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The diagnostic and prognostic significance of SCUBE1 levels in Crimean-Congo hemorrhagic fever

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SUMMARY

Background: The new biochemical marker, signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1), is secreted and cell surface glycoprotein expressed during early embryogenesis. The protein is found in platelet and endothelial cells. Crimean-Congo hemorrhagic fever (CCHF), which is caused by a tick-borne virus belonging to the *Bunyaviridae* family, may present with a mild clinical course or may exhibit a severe profile with potentially fatal hemorrhaging. The aim of this study was to determine the diagnostic and prognostic significance of SCUBE1 levels in CCHF.
Methods: This study was conducted with patients with CCHF. SCUBE1 levels in patients with CCHF were determined using an ELISA.
Results: SCUBE1 titers of CCHF patients were significantly higher compared to those of the control group ($p = 0.0001$). SCUBE1 levels of patients with hemorrhage were significantly higher than those of patients without hemorrhage ($p = 0.0001$). SCUBE1 values of patients who died were significantly higher than those of the survivors ($p = 0.012$).
Conclusions: SCUBE1 levels are a new biomarker that can be used in the differential diagnosis and monitoring of patients hospitalized with suspected CCHF. These levels are also significant as potential predictors of mortality.
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1. Introduction

The new biochemical marker, signal peptide-CUB-EGF domain-containing protein 1 (SCUBE1), is secreted and cell surface glycoprotein expressed during early embryogenesis. The protein is found in platelet and endothelial cells.[1] It contains nine EGF-like domains and one CUB domain at the carboxyl terminus. Secreted SCUBE1 can form oligomers and stably associates with the cell surface. Tu et al. reported that SCUBE1 is stored in the alpha-granules and is translocated to the surface upon platelet stimulation. SCUBE genes are present in the human, mouse, and zebrafish, suggesting that they are evolutionarily conserved and may have critical biological functions.[2] Potential roles and applications of plasma SCUBE1 levels in acute coronary syndrome and acute ischemic stroke have been shown by Dai et al.[3]

Crimean-Congo hemorrhagic fever (CCHF), which is caused by a tick-borne virus belonging to the *Bunyaviridae* family, may present with a mild clinical course or may exhibit a severe profile with potentially fatal hemorrhaging.[4,5] CCHF is a fatal viral infection described in parts of Africa, Asia, Eastern Europe, and the Middle East.[6] Patients mostly present with complaints such as fever, fatigue, generalized pain, myalgia, nausea, and vomiting, while severe cases exhibit epistaxis, hematemesis, melena, hematuria, gingival bleeding, vaginal bleeding, petechiae, or ecchymosis.[4,7] The main target of the CCHF virus is the mononuclear phagocyte system and endothelial cells.[4]

SCUBE1 may therefore be considered a diagnostic and prognostic marker for infectious diseases that impact on endothelial cells and platelets. The aim of this study was to determine the diagnostic and prognostic significance of SCUBE1 levels in CCHF.

2. Materials and methods

2.1. Study population

This retrospective study was conducted with patients with CCHF hospitalized between 2008 and 2011 at the Department of Infectious Diseases and Clinical Microbiology of the Faculty of

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Medicine, Karadeniz Technical University, Turkey. Informed consent was obtained from all patients and controls, and the study was approved by the local ethics committee. Blood samples were taken from subjects who were admitted to the hospital with a suspicion of CCHF, and patients who were diagnosed as having CCHF were included in the study. Blood samples were taken and collected until the day of measurement. SCUBE1 measurements were performed only in these blood samples. Patients whose CCHF diagnoses were confirmed through the detection of IgM using an ELISA and/or the genomic segment of the virus using real-time PCR (RT-PCR) in the Virology Laboratory of the Refik Saydam National Hygiene Center of the Turkish Ministry of Health, were enrolled. The presence of CCHF-specific serum IgM antibodies was determined using an in-house ELISA method. The antigens used in the ELISA tests were obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). Demographic characteristics, occupation, tick exposure history, incubation time, symptoms, duration between onset of symptoms and admission, clinical findings, and laboratory tests (white blood cell count (WBC), platelet count (PLT), hemoglobin (Hb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), myoglobin (Mb), blood urea nitrogen (BUN), plasma creatinine (Cr), C-reactive protein (CRP), prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), and D-dimer) of all patients were recorded on forms at admission. The control group was formed of companions of the CCHF patients in our clinic in 2011.

