Increased Irisin Concentrations in Patients with Crimean- Congo Hemorrhagic Fever

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SUMMARY: Crimean-Congo Hemorrhagic Fever (CCHF) is a life-threatening viral infection. The pathogenesis of the disease is not well understood. The aim of this study was to determine the change in irisin concentrations in patients with CCHF. The study included a total of 30 patients with CCHF and 30 control participants. Irisin concentrations were determined using a commercial ELISA kit. Median irisin concentrations were 9.03 (5.81–12.22) µg/mL and 4.2 (3.39–7.62) µg/mL, respectively, in each group. There was no correlation between irisin and disease severity. Any correlations between irisin, and lactate dehydrogenase (LDH), international normalization ratio (INR), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelets, activated partial thromboplastin time (aPTT), D-dimer and hemoglobin, were also investigated. There were statistically significant positive correlations between the values of irisin, and platelet (p = 0.005, r: 0.369), ALT (p = 0.049, r: 0.261), INR (p = 0.006, r: 0.359) and aPTT values (p = 0.002, r: 0.405). A negative correlation was also found between the values of irisin and LDH (p = 0.008, r: −0.348). No correlations were determined between the values of irisin, and AST, hemoglobin and D-dimer. These results suggest that irisin may have a role in CCHF.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by the CCHF virus (CCHFV), a tick-borne virus (Nairovirus genus) of the Bunyaviridae family (1). The disease is endemic in Africa, the Balkans, the Middle East, and Asia (2). CCHF has different non-specific symptoms such as high fever, muscle pain, dizziness, abnormal sensitivity to light, abdominal pain, and vomiting (3). The main clinical abnormality is hemorrhage due to increased vascular permeability, which together with the replication of the virus, proinflammatory response, endothelial damage, activation of the intrinsic coagulation cascade, dissemination of intravascular coagulation, and multi-organ failure, contribute to the pathogenesis of CCHF (4).

Proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, IL-10, and tumor necrosis factor-alpha, soluble tumor necrosis factor receptors, and interferon-gamma have an important role in the pathogenesis of CCHF (5,6). Several cytokines and peptides are produced, expressed, and released by muscle fibers and adipokines. These cytokines exert either paracrine or endocrine effects. One of these cytokines is irisin (fibronectin type III domain containing protein 5 [FNDC5]) (7). It is mainly released from adipocytes and muscles (8,9). Irisin is associated with different physiological and pathological conditions such as type 2 diabetes mellitus and renal disease (10,11). All of these studies indicate that irisin can also upregulate during acute or chronic inflammatory conditions (8).

To the best of our knowledge, there has been no study on the changes in irisin concentration in patients with CCHF. The purpose of this study was to investigate the concentration of irisin in patients with CCHF compared to a healthy control group. This study provides an important opportunity to advance the understanding of the relationship between irisin concentrations and CCHF.

MATERIAL AND METHODS

Patients and controls: This prospective study was carried out in Cumhuriyet University, Turkey. Patients admitted to our hospital from January to August 2016 with CCHF confirmed by the national reference Virology laboratory of Refik Saydam Hygiene Center in Ankara, Turkey, were enrolled in this study. CCHF confirmed by the presence of CCHFV RNA by reverse transcriptase-polymerase chain reaction and/or CCFV-specific IgM by ELISA in blood were defined as confirmed CCHF cases. The study participants comprised 30 CCHF patients and 30 healthy controls. Eleven patients (36%) exhibited hemorrhage, petechial-purpura and ecchymosis, and 2 patients had both hepatomegaly and splenomegaly. None of the patients had organ failure, and no mortality developed. No patient was taking ribavirin. Data were obtained from the records of Cumhuriyet University Hospital laboratory information system, including age, gender, and the concentrations of lactate dehydrogenase (LDH), international normalization ratio (INR), aspartate aminotransferase (AST), prothrombin time (PT), platelets, activated partial thromboplastin time (aPTT), D-dimer and hemoglobin (Hb). Patients were classified according to Bakır’s score (12) and then correlations

Received October 9, 2016. Accepted January 20, 2017. J-STAGE Advance Publication February 28, 2017. DOI: 10.7883/jyen.2016.432.

