Evaluation of ghrelin levels and endothelial functions in patients with coronary slow flow phenomenon

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Abstract: Background: Ghrelin has recently been reported to have beneficial effects on cardiac contractile functions and coronary blood flow. The main purpose of this study was to investigate the role of ghrelin in the pathogenesis of coronary slow flow (CSF) together with endothelial functions. Methods: Twenty-five patients having normal coronary arteries with CSF and 25 controls with normal coronary flow were included into the study. The quantitative measurement of coronary blood flow was performed for each coronary artery using the thrombolysis in myocardial infarction (TIMI) frame count (TFC) method. Ghrelin levels were measured using the enzyme-linked immunosorbent assay method from venous blood samples. Endothelial functions were evaluated from the brachial artery with the flow-mediated dilation (FMD) and nitrate-related dilation methods. Results: There was a significant difference in terms of mean TFC values between the control and CSF groups (p < 0.001 for all coronary arteries). The mean FMD percentage among patients with CSF was lower than that of the control group (5.9 ± 0.8 vs. 10.7% ± 1.1%; p < 0.001). A moderate negative correlation was observed between the FMD percentages and the TFCs. There was no relationship between the TFC and ghrelin levels. Conclusion: Plasma ghrelin levels seem to be uninfluential while impaired endothelial functions play an important role in the etiopathogenesis of CSF.

Keywords: coronary artery, coronary slow flow, endothelial dysfunction, ghrelin, TIMI frame count

Introduction

In patients with angiographically normal coronary arteries, the coronary slow flow (CSF) phenomenon is described as the filling and discharge of contrast material in the distal portion of the coronary arteries at a reduced speed. Endothelial dysfunction is considered as the most important factor in the pathogenesis of CSF [1]. The impairment of endothelium function is the earliest marker that indicates coronary artery disease. In many studies, the deterioration of endothelial functions is identified prior to the formation of atheroma plaques [2, 3]. In several recently conducted studies, demonstration of the imbalance between nitric oxide (NO) and endothelin-1 (ET-1) release in patients with CSF supports the consideration regarding the involvement of endothelial dysfunction in CSF etiopathogenesis [4, 5].

Ghrelin, a peptide that is primarily produced by the stomach and the small intestinal cells, is believed to have beneficial effects on left ventricular contraction, coronary blood flow, heart rate, left ventricular systolic and diastolic pressure, left ventricular dysfunction, left ventricular remodeling, ischemia, and reperfusion damage [6–8]. However, the role of ghrelin in the pathogenesis of CSF and its effects on endothelial functions has not been investigated yet.
In this study, we aimed to evaluate the plasma ghrelin levels in patients with CSF, as well as the relationship between CSF and the endothelial dysfunction assessed by the flow-mediated dilation (FMD) method.

**Methods**

**Patient selection**

Twenty-five angiographically identified patients with normal coronary arteries and CSF (16 males, mean age: 54.1 ± 6.1 years), along with 25 angiographically normal coronary flow patients with a similar risk profile and demographic characteristics (14 males, mean age: 54.9 ± 7.1 years), were included in the study. This study protocol was approved by local ethics committee, and detailed informed consent forms were obtained from all patients. Patients with diagnosed coronary artery disease, history of myocardial infarction, left ventricular dysfunction (left ventricular ejection fraction <50%), severe heart valve disease, cardiomyopathy, arrhythmia, left ventricular hypertrophy, uncontrolled hypertension, diabetes mellitus, connective tissue disease, liver, kidney or thyroid dysfunction, and using any medication were excluded from the study.

**Determination of thrombolysis in myocardial infarction (TIMI) frame count (TFC)**

The quantitative measurement of the coronary blood flow was performed by two cardiologists with no prior knowledge regarding the patients' diagnosis and condition using the TFC method [9]. The starting point was considered as the moment the contrast material contacted both sides of the coronary artery and began to advance. The end point was considered as the moment when the contrast material reached the distal branching point, known as the moustache in the left anterior descending (LAD) coronary artery, appeared on the first side branch of the posterolateral artery in the right coronary artery (RCA), and could be imaged in the distal bifurcation of the circumflex (Cx) coronary artery. As the LAD is notably longer than the other arteries, its measured TFC was divided by 1.7 (corrected TFC). By taking the exclusion criteria into account, patients with at least one coronary artery with a frame count above 36.2 for the LAD, 22.2 for the Cx, and 20.4 for the RCA were determined as having CSF [10].

