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Original article

Plasma homocysteine is associated with ischemic findings without organic stenosis in patients with slow coronary flow

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ABSTRACT

Aim: To investigate the plasma concentrations of homocysteine (Hcy) in slow coronary flow (SCF) patients before and at the end of the exercise test and compare with the values of healthy controls.
Methods: Study population consisted of 41 patients with SCF [68% men, aged 49 ± 8 years], and 41 subjects with normal epicardial coronary arteries [56% men, aged 50 ± 9 years]. Exercise test was performed in all study participants. Blood samples were drawn at rest and immediately at the end of exercise testing after 12 h of overnight fasting.
Results: The baseline Hcy value of the SCF patients was higher than that of the control subjects ($p < 0.0001$), and this difference continued after exercise test between the groups ($p < 0.0001$). Median post-exercise increases in Hcy levels were higher in the SCF group than in the control group, without a significant difference ($p = 0.088$). In the SCF group after exercise, Hcy levels in 17 patients with angina and 18 patients with ST depression were higher than those without angina and ST depression ($p < 0.0001$ and $p < 0.0001$, respectively). In addition, Hcy values in patients with both angina and ST depression were greater than those with either angina ($p < 0.05$) or ST depression ($p < 0.05$).
Conclusion: The results of this study show that there is an important pathophysiologic link between the increased levels of plasma Hcy, the degree of ischemic findings, and the severity of slow flow in SCF patients.

Introduction

Slow coronary flow (SCF) is an angiographic finding characterized by the slow movement of contrast throughout the coronary lumen in the absence of epicardial coronary obstructive disease. Since its first description, although a number of factors such as endothelial, vasomotor, and microvascular dysfunction, and diffuse coronary atherosclerosis and increased platelet activity have been responsible for SCF [1–8], its etiopathogenesis is still not fully elucidated.

Homocysteine (Hcy) is a sulfhydryl-containing amino acid that might give rise to endothelial damage and generate free radicals that induce oxidative damage and stress. Hyperhomocysteinemia has been determined as emerging independent risk factor for cardiovascular system and related diseases [9–12].

In this study, we aimed to assess the plasma Hcy levels of SCF patients before and after very short time exercise, and compared with those of controls. As far as we know, there is no study investigating the plasma Hcy levels both at baseline and post-exercise in SCF patients.

Materials and methods

Study population

Among 2397 coronary angiograms performed between February 2011 and March 2012 at cardiology clinics of Lokman Hekim Hospital and Mersin University by reason of suspected coronary artery disease, 217 (9.1%) had normal epicardial coronary arteries, i.e. showing no evidence of any coronary stenosis, spasm, and ectasia. Physical examination and medical history were assessed. Echocardiographic and electrocardiographic analyses were performed on all 217 patients. Patients with valvular heart and/or myocardial disease, systolic and/or diastolic dysfunction, left ventricular hypertrophy and enlargement detected by the echocardiographic data were excluded from the study. Patients

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who suffered from one of the following diseases or related disorders were also excluded: those with a history of acute coronary syndrome, acute myocardial infarction, percutaneous coronary intervention, and coronary artery bypass graft surgery, arrhythmia, renal dysfunction, connective tissue disease, thyroid disorders, and other systemic diseases, and those contraindicated for exercise testing. SCF was determined in 41 of the 217 patients. The control group, consisting of 41 subjects matched for demographic characteristics, was selected in a consecutive manner from the catheterized patients without exclusion criteria during the same study period. All control subjects, who were proved to have normal epicardial coronary arteries without SCF, had normal exercise and echocardiographic parameters. After signed informed consent was obtained, all concomitant medications, which may affect both results of the exercise test and Hcy levels such as beta blockers and lipid-lowering drugs, were discontinued for at least 30 days before the test. The trial was performed according to the principles of the Declaration of Helsinki and approved by the Investigational Review Board of Mersin University, School of Medicine.

Thrombolysis in myocardial infarction frame count

Coronary flow rates of the all subjects were determined by the thrombolysis in myocardial infarction (TIMI) frame count (TFC) method. The coronary TFCs were calculated separately for each major coronary artery and their average was determined as the mean TFC for each subject according to the method described by Gibson et al. [13]. Due to different durations required for normal visualization of coronary arteries, the corrected cut-off values were 36.2 ± 2.6 frames for left anterior descending coronary artery, 22.2 ± 4.1 frames for left circumflex artery, and 20.4 ± 3 frames for right coronary artery, as has been reported in the literature. The subjects with a TFC greater than 2 standard deviations (SD) from these thresholds for the particular vessel were accepted as having SCF in the present study. All TFCs were measured in matched projections using Siemens Medical Solution (version Artis zee, Erlangen, Germany).

