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Plasma Big-Endothelin Levels, Cardiac Autonomic Neuropathy, and Cardiac Functions in Patients with Insulin-Dependent Diabetes Mellitus

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Summary

Background: The alteration of endothelin (ET) levels in diabetic patients with cardiac autonomic neuropathy (CAN) has not been studied extensively and its correlation with cardiac function parameters has not been discussed.

Hypothesis: The aim of the present study was to discuss the correlation between the degree of cardiac autonomic neuropathy, plasma big-ET levels, and cardiac functions in diabetic patients who were clinically free of cardiovascular disease.

Methods: Twenty subjects (32.1 ± 7.8 years, 11 men, 9 women) with insulin-dependent diabetes mellitus (IDDM) were studied to evaluate the relationship between circulating big-endothelin (big-ET1) levels, CAN, and cardiac functions. The severity of CAN was scored according to Ewing's criteria. Cardiac functions were assessed using Doppler echocardiography.

Results: Left ventricular systolic function in the patient group was within normal limits and comparable with the values of the control group (n = 10). The mean E/A values of diabetics with CAN (1.15 ± 0.33, p = 0.004) and without CAN (1.34 ± 0.17) were significantly lower than those of controls (1.57 ± 0.27). Diabetics with CAN had significantly higher big-ET1 values (81.1 ± 94 pg/ml) compared with others (12.4 ± 5.9 and 21.1 ± 17.7 pg/ml, p = 0.04). Circulating big-ET1 levels showed a significant correlation with E/A values in the control group (p = 0.01, r = −0.7) and with peak A values (p = 0.003, r = 0.64) in diabetics. The CAN score correlated negatively with E/A values (p = 0.01, r = 0.54).

Conclusions: High big-ET levels might have an important role in the pathogenesis or consequences of diastolic dysfunction in diabetics with CAN. Their role in cardiac autonomic neuropathy and diastolic dysfunction should be investigated further.

Key words: diabetes mellitus, endothelin, cardiac autonomic neuropathy, cardiac functions

Introduction

Metabolic abnormalities, microangiopathic changes, fibrosis of myocardium, and cardiac autonomic neuropathy (CAN) influence the development and progression of diabetic cardiomyopathy. In addition, hypertension and ischemic heart disease may aggravate various disturbances in left ventricular function. Ventricular diastolic dysfunction is known to exist before clinical symptoms and to precede systolic function impairment. Noninvasive imaging methods, such as Doppler echocardiography and radionuclide ventriculography, allow for quantification of diastolic as well as systolic function.[1,2]

Endothelin (ET), a recently described peptide, is a potent vasoconstrictor substance secreted by endothelial cells.[3] Its elevated levels in diabetes mellitus have been suggested to contribute to the development of vascular complications.[4–7] In addition to its vasoconstrictive effect, its inotropic and chronotropic effects were shown experimentally in guinea pig atrial strips.[8] High-affinity endothelin receptors were identified in mammalian atria and ventricles.[9–11]

The alteration of ET levels in diabetic patients with CAN has not been studied extensively and its correlation with cardiac function parameters has not been discussed. Therefore, the aim of the present study was to discuss the correlation between the degree of CAN, plasma ET levels, and cardiac functions in diabetic patients who were clinically free of cardiovascular disease.

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Materials and Methods

Twenty subjects (11 men, 9 women, mean age = 32.1 ± 7.8 years) with insulin-dependent diabetes mellitus (IDDM) were studied. They had no previous history of coronary artery disease, known cardiomyopathy, or any illness and/or medication affecting cardiac function. All patients were on insulin therapy. They had normal electrocardiograms (ECG) and all were normotensive. Each patient's blood pressure levels were measured in supine position after 5 min rest from the right arm. Hypertension was defined by blood pressure levels > 140 mmHg for systolic and > 90 mmHg for diastolic value. Body mass index (BMI) was calculated as weight(kg)/height(m^2). Retinopathy was graded as none, background, or proliferative on the basis of an ophthalmologic assessment. The diagnosis of peripheral neuropathy was made when two or more of the following four evaluations were abnormal: symptoms, conduction studies showing abnormality in at least two different limb nerves, ankles reflexes, and vibration perception threshold. Nephropathy was assessed by the determination of urine microalbumin levels. Microalbuminuria was defined by the presence of persistent urinary albumin excretion between 30 and 300 mg/day in sterile urine.

