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The Relationship Between Flow-Mediated Dilatation and Left Ventricular Function in Type 2 Diabetic Patients with Microalbuminuria

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Objective: **The aim of this study was to assess the relationship between flow-mediated dilatation (FMD) and left ventricular (LV) systolic and diastolic function in type 2 diabetic patients with or without microalbuminuria.**

Research Design and Methods: **We prospectively evaluated 68 consecutive patients (36 women, 32 men; mean age 57 ± 11 yr) with type 2 diabetes mellitus (DM). Patients were divided into two groups according to whether or not they had microalbuminuria: group 1 (*n* = 29, mean age 58 ± 10 yr) with microalbuminuria and group 2 (*n* = 39, mean age 56 ± 10 yr) without microalbuminuria. LV function was assessed by classical methods and Doppler tissue imaging (DTI). Left ventricular ejection fraction (EF), interventricular (IVS) and posterior wall (PW) thickness, peak early (E) and late (A) transmitral filling velocities, their ratio (E/A) and deceleration time of the mitral E wave (DT), LV isovolumetric relaxation time (IVRT), flow propagation of velocity (Vp), and E/Vp were evaluated by conventional echocardiography. Early diastolic (Em), late diastolic (Am), and peak systolic (Sm) mitral annular velocities were measured. Em/Am and the ratio of early diastolic mitral inflow velocity to Em (E/Em), which is a reasonably good index for predicting elevated LV filling pressure, were calculated by DTI. Endothelial function, measured as flow-mediated dilatation of the brachial artery using ultrasound, was calculated in two groups.**

Results: **FMD was lower in those with microalbuminuria than those without (8.8 ± 6.44% vs 12.6 ± 7.24%, *p* = 0.03). Group 1 had longer DT (223 ± 39 ms vs 199 ± 37 ms, *p* = 0.01) and longer IVRT (109 ± 13 ms vs 100 ± 13 ms, *p* = 0.03) than that of group 2 with conventional echocardiography. Group 1 had significantly lower Em/Am (0.79 ± 0.27 cm/s vs 1.02 ± 0.44 cm/s, *p* = 0.01), lower Vp (40.4 ± 9.98 vs 50.4 ± 19.01 cm/s, *p* = 0.01) than that of group 2. Group 1 had significantly higher serum creatinine (1 ± 0.33 mg/dL vs 0.7 ± 0.19, *p* = 0.001). In logistic regression analysis, FMD was the only variable independently related to microalbuminuria. FMD was positively correlated with EF (*r* = 0.43, *p* = 0.02) and E/A (*r* = 0.40, *p* = 0.03), and negatively correlated with E/Em (*r* = 0.41, *p* = 0.04) and E/Vp (*r* = 0.41, *p* = 0.04) only in patients with microalbuminuria.**

Conclusion: **It was found that left ventricular diastolic function and FMD are impaired in type 2 diabetic patients with microalbuminuria. FMD may be related to LV diastolic dysfunction only in patients with microalbuminuria.**

Key Words: Type 2 diabetes mellitus; microalbuminuria; left ventricular function; flow-mediated dilatation.

Introduction

Microalbuminuria predicts cardiovascular (CV) morbidity and mortality in patients with diabetes mellitus (DM) independent of conventional cardiovascular risk factors, including age, hypercholesterolemia, and hypertension *(1–3)*. The mechanism of the association of microalbuminuria with cardiac events is not entirely understood. It is possible that increased vascular permeability, accumulation of lipids, and thrombus formation lead to renal dysfunction, and thus contribute to CV disease *(4,5)*.

Endothelial dysfunction is an early phase of atherosclerosis, and is associated with abnormal nitric oxide physiology *(6)*. Endothelial dysfunction can be measured noninvasively using high-resolution ultrasonography to measure postischemic flow-mediated dilatation (FMD) of conduit arteries *(7)*. Impaired FMD is an early marker of atherosclerotic degeneration and has been shown to be correlated with coronary endothelial dysfunction *(8)*.

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Studies on cardiac dysfunction in DM deal largely with type 1 DM, although reports on diastolic dysfunction in type 2 DM are also available *(9,10)*. Early determination of myocardial manifestations of DM is important, because myocardial involvement considerably influences the prognosis of diabetic patients.

