Evaluation of homocystein and asymmetric dimethyl arginine levels in patients with coronary slow flow phenomenon

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Abstract: *Background:* Previous studies have demonstrated that homocysteine and asymmetric dimethyl arginine (ADMA) levels were strongly associated with cardiovascular diseases including coronary artery disease. The aim of this study was to investigate the role of plasma homocysteine and ADMA levels in the pathogenesis of coronary slow flow (CSF) phenomenon. *Methods:* Twenty-three patients with CSF and 25 controls with normal coronary flow were included in this study. The quantitative measurement of coronary blood flow was performed using the thrombolysis in myocardial infarction frame count method. Plasma homocysteine and ADMA levels were determined using enzymatic assays from venous blood samples. *Results:* The patients with CSF had significantly higher plasma homocysteine levels than controls (16.2 ± 7.6 vs. 12.2 ± 2.2 μM/L; $p = 0.023$). The uric acid levels were significantly higher in CSF group than controls (5.4 ± 1.1 vs. 4.6 ± 0.9 mg/dl; $p = 0.011$). Plasma ADMA levels were also higher in the CSF group; however, this was not statistically significant (0.6 ± 0.1 vs. 0.5 ± 0.2 μM/L; $p = 0.475$). *Conclusions:* Increased homocysteine and uric acid levels may play an important role in the pathogenesis of CSF. Further large scale studies are required to determine the relationship between ADMA levels and CSF.

Keywords: asymmetric dimethyl arginine, coronary artery, coronary slow flow, endothelial dysfunction, homocystein

Introduction

The phenomenon of coronary slow flow (CSF) is described as filling and discharge of contrast material in the distal portion of the coronary arteries at a reduced speed in patients with angiographically normal coronary arteries [1]. The prevalence of CSF varies from 1% to 5% among patients undergoing coronary angiogram for stable angina pectoris [2]. CSF is related to various clinical events, such as acute myocardial infarction, angina pectoris, life-threatening arrhythmias, and sudden death [3]. The exact mechanism associated with this interesting phenomenon remains obscure, but several mechanisms have been proposed including enhanced oxidative stress and inflammation, microvascular dysfunction, impaired endothelial functions, and diffused atherosclerosis [4–7].

Previous studies have demonstrated that homocysteine was strongly associated with cardiovascular diseases including coronary artery disease and heart failure [8]. In general, the effects of homocysteine in cardiovascular diseases appear to be a consequence of oxidative process in the vascular endothelium resulting in disturbed nitric oxide (NO) synthesis and vasodilatation [9].

NO is well known to be a vasodilator that relaxes vascular smooth muscle via the cyclic guanosine monophosphate-dependent pathway. NO is produced from its precursor, L-arginine, by endothelial NO synthase enzymes. Asymmetric dimethyl arginine (ADMA) is an endogenous inhibitor of all major isoforms of NO

synthase, which reduces NO release from the endothelium, and has been associated with endothelial dysfunction and atherosclerosis [10]. Increased ADMA level is also an independent risk factor for progression of atherosclerosis and total mortality in coronary artery disease [11]. The association of homocysteine with ADMA is particularly of interest, since homocysteine increases ADMA level by inhibiting dimethylaminohydrolase, which hydrolyzes and degrades ADMA [12].

The role of homocysteine and ADMA 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 levels of homocysteine and ADMA in patients with CSF.

Methods

Study population

This single-center study enrolled 23 angiographically identified patients with normal coronary arteries and CSF (13 males, mean age: 50.5 ± 11.1 years), along with 25 angiographically normal coronary flow patients with a similar risk profile and demographic characteristics (9 males, mean age: 53.7 ± 10.0 years). 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 and liver, kidney, or thyroid dysfunction were excluded from the study. All patients underwent transthoracic echocardiography. Complete blood count and blood chemistry tests were performed in all patients during admission. All demographic, laboratory, and echocardiographic parameters were recorded into a data set and compared between CSF patients and controls. All patients provided a written informed consent and the study protocol was approved by the local ethics committee of the hospital in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

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 [13]. The initial 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 coronary artery (LAD), 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 longest branch of the circumflex coronary artery (Cx). 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 [14].

