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Correlations between homocysteine levels and atherosclerosis in Japanese type 2 diabetic patients

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Abstract

Elevated total plasma homocysteine (tHcy) level and aortic stiffness are associated with high mortality in type 2 diabetic patients. We tested the hypothesis that tHcy correlates with aortic stiffness and insulin resistance in type 2 diabetic patients. The study consisted of 40 Japanese patients with type 2 diabetes mellitus and high tHcy levels (mean age ± SD, 57 ± 7 years) and a control group of 45 age-matched patients with normal tHcy levels (mean age ± SD, 57 ± 6 years). Brachial-ankle pulse wave velocity (BaPWV) was measured by an automatic oscillometric method. Brachial-ankle pulse wave velocity was used as an index of atherosclerosis. Body mass index values ($P < .05$), waist circumferences ($P < .05$), and the waist-to-hip ratios ($P < .05$) were larger in the high-tHcy group than in the normal-tHcy group. The BaPWV was higher in the high-tHcy group than in the normal-tHcy group ($P < .0001$). Fasting plasma glucose ($P < .005$) and insulin concentrations ($P < .0001$), and the homeostasis model assessment (HOMA) index ($P < .0001$) were higher in the high-tHcy group than in the normal-tHcy group. Multiple regression analysis showed that tHcy levels were independently predicted by BaPWV and the HOMA index. In conclusion, our results indicate that the elevated level of tHcy in Japanese patients with type 2 diabetes mellitus is characterized by increased aortic stiffness and insulin resistance, and that the BaPWV and the HOMA index are independent predictors of tHcy.

1. Introduction

It has been reported that an elevated level of total plasma homocysteine (tHcy) is a risk factor for cardiovascular disease [1,2].

Pulse wave velocity (PWV) reflects arterial stiffness, and it has been demonstrated that carotid-femoral PWV relates to the severity of atherosclerosis [3] and predicts future atherosclerotic cardiovascular events [4]. Recently, a simple method of measuring brachial-ankle PWV (BaPWV) has been reported [5-7]. Moreover, BaPWV is a marker of severity of atherosclerosis [6,7], and increased BaPWV is a risk factor for cardiovascular disease [7] and prognosis in patients with acute coronary syndrome [8].

Insulin resistance is linked to established risk factors for atherosclerosis such as hypertension, hyperlipidemia, and obesity, which subsequently accelerate the development and progression of atherosclerosis [9,10].

Although tHcy is reported to be associated with insulin resistance in nonobese healthy subjects [11] and type 2 diabetic patients [12], the significance of tHcy in diabetic aortic stiffness has not been adequately investigated.

We hypothesized that increased levels of tHcy are associated with BaPWV and insulin resistance in type 2 diabetic patients. To test our hypothesis, we compared BaPWV in addition to metabolic profiles of Japanese type 2

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diabetic patients with normal tHcy levels and those with high tHcy levels; independent predictors of tHcy in these populations were evaluated.

2. Subjects and methods

One hundred-sixty five Japanese patients with type 2 diabetes mellitus who were admitted to our department in 2006 were screened.

Among these subjects, we enrolled 108 patients who did not have organic heart disease as determined by physical examination and routine laboratory tests, including serum electrolytes, serum creatinine, serum urea nitrogen, fasting plasma glucose, fasting immunoreactive insulin, chest x-ray, 12-lead electrocardiogram, echocardiography, treadmill exercise electrocardiogram, and thallium 201 cardiac scintigraphy.

All patients underwent clinical examination to rule out the presence of secondary hypertension. Essential hypertension was defined as a diastolic blood pressure of 90 mm Hg or higher, systolic blood pressure of 140 mm Hg or higher, or self-reported use of antihypertensive medication [13].

