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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

details of the measurement, validity, and reproducibility of this method have been reported previously [5-7]. Briefly, the subject was examined while resting in the supine position, with electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The cuffs were connected to the plethysmograph sensor that determines the volume pulse form and the oscillometric pressure sensor that measures blood pressure. The volume waveforms for the brachium and ankle were stored. The stored sample included sufficient waveform data. The characteristic points of waveforms were determined automatically according to the phase velocity theory. The components over 5 Hz were stored using a pass filter and the wave front was determined. The time interval between the wave front of the brachial waveform and that of the ankle waveform was defined as the time interval between the brachium and ankle (ΔTba). The distance between sampling points of BaPWV was calculated automatically according to the height of the subject. The path length from the suprasternal notch to the brachium (Lb) was obtained from superficial measurements and was expressed using the following equation: Lb = [0.2195 × height of the subject (in centimeters) − 2.0734]. The path length from the suprasternal notch to the ankle (La) was obtained from superficial measurements and was expressed using the following equation: La = [0.8129 × height of the subject (in centimeters) + 12.328]. Finally, the following equation was used to obtain BaPWV: BaPWV = [(La − Lb)/ΔTba]. In all the studies, BaPWV was measured after at least 5 minutes of rest. The interobserver coefficient of variation was 8.4% and the intraobserver coefficient of variation was 10.0% [7].

2.3. Anthropometric and body composition measurement

The anthropometric and body composition characteristics of the patients were evaluated using the following parameters: height, body weight, body mass index (BMI), waist circumference, hip circumference, and waist-to-hip ratio. Body mass index was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). The waist circumference was measured midway between the lower rib margin and the iliac crest, and the hip circumference was measured at the widest circumference over the trochanter in standing subjects after normal expiration.

2.4. Statistical analysis

Data are presented as mean ± SD. Differences between 2 groups were analyzed using the unpaired Student *t* test, χ^2 test, or by the Fisher exact probability test.

A *P* value of less than .05 was considered statistically significant. Simple (Spearman rank) correlation coefficients between tHcy and various parameters were calculated. Stepwise multiple regression analysis was then used to evaluate the association between the levels of homocysteine and other factors, such as BMI, waist circumference, waist-to-hip ratio, triglyceride levels, HDL-C levels, uric acid levels, fasting plasma glucose concentrations and plasma insulin concentrations, HOMA index values, urinary albumin excretion, creatinine level, creatinine clearance rates, and BaPWV. In our multivariate analysis, F values of 4 or higher were considered significant.

3. Results

As shown in Table 1, the mean ages of the high and normal-tHcy groups were similar, and there were no significant differences between the groups with respect to sex, duration of diabetes, or administered medications. The BMI values, waist circumferences, and the waist-to-hip ratios were larger in the high-tHcy group than in the normal-tHcy group (*P* = .0371, *P* = .0466, and *P* = .0127, respectively).

The resting heart rate and systolic and diastolic blood pressures were not significantly different between the 2 groups. Regarding glucose metabolism, fasting plasma glucose and insulin concentrations and HOMA index values were higher in the high-tHcy group than in the normal-tHcy group (*P* = .0012 and *P* < .0001, and *P* < .0001, respectively). However, there was no significant difference in hemoglobin A_{1c} between the 2 groups. With regard to lipid metabolism, the concentration of serum triglyceride was higher, and the concentration of serum HDL-C was lower in the high-tHcy group than in the normal-tHcy group (*P* = .0117 and *P* = .0002, respectively), whereas serum total cholesterol and LDL-C levels were not significantly different between the groups. The concentration of uric acid was higher in the

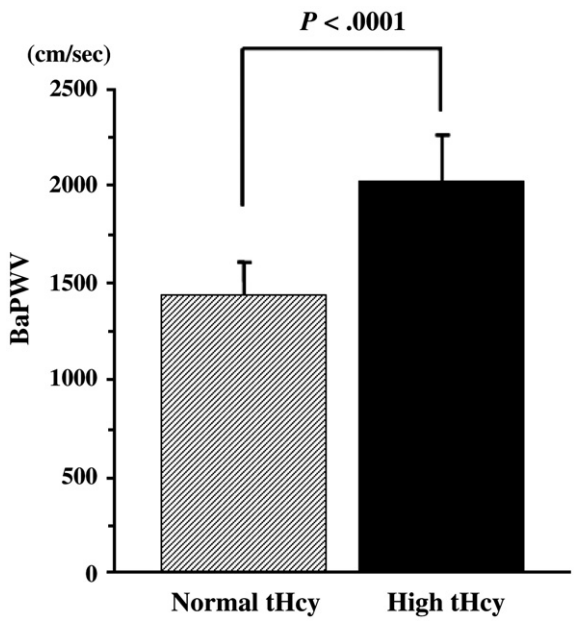

Fig. 1. Comparison of BaPWV between type 2 diabetic patients with normal homocysteine (normal tHcy) and those with high homocysteine (high tHcy). Data are expressed as mean ± SD.