Left ventricular diastolic dysfunction has been described as an early sign of diabetic heart muscle disease preceding the systolic damage as assessed by echocardiography *(11–13)*. Several conventional echocardiographic variables are used to assess left ventricular (LV) systolic and diastolic function, including ejection fraction (EF), peak E and late A transmitral filling velocities, their ratio (E/A), deceleration time of the mitral E wave (DT), and isovolumetric relaxation time (IVRT). Doppler tissue imaging (DTI) records the myocardial wall motion velocities during cardiac cycle *(14,15)*. Mitral annulus velocity determined by DTI is a relatively preload-independent variable and superior to conventional mitral Doppler indexes *(16)*. Early diastolic mitral annular velocity (Em), late diastolic mitral annular velocity (Am), and their ratio (Em/Am) are the commonly used variables to evaluate LV diastolic function. Peak systolic mitral annular velocity (Sm) is used to evaluate LV systolic function. The ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/Em) is relatively simple to obtain and conceptually has the potential for providing a reasonable estimate of filling pressures throughout a wide range of relaxation abnormalities *(17,18)*. In addition, color M-mod flow propogation velocity (Vp) is a new Doppler index of LV relaxation, which has been suggested to be less dependent on preload *(19,20)*.

To our knowledge, the relationship of FMD with LV function assessed by DTI has not been previously evaluated in type 2 diabetic patients with or without microalbuminuria. The aim of this study was to determine the correlation with FMD and LV function in patients with microalbuminuria, and also to evaluate FMD and the utility of conventional and DTI-derived parameters in the assessment of LV function in type 2 diabetic patients with or without microalbuminuria.

Results

The beseline characteristics of the patients are listed in Table 1. There were no significant differences between group 1 and group 2 regarding age, gender, hypertension, smoking, hyperlipidemia, therapies, body mass index, and hemoglobin A1c. The blood pressure during the test in both groups was similar (blood pressure was 130/80 mmHg in group 1 and 132/81 mmHg in group 2, $p > 0.05$). The levels of serum creatinine in patients with microalbuminuria were higher than those in patients with no albuminuria. The amounts of secreted albumin into urine were 185 ± 17 mg/d in patients with microalbuminuria, 8.2 ± 3.1 mg/d with no microalbuminuria.

Table 1
Clinical and Laboratory Characteristics of Study Population

	Group 1 (n = 29)	Group 2 (n = 39)	p value
Age (yr)	58 ± 10	56 ±10	NS
Sex (men/women)	16/13	19/20	NS
Body mass index (kg/m^2)	27 ± 4.3	28 ± 4.2	NS
Hypertension (%)	14 (48)	19 (49)	NS
Smoking (%)	8 (27)	10 (25)	NS
Hemoglobin A1c	8.6 ± 2.6	8.7± 2	NS
Duration of DM (yr)	12 ± 6	7 ± 6	0.001
Total cholesterol (mg/dL)	197 ± 61	188 ± 49	NS
Triglycerides (mg/dL)	173 ± 99	158 ± 81	NS
HDL-C (mg/dL)	47 ± 13	46 ± 8	NS
LDL-C (mg/dL)	143 ± 48	128 ± 29	NS
Serum creatinine (mg/dL)	1 ± 0.33	0.7 ± 0.19	0.001
Antidiabetic drugs (%)			
Insulin	45	40	NS
Oral antidiabetics	41	43	NS
Beta-blockers (%)	28	27	NS
ACE-inhibitors (%)	34	31	NS
AT II blockers	28	23	NS
Diuretics (%)	8	9	NS
Ca antagonists (%)	11	12	NS
Statins (%)	28	27	NS

NS, $p > 0.05$; DM, diabetes mellitus; ACE-inhibitor, angiotensin converting enzyme inhibitor; AT, angiotensin; Ca, calcium; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Two-Dimensional Doppler Echocardiography

Two-dimensional and pulsed wave Doppler echocardiographic variables of LV systolic and diastolic function are compared between groups in Table 2. LV dimensions, left atrial diameter, and EF were all within normal limits. In group 1, DT was longer than that in group 2 ($p = 0.01$). IVRT was longer in group 1 than group 2 ($p = 0.03$). Vp was lower in patients with microalbuminuria than with no microalbuminuria ($p = 0.01$). There were no significant differences in E, A, E/A, E/Vp, and E/Em among the groups. Left ventricular hypertrophy was not found in either group.

