cytokines and initiation of the immune response to microorganisms. In the course of inflammation, the soluble form of CD14 (sCD14) is degraded by plasma proteases, and fragments called presepsin are formed (10). An increase in presepsin levels due to gram-negative microorganisms has been reported, particularly in septic patients (11). Recent studies have indicated that CD14 plays a role in the pathogenesis of various viral diseases. However, no study has investigated presepsin levels in patients with CCHF.

MATERIALS AND METHODS

Study population: This prospective study was conducted at Cumhuriyet University Hospital, Sivas, Turkey. The protocol was approved by the Cumhuriyet University Ethical Committee. The study included 59 patients with CCHF (64.41% man) and 28 healthy controls (60.71% man). Diagnosis was made on the basis of clinic and laboratory findings. Only those patients whose CCHF diagnosis was confirmed at the National Reference Virology Laboratory of Refik Saydam Hygiene Center, Ankara, Turkey, were enrolled in the study. Patients' demographic and clinical characteristics, such as age, sex, occupation, city of residence, history of tick bite or of tick removal, smoking, and clinical outcome were obtained from the hospital information system. The most frequently observed clinical symptom was fever, which was found in 42 patients (71.19%). Other clinical findings were fatigue, myalgia, headache, tonsillopharyngitis, nausea, vomiting, som-

nolence, and agitation. Petechial-purpura and ecchymosies were found in 35 patients (59.3%), mucosal hemorrhages in 11 patients (18.6%), and internal bleeding in 2 patients (3.4%). Hepatomegaly was found in 1 patient (1.7%) and splenomegaly in 1 (1.7%). No patients had organ failure or died.

Patients with autoinflammatory diseases, chronic diseases such as chronic renal failure and liver disease, and malignancy were excluded, as were pregnant patients. Controls and patients were similar in terms of age and sex ($p > 0.05$). Control subjects were randomly recruited from a group of healthy volunteers who had been admitted to the hospital for routine checks. Basic laboratory tests (routine biochemistry analyses, complete blood counts, and coagulation tests) and physical examinations were carried out on controls and no pathological characteristics. Exclusion criteria in the study included clinical suspicion of infections (body temperature outside the range of 36–38°C, heart rate >90 beats/min, respiratory rate >20 breaths/min, white blood cell (WBC) count >12,000 cells/mm^3 or <4,000 cells/mm^3), presence of liver disease, kidney disease, malignancy, pregnancy, and smoking in healthy controls.

Laboratory analysis: All measurements were obtained from venous blood specimens collected from patients and controls. Blood samples of the patients were collected on the first morning of admission. An empty tube with gel was used for presepsin measurement and routine biochemistry analysis, a citrated tube for coagulation test analysis, and a tube containing K_2EDTA for complete blood count (all tubes were manufactured by Becton Dickinson, Franklin Lakes, NJ, USA). Plasma and serum specimens were obtained after centrifugation of the blood samples. Routine biochemistry analyses, complete blood counts and coagulation tests were immediately carried out on specimens. Serum samples for presepsin analysis were aliquoted, frozen, and kept at −20°C until testing.

Presepsin levels were determined in serum samples by using an enzyme-linked immunosorbent assay (ELISA) kit (Abbexa, Cambridge, UK) on a Triturus Analyser (Diagnostics Grifols, Barcelona, Spain). The range of the assay was 65–3,000 pg/ml.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine levels were determined in serum samples spectrophotometrically on an AU5800 Clinical Chemistry System (Beckman Coulter, Brea, CA, USA). The prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR) values were determined in plasma samples by means of a clotting time assay on an ACL TOP 300 CTS analyzer (Instrumentation Laboratory, Bedford, MA, USA). The same analyzer was used to determine D-dimer values in plasma samples by turbidimetric assay. The leukocyte (WBC), thrombocyte (PLT), hemoglobin (Hb), and monocyte counts (MON) were determined in whole blood samples on a BC 6800 Hematology Auto Analyzer (Mindray, Shenzen, China).

Statistical analysis: All analyses were conducted using SPSS Statistics for Windows ver. 22.00. Continuous variables were expressed as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to check the normality of distribution. In intergroup comparisons, Student's t, one-way analysis of variance (ANOVA), and Tukey's range test, which are parametric tests, and the Mann-Whitney U test and chi-square test, which are non-parametric tests, were used. Pearson correlation analysis was used to determine correlation. A p-value of <0.05 was considered significant.

RESULTS

Basic characteristics of the study population and laboratory results are given in Table 1. The mean presepsin level was determined to be 1,499.46 ± 411.96 pg/ml (range, 621–2,109 pg/ml) and 430.68 ± 61.21 pg/ml (range, 345–559 pg/ml) in the patient and control group, respectively. There was a significant difference in presepsin levels between the patient and control groups ($p < 0.05$) (Table 1). Patients with CCHF were allocated to a mild (group 1), moderate (group 2), or severe group (group 3) on the basis of their severity grading score (SGS) (12), and serum presepsin levels were compared among these 3 subgroups and controls. A significant difference in presepsin levels was found among the 3 patient subgroups and the control group ($p < 0.001$) (Table 2).

