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ORIGINAL ARTICLE

Diabetic retinopathy is associated with insulin resistance and cardiovascular autonomic dysfunction in type 2 diabetic patients

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Diabetic retinopathy (DR) and cardiovascular autonomic dysfunction are associated with high mortality in type 2 diabetic patients. This preliminary study was therefore designed to test the hypothesis that DR is associated with insulin resistance and cardiovascular autonomic dysfunction in type 2 diabetic patients without insulin treatment. Seventy persons were diagnosed to have type 2 diabetes in the examination from June 2004 to May 2006. The study group consisted of 29 type 2 diabetic patients with DR (age: 58 ± 6 years, mean ± s.d.) and 41 type 2 diabetic patients with no DR (NDR) (n=41, 58 ± 5 years). Cardiovascular autonomic function was assessed by baroreflex sensitivity (BRS), heart rate variability, plasma norepinephrine concentration and cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphic findings. DR patients had lower BRS, early and delayed ^{123}I-MIBG myocardial uptake values and higher percent washout rate (WR) of ^{123}I-MIBG than the NDR patients. With respect to metabolic findings, DR patients had higher fasting plasma insulin concentration (P<0.0001) and higher homeostasis model assessment (HOMA) index (P<0.00001) than the NDR patients. Multiple logistic regression analysis revealed that the presence of DR was independently predicted by HOMA index and the percent WR of ^{123}I-MIBG (P<0.01 and P<0.05, respectively). Our results suggest that DR is associated with depressed cardiovascular autonomic function and insulin resistance and that HOMA index and the percent WR of ^{123}I-MIBG are independently associated with DR in Japanese patients with type 2 diabetes mellitus.
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Keywords: baroreflex sensitivity; diabetic retinopathy; ^{123}I-metaiodobenzylguanidine scintigraphy; insulin resistance

INTRODUCTION

There is increasing evidence that micro- and macrovascular complications of diabetes share certain pathophysiological mechanisms. This may explain why microangiopathy has been associated with macroangiopathy and mortality.[1,2]

For example, diabetic retinopathy (DR) has been associated with increased cardiovascular and mortality risk from all causes, particularly in type 2 diabetes.[3,4]

Impaired autonomic nervous activity has been recognized as a crucial component of cardiac dysfunction and is strongly associated with harmful events and overall mortality in diabetic patients.[5,6] Recently, we have reported that depressed cardiovascular autonomic function is related to insulin resistance in type 2 diabetic patients.[7–9] Furthermore, DR is reported to be associated with insulin resistance in type 2 diabetic patients.[10,11] Although these results strongly suggest that DR, insulin resistance and autonomic dysfunction are related to each other, the significance of DR for diabetic cardiovascular autonomic function has not been adequately investigated.

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%.[12] 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.[13] Cardiac ^{123}I-MIBG scintigraphy is a sensitive method for detecting sympathetic dysfunction in many clinical disorders, including diabetes mellitus.[14,15]

We hypothesized that an increased severity of DR is associated with cardiovascular autonomic dysfunction and insulin resistance in type 2 diabetic patients. To test our hypothesis, we compared BRS, HRV, plasma norepinephrine concentrations and cardiac ^{123}I-MIBG scintigraphy in addition to the metabolic profiles in Japanese type 2 diabetic patients with and without DR, and independent predictors of the DR in these populations were evaluated.

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METHODS

Study population

We screened 155 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to our department between June 2004 and May 2006.

Among them, 70 patients (37 men and 33 women), with a mean s.d. age of 58 ± 6 years, fulfilled the inclusion criteria and were enrolled in this study. The inclusion criteria were as follows:

(1) Organic heart disease was not determined by treadmill exercise electrocardiography (ECG). We excluded subjects who have ST-T abnormal change by treadmill exercise ECG.
(2) An absence of causes of secondary hypertension (that is, primary aldosteronism, renal vascular hypertension, hyperthyroidism and pheochromocytoma).
(3) No past history of chronic diseases, such as renal failure (creatinine > 1.5 mg per 100 ml), pulmonary disease, liver dysfunction (aminotransferase > 50 IU l^{-1}), arteriosclerosis obliterans, sleep apnea syndrome and symptomatic cerebrovascular disease, was noted.
(4) The patient was not currently receiving treatment with insulin. (For this reason, insulin therapy patients were not measured by the homeostasis model assessment; HOMA index.)
(5) Female patients who were pregnant or treated with any postmenopausal hormone replacement therapy.