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Between irisin and disease severity were investigated. Correlations between irisin, and LDH, INR, ALT, AST, platelets, aPTT, D-dimer, and Hb, were also investigated. Patients with impaired renal and thyroid function, diabetes mellitus, rheumatic disease, intestinal disease, or malignancy were excluded from the study. For the control group, the exclusion criteria also included clinical suspicion of infections (body temperature out of the 36–38°C range, heart rate > 90 beats/min, respiratory rate > 20 beats/min, white blood count > 12 × 10³ mcL or < 4 × 10³ mcL), the presence of liver disease, kidney disease, rheumatic disease, malignancy, pregnancy or smoking. The protocol was approved by the Cumhuriyet University Ethics Committee. Informed consent was obtained from all individual participants included in the study.

**Samples:** Overnight fasting blood samples were collected from all participants into red top tubes (Becton Dickinson, Oxford, UK). Samples were taken on the day of admission to the hospital. The mean time from the disease onset to blood collection in patients was 2 days (minimum time: 1st day and maximum time: 4th day). The serum sample tubes were allowed to clot before centrifugation. After centrifugation at 4°C for 15 min at 3,500 rpm, the serum was aliquoted and immediately frozen at −40°C.

**Biochemical analysis:** Irisin concentrations were determined using commercially available ELISA kits (YH Bioscience, Shanghai, China). The intra-assay and inter-assay confidence intervals were < 10% and < 12% respectively. The limit of detection of ELISA was 0.1 ng/mL. LDH and AST concentrations were determined using the colorimetric enzymatic method (Beckman Coulter AU5,800, Brea, CA,USA). PT, aPTT and D-dimer values were determined using an autoanalyzer (ACLTOP 700, Instrumentation Laboratory, Bedford, MA, USA). Hemogram analysis was performed using an auto hematology analyzer (Mindray BC6,800, Shenzhen, China).

**Statistical analysis:** Analyses were performed using IBM SPSS software (release 20.0, IBM, SPSS Inc., Chicago, IL, USA). Conformity of data to normal distribution was assessed using the Shapiro-Wilk test. The Mann-Whitney U test was used to compare the differences in categorical variables. Pearson correlation coefficients were calculated to determine the relationship between irisin, and LDH, INR, AST, PT, platelets, aPTT, D-dimer and Hb. Chi-square analysis was used to compare the differences in categorical variables. Pearson correlation coefficients were calculated to determine the relationship between irisin, and LDH, INR, AST, PT, platelets, aPTT, D-dimer and Hb. A value of $p < 0.05$ was accepted as statistically significant.

**RESULTS**

The baseline characteristics of the study population are shown in Table 1. Median irisin concentrations were 9.03 (5.81–12.22) and 4.52 (3.39–7.62) ng/mL in the patients and control groups, respectively (Fig.1). A statistically significant difference was determined between the control group and the patient group in terms of irisin concentrations ($p < 0.001$). No correlation was seen between irisin and disease severity. Statistically significant positive correlations were determined between the values of irisin, and platelets ($p = 0.005$, r: 0.369), ALT ($p = 0.049$, r: 0.261), INR ($p = 0.006$, r: 0.359) and aPTT ($p = 0.002$, r: 0.405). A negative correlation was seen between irisin and disease severity.