Six patients had retinopathy (four background and two proliferative) and seven had peripheral neuropathy. Four patients had both retinopathy and peripheral neuropathy. Their microalbuminuria levels were within normal limits, except in two patients (mean = 25.5 ± 48.3, range = 1.3–194 mg/day). Ten healthy volunteers from medical staff were chosen to comprise the control group. They were age- and gender-matched and normotensive. They were not taking any drug. Both patients and volunteers gave their informed consent to take part in the study in accordance with the Helsinki II declaration.

Testing for Cardiac Autonomic Neuropathy

Diabetics were subdivided into two groups: Group 1: Twelve diabetic patients with CAN confirmed by their cardiovascular response to a series of autonomic tools (Valsalva maneuver, deep breathing, change of position from lying to standing, sustained hand-grip, fall in blood pressure on standing), recommended and scored by Ewing *et al.*,[12] and Group 2: eight patients without CAN. Of the 12 patients with CAN, 4 had retinopathy and 5 had peripheral neuropathy.

Cardiac Functions

Cardiac functions of the subjects were assessed by Doppler echocardiography (DE), using a Toshiba Sonolayer SSH 60 system (Toshiba, Nasu, Japan) combining pulsed and continuous waves. A 2.5 MHz duplex transducer was used which allowed both two-dimensional (2-D) echocardiographic and Doppler echocardiographic views. The patients were examined in the supine or left lateral decubitis positions. The transducer was positioned at the parasternal long axis, parasternal short axis, and apical four-chamber windows. Valve functions were evaluated first by M-mode and then by 2-D echocardiography. Left ventricular end-systolic and end-diastolic dimensions and intraventricular septal and posterior wall thicknesses were measured as recommended by the American Society of Echocardiography.[13] In addition, end-diastolic and end-systolic volumes, stroke volume, and ejection fraction values were calculated. Later, the sample volume of pulsed DE was placed between the tips of the mitral leaflets where the maximal flow velocity in early diastole was obtained. With the guidance of audio signals, attempts were made to obtain the best mitral flow velocity spectrum. The following measurements and calculations were obtained: peak velocity of the early diastolic filling wave (E), peak velocity of the atrial filling wave (A), and the ratio of E/A.

Plasma Big-Endothelin Levels

The subjects fasted for one night before sampling. Peripheral venous blood for the determination of big-ET was taken in the morning and on the same day as echocardiographic examination. Before the sampling, subjects rested for 20 min in supine position. Circulating plasma big-ET levels were determined using the radioimmunoassay (RIA) method. Plasma samples were collected in tubes containing 7.5 mM/ml ethylene diamine tetraacetic acid (EDTA) and 500 KIU/ml aprotinin, placed on ice, and promptly centrifuged at 3000 rpm for 15 min at 4°C. Plasma was then frozen and stored at −40°C until the assay was performed. Determinations were taken within 1 month after the sampling. The RIA was carried out using the big ET-1 {125 I} assay system (Peninsula Laboratories, Inc. San Carlos, Calif., USA). Big-ET was extracted from plasma using Sep-column and dried under nitrogen. Then, it was reconstituted in 200 µl assay buffer, and 100 µl of the sample was taken for the analysis. For the RIA procedure, standard synthetic ET dilutions ranging from 0.5 to 128 were prepared. Standard solutions (100 µl) and plasma extracts were pipetted into appropriate tubes and incubated for 24 h at + 4°C after addition of 100 µl of rabbit anti-ET serum to equal amounts of plasma. Then, 100 µl of 125 I-ET-1 was pipetted into all tubes and a second incubation for 24 h at + 4°C was made. After addition of 100 µl of goat anti-rabbit serum and 10 min incubation at room temperature, antibody-bound fraction was separated by magnetic separator in 15 min. The radioactivity present in each tube was determined by counting for 60 s in a gamma scintillation counter. The sensitivity of the assay is about 10 pg/tube. Plasma Big ET-1 levels were expressed as pg/ml. The cross reactivity with human ET-1, ET-2, and ET-3 was lower than 0.001%.