Of the 155 screened patients, 85 were excluded from further evaluation due to extenuating circumstances. Of those excluded, 35 patients were being treated with insulin, 7 patients had angina pectoris, 6 patients had renal failure and 6 patients had symptomatic cerebrovascular disease, 6 patients being treated with β-blockers.

Five patients had arteriosclerotic obliterans, five patients had sleep apnea syndrome, four patients being treated with antiarrhythmia drugs, four patients had secondary hypertension (two patients had primary adlosteronism, one patient had renal vascular hypertension and one patient had hyperthyroidism), three patients had liver dysfunction (one patient had hepatitis B and two patients had hepatitis C), two patients had lung cancer and two patients were treated with postmenopausal hormone replacement therapy. Therefore, only 70 patients were selected for the study.

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.

Assessment of DR

Ophthalmologic records, including ophthalmologic charts, fundus photography and fluorescein retinal angiography, were reviewed to evaluate patient's retina. According to a modification of the Diabetic Retinopathy Study and Early Treatment Diabetic Retinopathy Study grading scale, the severity in the worst affected eye was used, and the patients with retinopathy were grouped into three categories of retinopathy: (1) those with mild-to-moderate nonproliferative DR (NPDR) (only microaneurysms or microaneurysms plus one or more of the following: retinal hemorrhages, soft exudates, hard exudates, intraretinal microvascular abnormalities or venous beading), (2) those at a severe stage of NPDR (at least three of the following: extensive retinal hemorrhages or microaneurysms, soft exudates, intraretinal microvascular abnormalities and venous beading in two or more quadrants) and (3) those with proliferative DR (PDR) (the presence of new vessels, preretinal or vitreous hemorrhages, panretinal photocoagulation scars and a history of vitrectomy).[16,17]

Definition of hypertension

Hypertension was defined by performing BP measurement, registered as the average of three measurements obtained with a mercury-column sphygmomanometer after 10 min of physical resting by the patients. Essential hypertension was defined as diastolic blood pressure ≥ 90 mm Hg, systolic blood pressure ≥ 140 mm Hg or self-reported use of antihypertensive medication.[18]

Laboratory methods

Blood was taken at 0700 hours 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 HOMA index: (fasting plasma insulin [μU ml^{-1}] × fasting plasma glucose [mmol l^{-1}])/22.5.[19] Dyslipidemia was defined as fasting triglyceride levels ≥ 200 mg per 100 ml or a HDL-C concentration < 45 mg per 100 ml for women and < 35 mg per 100 ml for men.[20]

Echocardiography

M-mode and two-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 $1.04 \times ([\mathrm{LVIDd+IVSTd+PWTd}]^3 - \mathrm{LVIDd}^3) - 14$ 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 four-chamber view. Mitral inflow velocity was recorded with the sample volume at the mitral annulus level, taking the average from at least three 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.

Cardiovascular autonomic function tests

Autonomic function was assessed according to methods described in earlier studies.[9–11] During the tests, which were performed between 0900 and 1100 hours, 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 h) fast. For measurement of the plasma norepinephrine concentration, a blood sample was obtained from a catheter inserted in the right cubital vein 30 min before sampling. We measured plasma norepinephrine concentration according to the methods described in an earlier study.[7–9] Arterial blood pressure was recorded noninvasively through a tonometric sensor attached over the left radial artery (Jentow-7700; Nihon Colin, Komaki, Japan). The accuracy of continuous blood pressure monitoring has been demonstrated earlier.[21] Arterial blood pressure and a standard 12-lead ECG were monitored simultaneously; data were stored in a PCM data recorder (RD-200T; TEAC, Tokyo, Japan). Three-lead precordial Holter ECG recordings (model 459; Del Mar Avionics, Irvine, CA, USA) were also obtained throughout the procedure for analysis of HRV.

