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Hepatocyte growth factor levels are associated with the results of ^{123}I-metaiodobenzylguanidine myocardial scintigraphy in patients with type 2 diabetes mellitus

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Abstract

Elevated hepatocyte growth factor (HGF) levels and cardiovascular autonomic dysfunction are associated with a high mortality rate in patients with type 2 diabetes mellitus. We tested the hypothesis that elevated HGF is associated with insulin resistance and cardiovascular autonomic dysfunction in patients with type 2 diabetes mellitus not receiving insulin treatment. The study group consisted of 21 type 2 diabetes mellitus patients with high HGF levels (>0.26 ng/mL, 58 ± 5 years old, high-HGF group). The control group consisted of 25 type 2 diabetes mellitus patients with normal HGF levels (≤0.26 ng/mL, 58 ± 9 years old, normal-HGF group). Cardiovascular autonomic function was assessed by baroreflex sensitivity, heart rate variability, plasma norepinephrine concentrations, and cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy. Early and delayed ^{123}I-MIBG myocardial uptake values were lower ($P < .005$ and $P < .01$, respectively) and the percentage of washout rate of ^{123}I-MIBG was higher ($P < .001$) in the high-HGF group than in the normal-HGF group. The fasting plasma insulin concentrations ($P < .0001$) and the homeostasis model assessment index values ($P < .0001$) were higher in the high-HGF group than in the normal-HGF group. Multiple regression analysis revealed that the level of HGF was independently predicted by the homeostasis model assessment index values and the myocardial uptake of ^{123}I-MIBG at the delayed phase. Our results demonstrate that high levels of HGF are associated with depressed cardiovascular autonomic function and insulin resistance in patients with type 2 diabetes mellitus.

1. Introduction

Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor that regulates cell growth and motility and morphogenesis of various cell types [1]. It has the unique ability to stimulate endothelial cell growth without affecting vascular smooth muscle cell growth [1]. Furthermore, HGF exerts an antiapoptotic effect on the endothelium [2]. Through these functions, tissue HGF has been shown to play an antiatherogenic role [3]. Although its origin is not fully known, HGF is present in the circulation [4-8]. The circulating level of HGF has been shown to be increased in cardiocerebrovascular disease, including hypertension [4], atherosclerosis [5], and myocardial infarction [6], as well as cerebral infarction [7] and end-stage renal disease [8].

Impaired autonomic neural activity has been recognized as a crucial risk factor of cardiac dysfunction and is strongly associated with an increased risk for harmful events and overall mortality in diabetic patients [9-11]. We have reported that depressed cardiovascular autonomic function is related to insulin resistance in patients with type 2 diabetes mellitus [12-14]. Furthermore, elevated HGF levels are reported to be associated with fasting plasma glucose and insulin in the general population [15]. Although these results strongly suggest that HGF levels, insulin resistance, and autonomic dysfunction are related, the significance of increased HGF levels for diabetic cardiovascular autonomic function has not been adequately investigated.

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Technical advances, including measurements of baroreflex sensitivity (BRS), heart rate variability (HRV), and the concentration of norepinephrine, allow cardiac autonomic function to be assessed. The reliability coefficients of these parameters, however, were shown to be around 50% [16]. A reduction in myocardial uptake of ^{123}I-metaiodobenzylguanidine (MIBG) reflects a reduction in the concentration of norepinephrine at presynaptic sites or a reduction in the neural density, whereas an enhanced washout rate (WR) of ^{123}I-MIBG reflects enhanced release of norepinephrine from presynaptic sites [17]. Cardiac ^{123}I-MIBG scintigraphy is a sensitive method for detecting sympathetic dysfunction in many clinical disorders, including diabetes mellitus [18,19].

We hypothesized that increased levels of HGF are associated with cardiovascular autonomic dysfunction and insulin resistance in patients with type 2 diabetes mellitus. To test our hypothesis, we compared BRS, HRV, plasma norepinephrine concentrations, and cardiac ^{123}I-MIBG scintigraphy in addition to the metabolic profiles of Japanese type 2 diabetes mellitus patients with normal HGF and those with high HGF; and independent predictors of the level of HGF in these populations were evaluated.

2. Subjects and methods

We screened 110 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to our department between December 2004 and May 2006.