Microalbuminuria levels were also determined by RIA. Glycemic control of the patients was assessed by glycosylated hemoglobin (HbA1) measurements. Its levels were estimated using a quantitative colometric determination (Stanbio, San Antonio, Texas, USA). The normal limits were between 6 and 8%.

Statistical Analysis

All results are given as mean ± standard deviation. The Mann-Whitney-U test and analysis of variance (ANOVA)

were used to compare the results between two groups and controls. Sperman's rank test was used for measuring correlations. A p value < 0.05 was regarded as significant.

Results

The mean values of clinical parameters of diabetic patients and the control group are given in Table I. The mean cholesterol level in Group 1 was significantly higher than that in Group 2 (p = 0.04). The difference between the mean fasting blood glucose level in the groups was significant (p = 0.0008). Other parameters were comparable. Sixteen patients had poor metabolic control. Diabetics with CAN had higher big ET-1 levels, whereas diabetics without CAN had slightly lower big ET-1 levels than the control group (p = 0.04).

Plasma big ET-1 levels had a significant correlation with HbA1c (p = 0.03, r = 0.51), cholesterol (p = 0.01, r = 0.54), triglycerides (p = 0.01, r = 0.59), and very low-density lipoprotein (VLDL) (p = 0.03, r = 0.51) levels. Ejection fraction values did not differ between diabetics and control subjects (Table II). Cardiac hypertrophy or ventricular dilation was not observed. When comparing the echocardiographic parameters, there was no significant difference between the groups, except for peak A, and E/A ratio. The peak A values in Group 1 were significantly higher than those in the control group (p = 0.04). The mean E/A ratio in Group 1 was significantly lower than that in the control group (p = 0.004), indicating the more pronounced diastolic dysfunction in patients with CAN in the presence of normal systolic function.

Endothelin values had a significant correlation with E/A values in the control group (p = 0.01, r = −0.7) and with peak A values (p = 0.003, r = 0.64) in patients. In addition, in patients ET levels correlated with deceleration time (p = 0.008, r = 0.58). The CAN score correlated negatively with E/A values (p = 0.01, r = 0.54). However, no significant relationship between CAN score and ET levels was observed.

Discussion

In recent years, Doppler echocardiography has emerged as a useful tool for assessing diastolic cardiac function in both healthy persons and patients.[1] Left ventricular diastolic dysfunction is now recognized as a significant cause of cardiac symptoms even in patients with apparently normal systolic ventricular function. An increase in A value and decrease in E/A ratio has been accepted as a sign of diastolic dysfunction. In our study group, which consisted of diabetic subjects with normal systolic functions, the mean A value was significantly higher, especially in the diabetic subjects with CAN, than in the controls. In addition, the E/A values in the patients and in the subgroup with CAN were significantly lower. Both results indicate the presence of the diastolic dysfunction in diabetic patients with CAN, as reported previously by several authors.[14, 15]

Endothelial dysfunction in patients with diabetes mellitus is known to cause decreased synthesis of endothelium-dependent vasodilators and increased synthesis and release of vasoconstrictors, such as ET. It has been accepted to precede the

TABLE I The mean values of the clinical parameters in diabetic patients and in the control group