After an interval of 30 min to permit stabilization of the cardiovascular baroreflex mechanism, the patient was asked to breathe at a rate of 15 breaths per min using a metronome to stabilize the relationship between respiration and cardiovascular function. BRS was assessed by the phenylephrine method.[7–9] Briefly, phenylephrine (2–3 μg kg^{-1}) was injected for 15 s to obtain a 15- to 40-mm Hg rise in systolic blood pressure. BRS 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 two slopes was taken as the BRS value.

Heart rate variability was analyzed using Holter ECG recordings (Marquette Electronics Inc., Milwaukee, WI, USA), with methods described in an earlier study.[22] 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 two spectral bands: the normal-frequency (LF) component at 0.04–0.15 Hz and the high-frequency (HF) component at 0.15–0.40 Hz. On the basis of 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 min (early) and 4 h (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 percent WR of the tracer from the myocardium was determined over a 4-h period.

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. BMI was calculated as weight/(height^2) (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.

Statistical analysis

All data are classified into two groups, that is the normal and the DR, and are summarized as the means ± s.d. (Table 1). For each variable in Table 1, the two-sided test with a level of significance 0.05 was performed to test the difference between the two groups. The Student's *t*-test was used for continuous variables, and for categorical variables, the χ^2 test was carried out. Logistic regression analysis was used to assess the influence of explanatory variables on DR, where the explanatory variables were age, gender, BMI, duration of diabetes, hypertension, dyslipidemia, blood pressure, heart rate, T-cho, triglyceride, HDL-C, fasting plasma glucose (FPG), fasting immunoreactive isulin (F-IRI), HOMA index, hemoglobin A1c, uric acid, creatinine, ejection fraction, left ventricular mass index, *E*/*A* ratio, deceleration time, plasma norepinephrine, HF power, LF/HF, BRS, the percent WR of ^{123}I-MIBG and the *H*/*M* ratio at the early and delayed phases after^{123}I-MIBG administration, where gender, hypertension and dyslipidemia were dichotomized as 1 (presence) and 0 (absence) by cutoff values defined in the earlier section.

In the procedure of DR, positive was represented as 1 and negative as 0. To determine factors among all explanatory variables used, a backward elimination procedure was employed. In the procedure, the BMI, triglyceride, HDL-C, FPG, F-IRI, HOMA index, uric acid, BRS, the percent WR of ^{123}I-MIBG and the *H*/*M* ratio at the early and delayed phases after ^{123}I-MIBG administration were determined as significant factors influencing DR.

All the analyses were performed using a standard statistical package (JMP 6.0; SAS Institute, Cary, NC, USA).

Table 1 Fundus findings of type 2 diabetic patients

Fundus findings	*No. of patients (%)*
No retinopathy	41 (58.6)
Mild-to-moderate NPDR	14 (20.0)
Microaneurysms only	6
Microaneurysms, retinal hemorrhages and hard exudates	5
Microaneurysms, retinal hemorrhages and soft exudates	4
Severe stage of NPDR	9 (12.9)
Extensive microaneurysms, retinal hemorrhages and soft exudates	5
Extensive microaneurysms, retinal hemorrhages and soft exudates and intraretinal microvascular abnormalities	4
PDR	6 (8.6)
Neovascularization	2
Preretinal hemorrhage	1
Vitreous hemorrhage	1
Panretinal photocoagulation scar	1
History of vitrectomy	1
Total	70

Abbreviations: NPDR, nonproliferative diabetic retinopathy; PDR, proliferative retinopathy.

RESULTS

Fundus findings of the 70 diabetes patients are shown in Table 1. Forty one (58.6%) diabetic patients did not have retinopathy and 29 (41.4%) had DR. Among the 29 diabetic patients with retinopathy, 14 patients were grouped as having mild-to-moderate NPDR, 9 as having severe NPDR and 6 patients as having PDR.

As shown in Table 2, the mean ages of the DR and no DR (NDR) groups were similar, and there were no significant differences between the groups with respect to gender, duration of diabetes or administered medications. The BMI values, waist circumferences and waist-to-hip ratios were larger in the DR group than in the NDR group (P=0.0085, P=0.0011 and P=0.0113, respectively). Regarding glucose metabolism, fasting insulin concentrations and HOMA index were higher in the DR group than in the NDR group ($P<0.0001$ and $P<0.0001$, respectively).