**Laboratory evaluation**

Following 12 h of fasting in the morning, the venous blood samples were collected into ethylenediaminetetraacetic acid-containing tubes. 0.6 TIU/mL of the protease inhibitor aprotinin was added, and the ghrelin levels were measured using the enzyme-linked immunosorbent assay method. Complete blood counting, fasting blood glucose, urca, creatinine, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), sodium, potassium, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were also measured.

**Evaluation of endothelial functions**

Brachial artery B-mode ultrasonography evaluations were performed with the 10 MHz linear array transducer of the VingMed Five device (GE Medical Systems, Horten, Norway). The FMD technique was used to investigate endothelial functions in the brachial artery. The technique was based on the guide by previously published paper [11]. The diameter of the brachial artery (from intima to intima) was measured thrice and the average of these three measurements was recorded as the basal diameter. These measurements obtained from the brachial artery were collected at the end of the diastole accompanied by electrocardiography monitoring. To provide a stimulus for the flow in the brachial artery, the cuff of the sphygmomanometer was placed above the right antecubital fossa. After the basal measurements were recorded, the cuff pressure was raised 50 mmHg above the patient’s systolic blood pressure to fully block the artery flow, and the pressure cuff was maintained in this position for 5 min. Ischemia was induced by interrupting the antegrade blood flow. After the cuff was removed, two-dimensional images of the brachial artery of the longitudinal plane were obtained up until 60 s. The average of three different measurements was recorded as the post-flow brachial artery lumen diameter ($D_3$). FMD was expressed as a percentage increase with respect to the baseline diameter ($D_1$). Endothelium-dependent post-ischemic FMD was calculated with the formula: $\text{FMD} = \left[ (D_3 - D_1)/D_1 \right] \times 100$. For the measurement of endothelium-independent vasodilation, a 10-min waiting period was allowed after the cuff was deflated to provide basal conditions once again, and sublingual nitroglycerine spray (0.4 mg = 1 puff) was administered. Sublingual nitrate was not provided to individuals with clinically distinct hypotension or brachycardia. Five minutes later, the brachial artery lumen diameter ($D_3$) was measured from three different points and the average of these values was taken. The percentage of endothelium-independent vasodilation, also known as nitrate-mediated vasodilation (NMD), was calculated with the formula: $\text{NMD} = \left[ (D_3 - D_1)/D_1 \right] \times 100$. 

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Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY). The variables were investigated using analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test) to determine whether or not they were approximately normally distributed. Descriptive statistics were reported as mean with standard deviation for continuous variables with normal distribution, median and 25th–75th percentile values for continuous variables without normal distribution, and frequencies with percentages for the categorical variables. Group comparisons for continuous variables were tested using Student’s t-test when data distribution was normal and Mann–Whitney U test when data distributions were not normal. Comparisons for categorical variables were evaluated by χ² test. The linear relation between two variables was evaluated with the Pearson’s (in case the data showed normal distribution) and Spearman’s (for data without normal distribution) correlation analyses. Significance level was accepted as p < 0.05 in all statistical analyses.

Results

A total of 50 patients, composed of 25 patients with CSF and 25 patients with normal coronary flow, were evaluated in this study for endothelial functions and plasma ghrelin levels. The basic demographic and clinical data of both groups are shown in Table I.

Comparison of laboratory parameters between CSF group and controls were included in Table II. There was no significant difference in terms of white blood cells, platelets, hemoglobin, fasting blood glucose, urea, creatinine, uric acid, AST, ALT, sodium, potassium, TC, HDL, LDL, TG, ESR, and CRP levels between the groups. The FMD and NMD percentages for both groups are shown in Table I. The FMD percentage in CSF patients was found to be significantly lower that those of patients without CSF (5.9 ± 0.8 vs. 10.7 ± 1.1, p < 0.001). Although the ghrelin levels were found to be lower in patients with CSF, the difference was not statistically significant (1.7 ± 0.6 vs. 1.19 ± 0.6 ng/mL, p = 0.275) (Fig. 1).