Laboratory analysis

In order to detect plasma ADMA levels, venous blood samples were collected after 12 h of fasting by a clean puncture of an antecubital vein from all patients. Blood samples were taken into ethylenediaminetetraacetic acid containing tubes and centrifuged at 3,000 rpm for 10 min at room temperature. The plasma samples, obtained after centrifugation, were stored at –20 °C for further analysis. Plasma ADMA levels were measured by enzyme linked immunosorbent assay method using an Immunodiagnostic human ADMA kit (Immunodiagnostic AG, Bensheim, Germany). Plasma homocysteine levels were quantitatively determined using homocysteine enzymatic assay kit (Recipe ClinRep, Munich, Germany) according to the manufacturer's recommendation. Complete blood countings were measured on Sysmex XT2000i analyzer (Sysmex Corporation, Kobe, Japan). Fasting blood glucose, urea, 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 on an autoanalyzer (Siemens Advia 2400 Chemistry System, Siemens Diagnostic, Tarrytown, USA).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 19.0. (IBM Corp., Armonk, NY, USA). 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 using Mann–Whitney *U* test when data distributions were not normal. Comparisons

for categorical variables were evaluated by χ^2 test or Fisher's exact test as appropriate. Significance level was accepted as $p < 0.05$ in all statistical analyses.

Results

The clinical and demographical characteristics of patients with and without CSF were presented in *Table I*. There was no significant difference between the groups in terms of age, gender, body mass index, frequency of diabetes mellitus, hypertension, dyslipidemia, smoking status, and family history of coronary artery disease. Heart rate, systolic blood pressure, diastolic blood pressure, and left ventricular ejection fraction were also similar between the groups *(Table I)*.

Comparison of laboratory parameters between CSF group and controls was included in *Table II*. There was no significant difference in terms of white blood cells, platelets, hemoglobin, fasting blood glucose, urea, creatinine, AST, ALT, sodium, potassium, TC, HDL, LDL, TG, ESR, and CRP levels between the groups. The uric acid levels were significantly higher in CSF group than controls (5.4 ± 1.1 vs. 4.6 ± 0.9 mg/dl; $p = 0.011$). The patients with CSF had significantly higher plasma homocysteine levels than controls (16.2 ± 7.6 vs. 12.2 ± 2.2 μM/L; $p = 0.023$). Plasma ADMA levels were also higher in the CSF group; however, this was not statistically significant (0.6 ± 0.1 vs. 0.5 ± 0.2 μM/L; $p = 0.475$) *(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) *(Table III)*. There was a weak but significant positive correlation between the avarage TFCs and plasma homocysteine levels ($r = 0.371$; $p = 0.009$). There was no significant correlation between the TFCs, plasma ADMA, and uric acid levels.

Discussion

In this study, we have focused on the role of homocysteine, uric acid, and ADMA levels in patients with CSF. The results demonstrated that increased homocysteine and uric acid levels may play a significant role in the pathogenesis of CSF. However, further large-scale studies are required to determine the relationship between ADMA levels and CSF.

CSF is a well-known terminology by the interventional cardiologists in which opacification of major epicardial coronary arteries has been delayed at the distal segments without any atherosclerotic stenosis [15]. In addition to its simple definition, the exact etiopathogenesis is unclear. However, various mechanisms have been suggested in the development of SCF including early atherosclerosis, inflammation, oxidative stress, impaired platelet function, coronary vasomotor dysfunction, and endothelial dysfunction [14, 16].

The TFC technique has been successfully used for the assessment of coronary flow velocity using coronary angiograms. It is a simple, reproducible, objective, and quantitative index of coronary blood flow. In this method, the number of cineangiographic frames from initial contrast material opacification of the proximal portion of the coronary artery to opacification of the distal arterial landmarks with contrast material is counted [17]. TFC has been widely used in the evaluation of CSF patients.