2.2. Determination of SCUBE1 levels

Levels of SCUBE1 were determined using an ELISA kit (Cusabio Biotech Co., Catalog No. CSB-E15005 h, PR China), in accordance with the manufacturer's instructions. The absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (Molecular Devices, CA, USA). The results were expressed in ng/ml. The minimum detectable dose of human SCUBE1 is typically less than 0.16 ng/ml.

2.3. Statistical analysis

A descriptive statistical analysis was performed for all parameters. The Kolmogorov–Smirnov test was used for the eligibility of variables. Data in conformity with normal distribution were analyzed using the Student's *t*-test, and those not conforming to normal distribution using the Mann–Whitney *U*-test. Data obtained by measurements are given as the mean ± standard deviation. Data obtained by counting are given as numbers with percentages; analyses were performed using the Chi-square test. The area under the receiver operating characteristics (ROC) curve was used to calculate the discriminative ability of SCUBE1 to determine patients with CCHF. Sensitivity, specificity, the negative predictive value (NPV), and the positive predictive value (PPV) were calculated according to ROC curves for SCUBE1. A p-value of <0.05 was considered significant.

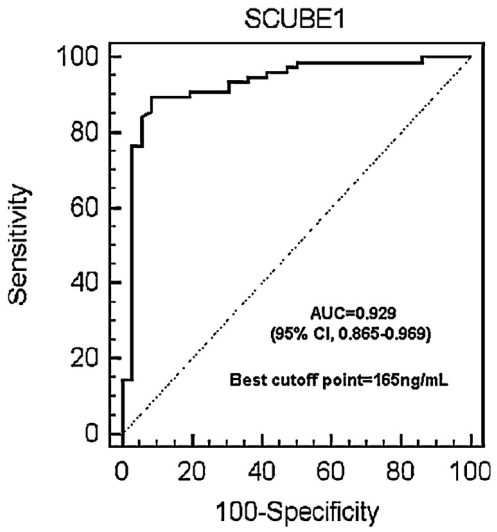


Figure 1. ROC curve analysis of CCHF patients' SCUBE1 values.

3. Results

Two hundred five consecutive patients with CCHF, 103 female and 102 male, with a mean age of 48.3 ± 16.7 years, were included in the study. The control group consisted of 76 individuals (41 female and 35 male), with a mean age of 42.2 ± 13.4 years. One hundred

Table 1
Correlation analysis between SCUBE1 and other laboratory parameters

Biochemical markers	Mean ± SD	Correlation analysis (SCUBE1)
SCUBE1 (ng/ml)	272.0 ± 89.0	$r = 1$
WBC ($\times 10^9$/l)	2.852 ± 2.019	$r = -0.096$; $p = 0.409$
Hb (g/dl)	13.7 ± 1.7	$r = -0.039$; $p = 0.736$
PLT ($\times 10^9$/l)	82.900 ± 48.129	$r = -0.488$; $p = 0.0001$
AST (U/l)	211 ± 403	$r = 0.282$; $p = 0.014$
ALT (U/l)	113 ± 163	$r = 0.341$; $p = 0.003$
CPK (U/l)	672 ± 1058	$r = 0.348$; $p = 0.002$
LDH (U/l)	776 ± 560	$r = 0.370$; $p = 0.001$
Mb (g/dl)	156 ± 291	$r = 0.145$; $p = 0.227$
BUN (mg/dl)	17.8 ± 13.4	$r = 0.357$; $p = 0.002$
Cr (mg/dl)	0.9 ± 0.3	$r = 0.456$; $p = 0.0001$
CRP (mg/dl)	2.0 ± 2.7	$r = -0.002$; $p = 0.985$
PT (s)	13.9 ± 2.6	$r = 0.188$; $p = 0.104$
aPTT (s)	35.8 ± 9.1	$r = 0.421$; $p = 0.0001$
INR	1.2 ± 0.2	$r = 0.161$; $p = 0.165$
D-dimer (μg/ml)	5.5 ± 9.2	$r = 0.384$; $p = 0.001$

ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Hb, hemoglobin; Cr, plasma creatinine; CRP, C-reactive protein; INR, international normalized ratio; LDH, lactate dehydrogenase; Mb, myoglobin; PLT, platelet count; PT, prothrombin time; SCUBE1, signal peptide-CUB-EGF domain-containing protein 1; SD, standard deviation; WBC, white blood cell count.