Among them, 46 patients (23 men and 23 women), with ages ranging from 45 to 65 years with a mean SD age of 57 ± 6 years, fulfilled the inclusion criteria and were enrolled in the present study. The inclusion criteria were as follows: (1) Organic heart disease was not determined by treadmill exercise electrocardiography (ECG). Treadmill exercise ECG did not show ST-T abnormal changes. (2) Absence of causes of secondary hypertension (ie, primary aldosteronism, renal vascular hypertension, hyperthyroidism, pheochromocytoma). (3) No history of chronic disease, such as renal failure (creatinine >1.5 mg/dL), pulmonary disease, liver dysfunction (aspartate aminotransferase >50 IU/L), arteriosclerotic obliterans, sleep apnea syndrome, and symptomatic cerebrovascular disease, was noted. (4) The patient was not currently receiving treatment with insulin. (5) Female patients who were pregnant or treated with any postmenopausal hormone replacement therapy were excluded.

Of the 110 enrolled patients, 64 were excluded from further evaluation because of extenuating circumstances. These include the following: 22 patients were being treated with insulin, 8 patients had angina pectoris, 7 patients had renal failure, 6 patients had symptomatic cerebrovascular disease, 5 patients had arteriosclerotic obliterans, 5 patients had sleep apnea syndrome, 4 patients had secondary hypertension (2 patients had primary aldosteronism, 1 patient had renal vascular hypertension, 1 patient had hyperthyroidism), 3 patients had liver dysfunction (1 patient had hepatitis B, 2 patients had hepatitis C), 2 patients had lung cancer, and 2 patients were being treated with postmenopausal hormone replacement therapy. Therefore, only 46 patients were selected for the study.

Serum HGF concentrations were measured with an enzyme-linked immunosorbent assay kit (Otsuka Assay Laboratories, Osaka, Japan) using an anti–human HGF monoclonal antibody for the solid phase and an anti–human HGF rabbit polyclonal antibody for the liquid phase [20]. Using this assay, 21 patients were determined to have high HGF levels (>0.26 ng/mL; high-HGF group). We also included 25 patients who had normal levels of HGF (≤0.26 ng/mL; normal-HGF group), the classification of which has previously been validated [21]. The coefficient of variation of HGF was less than 5.5% for 1 ng/mL in this study. The clinical characteristics of patients in the normal- and high-HGF groups are summarized in Table 1.

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 University Hospital.

Table 1
Clinical characteristics of the studied patients

	Normal-HGF group	High-HGF group	*P* value
Age (y)	57 ± 6	57 ± 5	NS
Sex (male/female)	13/12	10/11	NS
HGF levels (ng/mL)	0.19 ± 0.05	0.51 ± 0.16	<.0001
Duration of diabetes (y)	7.6 ± 4.6	8.0 ± 5.3	NS
Hypertension (%)	64	67	NS
Dyslipidemia (%)	36	38	NS
Drug use (%)			
Sulfonylurea	44	48	NS
α-Glucosidase inhibitors	36	43	NS
Pioglitazone	12	14	NS
Statin	32	29	NS
Calcium channel antagonists	36	38	NS
ACE inhibitors	16	19	NS
Angiotensin receptor blocker	40	38	NS
Diuretic	12	14	NS
BMI (kg/m^2)	25.2 ± 2.9	27.3 ± 3.7	.0392
Waist circumference (cm)	84.5 ± 9.1	94.7 ± 11.4	.0016
Hip circumference (cm)	94.5 ± 8.4	98.8 ± 7.8	NS
Waist-to-hip ratio	0.90 ± 0.07	0.96 ± 0.11	.0188
Systolic blood pressure (mm Hg)	128 ± 10	133 ± 15	NS
Diastolic blood pressure (mm Hg)	76 ± 8	78 ± 9	NS
Heart rate (beats/min)	68 ± 6	69 ± 7	NS
Total cholesterol (mg/dL)	198 ± 34	209 ± 40	NS
Triglyceride (mg/dL)	125 ± 37	148 ± 33	.0366
HDL-C (mg/dL)	47 ± 9	40 ± 7	.0101
Fasting plasma glucose (mg/dL)	137 ± 23	152 ± 29	.0285
Fasting immunoreactive insulin (μU/mL)	6.0 ± 1.6	8.3 ± 1.9	<.0001
Homeostasis model assessment index	2.0 ± 0.6	3.1 ± 0.9	<.0001
Hemoglobin A_{1c} (%)	7.6 ± 1.1	7.7 ± 0.9	NS
Uric acid (mg/dL)	5.6 ± 1.4	6.7 ± 1.5	.0076
Creatinine (mg/dL)	0.74 ± 0.20	0.83 ± 0.16	NS

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

2.1. Definition of blood pressure

Hypertension was defined by performing blood pressure measurement, registered as the average of 3 measurements obtained with a mercury-column sphygmomanometer after 10 minutes of physical resting by the patients. *Essential hypertension* was defined as diastolic blood pressure of at least 90 mm Hg, systolic blood pressure of at least 140 mm Hg, or self-reported use of antihypertensive medication [22].