Table I Comparison of demographic parameters and endothelial functions between patient groups with and without coronary slow flow phenomenon

	CSF ($n = 23$)	Controls ($n = 25$)	p value
Age (years)	50.5 ± 11.1	53.7 ± 10.0	0.309
Gender (male/female) [n (%)]	13/10 (56.5/43.5)	9/16 (36/64)	0.256
BMI (kg/m^2)	28.6 ± 5.07	29.1 ± 2.93	0.740
HT [n (%)]	9 (39.1)	13 (52)	0.546
DM [n (%)]	2 (8.7)	5 (20)	0.490
Dyslipidemia [n (%)]	6 (26.1)	4 (16)	0.487
Smoking [n (%)]	14 (60.9)	9 (36)	0.152
Family history of CAD [n (%)]	9 (39.1)	8 (32)	0.831
SBP (mmHg)	126.5 ± 14.3	130.4 ± 17.1	0.402
DBP (mmHg)	83.4 ± 10.6	82.6 ± 6.6	0.730
HR (bpm)	69 (56–78)	74 (60–79)	0.140
LVEF (%)	61.7 ± 3.6	62.1 ± 3.1	0.673

BMI: Body mass index; CSF: coronary slow flow; CAD: coronary artery disease; DM: diabetes mellitus; HR: heart rate; HT: hypertension; LVEF: left ventricular ejection fraction; SBP: systolic blood pressure; DBP: diastolic blood pressure

Table II Comparison of laboratory parameters between patient groups with and without coronary slow flow phenomenon

	CSF ($n=23$)	Controls ($n=25$)	p value
White blood cell ($\times10^3$/ml)	7.29 ± 1.81	7.50 ± 2.49	0.737
Neutrophils ($\times10^3$/ml)	4.88 ± 1.70	4.94 ± 2.18	0.908
Lymphocytes ($\times10^3$/ml)	1.62 ± 0.72	1.58 ± 0.83	0.858
Platelets ($\times10^3$/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)	115 ± 64.9	120 ± 42.8	0.747
Urea (mg/dl)	16.6 ± 5.4	14.5 ± 4.2	0.146
Creatinine (mg/dl)	1.0 ± 0.1	0.9 ± 0.2	0.127
Aspartate transaminase (U/L)	29 (22–42)	31 (19.5–63)	0.393
Alanine transaminase (U/L)	17 (14–30.5)	17 (11.5–41)	0.719
Sodium (mEq/L)	137 (135–139)	137 (135–140)	0.953
Potasium (mEq/L)	4.42 ± 0.57	4.24 ± 0.54	0.242
Uric acid (mg/dl)	5.4 ± 1.1	4.6 ± 0.9	0.011
Total cholesterol (mg/dl)	181 (139–225)	195 (145–215)	0.115
High-density lipoprotein (mg/dl)	41.7 ± 9.8	45.6 ± 10.7	0.190
Low-density lipoprotein (mg/dl)	112 ± 23	121 ± 23	0.195
Triglyceride (mg/dl)	126 ± 58.1	157 ± 106	0.230
C-reactive protein (mg/L)	3.5 ± 0.9	3.6 ± 1.2	0.918
Sedimentation (mm/h)	22 (10.5–45)	18 (8.5–37)	0.290
Homocysteine (μM/L)	16.2 ± 7.6	12.2 ± 2.2	0.023
Asymmetric dimethyl arginine (μM/L)	0.6 ± 0.1	0.5 ± 0.2	0.475