The TFC values were calculated separately for each coronary artery in two groups. The TFCs in LAD, Cx, and RCA were significantly lower in CSF patients than the controls (p < 0.001 for each coronary artery) (Fig 2, Table III). A mild to moderate negative correlation was observed between the FMD percentage and the TFCs (r = −0.734, p < 0.001; r = −0.489, p < 0.001; r = −0.644, p < 0.001 for LAD, Cx, and RCA, respectively) (Fig. 3). No correlation was identified between the ghrelin levels and the TFCs. There was no relation between the FMD and NMD percentages and the ghrelin levels (r = 0.19, p = 0.199 and r = 0.006, p = 0.677, respectively).

Discussion

This study demonstrated the role of endothelial dysfunction in the etiopathogenesis of CSF. There is a correlation between the extent of disruption in endothelial function and the CSF level. However, there was no significant difference between the ghrelin levels of patients with and without CSF indicating that ghrelin levels may not have an effect on CSF etiopathogenesis.

Tambe et al. [12], who first defined CSF in 1972, had proposed earlier that the CSF phenomenon could be related to anomalies in microcirculation. Among the factors involved in the CSF phenomenon, a particular emphasis has been placed on atherosclerosis and endothelial function [1, 13–15]. Endothelial dysfunction is one of the most significant early indicators of atherosclerotic processes [2, 3, 16]. The normal function of the endothelium layer is dependent on the balance between the endothelium-derived relaxing factor and the endothelium-derived constrictor factor. When this
balance is altered, this situation is described as an endothelium dysfunction [17]. The most important of the endothelium-derived mediators is NO, which is also designated as the “endogenous anti-atherosclerotic molecule.” A decrease in NO production or activity, accompanied by an increase in the synthesis of oxygen

|                  | CSF (n = 25) | Controls (n = 25) | P value |
|------------------|--------------|-------------------|---------|
| WBC (×10³/mL)    | 7.29 ± 1.81  | 7.50 ± 2.49       | 0.737   |
| Neutrophils (×10⁷/mL) | 4.88 ± 1.70 | 4.94 ± 2.18       | 0.908   |
| Lymphocytes (×10⁹/mL) | 1.62 ± 0.72 | 1.58 ± 0.83       | 0.858   |
| Platelets (×10³/mL) | 233.84 ± 59.06 | 230.96 ± 58.21   | 0.863   |
| Hemoglobin (mg/dL) | 11.28 ± 2.01 | 11.99 ± 1.81      | 0.193   |
| Glucose (mg/dL)   | 90 (85–99)   | 90 (85.5–96.5)    | 0.627   |
| Urea (mg/dL)      | 36 (28–51.5) | 34 (26–53)        | 0.846   |
| Creatinine (mg/dL)| (0.7–1)      | 0.8 (0.7–1)       | 0.769   |
| AST (u/L)         | 29 (22–42)   | 31 (19.5–63)      | 0.393   |
| ALT (u/L)         | 17 (14–30.5) | 17 (11.5–41)      | 0.719   |
| Sodium (mEq/L)    | 137 (135–139)| 137 (135–140)     | 0.953   |
| Potassium (mEq/L) | 4.42 ± 0.57  | 4.24 ± 0.54       | 0.242   |
| Uric acid (mg/dL) | 5.5 (4.5–6.4) | 5.7 (4.3–6.3)    | 0.861   |
| Total cholesterol (mg/dL) | 171.32 ± 37.78 | 163.04 ± 31.39  | 0.404   |
| HDL (mg/dL)       | 37.84 ± 8.42 | 34.80 ± 6.72      | 0.165   |
| LDL (mg/dL)       | 110.56 ± 24.58 | 107.24 ± 26.65   | 0.649   |
| Triglyceride (mg/dL) | 120.04 ± 58.28 | 112.72 ± 43.95  | 0.618   |
| CRP (mg/L)        | 0.5 (0.2–0.9)| 0.6 (0.2–1.4)     | 0.372   |
| ESR (mm/h)        | 22 (10.5–45) | 18 (8.5–37)       | 0.290   |
| Plasma ghrelin (ng/mL) | 1.17 ± 0.6  | 1.19 ± 0.6        | 0.275   |

AST: aspartate transaminase; ALT: alanine transaminase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WBC: white blood cell

Fig. 1. Comparison of box-plot graphs of ghrelin levels. Although the ghrelin levels were found to be lower in patients with coronary slow flow (CSF), the difference was not statistically significant.