2.2. 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 (in microunits per milliliter) × fasting plasma glucose (in millimoles per liter)]/22.5 [23]. *Dyslipidemia* was defined as fasting triglycerides levels of at least 200 mg/dL or an HDL-C concentration of less than 45 mg/dL for women and less than 35 mg/dL for men [22].

2.3. Echocardiography

M-mode and 2-dimensional echocardiography and cardiac Doppler recordings were obtained using a phase-array echo-Doppler system. Echocardiograms were obtained using standard parasternal, short axis, and apical views. The left ventricular mass was calculated as $1.04 \times ([\mathrm{LVIDd} + \mathrm{IVSTd} + \mathrm{PWTd}]^3 - \mathrm{LVIDd}^3) - 14$ g, where LVIDd is the left ventricular internal diameter at the end diastole, IVSTd is the intraventricular septal thickness at the end diastole, and PWTd is the posterior wall thickness at the end diastole. The left ventricular mass was divided by the body surface area to calculate the left ventricular mass index. Pulsed Doppler recordings were made from a standard apical 4-chamber view. Mitral inflow velocity was recorded with the sample volume at the mitral annulus level, taking the average from at least 3 cardiac cycles. The peak velocity of early (E) and late ventricular filling (A) was determined, and the ratio (E/A) and deceleration time were recorded.

2.4. Cardiovascular autonomic function tests

Autonomic function was assessed according to methods described in previous studies [12-14]. During the tests, which were performed between 9:00 AM and 11:00 AM, all subjects were in a supine position in a quiet room with dimmed lights. Autonomic function tests were performed in the morning after an overnight (≥12 hours) fast. For measurement of the plasma norepinephrine concentration, a blood sample was obtained from a catheter inserted in the right cubital vein 30 minutes earlier. We measured plasma norepinephrine concentration according to methods described in previous study [12-14]. Arterial blood pressure was recorded noninvasively through a tonometric sensor attached over the left radial artery (Jentow-7700; Nihon Colin, Komaki, Japan). The tonometric sensor was attached over the left radial artery. The accuracy of continuous blood pressure monitoring has been demonstrated previously [24]. Arterial blood pressure and a standard 12-lead ECG were monitored simultaneously; data were stored in a pulse code modulation (PCM) data recorder (RD-200T; TEAC, Tokyo, Japan). Three-lead precordial Holter ECG recordings (model-459; Del Mar Avionics, Irvine, CA) were also obtained throughout the procedure for analysis of HRV.

After an interval of 30 minutes to permit stabilization of the cardiovascular baroreflex mechanism, the patient was asked to breathe at a rate of 15 breaths per minute using a metronome to stabilize the relationship between respiration and cardiovascular function. Baroreflex sensitivity was assessed by the phenylephrine method [12-14]. Briefly, phenylephrine (2-3 μg/kg) was injected for 15 seconds to obtain a 15– to 40–mm Hg rise in systolic blood pressure. Baroreflex sensitivity was calculated as the slope of the linear regression function relating systolic blood pressure changes to changes in the RR interval. Regression lines with more than 20 data points and a correlation coefficient (r) greater than 0.8 were accepted for analysis. The average of the 2 slopes was taken as the BRS value.

Heart rave variability was analyzed using Holter ECG recordings (Marquette Electronics, Milwaukee, WI), with methods described in a previous study [25]. The power spectrum of the RR interval was computed by a fast Fourier transformation and expressed as the area under the power spectrum. We calculated the power of 2 spectral bands: the normal-frequency (LF) component at 0.04 to 0.15 Hz and the high-frequency (HF) component at 0.15 to 0.40 Hz. Based on their skewed distribution, the measured values of HRV were transformed to natural logarithmic values. The ratio of LF to HF (LF/HF) also was computed.

Planar and single-photon emission computed tomography studies were performed 15 minutes (early) and 4 hours (delayed) after the injection of 111 MBq of ^{123}I-MIBG using a rotating gamma camera (ZLC 7500; Siemens, Munich, Germany). Data were analyzed with analysis software (SCINTIPAC; Shimadzu, Kyoto, Japan). The anterior planar images from early and delayed ^{123}I-MIBG studies were analyzed visually. For semiquantitative analysis, regions of interest were identified within the whole heart; and a 10 × 10-mm area over the upper mediastinum on the early and delayed planar images was used to calculate the mean heart-to-mediastinum (H/M) ratio. After correcting for the physical decay of ^{123}I, the percentage of WR of the tracer from the myocardium was determined over a 4-hour period. Two cardiovascular physiologists interpreted the ^{123}I-MIBG myocardial scans of the subjects.