Uric acid was higher in the DR group than in the NDR group (P=0.0257). However, there was no significant difference in hemoglobin A1c. With regard to lipid metabolism, serum triglyceride was higher and serum HDL-C was lower in the DR group than in the NDR group (P=0.0321 and P=0.0189, respectively), whereas serum total cholesterol showed no significant difference between the two groups. Regarding the renal function, there was no significant difference in the serum creatinine concentration. The hemodynamic data listed in Table 2 were obtained immediately before BRS assessment. The resting

Table 2 Clinical characteristics of studied patients

	NDR	*DR*	P-*value*
Age (years)	58 ± 7	58 ± 5	NS
Gender (men/women)	22/19	15/14	NS
Duration of diabetes (year)	6.3 ± 3.6	6.8 ± 3.3	NS
Hypertension (%)	63	66	NS
Dyslipidemia (%)	41	45	NS
Drug use (%)			
Sulfonylurea	41	45	NS
α-Glucosidase inhibitors	37	34	NS
Statin	32	34	NS
Aldose reductase inhibitor epalrestat	25	28	NS
Calcium channel antagonists	39	41	NS
ACE inhibitors	22	21	NS
Angiotensin receptor blocker	44	45	NS
Body mass index (kg/m^2)	24.9 ± 2.3	26.5 ± 2.6	0.0085
Waist circumferences (cm)	84.2 ± 7.3	90.9 ± 8.5	0.0011
Hip circumferences (cm)	96.2 ± 5.5	97.8 ± 7.9	NS
Waist-to-hip ratio	0.88 ± 0.10	0.93 ± 0.82	0.0113
Systolic blood pressure (mm Hg)	129 ± 17	132 ± 12	NS
Diastolic blood pressure (mm Hg)	76 ± 8	77 ± 7	NS
Heart rate (b.p.m.)	67 ± 6	68 ± 8	NS
Total cholesterol (mg per 100 ml)	201 ± 27	210 ± 33	NS
Triglyceride (mg per 100 ml)	125 ± 41	149 ± 50	0.0321
HDL cholesterol (mg per 100 ml)	48 ± 11	42 ± 9	0.0189
Fasting plasma glucose (mg per 100 ml)	142 ± 18	153 ± 22	0.0187
Fasting immunoreactive insulin (μU ml^{-1})	5.8 ± 1.1	8.1 ± 2.7	<0.0001
HOMA index	2.0 ± 0.4	3.1 ± 1.1	<0.0001
Hemoglobin A1c (%)	7.5 ± 1.3	7.8 ± 1.1	NS
Uric acid (mg per 100 ml)	5.7 ± 1.2	6.4 ± 1.0	0.0257
Creatinine (mg per 100 ml)	0.7 ± 0.2	0.8 ± 0.2	NS

Abbreviations: ACE, angiotensin-converting enzyme; DR, diabetic retinopathy; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; NDR, no diabetic retinopathy; NS, not significant.
Data are mean ± s.d.

heart rate, systolic and diastolic blood pressures were not significantly different between the two groups.

Table 3 presents a summary of echocardiographic findings. The left ventricular dimensions at end diastole and end systole, intraventricular septal and posterior wall thickness at end diastole, ejection fraction and left ventricular mass index were essentially similar in the two groups. With regard to the left ventricular diastolic function, the ratio of peak velocities of early to late ventricular filling (*E/A* ratio) was lower in the DR group than in the NDR group (P=0.0476). Deceleration time was longer in the DR group than in the NDR group (P=0.0485).