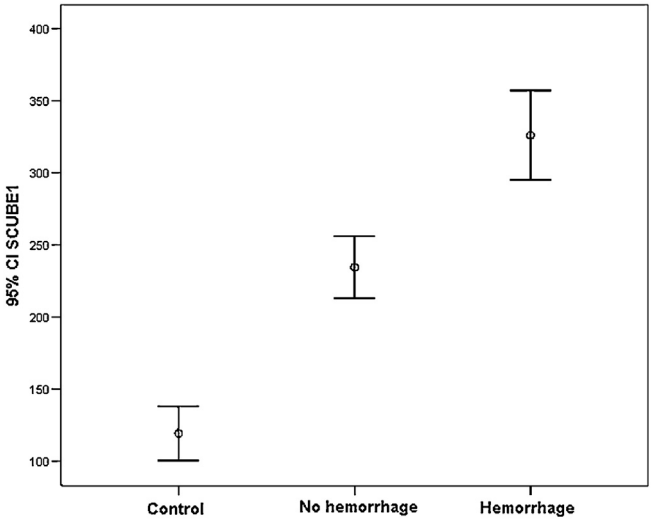


Figure 2. SCUBE1 values for the control group and CCHF patients with and without hemorrhage.

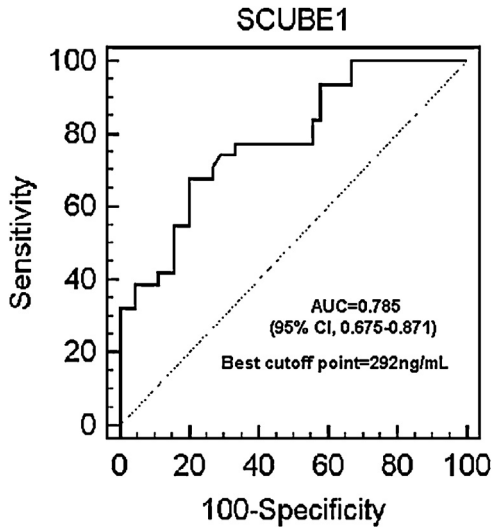


Figure 3. ROC curve analysis of CCHF patients' SCUBE1 values by presence of hemorrhage.

sixty-two patients worked in farming or animal husbandry. A history of tick bite was established in 147 patients. Fever (88.8%), headache (68.3%), nausea (68.3%), myalgia (81.5%), vomiting (28.3%), and bleeding (21.0%) were observed in these patients. A SCUBE1 value of 272.0 ± 89.0 ng/ml was determined in the CCHF patients and of 119.4 ± 55.7 ng/ml in the control group ($p = 0.0001$). While the SCUBE1 value was negatively correlated with PLT, it was positively correlated with AST, ALT, CPK, LDH, BUN, Cr, aPTT, and D-dimer values (Table 1). When the SCUBE1 results were analyzed using the ROC curve method, the optimum diagnostic cut-off point was 165 ng/ml, the area underneath the ROC curve (AUC) was 0.929 (95% confidence interval (CI) 0.865–0.969), sensitivity 89.5%, specificity 91.7%, PPV 95.8%, and NPV 80.5% (Figure 1).

The mean SCUBE1 value in patients with hemorrhage was 326.3 ± 84.5 ng/ml, compared to 234.7 ± 71.7 ng/ml in patients without hemorrhage ($p = 0.0001$) (Figure 2). On ROC curve analysis of SCUBE1 values, the optimum diagnostic cut-off point was determined to be 292 ng/ml and AUC was 0.785 (95% CI 0.675–0.871). This cut-off point SCUBE1 value had a sensitivity of 67.7%, specificity 80%, PPV 70%, and NPV 78.3% for potentially identify hemorrhagic patients (Figure 3).