2.5. 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 weight/(height^2) (kilograms per square meter). 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.

2.6. Statistical analysis

First, data are summarized with means of the variables used and their standard errors (Table 1). For each variable, the difference between 2 groups is compared by a 2-sided t test with level of significance at .05. Second, correlations between HGF and the other variables are tested by using the simple correlation coefficient (Table 2). Finally, multiple regression analysis is performed; and to determine significant variables associated with HGF, the most parsimonious model is selected by using a backward elimination procedure (Table 3).

3. Results

As shown in Table 1, the mean ages of the high- and normal-HGF groups were similar; and there were no significant differences between the groups with respect to sex, duration of diabetes, and administered medications. The BMI values, waist circumferences, and waist-to-hip ratios were larger in the high-HGF group than in the normal-HGF group (P = .0392, P = .0016, and P = .0188, respectively). Regarding glucose metabolism, fasting plasma glucose and insulin concentrations and HOMA index values were higher in the high-HGF group than in the normal-HGF group (P = .0285, P < .0001, and P < .0001, respectively). There, however, was no significant difference in the levels of hemoglobin A_{1c}. With regard to lipid metabolism, the concentration of serum triglyceride was higher and the concentration of serum HDL-C was lower in the high-HGF group than in the normal-HGF group (P = .0336 and P = .0101, respectively), whereas serum total cholesterol levels were not significantly different between the groups. The concentration of uric acid was higher in the high-HGF group than in the normal-HGF group (P = .0076).

Table 2
Correlations of HGF levels to other variables

Parameters	Univariate analysis	
	r	P value
Age	0.208	.1652
Duration of diabetes mellitus	0.240	.1075
BMI	0.349	.0174
Waist circumference	0.352	.0163
Hip circumference	0.060	.6939
Waist-to-hip ratio	0.410	.0047
Systolic blood pressure	0.134	.3755
Diastolic blood pressure	0.193	.1976
Heart rate	0.196	.1920
E/A ratio	−0.326	.0270
Deceleration time	0.284	.0555
Total cholesterol	0.213	.1546
Triglyceride	0.363	.0131
HDL-C	−0.374	.0105
Uric acid	0.364	.0129
Fasting plasma glucose	0.406	.0051
Fasting immunoreactive insulin	0.487	.0006
HOMA index	0.586	<.0001
Hemoglobin A_{1c}	0.117	.4383
Creatinine	0.200	.1824
BRS	−0.365	.0127
Plasma norepinephrine	0.255	.0877
HF power	−0.201	.1835
LF/HF	−0.144	.3409
H/M ratio at early phase	−0.460	.0013
H/M ratio at delayed phase	−0.516	.0002
WR	0.350	.0170

Table 3
Stepwise regression analysis between HGF levels and other parameters

Independent variable	Regression coefficient	Standard error	Standard regression coefficient	F value
To HGF intercept	0.628			
H/M ratio at the delayed phase	−0.269	0.108	−0.319	6.142
HOMA index	0.094	0.027	0.446	11.990

Renal function tests showed no significant difference in serum concentration between the 2 groups. The hemodynamic data listed in Table 1 were obtained immediately before BRS assessment. The resting heart rate and the systolic and diastolic blood pressures were not significantly different between the 2 groups.

The echocardiographic findings are as follows: The left ventricular dimensions at the end diastole and end systole, the intraventricular septal and posterior wall thicknesses at the end diastole, the ejection fraction, and the left ventricular mass index values were similar in the 2 groups. With regard to left ventricular diastolic function, the E/A ratio was lower in the high-HGF group compared with the normal-HGF group (0.79 ± 0.16 vs 0.92 ± 0.17, P = .0109). The deceleration time was longer in the high-HGF group than in the normal-HGF group (252 ± 28 vs 236 ± 24 milliseconds, P = .0208).

Fig. 1 summarizes the results of the cardiovascular autonomic function tests. Baroreflex sensitivity was lower in the high-HGF group than in the normal-HGF group (8.6 ± 3.6 vs 11.4 ± 3.3 ms/mm Hg, P = .0069; Fig. 1A). The plasma norepinephrine concentrations were similar in both groups (high-HGF group: 242 ± 89 pg/mL; normal-HGF group: 203 ± 62 pg/mL; P = not significant; Fig. 1B). Furthermore, analysis of HRV in the high- and low-HGF groups revealed that the HF power (3.4 ± 1.0 and 3.7 ± 1.1 ln-millisecond^2, respectively; P = not significant) and the LF/HF ratios (1.4 ± 0.7 and 1.7 ± 1.0, respectively; P = not significant; Fig. 1C) were not significantly different between the 2 groups. Cardiac ^{123}I-MIBG scintigraphy disclosed that