Figure 1 summarizes the results of the cardiovascular autonomic function tests. BRS was lower in the DR group than in the NDR group (DR group, 8.8 ± 4.0 ms per mm Hg *vs.* NDR group, 11.4 ± 3.1 ms per mm Hg; P=0.0028; Figure 1a). Plasma norepinephrine concentration was similar in both groups (DR group, 229 ± 80pg ml^{-1} *vs.* NDR group, 212 ± 90 pg ml^{-1}; P=0.5146; Figure 1b). Furthermore, analysis of HRV in the DR and NDR groups revealed that the HF power (3.5 ± 1.4 and 4.0 ± 1.6 ln-ms^2, respectively; P=0.1803) and the LF/HF ratios (1.3 ± 1.0 and 1.5 ± 1.1, respectively; P=0.3423; Figure 1c) were not significantly different between the two groups. Cardiac ^{123}I-MIBG scintigraphy disclosed that the *H/M* ratios at early and delayed phases in the DR group were significantly smaller than those in the NDR group (early phase: 2.05 ± 0.27 *vs.* 2.26 ± 0.22, respectively; P=0.0004; delayed phase: 1.91 ± 0.29 *vs.* 2.18 ± 0.21, respectively; P<0.0001; Figure 1d).

The percent WR of ^{123}I-MIBG was higher in the DR group than in the NDR group (45.1 ± 8.9 *vs.* 34.5 ± 9.6%, P<0.0001; Figure 1d).

In univariate logistic regression analysis, the DR was associated with triglyceride (odds ratio (OR) 1.01, 95% CI=1.00–1.03; P=0.0382), HDL-C (OR 0.94, 95% CI=0.90–0.99; P=0.0207), fasting plasma glucose (OR 1.03, 95% CI=1.00–1.06; P=0.0227), fasting plasma insulin (OR 1.87, 95% CI=1.32–2.65; P=0.0004), HOMA index (OR 6.05, 95% CI=2.28–16.1; P=0.0003), uric acid (OR 1.65, 95% CI=1.05–2.60; P=0.0310), BRS (OR 0.81, 95% CI=0.69–0.94; P=0.0055), *H/M* ratio at early phase (OR 0.63, 95% CI=0.42–0.86; P=0.0039), *H/M* ratio at delayed phase (OR 0.55, 95% CI=0.31–0.83; P=0.0008) and the percent WR of ^{123}I-MIBG (OR 1.13, 95% CI=1.06–1.20; P=0.0002) as the dependent lipid and glucose metabolic and cardiovascular autonomic function parameters in type 2 diabetic patients (Table 4).

Multivariate logistic analysis identified HOMA index (OR 4.36, 95% CI=1.53–12.5; P=0.0060) and the percent WR of ^{123}I-MIBG

Table 3 Echocardiographic findings

	NDR	*DR*	P-*value*
Ejection fraction (%)	70 ± 6	68 ± 5	NS
LVIDd (mm)	47 ± 3	49 ± 4	NS
LVIDs (mm)	30 ± 3	32 ± 4	NS
IVSTd (mm)	8.7 ± 1.1	9.0 ± 1.3	NS
PWTd (mm)	9.2 ± 1.2	9.5 ± 1.0	NS
LVMI (g m^{-2})	109 ± 19	116 ± 22	NS
E/A ratio	0.96 ± 0.18	0.87 ± 0.15	0.0476
Deceleration time (ms)	236 ± 26	249 ± 31	0.0485

Abbreviations: DR, diabetic retinopathy; *E/A*, the ratio of peak velocities of early to late ventricular filling; IVSTd, interventricular septal thickness at end diastole; LVIDd, left ventricular internal dimension at end diastole; LVIDs, left ventricular internal dimension at end systole; LVMI, left ventricular mass index; NDR, no diabetic retinopathy; NS, not significant; PWTd, posterior wall thickness at end diastole.
Data are mean ± s.d.