Exercise treadmill testing

The 12-leads maximal exercise test was carried out using standard graduated treadmill protocols consistent with American Heart Association (AHA) guidelines [14]. Patients were encouraged to give their maximal effort, but not to allow their angina to reach levels higher than previously experienced. The results were analyzed and reported using a computerized database (EXTRA; Mosby Publishers; Chicago, IL, USA) [15]. The ST segment response considered was the most horizontal or downsloping ST-segment depression in any lead, except aVR, during exercise or recovery. An abnormal response was defined as $\geq$0.1 mV at 0.08 s after the J point of horizontal or downsloping ST-segment depression and angina (pain felt as pressure, heaviness, and squeezing across the chest) during exercise or recovery.

Laboratory analysis

All blood samples were drawn after 12 h of overnight fasting for total cholesterol (TC), triglyceride (TG), high-density lipoprotein–cholesterol (HDL-C), complete blood count (CBC), and creatinine. Assays for TC, TG, HDL-C, and creatinine were performed using a Cobas Integra 800 automated analyzer (Roche Diagnostics, Mannheim, Germany). Assays for CBC were carried out using a Coulter LH 750 analyzer (Beckman Coulter, Galway, Ireland). The serum low-density lipoprotein–cholesterol (LDL-C) was calculated according to Friedewald's formula [16]. The glomerular filtration rate (GFR) was estimated as a function of age, serum creatinine, and race using the simplified Modified Diet in Renal Disease (MDRD) equation [eGFR $(\text{mL/min}/1.73\,\text{m}^2) = 186.3 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times (0.742$ if female)] [17].

Analysis of homocysteine levels

Blood samples for Hcy were drawn from the forearm vein at rest and immediately after the end of exercise testing. After centrifugation, the obtained plasma was stored at $-20\,^{\circ}$C. Plasma Hcy levels were determined by using reagent kit for high-performance liquid chromatography (HPLC) analysis of Hcy in serum/plasma (Chromosystems GmbH, Munich, Germany). Analyses were performed with isocratic HPLC system with fluorescence detector (HP 1100). The HPLC condition for Hcy: injection volume: 20 μI, flow rate: 1.7 mL/min, room temperature 25 °C, wavelength: EX 385, EM, 515. High plasma Hcy concentrations were accepted as >12 μmol/L [11].

Statistical analysis

Continuous variables were expressed as means ± SD. Categorical variables were expressed as percentage. The distribution of variables in both groups was assessed by the Kolmogorow–Smirnov test. To compare the two groups according to changes after the exercise in the variables that exhibit normal distribution and according to measurements before and after exercise, independent *t*-test was used. In contrast, for the variables that do not show normal distribution, Mann–Whitney *U* test was used. Also in the patients according to these measurements for comparison of that with angina and no angina; and also for comparison of those with ST segment depression and without ST depression, independent *t*-test was used. Paired *t*-test was used to determine the significance of changes observed before and after exercise in both patients and controls. ANOVA was used to compare more than two groups. Post hoc Tukey test was used when the ANOVA found a significant effect. Pearson and Sperman correlation analyses were used for the assessment of relation between variables in both groups. All hypothesis testing was 2-tailed. *p*-Values of <0.05 were regarded as significant. Statistical analysis was performed using SPSS software package (Version 15.0, SPSS Inc, Chicago, IL, USA).

Results

The clinical characteristics, risk factors, CBC, and TFC of both groups are delineated in Table 1. There were no statistically significant differences between the two groups in terms of clinical characteristics and risk factors. TFC was significantly higher in patients with SCF than in control subjects. SCF was detected to affect left anterior descending artery the most (82.9%), followed by right coronary artery (53.7%) and circumflex (51.2%). SCF was observed to have a tendency to affect two vessels (43.9%) or one vessel (34.1%), whereas three-vessel involvement was less common (22%) (Table 1). Plasma baseline Hcy levels of patients with SCF were found to be significantly higher than those of control subjects (14.9 ± 4.0 μmol/L vs 6.5 ± 4.8 μmol/L; $p < 0.0001$, respectively) (Table 2). Post-exercise plasma Hcy levels of SCF patients were also higher (16.4 ± 4.9 μmol/L vs 7.1 ± 5.2 μmol/L; $p < 0.0001$). The highest Hcy level was determined in patients with SCF in all of 3 coronary arteries both at baseline and post-exercise, when compared to that of the controls, one-vessel, and two-vessel SCF patients (Table 2) (ANOVA $p < 0.05$ vs control, $p < 0.05$ vs one-vessel SCF, and $p < 0.05$ vs two-vessel SCF, respectively). There was no significant difference for Hcy level between the one-vessel and two-vessels both at baseline and post-exercise in SCF patients ($p > 0.05$). Moreover, the number of vessels with slow flow was