Table 2
Correlations between homocysteine and various parameters

Parameters	Univariate analysis	
	r	*P*
Age	0.064	.6948
Duration of diabetes mellitus	0.037	.7398
BMI	0.230	.0346
Waist circumference	0.258	.0173
Hip circumference	0.091	.4065
Waist-to-hip ratio	0.276	.0107
Systolic blood pressure	0.015	.8948
Diastolic blood pressure	0.060	.5881
Heart rate	0.179	.1003
Total cholesterol	0.144	.1885
Triglyceride	0.242	.0254
HDL-C	−0.259	.0169
LDL-C	0.134	.2221
Uric acid	0.249	.0214
Fasting plasma glucose	0.314	.0034
Fasting immunoreactive insulin	0.469	<.0001
HOMA index	0.551	<.0001
Hemoglobin A_{1c}	0.032	.7722
Urinary albumin excretion	0.290	.0072
Creatinine	0.244	.0242
Creatinine clearance	−0.273	.0116
BaPWV	0.519	<.0001

high-tHcy group than in the normal-tHcy group ($P = .0252$). Parameters measuring renal function, including the serum creatinine concentration, the creatinine clearance, and urinary albumin excretion, were greater in the in the high-tHcy group than in the normal-tHcy group ($P = .0024$, $P < .0001$, and $P < .0001$, respectively).

Fig. 1 shows the BaPWV in the normal-tHcy group and in the high-tHcy group of type 2 diabetic patients. The BaPWV was higher in the high-tHcy group than in the normal-tHcy group (2013 ± 243 vs 1420 ± 173 cm/s, $P < .0001$).

Table 2 depicts the correlation between the tHcy level and age, BMI, and various other variables in both the high-tHcy and the normal-tHcy group. Total Hcy levels were positively correlated with BMI values, waist circumference, waist-to-hip ratio, triglyceride levels, fasting plasma glucose, fasting plasma insulin concentration, uric acid levels, HOMA index values, creatinine levels, urinary albumin excretion, and BaPWV and were negatively correlated with HDL-C levels and creatinine clearance rate.

Multiple regression analysis was performed using the stepwise procedure. The level of tHcy was independently predicted by BaPWV (F value = 7.177) and HOMA index (F value = 11.563).

4. Discussion

In the present study, type 2 diabetic patients with elevated tHcy manifested increased arterial stiffness evaluated by BaPWV. Among the metabolic parameters, fasting plasma concentrations of glucose and insulin and the HOMA index were higher in patients with high tHcy than in those with normal tHcy. In addition, multiple regression analysis revealed that the levels of tHcy in the patients could be independently predicted by the HOMA index values and BaPWV in Japanese patients with type 2 diabetes mellitus.

What are the main mechanisms of the relationship between tHcy and aortic stiffness? Elevated tHcy induces oxidative injury to vascular endothelial cells and impairs the production of nitric oxide, a strong vascular relaxing factor, by the endothelium [18,19]. Hyperhomocysteinemia also enhances platelet adhesion to endothelial cells [20], promotes growth of vascular smooth muscles cells [21], and is associated with higher levels of prothrombotic factors such as β-thromboglobulin, tissue plasminogen activator, and factor VIIc [22]. In addition, Chowdhary et al [23] showed that oral folate supplementation improves the arterial endothelium-dependent vascular function of the brachial artery in healthy subjects with mild hyperhomocysteinemia. In another study, administration of folate and vitamin B_{12} for 9 weeks to patients with coronary heart disease and hyperhomocysteinemia improved vascular endothelial function as assessed by brachial artery flow-mediated dilatation [24]. Based on these results, tHcy appears to be not only a risk factor for arterial sclerosis but possibly could act as a pathophysiologic modulator causing other endothelial dysfunctions.