Parameter	All patients (n = 20)	Group 1 (n = 12)	Group 2 (n = 8)	Control (n = 10)
Age (years)	32.1 ± 7.8	32.2 ± 7.3	30.5 ± 8.7	31.5 ± 8.5
Duration of disease (years)	11.8 ± 5.5	12.5 ± 5.6	10.8 ± 5.6	—
BMI (kg/m^2)	22.5 ± 2.6	23.1 ± 2.6	21.6 ± 2.4	23.5 ± 3.2
SBP (mmHg)	122.7 ± 17	125.4 ± 14.3	118.7 ± 2.4	119.5 ± 12.7
DBP (mmHg)	70 ± 10	73.3 ± 10.7	65 ± 7.5	77.2 ± 6.4
FBG (mg/dl)	208.2 ± 99[a]	237 ± 98[a]	168.6 ± 92.7	89.2 ± 12.8
HbA1 (%)	9.4 ± 1.7	9.7 ± 2.06	8.8 ± 1.1	—
CAN score	3.4 ± 2.06	4.66 ± 1.61	1.5 ± 0.75	—
BUN (mg/dl)	13.7 ± 3.3	14.2 ± 3.4	13 ± 3.2	13.2 ± 2.4
Cr (mg/dl)	1 ± 0.3	0.91 ± 0.14	1.125 ± 0.44	0.99 ± 0.22
Cholesterol (mg/dl)	164.1 ± 34.8	177.4 ± 36.7[b]	141.3 ± 14	175.3 ± 44
Triglycerides (mg/dl)	77.7 ± 70	90.7 ± 81.8	51.8 ± 26	103.4 ± 40
HDL (mg/dl)	48.8 ± 17.7	51 ± 19	44.5 ± 15	45.6 ± 16.5
LDL (mg/dl)	99.8 ± 31.8	108.3 ± 34	83.1 ± 18.6	108.8 ± 30
VLDL (mg/dl)	17 ± 14	18 ± 16	14.8 ± 11	19.9 ± 8
Big-endothelin (pg/ml)	55.8 ± 81.3	81.1 ± 94[b]	12.4 ± 5.9	21.2 ± 17.7
Range	(1.25–320)	(1.25–320)	(4.75–20.3)	(7.25–62.5)

[a] $p<0.05$ compared with control group.

[b] $p<0.05$ compared with Group 2.

Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, FBG= fasting blood glucose, HbA1 = glycosylated hemoglobin, CAN = cardiac autonomic neuropathy, BUN = blood urea nitrogen, Cr = creatine, HDL = high-density lipoprotein, LDL = low-density lopoprotein, VLDL = very low-density lipoprotein.

TABLE II The mean values of the echocardiographic parameters

Parameter	All patients (n = 20)	Group 1 (n = 12)	Group 2 (n = 8)	Control (n = 10)
Heart rate (beats/min)	78.2 ± 6.3	80.4 ± 6.3	75 ± 5.2	76 ± 4
M-mode echocardiography				
EF(%)	68.6 ± 6.6	69.2 ± 7.7	67.6 ± 5	72.9 ± 4.7
FS (%)	38.7 ± 4.5	39.6 ± 4.8	37.5 ± 4.1	41.6 ± 3.55
IVS (cm)	0.81 ± 0.17	0.81 ± 0.19	0.81 ± 0.15	0.82 ± 0.1
LVIDd (cm)	4.6 ± 0.35	4.6 ± 0.3	4.7 ± 0.4	4.5 ± 0.3
LVIDs /cm)	2.86 ± 0.37	2.8 ± 0.4	2.9 ± 0.3	2.6 ± 0.2
LVPW (cm)	0.78 ± 0.09	0.77 ± 0.08	0.81 ± 0.12	0.81 ± 0.14
EDV (cm^3)	99.5 ± 17.8	97.5 ± 16.3	102.5 ± 20.6	95.2 ± 16.5
ESV /cm^3)	31.5 ± 9.2	29.5 ± 8.6	33.6 ± 10	25.7 ± 5.5
SV (cm^3)	67.4 ± 11	68 ± 11.4	68.8 ± 12.8	69.4 ± 13.7
Deceleration time (s)	140.3 ± 64	144.6 ± 80	133.8 ± 32.7	105.8 ± 51
Acceleration time (s)	78.4 ± 19	80.9 ± 13.1	74.6 ± 27.9	85 ± 11
Total diastolic time (s)	419.9 ± 99	398.5 ± 102	452 ± 92	409.2 ± 117
Doppler echocardiography				
Peak A (cm/s)	56.4 ± 14[a]	59.3 ± 15.8[a]	52 ± 11	46.2 ± 5.8
Peak E (cm/s)	66.7 ± 12	65.3 ± 13.7[a]	68.8 ± 11	69.4 ± 13.7
E/A	1.22 ± 0.22[a]	1.15 ± 0.33[a]	1.34 ± 0.17	1.57 ± 0.27

[a] $p < 0.05$ compared with control.