Fig. 2. The thrombolysis in myocardial infarction frame counts (TFCs) in left anterior descending (LAD) coronary artery, circumflex (Cx), and right coronary artery (RCA) were significantly lower in coronary slow flow (CSF) patients than the controls.
species and free radicals is the main mechanism for endothelial dysfunction and increases the risk for the development of atherosclerosis [18].

There have recently been many studies that evaluated the endothelium function of patients with CSF by non-interventional methods. Currently, the most highlighted among these methods is the FMD method. Sezgin et al. [1] have evaluated the presence and role of endothelium dysfunction in the pathogenesis of CSF by measuring the FMD of the brachial artery. In these patients, a close and negative relation between the TFC and the FMD was identified. The decrease in NO release by the endothelium was considered to be responsible for this observation. In this study, the lower FMD percentages observed in comparison with the control group supports the view regarding the role of endothelial dysfunction in CSF etiopathogenesis. The negative correlation identified between the FMD percentage and TFC gives rise to the thought that the level of slow flow is proportional to the degree of endothelium dysfunction.

Ghrelin is a 28 amino acid peptide with paracrine properties that is mostly produced in the stomach and small intestines [19]. Many studies have been conducted recently regarding the role of ghrelin in the cardiovascular system, and the evaluation of its effects was considered as worthwhile. In light of these studies, the beneficial effects of ghrelin on left ventricular contraction, coronary blood flow, heart rate, left ventricular systolic and diastolic pressure, left ventricular dysfunction, left ventricular remodeling, ischemia, and reperfusion damage have been demonstrated [6, 7].

Current knowledge on the effects of ghrelin on endothelium function is limited to only a few studies. In a study by Wiley and Davenport [20], it was claimed that ghrelin is a direct vasodilator agent independent of the endothelium. In the same study, strong dilation by ghrelin was observed on the human intermamarian artery vasoconstricted by ET-1. The vasodilator response caused by ghrelin is slow but long-termed.

In a study conducted by Hedayati et al. [21], it was demonstrated that ghrelin corrected endothelium functions in homocysteine-dependent endothelium dysfunction, which is an independent risk factor for atherosclerosis. It was demonstrated that ghrelin achieved this protective effect on the endothelium by blocking the homocysteine-induced endothelium dysfunction, by increasing the endothelial release of NO synthase, and by decreasing oxidative stress. In another important study conducted by Okumura et al. [22], the increase in blood flow in the forearm in response to the intraarterial infusion of ghrelin was evaluated using plethysmography procedures. In the results of the study, a dose-dependent increase in brachial blood flow was observed, and this effect lasted for more than 20 min. Based on available knowledge, ghrelin levels have not been investigated in CSF patients before. In this study, although the ghrelin levels were lower in CSF patients in comparison with the control group, the difference was not statistically significant. The reason for this observation might be the limited size of the patient population. However, ghrelin, which is known to have a regulatory effect on many cardiovascular functions, is not directly involved in the etiopathogenesis of CSF. The data from this study should be supported by conducting large-scale studies.

![Fig. 3](image-url) A mild to moderate negative correlation was observed between the flow-mediated dilatation (FMD) percentage and the thrombolysis in myocardial infarction frame counts (TFCs) in left anterior descending (LAD) coronary artery, circumflex (Cx), and right coronary artery (RCA).
**Limitations of the study**

There were a number of limitations to this study. First, the study population consisted of limited number of patients. In addition, the brachial artery FMD was used as an indicator of endothelium function. This method was far from being a gold standard, as the results obtained by this method are affected by the patients, the technical procedures employed, and by the performers.

**Conclusions**

The results of this study demonstrated the role of endothelial dysfunction in the etiopathogenesis of CSF. There is a correlation between the extent of disruption in endothelium function and the CSF level. However, the varying ghrelin values observed among patients leads to the consideration that ghrelin levels may not have an effect on CSF etiopathogenesis.

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**Authors’ contribution:** All of the authors were responsible for the planning, conduct, and reporting of the work. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Conflict of interest:** The authors declare no conflict of interest.

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