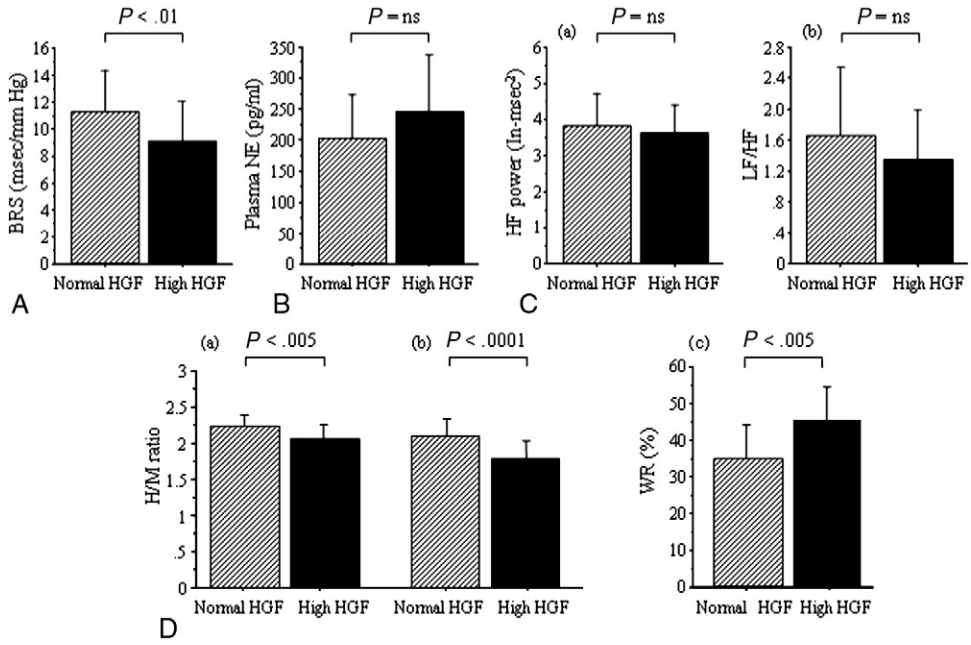

Fig. 1. Comparison of autonomic function tests of type 2 diabetes mellitus patients with normal plasma HGF levels or high HGF levels. A, Baroreflex sensitivity. B, Plasma norepinephrine concentration. C, Heart rate variability. Power of the HF component (0.15-0.40 Hz, a) and the ratio of the LF power (0.04-0.15 Hz) to HF power (LF/HF, b). The distribution of HRV values was skewed, and the values were thus transformed to natural logarithmic values. D, Cardiac ^{123}I-MIBG scintigraphic findings. Myocardial uptake of ^{123}I-MIBG at the early (a) and delayed (b) phases. Myocardial uptake of ^{123}I-MIBG is expressed as the mean H/M ratio. Percentage of WR of ^{123}I-MIBG (c). Data are mean ± SD. NE indicates norepinephrine; NS, not significant.

the H/M ratios at early and delayed phases in the high-HGF group were significantly smaller than those in the normal-HGF group (early phase: 2.05 ± 0.17 vs 2.26 ± 0.20, respectively, $P = .0004$; delayed phase: 1.84 ± 0.18 vs 2.10 ± 0.21, respectively, $P < .0001$; Fig. 1D). The percentage of WR of ^{123}I-MIBG was higher in the high-HGF group than in the normal-HGF group (44.5% ± 8.7% vs 35.4% ± 8.8%, $P = .0010$; Fig. 1D).

Table 2 depicts that the HGF levels were positively correlated with BMI values, waist circumference, waist-to-hip ratio, triglyceride levels, uric acid levels, fasting plasma insulin concentration, HOMA index values, and percentage of WR of ^{123}I-MIBG, and was negatively correlated with the HDL-C levels, E/A ratio, and H/M ratio at the early and the delayed phase.

Multiple regression analysis was performed using the stepwise procedure. The level of HGF was predicted from the HOMA index values and the H/M ratio at the delayed phase (Table 3).

4. Discussion

Elevated HGF levels have previously been reported to be associated with fasting plasma glucose and insulin in metabolic syndrome population [15]. Furthermore, there was a higher degree of metabolic abnormality in the greater qualities of HGF levels. In type 2 diabetes mellitus patients without organic heart diseases, we confirmed the relation of the level of HGF and indices of metabolic syndrome such as BMI, triglyceride levels, HDL-C levels, fasting plasma insulin concentration, and HOMA index values. In addition, multiple regression analysis revealed that the level of HGF in the patients could be independently predicted by the HOMA index values and the H/M ratio during the delayed phase by ^{123}I-MIBG scintigraphy.