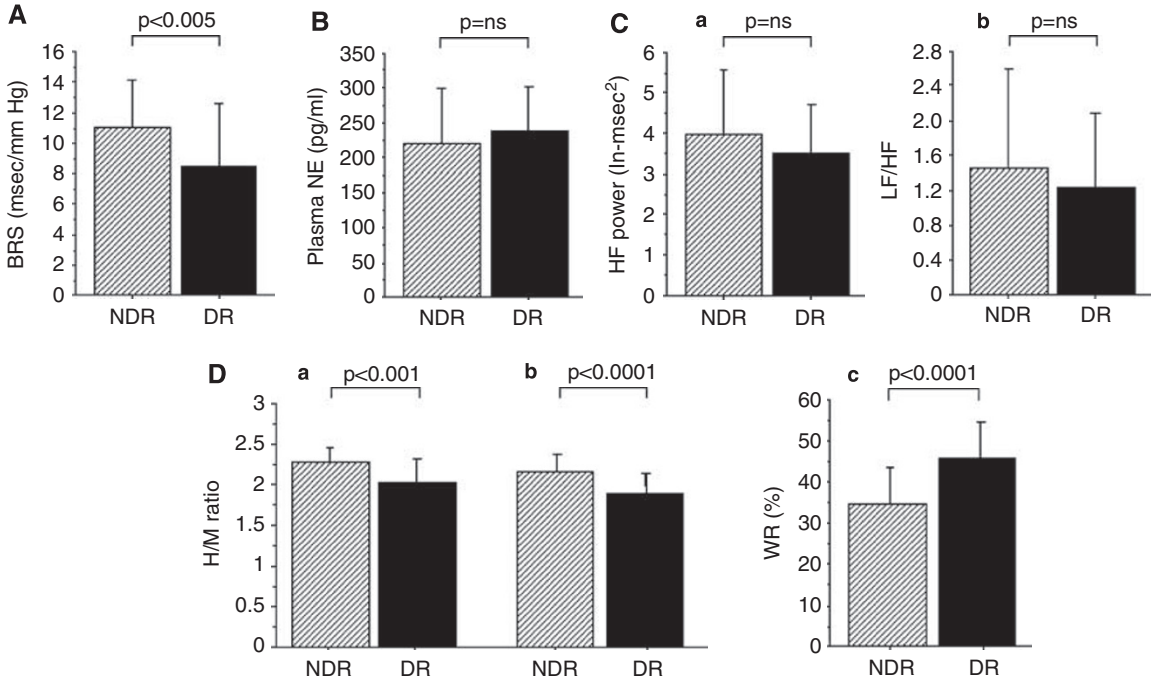

Figure 1 Comparison of autonomic function tests between type 2 diabetic patients with diabetic retinopathy (DR) and those with no diabetic retinopathy (NDR). (**A**) Baroreflex sensitivity (BRS). (**B**) Plasma norepinephrine (NE) concentration. (**C**) Heart rate variability (HRV). Power of high-frequency component (HF, 0.15–0.40 Hz, a) and the ratio of the low-frequency power (LF, 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-metaiodobenzylguanidine (MIBG) scintigraphic findings. Myocardial uptake of ^{123}I-MIBG at early (a) and delayed (b) phases. Myocardial uptake of ^{123}I-MIBG is expressed as the mean heart-to-mediastinum (*H/M*) ratio. (c) Percent washout rate (WR) of ^{123}I-MIBG. Data are mean ± s.d. NS=not significant.

Table 4 Univariate logistic regression analysis with diabetic retinopathy as the dependent variable in type 2 diabetes mellitus

	Diabetic retinopathy		
	Odds ratio	*95% CI*	P-*value*
Age	0.98	0.90–1.07	NS
Gender	2.15	0.63–7.36	NS
BMI	1.33	1.06–1.66	0.0128
Duration of diabetes	1.04	0.91–1.20	NS
Hypertension	0.85	0.36–2.59	NS
Dyslipidemia	1.13	0.53–2.65	NS
Systolic blood pressure	1.01	0.98–1.05	NS
Diastolic blood pressure	1.03	0.97–1.10	NS
Heart rate	1.02	0.95–1.09	NS
T-cholesterol	1.01	0.99–1.03	NS
Triglyceride	1.01	1.00–1.03	0.0382
HDL cholesterol	0.94	0.90–0.99	0.0207
Fasting plasma glucose	1.03	1.00–1.06	0.0227
F-IRI	1.87	1.32–2.65	0.0004
HOMA index	6.05	2.28–16.1	0.0003
Hemoglobin A1c	1.26	0.83–1.93	NS
Uric acid	1.65	1.05–2.60	0.0310
Creatinine	1.68	0.13–21.6	NS
Ejection fraction	0.96	0.86–1.08	NS
LVMI	1.02	0.99–1.05	NS
E/A ratio	0.79	0.61–1.08	NS
Deceleration time	1.03	0.99–1.05	NS
Plasma norepinephrine	1.00	0.98–1.01	NS
HF power	0.83	0.58–1.11	NS
LF/HF	0.78	0.51–1.26	NS
Baroreflex sensitivity	0.81	0.69–0.94	0.0055
H/M ratio at early phase	0.63	0.42–0.86	0.0039
H/M ratio at delay phase	0.55	0.31–0.83	0.0008
Wash out	1.13	1.06–1.20	0.0002