Pulsed Wave Doppler Tissue Imaging

Mitral annular Em/Am ratio was significantly lower in those with microalbuminuria than that in those without ($p = 0.01$). IVRT was longer in group 1 than in group 2 ($p = 0.03$). E/Em was higher in group 1 than in group 2, but the differences did not reach any statistical significance ($p > 0.05$).

Flow-Mediated Dilatation

Flow-mediated dilatation was significantly lower in patients with microalbuminuria than those patients with no microalbuminuria ($p = 0.03$).

For logistic regression analysis, independent variables including DT, IVRT, Em/Am, Vp, and FMD were selected.

Table 2
Two-Dimensional and Pulsed Wave Doppler Echocardiographic Measurements

	Group 1 (n = 29)	Group 2 (n = 39)	p value
IVS (cm)	1.10 ± 0.15	1.08 ± 0.13	NS
PW (cm)	1.06 ± 0.1	1.02 ± 0.1	NS
LVSD (cm)	2.7 ± 0.4	2.7 ± 0.3	NS
LVDD (cm)	4.6 ± 0.4	4.6 ± 0.5	NS
EF (%)	66 ± 6	66 ± 5	NS
LA (cm)	3.2 ± 0.2	3.2 ± 0.3	NS
E (cm/s)	56 ± 16	55 ± 19	NS
A (cm/s)	59 ± 13	58 ± 17	NS
E/A	0.94 ± 0.43	0.94 ± 0.35	NS
DT (ms)	223 ± 39	199 ± 37	0.01
IVRZ (ms)	109 ± 13	100 ± 13	0.03
Vp (cm/s)	40.4 ± 9.98	50.4 ± 19.01	0.01
E/Vp	1.7 ± 0.55	1.6 ± 0.58	NS

NS, $p > 0.05$; LVSD, left ventricular end-systolic diameter; LVDD, left ventricular end-diastolic diameter; EF, ejection fraction; LA, left atrium; E, early diastolic mitral inflow velocity; A, late diastolic mitral inflow velocity; DT, deceleration time of the mitral E wave; IVRT, isovolumetric relaxation time; Vp, E wave propagation velocity.

Table 3
Results of Logistic Regression Analysis

	OR	95% CI	p	β
FMD	0.879	0.790–0.977	0.016	–0.130
DT	1.002	0.984–1.022	0.804	0.002
IVRT	0.996	0.946–1.050	0.892	–0.004
Vp	0.961	0.905–1.020	0.189	–0.040
Em/Am	0.213	0.022–2.046	0.181	–1.544

FMD, flow-mediated vasodilatation; DT, deceleration time of the mitral E wave; IVRT, isovolumetric relaxation time; Vp, E wave propagation velocity; Em/Am, early diastolic and late diastolic mitral annular velocities ratio.

Regression analysis showed that FMD was the only significant predictor of microalbuminuria (OR = 0.88; 95% CI, 0.79–0.98; $p = 0.016$). DT (OR = 1; 95% CI, 0.98–1.02; $p = 0.8$), IVRT (OR = 0.99; 95% CI, 0.94–1.04; $p = 0.8$), Em/Am (OR = 0.21; 95% CI, –0.02–2.04; $p = 0.1$), and Vp (OR = 0.96; 95% CI, –0.90–1.02; $p = 0.1$). The results of logistic regression analyses are given in Table 3.

Mean values of the parameters derived by DTI and FMD are shown in Table 4.

Correlations of Flow-Mediated Dilatation and Left Ventricular Function

FMD positively correlated with EF ($r = 0.43$, $p = 0.02$), E/A ($r = 0.40$, $p = 0.03$), and negatively correlated E/Em ($r = –0.41$, $p = 0.04$) and E/Vp ($r = –0.41$, $p = 0.04$) in diabetic patients with microalbuminuria. In patients without microalbuminuria there was no significant correlation between FMD and LV function.