The novel and important finding of the present study is that high levels of HGF are associated with cardiac ^{123}I-MIBG scintigraphic findings in patients with type 2 diabetes mellitus.

The specific mechanism that links the HGF level and insulin resistance remains to be elucidated. Bell et al [26] demonstrated that serum HGF is regulated by changes in adipose tissue mass and that adipocytes from obese subjects secret greater amounts of HGF than those from lean subjects. Greater release of tumor necrosis factor (TNF) α and elevated levels of TNF-α messenger RNA in adipose tissue from obese humans are reported [26,27]. Furthermore, TNF-α levels are inversely correlated with insulin sensitivity in obese patients with type 2 diabetes mellitus [28]. These findings suggest a strong link between HGF concentrations and indices of obesity. We have recently reported that the HOMA index and the myocardial uptake of ^{123}I-MIBG at the delayed phase were independent predictors of visceral fat accumulation (VFA) [14]. Our findings may support a potential role of VFA in metabolic syndrome and the present study population.

The lower BRS has been established as a marker of depressed reflex vagal function. Decreased ^{123}I-MIBG uptake in the delayed phase reflects at least 3 events:

reduced neuron density, reduced uptake-1, or enhanced sympathetic drive. Impairment in uptake-1 by transporter reduction in diabetic cardiac sympathetic nerve has been implicated in heterogeneous accumulation of ^{123}I-MIBG [29]. Enhanced sympathetic activity is caused by insulin resistance associated with obesity, metabolic syndrome, or diabetes [14]. Cardiac MIBG scintigraphy provides direct information on the function and integrity of presynaptic nerve endings [30,31]. A reduction in the myocardial uptake of ^{123}I-MIBG reflects a reduction in the concentration of norepinephrine at presynaptic sites or a reduction in neural density. In fact, the decreased H/M ratio during the delayed phase is an independent predictor of long-term mortality; and MIBG myocardial scintigraphy is useful for predicting cardiac events and long-term mortality in patients with type 2 diabetes mellitus [32]. On the other hand, an enhanced WR on ^{123}I-MIBG reflects the enhanced release of norepinephrine from presynaptic sites [17]. Cardiac ^{123}I-MIBG scintigraphy is a sensitive method for detecting sympathetic dysfunction in many clinical disorders, including diabetes mellitus [18,19]. In the present study, HRV and the plasma norepinephrine levels were not different between the 2 groups. However, on the cardiac ^{123}I-MIBG scintigraphy, serum HGF levels were positively correlated with the percentage of WR and negatively correlated with the H/M ratio during the early and delayed phases. In addition, myocardial uptake of ^{123}I-MIBG during the delayed phase was an independent predictor of serum HGF levels. Together with our and others' previous studies [12-14,33], the present study also supported that ^{123}I-MIBG scintigraphy is useful and is one of the fairly sensitive methods for detecting cardiac sympathetic dysfunction in diabetic patients.

The precise mechanisms underlying the association of HGF levels and impaired cardiac autonomic function remain unclear. Hepatocyte growth factor may affect autonomic function through endothelial dysfunction and impairment of nitrate oxide system. In fact, nitric oxidant damage and endothelial dysfunction are associated with cardiac autonomic dysfunction and increased HRV [34]. Ma et al [35] reported that HGF is expressed in human atherosclerotic plaques and colocalizes with vascular smooth muscle cells, microvascular endothelial cells, and monocytes/macrophages. Furthermore, in a recent report demonstrating the association between increase of HGF, insulin resistance, and obesity, the authors stressed the central role of endothelial dysfunction [36]. However, Makondo et al [37] demonstrated that HGF directly stimulates endothelial nitric oxide synthase activity in vascular endothelial cells by a phosphoinositide 3-kinase/Akt-dependent phosphorylation. Hepatocyte growth factor has been reported to protect endothelial cells from apoptosis [2]. The effect of HGF on endothelial cells is still to be debated.

Taken together, it is possible that HGF concentration, insulin resistance, and autonomic dysfunction are markers of target organ effects in type 2 diabetes mellitus patients with the metabolic syndrome.

There were significant differences between cardiac diastolic function assessed by E/A ratio and HGF. It had been demonstrated that cardiac diastolic dysfunction is associated with insulin resistance and obesity [14,38]. In the present study, however, it remains unclear how diastolic dysfunction was related to ^{123}I-MIBG scintigraphy findings.