A statistically significant positive correlation was also found between the presepsin levels and the AST, LDH, PT, aPTT, D-dimer, and INR values ($p = 0.003$, correlation coefficient (r): 0.38; $p = 0.001$, r: 0.523; $p = 0.02$, r: 0.3; $p = 0.002$, r: 0.4; $p = 0.001$, r: 0.5; $p = 0.01$, r: 0.317, respectively). In addition, a negative correlation was observed between presepsin levels and PLT values ($p = 0.001$, r: −0.571) in the acute phase (Fig. 1). No correlation was observed between presepsin levels and the creatinine and BUN values ($p = 0.97$, r: −0.007; $p = 0.15$, r: −0.28, respectively).

DISCUSSION

In this study, we have found that presepsin levels in patients with CCHF were higher than those in the healthy control group. In addition, when CCHF patients were grouped in terms of disease severity, a significant inter-group difference was found with respect to presepsin levels (12). These results suggest that evaluation of presepsin levels in patients with CCHF should be considered.

CD14 is a myeloid cell receptor that binds to bacterial lipopolysaccharides, ensures intracellular transfer of endotoxins and thus stimulates the inflammatory response (13). The soluble form of CD14 is found in blood, and its production is thought to increase during infections. In a healthy population, the serum concentration of sCD14 is at microgram level (10), and is an indicator of macrophage and monocyte activation. Presepsin is an indirect sepsis marker that forms with degradation of sCD14 (11,14). Elevated levels of presepsin have been found in various infectious diseases such as acquired immunodeficiency syndrome (15), meningitis (16), hepatitis (17), sepsis (11), periodontitis (18), and malaria (19).

In viral diseases with hemorrhagic fever, including

Table 1. Baseline characteristics of study groups

	CCHF (n = 59)	Control (n = 28)	p value
Study marker			
Presepsin (pg/ml)	1,499.46 ± 411.96	430.68 ± 61.21	<0.005
Baseline characteristics			
Age (yr)	49.34 ± 13.52 (range, 22–71)	49.5 ± 11.94 (range, 27–71)	>0.05
Man/Woman	38/21	17/11	>0.05
Additional disease (hypertension)	7 (11.9%)		
Presence of tick exposure	41 (69.5%)		
Presence of livestock exposure	59 (100.0%)		
Duration of symptoms (days)	4.44 ± 1.94		
Laboratory analysis			
AST (IU/l)	235.6 ± 210.28	23.57 ± 8.37	<0.005
ALT (IU/l)	122.98 ± 103.16	19.21 ± 9.06	<0.005
LDH (IU/l)	692.54 ± 541.39	195.46 ± 31.86	<0.005
BUN (mg/dl)	17.27 ± 12.72	14.34 ± 3.20	>0.05
creatinine (mg/dl)	0.89 ± 0.41	0.82 ± 0.15	>0.05
Hb (g/dl)	14.13 ± 1.62	14.05 ± 1.06	>0.05
PLT (cells/μl)	76,677.97 ± 45,214.22	250,500 ± 43,780.17	<0.005
WBC (cells/μl)	3,890.51 ± 2,375.73	4,354.64 ± 676.59	>0.05
MON (cells/μl)	180 ± 140	160 ± 130	>0.05
aPTT (s)	40.51 ± 12.37	31.04 ± 4.63	<0.005
PT (s)	14.78 ± 2.89	11.68 ± 0.60	<0.005
INR	1.15 ± 0.30	1.03 ± 0.08	<0.05

Results are n (%) or mean ± standard deviation.
CCHF, Crimean-Congo hemorrhagic fever; AST, aspartate aminotransferase; ALT, alanine amino transferase; LDH, lactate dehydrogenase; BUN, blood urea nitnogen; Hb, hemoglobin; PLT, platelet; WBC, white blood cell; MON, monocyte; aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio.

Table 2. The presepsin level of patients with CCHF and control group

	CCHF			Control	p value
	Group 1[1)] (n = 15)	Group 2[1)] (n = 33)	Group 3[1)] (n = 11)	(n = 28)	
Presepsin level (pg/ml)	1,204.53 ± 371.18	1,464.21 ± 338.37	2,007.36 ± 82.18	430.68 ± 61.21	<0.001

[1)]: According to SGS system developed by Bakir et al. (12), patients with CCHF are classified in mild, moderate, and severe groups (group 1, 2, and 3, respectively).

CCHF, the virus proliferates in the regional lymphatic glands and local tissues after entering the body and spreads to other organs, in particular the spleen and liver, by means of monocytes (20). The main inflammatory cells which participate in these diseases are monocytes and neutrophils. A systematic inflammatory response occurs by the interaction of macrophages and endothelial cells in particular (8,21,22). It has been reported that cytokines such as TNF-α and IFN-γ, which are released from activated lymphocytes, could lead to co-activation of macrophages in the course of the disease (1,5,23–25). With respect to the current study, higher levels of presepsin observed in patients with CCHF compared to those in healthy controls are likely to be associated with macrophage and monocyte hyperactivation, which develops in disease pathogenesis. According to the results of some studies on viral meningitis and hepatitis, elevated levels of sCD14 are likely to be associated with macrophage activation (16,26).