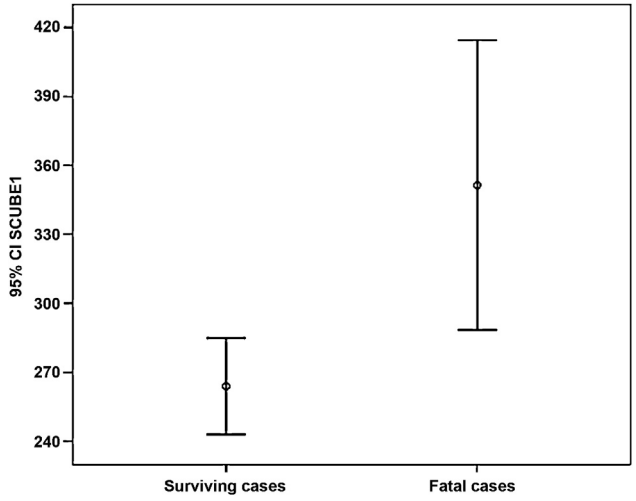


Figure 4. SCUBE1 values for the surviving and fatal CCHF cases.

Seven of the patients died. A SCUBE1 value of 351.3 ± 68.2 ng/ml was determined in the patients who died, and of 264.0 ± 87.3 ng/ml in the survivors ($p = 0.012$) (Figure 4). In terms of mortality, when the SCUBE1 results were analyzed using the ROC curve method, the optimum diagnostic cut-off point was determined to be 310 ng/ml, AUC 0.791 (95% CI 0.682–0.876), sensitivity 71.4%, specificity 72.5%, PPV 20.8%, and NPV 96.2%.

4. Discussion

SCUBE1 is a new biomarker, and no studies on its use in CCHF are as yet available. SCUBE1 appears to be expressed selectively in the endothelium.[8,9] In situ hybridization has demonstrated SCUBE1 expression in the vascular endothelium lining the arteries, veins, and microvessels of all organs examined.[8] Increased SCUBE1 levels have been reported in diseases such as acute coronary syndromes and acute ischemic stroke, which impact on the endothelium.[3] SCUBE1 levels were also found to be elevated in gastric cancer and hemodialysis patients compared to control groups.[10,11] In this study, the SCUBE1 values of patients with CCHF were higher than those of the controls ($p = 0.0001$). A cut-off value of 165 ng/ml for SCUBE1 was associated with 89.5% sensitivity and 91.7% specificity, with an NPV of 80.5% and PPV of 95.8%. While there was a negative correlation between SCUBE1 values and PLT, there was a positive correlation between SCUBE1 and AST, ALT, CPK, LDH, BUN, Cr, aPTT, INR, and D-dimer. Dai et al. determined that both acute coronary syndromes and acute ischemic stroke were independent predictors of elevated plasma SCUBE1 concentrations.[3] According to our results, high SCUBE1 levels would seem to be a marker for CCHF.

Patients with CCHF may present with a clinical profile spectrum varying from mild to fatal.[4,12,13] Mortality is particularly high following massive hemorrhages resulting from thrombocytopenia and endothelial dysfunction.[4,12] The prediction of hemorrhage is important for replacement of blood and blood products, supportive treatments, and the preparation of intensive care conditions. SCUBE1 levels in the patients when they first presented to the hospital were over 292 ng/ml, and this could predict possible hemorrhage with 67.7% sensitivity and 80% specificity. This will help clinicians to plan treatment and make efficient use of available equipment.

Increased SCUBE1 levels may also facilitate the prediction of mortality. In our study, SCUBE1 levels over 310 ng/ml indicated mortality with 71.4% sensitivity and 72.5% specificity. Dai et al. reported that SCUBE1 levels are a prognostic biomarker for acute coronary syndromes and acute ischemic stroke.[3] Prediction of the prognosis of the disease may be vital in ensuring intensive support treatment after the first day of hospitalization.

In conclusion, SCUBE1 levels may be a novel biomarker for the prediction of severity of disease in patients with CCHF when presenting to the hospital. The use of SCUBE1 levels for prognosis may be possible; further studies with SCUBE1 measurements taken at different periods of the disease are required. Additionally, SCUBE1 can predict the disease course in patients with CCHF and guide the clinician throughout treatment.

4.1. Limitations

The major limitation of the current study is the relatively small number of patients and controls involved. However, in terms of the novel finding that SCUBE1 is a new prognostic biomarker, our study can be considered a pioneering work in the field and can serve to prompt further comprehensive studies.

In conclusion, SCUBE1 levels may be a good biomarker for the prediction of the prognosis and severity of disease in patients with CCHF when presenting to the hospital.

Ethical approval: The study was approved by the local ethics committee (reference 2010-69).

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Conflict of interest: The authors had no personal relationships with other individuals or organizations that might have inappropriately influenced their work during the submission process.

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