Abbreviations: BMI, body mass index; CI, confidence interval; *E/A*, the ratio of peak velocities of early to late ventricular filling; *H/M* ratio, heart-to-mediastinum ratio; HDL, high-density lipoprotein; HF, high-frequency; HOMA, homeostasis model assessment; LF/HF, ratio of LF to HF; LVMI, left ventricular mass index; NDR, no diabetic retinopathy; NS, not significant. Significant predictors of diabetic retinopathy were explored among three parameters: gender (female=0, men=1), hypertension (absent=0, present=1) and dyslipidemia (absent=0, present=1).

Table 5 Multivariate logistic regression analysis with WML as dependent variable in type 2 diabetes mellitus

	White matter lesions		
	Odds ratio	*95% CI*	P-*value*
HOMA index	4.36	1.53–12.5	0.0060
The percent WR of ^{123}I-MIBG	1.45	1.08–2.37	0.0270

Abbreviations: CI, confidence interval; HOMA, homeostasis model assessment; MIBG, metaiodobenzylguanidine; WR, washout rate.

(OR 1.45, 95% CI=1.08–2.37; P=0.0270) in type 2 diabetic patients as independent parameters for DR (Table 5).

DISCUSSION

In our present study, type 2 diabetic patients with DR group had lower BRS, and lower myocardial uptake and enhanced clearance of ^{123}I-MIBG, relative to the values in type 2 diabetic patients with NDR group. Among the metabolic profiles, the fasting plasma insulin concentration and the HOMA index were higher in patients with DR group than in those with NDR group. Multiple logistic analyses revealed that the HOMA index and the percent WR of ^{123}I-MIBG were independent risk factors for the presence of DR in type 2 diabetic patients.

This study revealed the novel and important findings that the type 2 diabetic patients in the DR group had the higher percent WR of ^{123}I-MIBG and stronger insulin resistance than the NDR group.

There is a report indicating that DR is associated with insulin resistance in type 1 diabetes[10] and type 2 diabetes mellitus.[11]

Hadjadj *et al.*[10] investigated the association between DR and insulin resistance score using a WHO recommendation[23] (that is, hypertension, personal history of lipid disorders, personal history of type 2 diabetes and obesity were considered). They found a significant association of DR with insulin resistance in type 1 diabetic patients. Parvanova *et al.*[11] have reported that proliferative retinopathy is associated with insulin resistance using a hyperinsulinemic-euglycemic clamp in 115 patients with type 2 diabetes mellitus. In this study, the DR was associated with BMI, triglyceride levels, HDL-C levels, fasting plasma glucose, fasting plasma insulin concentration and the HOMA index values.

The mechanism by which DR increases insulin resistance remains to be elucidated. In our opinion, there are a couple of possible explanations for this observation. First, defective fibrinolysis caused by excess plasminogen activator inhibitor-1 activity and selective inhibition of some antiatherogenic effects of insulin may promote the occlusion of retinal capillaries and secondary ischemia-induced neovascularization.[24] Second, the ischemic damage can be further amplified by insulin resistance that has been related to a lower ability of insulin to induce vasodilatation through impaired nitric oxide endothelial production or accelerated inactivation.[25]

The relationship between DR and cardiovascular autonomic function has been examined in earlier studies using HRV analysis.[26,27] Schmidt *et al.*[26] have shown that DR is associated with heart rate response by electrocardiographical monitoring in type 2 diabetic patients. Duvnjak *et al.*[27] reported that DR is related to sympathetic and parasympathetic reactivity by HRV. In this study, HRV and the plasma norepinephrine concentrations were not different between the two groups. However, a significant difference was seen in ^{123}I-MIBG parameters. These results may suggest association of DR in diabetic patients with impairment of uptake-1 (norepinephrine transporter) as well as the acceleration of norepinephrine turnover in sympathetic nerve terminal. Impairment in uptake-1 in diabetic rats has been reported.[28] Our and the earlier studies by others[7–9,29] demonstrated that ^{123}I-MIBG scintigraphy is a fairly sensitive method for detecting cardiac sympathetic dysfunction in diabetic patients. The present results support potential of ^{123}I-MIBG scintigraphy in the diagnosis of cardiovascular autonomic dysfunction.