Although the mechanism of presepsin release is not fully understood, one stimulus that increases its level is the process of phagocytosis (1). In a study on human cell culture, phagocytosis was reported to stimulate presepsin release from monocytes (27). According to a study by Karti et al., reactive hemophagocytosis can develop in the course of CCHF (28).

The clinical severity of CCHF can change from mild to the manifestation of disseminated intravascular coagulation (DIC). It has been postulated that, in order to be able to make a judgment about the severity of the disease, factors such as old age, elevated AST, ALT, and LDH levels, increased WBC count, and the presence of bleeding and organ failure should be evaluated together (12). In our study, AST, ALT, LDH, and WBC levels in the patient group were higher than those in the control group. Moreover, a positive correlation was found between presepsin levels and AST, LDH, PT, aPTT, D-dimer, and INR values, whereas a negative correlation was found between presepsin levels

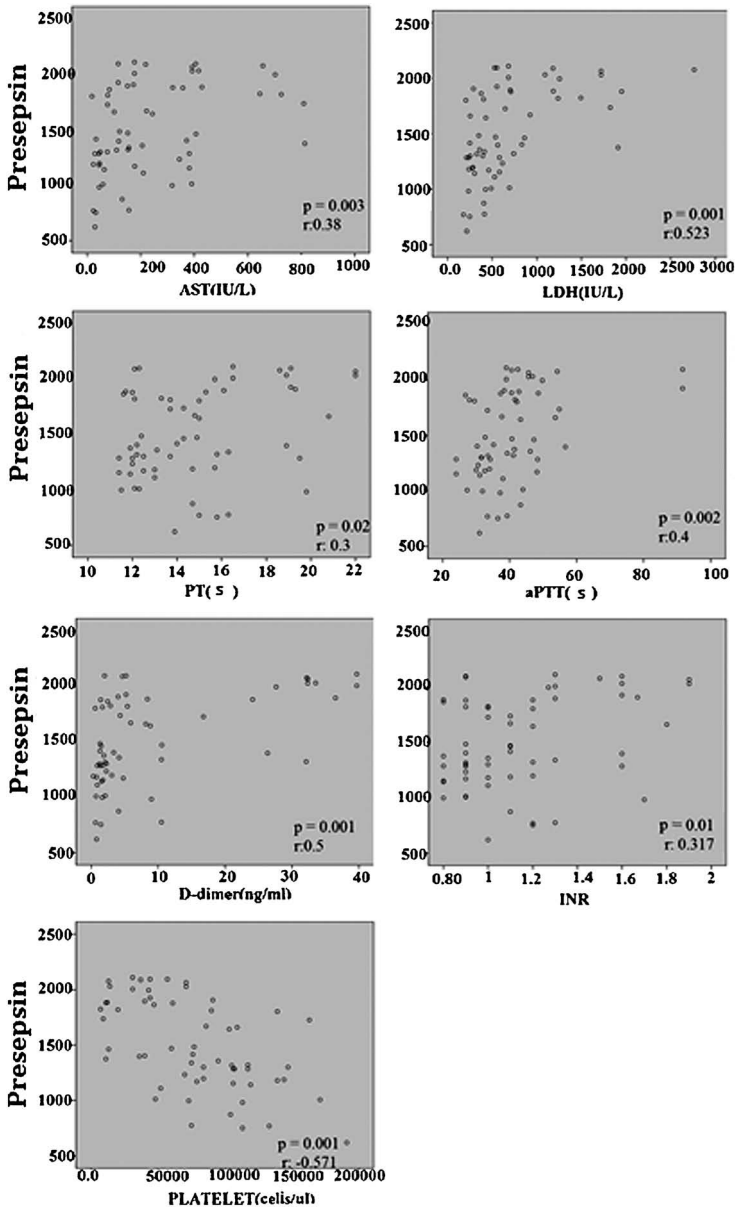

Fig. 1. Scatter plot matrix with Pearson correlation between presepsin and AST, LDH, PT, aPTT, D-dimer, INR, or platelet values.

and platelet values. We found that if the cases in the acute period of the disease are grouped based on the SGS, presepsin levels are observed to increase significantly as the severity score increases. When assessing the patient in the acute period, evaluating presepsin levels prior to obtaining all other laboratory results could help save time in the clinical approach to disease.

There is no available study investigating the usefulness of presepsin levels in assessing patients with CCHF. When the laboratory findings of CCHF patients were analyzed, it was determined that there were no observed changes in the levels of platelets, WBC, ALT, and AST in some cases (29). Thus, in addition to routine laboratory tests used for the diagnosis and follow-up of CCHF patients, we postulate that it would be beneficial to use presepsin as a biomarker for diagnosis and follow-up of the disease. Moreover, in order to obtain more information and to reveal the association between presepsin levels in CCHF patients, studies with a higher number of patients should be conducted.

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Conflict of interest None to declare.

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