Abbreviations: EF = ejection fraction, FS = fractional shortening, IVS = interventricular septum, LVIDd = left ventricular interdimension-diastolic, LVIDs = left ventricular interdimension-systolic, LVPW = left ventricular posterior wall, EDV = end-diastolic volume, ESV = end-systolic volume, SV = stroke volume.

vascular complications in diabetics. Elevated values of ET in DM with or without complications have been published.[4] Although some studies report normal ET levels in diabetic patients with or without complications,[5] more pronounced elevation of these levels has also been documented in association with diabetic complications.[6,7] In those reports, the presence of microangiopathic complications, such as retinopathy and nephropathy, correlated with high ET levels. In this study, we observed the high plasma levels of big-ET1 in diabetic subjects with CAN when compared with the control group. It is interesting that diabetics without CAN had slightly lower big-ET1 values than the control group. A similar finding was also reported by Smulders *et al.* for male diabetics.[16] They reported that ET levels were lower in men with uncomplicated diabetes than in male control subjects and concluded that ET concentrations might be influenced by gender. Their results were in agreement with the results of Polderman *et al.*[17] Tsunoda *et al.*[18] reported a decrease in the conversion of big-ET-1 to ET-1 in patients with DM and concluded that the activity of a putative ET-converting enzyme may be suppressed, resulting in increase in the plasma concentration of big-ET-1, but not ET-1. In this study, we determined the plasma big-ET-1 levels; therefore its elevated levels may be due to decrease conversion to ET-1. Another reason may be the large range of big-endothelin in the control group. The highest value (62.5 pg/ml) was excluded; the mean and standard deviation (SD) value of big-endothelin for the control group will be 16.1 ± 9.3, which is comparable with diabetics without CAN.

Plasma ET levels in diabetic subjects may also be influenced by insulin.[19] In addition to insulin, high glucose levels have also been known to alter ET levels.[20] In our study group, all patients were receiving insulin and 16 patients had poor metabolic control. The plasma ET levels were correlated with HbA1 levels, significantly supporting the elevating effect of glucose levels on ET concentrations.

Endothelin-A receptors are found on myocardial and vascular smooth muscle cells, and ET-B receptors on myocytes and vascular endothelial cells. Endothelin receptors on myocardial cells are regulated by different stimuli, as they are downregulated in response to increased plasma concentration of endothelin. Endothelins induce a positive inotropic effect on cardiac myocytes either by increasing cytosolic calcium or increasing contractile amplitude.[21] However, the circulating levels of ET, reported in disease states, seem unlikely to influence coronary perfusion and ventricular function, because more higher concentrations of ET are needed to modulate the cardiac functions. Endothelin operates as a local paracrine hormone more than through the systemic circulation.

In this study, we observed a significant negative correlation between plasma ET levels and E/A values in controls. In diabetics, a positive correlation was found with peak A values. The subjects with better diastolic function had lower ET values. In addition, ET levels of patients also correlated with deceleration time. The patients with longer deceleration time, indicating diastolic dysfunction, had higher ET levels. Although diastolic dysfunction parameters were related to high

ET levels, systolic function parameters were found to have no correlation with ET levels.

According to the results of this study, diabetic patients with CAN had more pronounced diastolic function impairment than diabetic patients without CAN. Those patients had elevated levels of circulating big-ET-1. A significant correlation between left ventricular diastolic dysfunction and ET levels was observed in the presence of normal left ventricular systolic function.

Conclusion

High ET levels might play an important role in the pathogenesis or consequences of diastolic dysfunction in diabetics with cardiac autonomic neuropathy. Its role in cardiac autonomic neuropathy and diastolic dysfunction should be investigated further.

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