Although the precise mechanisms underlying the interactions between DR and impaired autonomic function remain unclear, there are several explanations regarding the mechanisms. First, DR may affect autonomic function through endothelial dysfunction and impairment of the nitrate oxide system. DR is associated with endothelial dysfunction by oxidant stress.[30] Endothelial dysfunction is associated with cardiac autonomic dysfunction and increased HRV.[31,32] Moreover, we have recently reported that the HOMA index and the myocardial uptake of ^{123}I-MIBG at the delayed phase were independent predictors of adiponectin concentration.[7] In fact, in a recent report demonstrating the association between DR, insulin resistance and adiponectin, the authors stressed the central role of endothelial dysfunction.[33] Second, DR may cause dysregulation of autonomic nervous system through an enhanced release of free fatty

acid. Insulin resistance is strongly associated with DR and free fatty acids.[34]

Taken together, it is possible that DR, insulin resistance and autonomic dysfunction interact and reinforce each other through mechanisms associated with endothelial dysfunction.

Compared with the NDR group, patients in the DR group showed cardiac diastolic dysfunction. Consistent with these results, the earlier study demonstrated that cardiac diastolic dysfunction was associated with cardiovascular autonomic dysfunction and insulin resistance.[20] Annonu *et al*.[35] reported that an increased DR was associated with cardiac left ventricular diastolic dysfunction in diabetic patients. Although the precise mechanism is unclear, diastolic dysfunction may interact with cardiac sympathetic nervous function through insulin resistance.

There are several limitations to this study. First, subjects in this study population had essential hypertension, which was treated with one or more antihypertensive drugs. These characteristics of the patients' backgrounds have been reported to affect insulin resistance[36,37] and sympathetic nerve function.[38,39] Nonetheless, the results indicate that DR in diabetic patients is related to cardiac depression and insulin resistance. Second, there is a potential link of DR with an enhanced release of free fatty acid.[40] It remains uncertain whether free fatty acid status affected the risk of DR in type 2 diabetes patients, because we did not examine the free fatty acid concentration in this investigation. Third, an aldose reductase inhibitor, epalrestat, has been proven to improve peripheral sensory nerve impairments in diabetes mellitus.[41] Further clinical investigators are needed to determine the relationship between aldose reductase inhibitor, epalrestat, cardiac autonomic function and DR in type 2 diabetes patients. Fourth, we have reported earlier that visceral fat accumulation and hyperhomocysteine are associated with insulin resistance as well as cardiac sympathetic nerve function assessed by ^{123}I-MIBG.[8,9] Therefore, further studies are required to evaluate the association among the DR, visceral fat accumulation, hyperhomocysteine, HOMA index values and ^{123}I-MIBG parameters. In addition, the prognostic implications of cardiac autonomic function as assessed by ^{123}I-MIBG scintigraphy remain to be addressed, although there are prognostic studies of heart failure patients using cardiac ^{123}I-MIBG imaging.[42] Finally, compared with the mild-to-moderate to severe NPDR group, patients with PDR group showed impaired metabolic/cardiac parameters. The HOMA index values and ^{123}I-MIBG parameters were higher in the PDR group than in the mild-to-moderate to severe NPDR group (data not shown). It would be necessary for further studies to examine the independent factor between PDR and NPDR groups according to the impaired metabolic/cardiac parameters.

In conclusion, our findings suggest that DR in patients with type 2 diabetes is associated with both cardiovascular autonomic function assessed by the percent WR of ^{123}I-MIBG and insulin resistance. In the future, large cohort studies including other populations may be beneficial.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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