**Table 1. Baseline characteristics of study population**

| Characteristic | Patient (n = 30) | Control (n = 30) | P value |
|---------------|-----------------|-----------------|--------|
| Age (years)   | 42 ± 13         | 43 ± 14         | >0.05  |
| Male and female (n) | 19/11         | 14/16           | 0.691  |
| LDH (U/L)     | 356.00 (287.00-474.00) | 219.00 (188.75-233.50) | <0.001 |
| INR           | 1.10 (0.97-1.20) | 1.03 (0.97-1.08) | 0.066  |
| AST (U/L)     | 76.00 (49.00-173.00) | 19.50 (16.75-23.00) | <0.001 |
| PT (sec)      | 14.10 (12.10-15.70) | 11.80 (11.10-12.42) | <0.001 |
| ALT (U/L)     | 75.00 (24.00-122.00) | 19.00 (14.75-23.50) | <0.001 |
| Platelet (× 10³ mcL) | 91.00 (61.00-123.00) | 255.00 (180-294.75) | <0.001 |
| aPTT (sec)    | 37.30 (32.10-42.40) | 31.30 (27.60-33.32) | <0.001 |
| D-dimer (ng/mL) | 218.00 (123.00-387.00) | 112.00 (76.75-126.00) | <0.001 |
| Hb (g/dL)     | 14.00 (11.80-14.90) | 13.95 (13.05-14.80) | 0.482  |

LDH, Lactate dehydrogenase; INR, International normalization ratio; AST, Aspartate transaminase; PT, Prothrombin time; aPTT, Activated prothrombin time; ALT, Alanine aminotransferase; Hb, Hemoglobin. Results are expressed as median (25th-75th percentile) with 95% confidence intervals.
correlation was determined between the values of irisin and LDH \((p = 0.008, r: -0.348)\) (Fig. 2). There was no correlation between the values of irisin, and AST, hemoglobin and D-dimer.

**DISCUSSION**

Irisin is secreted from muscles after the proteolysis of fragments of the plasma membrane protein FNDC5. Normally, the well-known function of irisin is the conversion of white fat into beige fat to regulate thermogenesis. Recent studies have discovered a potential role of irisin in the central nervous system and in various inflammatory and metabolic diseases (9,13). In this study, the median irisin levels were higher in patients than in the control group. In a study by Lu et al. (2015), it was indicated that systemic administration of irisin improved endothelial dysfunction and decreased endothelial apoptosis through the activation of the AMPK-PI3K-Akt-Enos signaling pathway (14). The promoter effect of irisin on human umbilical vein endothelial cell proliferation was also reported by Song et al. (2014) (15). Endothelial cells are the major target of CCHFV, and endothelial damage is known to have an important role in the pathogenesis of CCHF (16). It is speculated that the reason for the presence of higher irisin levels in patients compared to control participants could be the response to increased endothelial damage.

In a recent study, Rivada-Roca et al reported that irisin is a potential adipokine, and is secreted by subcutaneous adipose tissue (17). Vykoukal et al. indicated that the changes in adipokine concentrations cause hypercoagulopathy (18). In the present study, higher concentrations of PT, aPTT, and D-dimer were determined in patients and higher concentrations of platelets in the control group. Therefore, it was
speculated that increased hemorrhage and bleeding might induce irisin secretion both in muscles and adipose tissues, to increase coagulation.

In the present study, despite the low ‘r’ value, a positive correlation was determined between the concentrations of ALT and irisin. However, no correlation was found between the concentrations of irisin and AST. Polyzos et al. also reported a positive correlation between ALT and irisin but no correlation between irisin and AST in patients with non-alcoholic fatty liver disease (19). Findings of the current study are in accordance with those results. It is a well-known fact that ALT is a commonly measured biomarker for the evaluation of liver health (20). It was hypothesized in the current study that irisin might be independently and positively associated with the presence of inflammation in the liver in patients with CCHF. In spite of the presence of the correlation between irisin and ALT, no correlation was observed between AST and irisin due to the differences in releasing time and half-life of liver enzymes.

The main limitation of this study was the low number of the study population. There was also a lack of information on body mass index.

In conclusion, the findings of the present study demonstrated increased concentrations of irisin in CCHF patients compared to healthy control participants. This is the first report to show increasing concentrations of irisin in CCHF patients. The findings of this study suggest that irisin may have an important role in endothelial function and hemorrhagic states in patients with CCHF. In addition, the concentrations of irisin may also be related to liver inflammation. However, there is a need for further studies with larger numbers of patients to evaluate the changes of irisin in CCHF disease.

Conflict of interest None to declare.

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