CSF: coronary slow flow

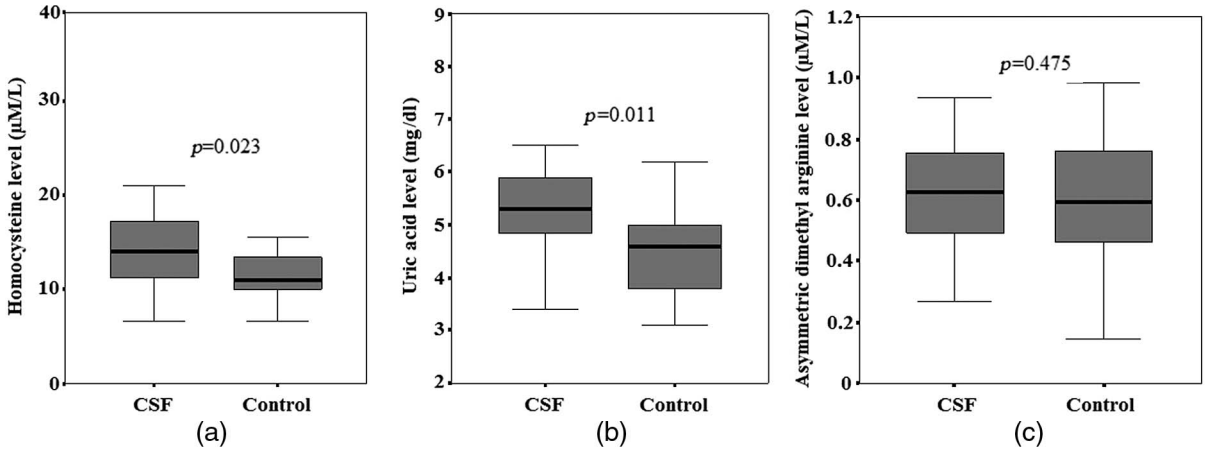

Fig. 1. Comparison of box-plot graphs of (a) homocysteine, (b) uric acid, and (c) asymmetric dimethyl arginine levels between patients with and without coronary slow flow (CSF)

Endothelial dysfunction is one of the most significant early indicators of atherosclerotic processes [18]. The normal function of the endothelium layer is dependent on the balance between the endothelium-derived relaxing factors and the endothelium-derived constrictor factors. The most important of the endothelium-derived mediators is NO. A decrease in NO production or activity accompanied by an increase in the synthesis of oxygen species free radicals is the main mechanism for endothelial dysfunction and increases the risk for the development of atherosclerosis [19].

Homocysteine is a sulfur containing amino acid, which is formed during methionine metabolism.

Table III Comparison of TIMI frame counts between patient groups with and without coronary slow flow phenomenon

TIMI frame count	CSF group ($n = 23$)	Control group ($n = 25$)	p value
LAD (cLAD)	46.9 ± 3.0	20.1 ± 1.9	<0.001
Cx	38.8 ± 4.9	18.4 ± 1.2	<0.001
RCA	38.3 ± 4.8	19.2 ± 1.4	<0.001

TIMI: thrombolysis in myocardial infarction; LAD: left anterior descending coronary artery; cLAD: corrected LAD; CSF: coronary slow flow; Cx: circumflex coronary artery; RCA: right coronary artery

Hyperhomocysteinemia is associated with increased risk of coronary, cerebral, and peripheral atherosclerotic disease independent of classical cardiovascular risk factors, such as hyperlipidemia, hypertension, or cigarette smoking [20]. The mechanisms of homocysteine-induced endothelial dysfunction remain poorly understood. Possible mechanisms may involve reduced release of NO by the endothelium due to direct toxic effects of homocysteine on endothelial cells or inactivation of NO via increased generation of reactive oxygen species [21, 22]. The adverse effect of homocysteine on endothelial function may also occur by an indirect mechanism. Hyperhomocysteinemia may cause a dose-dependent impairment of dimethylarginine dimethylaminohydrolase activity, which is the enzyme that degrades ADMA. Thus, endothelial dysfunction in hyperhomocysteinemia may be secondary to increased ADMA levels, which is a potent endogenous inhibitor of the endothelial NO synthase [12].

The uric acid is the final product of xanthine oxidase activity in purine metabolism. Adenosine, which is synthesized locally in vascular smooth muscle cells of the myocardial heart tissue, is rapidly degraded to uric acid in the endothelium. Several papers reported association of increased uric acid concentrations with increased incidence of cardiovascular diseases [23]. There is also evidence that increased uric acid levels can promote lipids metabolism impairment, and can stimulate the free radical formation as well as the occurrence of atherosclerotic plaque [24].

In this study, increased homocystein and uric acid levels were observed in patients with CSF, which have been shown to be strongly associated with endothelial dysfunction. Increased homocysteine and uric acid levels may play an important role in the pathogenesis of CSF. Plasma ADMA levels were also higher in CSF patients. However, due to the limited number of study population, the relationship between ADMA levels and CSF did not reach a statistically significant level.

Limitations

The primary limitation was that this was a non-randomized and single-center study with a limited number of patients.

Conclusions

The results of this study demonstrated that increased homocysteine and uric acid levels may play a major role in the pathogenesis of CSF. However, further large-scale studies are required to determine the relationship between ADMA levels and CSF.

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Authors' contribution: All the authors contributed to planning, conduct, and reporting of the work. They 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: All authors declare no conflict of interest.

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