Table 4
Pulsed-Wave Doppler Tissue Imaging and Flow-Mediated Dilatation Measurements

	Group 1 (n = 29)	Group 2 (n = 39)	p value
Em (cm/s)	9.7 ± 2.88	11.1 ± 3.24	NS
Am (cm/s)	13 ± 3.55	11.7 ± 3.22	NS
Em/Am	0.79 ± 0.27	1.02 ± 0.44	0.01
Sm (cm/s)	10.8 ± 3.13	11.2 ± 2.43	NS
E/Em	7.51 ± 2.62	7 ± 2.20	NS
IVRT (ms)	92 ± 33.2	64 ± 23.1	0.03
FMD (%)	8.8 ± 6.44	12.6 ± 7.24	0.03

NS, $p > 0.05$; Em, early diastolic mitral annular velocity; Am, late diastolic mitral annular velocity; Sm, peak systolic mitral annular velocity; E, early diastolic mitral inflow velocity; IVRT, isovolumetric relaxation time; FMD, flow-mediated vasodilatation.

Discussion

To our knowledge, this study is the first study to investigate FMD and LV systolic and diastolic function as assessed by DTI and the relationship between FMD and LV function in type 2 diabetic patients with or without microalbuminuria. In this study, we found that IVS and DT were higher, whereas Vp, Em/Am, and FMD were lower in diabetic patients with microalbuminuria than in patients with normoalbuminuria. FMD was positively correlated with EF, E/A, and negatively correlated with E/Em and E/Vp only in patients with microalbuminuric diabetic patients. Logistic regression analysis showed that only FMD was significantly associated with microalbuminuria in type 2 diabetic patients.

Previously, it was shown that type 1 diabetic patients with microalbuminuria had endothelial dysfunction associated with the degree of albuminuria *(21)*. Lekakis et al. *(22)* showed that type 1 diabetic patients with microalbuminuria had worse endothelial dysfunction and microalbuminuria correlated with impaired endothelial dysfunction. But, we demonstrated that FMD was impaired in type 2 diabetic patients with microalbuminuria compared with nonmicroalbuminuric diabetic patients. But, Hanereh et al. *(23)* found there was no association between FMD and microalbuminuria. Shivalkar et al. *(24)*, showed that FMD is associated with abnormal segmental cardiac dysfunction in type 1 DM. But, in their study, in contrast to our study, microalbuminuria did not emerge as a significant parameter. In our study, logistic regression analysis showed that FMD was the only significant predictor of microalbuminuria.

We evaluated LV function in type 2 diabetic patients with or without microalbuminuria. Previous studies showed that a higher percentage of diabetic patients had a diastolic dysfunction, whereas the systolic dysfunction was normal *(25,*

26). Liu et al. *(27)* showed that DT was longer in groups with albuminuria than in the group with no albuminuria. In this study, we found that DT was longer, Em/Am and Vp were lower in patients with microalbuminuria than those without microalbuminuria. In diabetic patients with no cardiac disease, LV abnormalities may be related to both albuminuria and hypertension *(28)*. In our study, there was no significant difference in the percentage of hypertension among the groups.

The flow propagation velocity (Vp) of early LV filling measured by color M-mode is a new Doppler index of LV relaxation, which has been suggested to be less dependent on preload and may differentiate in patients with abnormal LV diastolic function and pseudonormal or restrictive Doppler indexes *(29)*. Normal systolic function and delayed relaxation have lower Vp (45 cm/s) *(30)*. In our study, we found that Vp is reduced in diabetic patients with microalbuminuria.

Myocardial velocities assessed by DTI are relativeley preload independent *(16)*. The results of our study indicate that Em/Am, which has been demonstrated in LV diastolic dysfunction measured by DTI, was significantly lower in those with microalbuminuria than in those without. But the E/A ratio was not significantly different in two groups. This finding showed that Em/Am is superior to conventional mitral Doppler indexes in the assessment of LV diastolic dysfunction in patients with microalbuminuria.

The peak early diastolic filling velocity and flow propagation velocity (E/Vp) ratio by color M-mode Doppler provides a better estimate of pulmonary capillary wedge pressure than transmitral or pulmonary venous flow. It has been shown that E/Vp has related well to LV filling pressures in previous investigations in sinus rhythm and atrial fibrillation *(31)*. Patients with higher E/Em ratios can be considered as having elevated filling pressures. The ratio E/Em can correct for the influence of relaxation on transmitral E and relates strongly to filling pressures *(20)*. Saraiva et al. *(32)*, reported the presence of higher E/Em ratio in diabetics with diastolic dysfunction.