There are several reports indicating that an elevated tHcy concentration is associated with insulin resistance in nonobese healthy subjects [11] and in patients with type 2 diabetes mellitus [12]. Giltay et al [11] investigated the association between plasma tHcy levels and insulin resistance using a hyperinsulinemic-euglycemic clamp in 24 nonobese healthy subjects. They found a significant increase in the plasma tHcy levels of healthy subjects with insulin resistance. Emoto et al [12] demonstrated that insulin resistance was an independent determinant of elevated plasma tHcy levels in 75 type 2 diabetic patients. In the present study, the level of tHcy correlated with BMI, triglyceride levels, HDL-C levels, fasting plasma insulin concentration, and HOMA index values.

Although the specific mechanism that links the tHcy level and insulin resistance remains to be elucidated, several mechanisms could explain our observations. Homocysteine may induce and/or enhance insulin resistance through oxidant stress. Homocysteine causes angiotoxicity and inhibits the nitric oxide system by inducing oxidant stress [25]. Oxidant stress, in turn, reduces insulin responsiveness in vitro by interrupting insulin signaling [26]. Moreover, Najib and Sanchez-Margalet [27] reported that homocysteine induces insulin resistance in vitro at high concentrations (50 μmol/L) by inhibiting insulin signaling, and this effect may be mediated by oxidative stress because it can be prevented by glutathione administration.

On the other hand, hyperinsulinemia associated with insulin resistance may affect the activities of enzymes involved homocysteine metabolism and thereby influence

plasma homocysteine levels. Jacobs et al [28] have also reported that tHcy levels decreased because of the increase in the activity of cystathionine β-synthase in streptozotocin-induced diabetic rats but returned to the initial plasma levels after administration of insulin. Fonseca et al [29] have recently shown that hyperinsulinemia in the high fat-sucrose–fed rat with insulin resistance induced a decrease in cystathionine β-synthase activity and compensatorily increased the activity of 5,10-methylene tetrahydrofolate reductase, which was followed by hyperhomocysteinemia.

Taken together, this is the first study to report that homocysteine levels correlate with BaPWV, which may relate to insulin resistance in type 2 diabetic patients.

Compared with the normal-tHcy group, patients with high tHcy levels showed cardiac diastolic dysfunction, although no significant difference was found between the 2 groups in the left ventricular mass index. Cardiac diastolic dysfunction is associated with cardiovascular autonomic dysfunction and insulin resistance [13]. Consistent with the present results, Wheeler et al [30] reported that elevated homocysteine levels were associated with cardiac left ventricular diastolic dysfunction in diabetic patients.

It has also been clearly documented that increased levels of tHcy occur in association with marked degrees of renal dysfunction [17]. Davies et al [17] demonstrated the relationship between plasma tHcy levels and urinary albumin excretion due to associated changes in renal function as defined by the creatinine clearance rate in type 2 diabetic patients. Emoto et al [12], however, demonstrated that insulin resistance was an independent determinant of the plasma tHcy level in type 2 diabetic patients, even when the group of patients with type 2 diabetes mellitus was limited to those with normal renal function (creatinine clearance rate >60 mL/min). As in these studies, the present results demonstrated that both insulin resistance and renal dysfunction were associated with high tHcy levels, and insulin resistance was an independent predictor of the concentration of tHcy in type 2 diabetes mellitus.

There are several limitations to this study. Firstly, subjects in the present study population had essential hypertension, which was treated with 1 or more antihypertensive drugs. These characteristics of the patients' backgrounds have been reported to affect insulin resistance [31,32] and aortic stiffness [33,34].

Secondly, serum concentrations of folate and vitamins B_6 and B_{12} affect homocysteine metabolism [35]. It remains uncertain whether nutritional status affected the risk of aortic stiffness in type 2 diabetic patients, although we did not monitor the folate and vitamin concentrations in this investigation.

Finally, the most frequent genetic defect in homocysteine metabolism involves the enzyme methylene tetrahydrofolate reductase [36]. A common polymorphism (C677T) is associated with hyperhomocysteinemia, the highest levels of homocysteine being found in those with the TT genotype. Recently, it was reported that the frequency of the C677T in Japan was 0.42 and that for whites, 0.34 [37]. We did not investigate this genetic factor in the present study. Further clinical investigations are needed to determine the relationship between genetic factors and aortic stiffness in type 2 diabetic patients.

In conclusion, our findings suggest that higher levels of tHcy are associated with aortic stiffness and insulin resistance, and that BaPWV and the HOMA index are independent predictors of tHcy in type 2 diabetic patients.

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