2.1. Laboratory methods

Blood was taken at 7:00 AM from the antecubital vein with the patient in the recumbent position after an overnight fast. All patients underwent routine laboratory tests including assays for serum electrolytes, serum total cholesterol, serum triglycerides, serum high-density lipoprotein cholesterol (HDL-C), fasting plasma glucose, and fasting immunoreactive insulin. Insulin resistance was evaluated by the homeostasis model assessment (HOMA) index: (fasting plasma insulin [μU/mL] × fasting plasma glucose [mmol/L])/22.5 [14]. Low-density lipoprotein cholesterol (LDL-C) concentrations in serum were measured by the Friedewald formula [15] from concentrations of total cholesterol, triglycerides, and HDL-C. Serum tHcy levels were determined using the homocysteine microplate enzyme immunoassay assay (Bio-Rad Laboratories, Oslo, Norway) [16]. Forty patients were determined to have high tHcy levels (>15 mmol/L; high-tHcy group) using this assay. We also included 45 age-matched patients from the original 108 enrolled patients who had normal levels of tHcy (≤15 mmol/L; normal-tHcy group); this cutoff for elevated homocysteine has been used by others [17]. The clinical characteristics of patients in the normal- and high-tHcy groups are summarized in Table 1. Twenty-seven of the 40 patients in the high-tHcy group and 29 of the 45 patients in the normal-tHcy group met the criteria for essential hypertension, and all of these patients were being treated with calcium channel antagonists, angiotensin-converting enzyme inhibitors, and/or angiotensin II receptor blockers with diuretics. None of the patients were being treated with β-blockers or insulin. Dyslipidemia was defined as fasting triglyceride levels ≥200 mg/dL or a HDL-C concentration <45 mg/dL for women and <35 mg/dL for men [13]. Eleven of the 30 patients in the high-tHcy group and 14 of the 41 patients in the normal-tHcy group met the criteria for dyslipidemia. Patients treated with insulin were also excluded. Female patients who were pregnant or treated with any postmenopausal hormonal replacement or contraceptives were also excluded. Urinary albumin excretion was measured in urine collected during a 24-hour period. Patients with abnormal plasma creatinine concentrations (≥1.5 mg/dL) were excluded from the study.

Table 1
Clinical characteristics of the studied patients

	Normal-tHcy group	High-tHcy group	*P*
Age (y)	57 ± 6	57 ± 7	NS
Sex (male/female)	22/23	21/19	NS
Homocysteine levels (μmol/L)	10.1 ± 3.2	22.8 ± 6.9	<.0001
Duration of diabetes (y)	7.7 ± 3.0	8.1 ± 4.5	NS
Hypertension (%)	64	68	NS
Dyslipidemia (%)	36	40	NS
Drug use (%)			
Sulfonylurea	44	48	NS
α-Glucosidase inhibitors	38	35	NS
Pioglitazone	9	8	NS
Statin	33	35	NS
Calcium channel antagonists	38	40	NS
ACE inhibitors	18	20	NS
Angiotensin receptor blocker	40	43	NS
BMI (kg/m^2)	25.8 ± 2.1	27.1 ± 3.6	.0371
Waist circumference (cm)	84.7 ± 9.3	89.4 ± 11.7	.0466
Hip circumference (cm)	96.6 ± 8.6	97.1 ± 10.5	NS
Waist-to-hip ratio	0.88 ± 0.06	0.92 ± 0.10	.0127
Systolic blood pressure (mm Hg)	128 ± 11	133 ± 14	NS
Diastolic blood pressure (mm Hg)	76 ± 8	78 ± 9	NS
Heart rate (beats/min)	69 ± 6	70 ± 7	NS
Total cholesterol (mg/dL)	199 ± 28	209 ± 40	NS
Triglyceride (mg/dL)	125 ± 50	150 ± 35	.0117
HDL-C (mg/dL)	47 ± 9	41 ± 7	.0002
LDL-C (mg/dL)	127 ± 30	138 ± 41	NS
Fasting plasma glucose (mg/dL)	138 ± 21	152 ± 30	.0012
Fasting immunoreactive insulin (μU/mL)	5.7 ± 1.7	8.5 ± 2.0	<.0001
HOMA index	1.9 ± 0.6	3.2 ± 0.8	<.0001
Hemoglobin A_{1c} (%)	7.6 ± 1.2	7.8 ± 1.0	NS
Uric acid (mg/dL)	5.7 ± 1.3	6.5 ± 1.6	.0252
Creatinine (mg/dL)	0.79 ± 0.23	0.92 ± 0.15	.0024
Creatinine clearance (mL/min)	105 ± 32	63 ± 16	<.0001
Urinary albumin excretion (mg/d)	60 ± 43	220 ± 85	<.0001

Data are expressed as means ± SD. NS indicates not significant; ACE, angiotensin-converting enzyme.

All subjects gave their written informed consent to participate in the study, and the study protocol was approved by the ethics committee of the Oita Red Cross Hospital (Oita, Japan).

2.2. Measurement of PWV

Brachial-ankle PWV was measured using a volume plethysmograph (Form/ABI, Colin, Komaki, Japan). The