There are several limitations in this study. Firstly, for semiquantitative analysis on ^{123}I-MIBG uptake, regions of interest were identified within the whole heart. It is noted that subjective rather than objective criteria may be applied to selection of heart areas for comparison. Secondly, it has been recognized that there is a sex difference in various aspects of cardiovascular autonomic function and metabolism. In the present study, there was no significant difference in these measures between male and female subjects (data not shown). A large-scale study is needed to clarify the sex difference. Finally, we have previously reported that homocysteine and abdominal VFA are associated with insulin resistance as well as cardiac sympathetic nerve function assessed by ^{123}I-MIBG [13,14]. Therefore, further studies are required to evaluate the association among the levels of homocysteine, VFA, HOMA index values, and ^{123}I-MIBG parameters. In addition, the prognostic implication of cardiac autonomic function assessed by ^{123}I-MIBG scintigraphy remains to be addressed, although there are prognosis studies of heart failure patients using cardiac ^{123}I-MIBG imaging [39].

In conclusion, multiple regression analysis revealed that the level of HGF in the patients is independently predicted by the HOMA index values and the H/M ratio at the delayed phase. Our findings suggest that higher levels of HGF in patients with type 2 diabetes mellitus are associated with depressed cardiovascular autonomic function and insulin resistance.

References

[1] Morishita R, Moriguchi A, Higaki J, et al. Hepatocyte growth factor (HGF) as a potential index of severity of hypertension. Hypertens Res 1999;22:161-7.

[2] Yamamoto K, Morishita R, Hayashi S. Contribution of Bcl-2, but not Bcl-xL and Bax, to antiapoptotic actions of hepatocyte growth factor in hypoxia-conditioned human endothelial cells. Hypertension 2001;37: 1341-8.

[3] Hayashi K, Nakamura S, Morishita R. In vivo transfer of human hepatocyte growth factor gene accelerates re-endothelialization and inhibits neointimal formation after balloon injury in rat model. Gene Therapy 2000;7:1664-71.

[4] Hayashi Y, Saitoh S, Takagi S. Hepatocyte growth factor and 24-hour ambulatory blood pressure monitoring. Hypertens Res 2002;25: 655-60.

[5] Satani K, Konya H, Hamaguchi T, et al. Clinical significance of circulating hepatocyte growth factor, a new risk marker of carotid atherosclerosis in patients with type 2 diabetes. Diabetic Med 2006;23: 617-22.

[6] Hata N, Matsumori A, Yokoyama S, et al. Hepatocyte growth factor and cardiovascular thrombosis in patients admitted to the intensive care unit. Cir J 2004;68:645-9.

[7] Matsumori A, Takano H, Obata J. Circulating hepatocyte growth factor as a diagnostic marker of thrombus formation in patients with cerebral infarction. Cir J 2002;66:216-8.
[8] Malatino LS, Cataliotti A, Benedetto FA, et al. Hepatocyte growth factor and left ventricular geometry in end stage renal disease. Hypertension 2003;41:88-92.
[9] Tsuji H, Larson MG, Venditti Jr FJ. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Study. Circulation 1996;94:2850-5.
[10] La Rovere MT, Bigger Jr JT, Marcus FI, et al. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infraction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infraction) Investigators. Lancet 1998;351:478-84.
[11] Reichard P, Pihl M. Mortality and treatment side-effects during long-term intensified conventional insulin treatment in the Stockholm Diabetes Intervention Study. Diabetes 1994;43:313-7.
[12] Takahashi N, Nakagawa M, Saikawa T. Effect of essential hypertension on cardiac autonomic function in type 2 diabetic patients. J Am Coll Cardiol 2001;38:232-7.
[13] Anan F, Yonemochi H, Masaki T, et al. Homocysteine levels are associated with the results of ^{123}I-metaiodobenzylguanidine myocardial scintigraphy in type 2 diabetic patients. Eur J Nucl Med Mol Imaging 2007;34:28-35.
[14] Anan F, Masaki T, Yonemochi H, et al. Abdominal visceral fat accumulation is associated with the results of ^{123}I-metaiodobenzylguanidine myocardial scintigraphy in type 2 diabetic patients. Eur J Nucl Med Mol Imaging 2007;34:1189-97.
[15] Hiratsuka A, Adachi H, Fujiura Y, et al. Strong association between serum hepatocyte growth factor and metabolic syndrome. J Clin Endocrinol Metab 2005;90:2927-31.
[16] Gerritsen J, TenVoorde BJ, Dekker JM, et al. Measures of cardiovascular autonomic dysfunction nervous function: agreement, reproducibility, and reference values in middle age and elderly subjects. Diabetologia 2003;46:330-8.
[17] Sakata K, Shirotani M, Yoshida H, et al. Cardiac sympathetic nervous system in early essential hypertension assessed by ^{123}I-MIBG. J Nucl Med 1999;40:6-11.
[18] Spallone V, Menzinger G. Diagnosis of cardiovascular autonomic neuropathy in diabetes. Diabetes 1997;46:S67-76.
[19] Watanabe K, Sekiya M, Tsuruoka T, et al. Relationship between insulin resistance and cardiac sympathetic nervous function in essential hypertension. J Hypertens 1999;17:1161-8.
[20] Tsubouchi H, Niitani Y, Hirono S. Levels of human hepatocyte growth factor in serum of patients with liver disease determined by an enzyme-linked immunosorbent assay. Hepatology 1991;13:1-5.
[21] Tanigawa N, Segawa Y, Maeda Y, et al. Serum hepatocyte growth factor/scatter factor levels in small cell lung cancer patients. Lung Cancer 1997;7:211-8.
[22] Mancia G, De Backer G, Dominiczak A, et al. 07 ESH-ESC practice guidelines for the management of arterial hypertension: ESH-ESC task force on the management of arterial hypertension. J Hypertens 2007; 25:1751-62.
[23] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-9.
[24] Sato T, Nishinaga M, Kawamoto A, et al. Accuracy of a continuous blood pressure monitor based on arterial tonometry. Hypertension 1993;21:866-74.
[25] Milliani A, Pagani M, Lombardi F, et al. Cardiovascular neural regulation explored in the frequency domain. Circulation 1991;84: 482-92.
[26] Bell LN, Ward JL, Degawa-Yamauchi M, et al. Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. Am J Physiol Endocrinol Metab 2006;291:E843-8.
[27] Hotamisligil GS, Arner P, Caro JF, et al. Increased adipose tissue expression of tumor necrosis factor–α in human obesity and insulin resistance. J Clin Invest 1995;95:2409-15.
[28] Katsuki A, Sumida Y, Murashima S, et al. Serum levels of tumor necrosis factor–α are increased in obese patients with noninsulin-dependent diabetes mellitus. Clin Endocrinol Metab 1998;83:859-62.
[29] Kinjyo Y, Kajiyama S, Fujiwara H, et al. Influence of the polyol pathway on transporter reduction in diabetic cardiac sympathetic nerve: implication for heterogeneous accumulation of MIBG. Eur J Nucl Med Mol Imaging 2005;32:438-42.
[30] Schofer J, Spielmann R, Schuchert A, et al. Iodine-123 metaiodobenzylguanidine scintigraphy a noninvasive method to demonstrate myocardial adrenergic nerve system disintegrity in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1988;12:1252-8.
[31] Imamura Y, Ando H, Mitsuoka W, et al. Iodine-123 metaiodobenzylguanidine images reflect intense myocardial adrenergic nervous activity in congestive heart failure independent of underlying cause. J Am Coll Cardiol 1995;26:1594-9.
[32] Nagamachi S, Fujita S, Nishii R, et al. Prognostic value of cardiac I-123 metaiodobenzylguanidine images in patients with non–insulin-dependent diabetes mellitus. J Nucl Cardiol 2006;13:34-42.
[33] Mantysaari M, Kuikka J, Mustonen J. Noninvasive detection of cardiac sympathetic nervous dysfunction in diabetic patients using [^{123}I] metaiodobenzylguanidine. Diabetes 1992;41:1069-75.
[34] Meigs J, Jacques PF, Selhub J, et al. Framingham Offspring Study. Fasting plasma homocysteine levels in the insulin resistance syndrome. The Framingham Offspring study. Diabetes Care 2001;24:1403-10.
[35] Ma H, Cadleron TM, Fallon JT, et al. Hepatocyte growth factor is a survival factor for endothelial cells and is expressed in human atherosclerotic plaques. Atherosclerosis 2002;164:79-87.
[36] Rehman J, Considine RV, Bovenkerk JE, et al. Obesity is associated with increased levels of circulating hepatocyte growth factor. J Am Coll Cardiol 2003;41:1408-13.
[37] Makondo K, Kimura K, Kitamura N. Hepatocyte growth factor activates endothelial nitric synthase by Ca^{2+}- and phosphoinositide 3-kinase/AKT-dependent phosphorylation in aortic endothelial cells. Biochem J 2003;374:63-9.
[38] Watanabe K, Sekiya M, Tsuruoka T, et al. Effect of insulin resistance on left ventricular hypertrophy and dysfunction in essential hypertension. J Hypertens 1999;17:1153-60.
[39] Yamashina S, Yamazaki J. Role of MIBG myocardial scintigraphy in the assessment of heart failure: the need to establish evidence. Eur J Nucl Med Mol Imaging 2004;31:1353-5.