Previous studies evaluated diastolic dysfunction but did not assess the relation between FMD and LV function. Potential associations of FMD and EF, E/A, E/Em and E/Vp may be relevant to understand the associations between FMD and LV functions in type 2 diabetic patiens with microalbuminuria. A number of confounding factors may impact on the correlations of FMD and LV systolic and diastolic dysfunction, including subclinical coronary artery disease and hypertension. In order to minimize the the impact of confounding factors, we selected a group of patients with normal ECG, no angina. There was no statistical difference of hypertension between group 1 and group 2. Our findings showed that FMD was negatively associated with E/Em, E/Vp. Thus, the inverse association of FMD and LV E/Em and E/Vp and positive association E/A suggest that impaired FMD may play a role in the pathogenesis of diastolic dysfunction in type 2 diabetics with microalbuminuria. The present study provides new and potentially important findings, identifying associations of FMD with LV diastolic function, including E/Em, E/Vp, and E/A ratio.

Limitations

The existence of coronary heart disease cannot be ruled out, because a noninvasive stress test or angiography was not performed. However, there was no clinical, electrocardiographic, or echocardiographic ischemic evidence. ACE inhibitors may affect endothelial function, but there was no significant difference regarding the ACE-inhibitors therapy. Another limitation of this study is that it involves a small number of patients. Therefore, large prospective studies are needed to establish the association between DTI parameters and FMD diabetic patients with microalbuminuria.

In conclusion, the main finding of this study was that type 2 diabetic patients with microalbuminuria had impaired FMD compared with those patients without microalbuminuria. FMD was significantly and negatively associated with E/Em, E/Vp and positively correlated with E/A ratio and EF only in microalbuminuric patients. FMD was the only significant predictor of microalbuminuria. Measurement of endothelial function of the brachial artery could be useful in idendifying a high-risk group of type 2 diabetic patients who might benefit from more intensive conventional and new treatments. Strategies to reduce or retard endothelial dysfunction in these patients may lead to decreased CV morbidity and mortality.

Methods

Patients

We prospectively evaluated 68 consecutive patients (36 women, 32 men; aged 55 ± 11 yr) with type 2 diabetes mellitus (DM). We included patients with an EF > 50% and no history of angina pectoris, myocardial infarction, or congestive heart failure. In addition, the patients had no complaints or physical signs of congestive heart failure, or coronary heart disease. Electrocardiography showed sinus rhythm without any signs of ischemia in all of the patients. We excluded patients with bundle brunch block, atrial fibrillation, paced rhythm, atrioventricular block, moderate–severe valvular heart disease, restrictive, hypertrophic, or dilate cardiomyopathies, congenital heart disease, hyperthyroidism, hypothyroidism, and poor echocardiographic image.

The patients were divided into two groups according to the presence or absence of microalbuminuria. Group 1 consisted of 29 patients with microalbuminuria (mean age 58 ± 10 yr, 16 women, 13 men); and group 2 had 39 patients without microalbuminuria (aged 56 ± 10 yr, 20 women, 19 men). The study was approved by the local scientific ethical committee.

Echocardiography

All patients were evaluated by two-dimensional, pulsed-wave Doppler and DTI echocardiography. All examinations were performed with the HP SONOS 5500 machine with a 2.5-MHz transducer. Two echocardiographers who were blinded to the patients' clinical and laboratory data interpreted each echocardiographic examination independently. All Doppler echocardiographic and DTI recordings were obtained during normal respiration.

Two-Dimensional Doppler Echocardiography

Left ventricular ejection fraction was assessed by the modified biplane Simpson method *(33)*, and the mean of three measurements was used. LV diastolic filling patterns were determined by the mitral inflow pulsed-wave Doppler examination. In the apical four-chamber view, the Doppler sample volume was placed in the middle of the LV inflow tract 1 cm below the plane of mitral annulus between the mitral leaflet tips, where maximal flow velocity in early diastole was recorded. Special care was taken to align the sample volume as close to perpendicular as possible to the mitral annular plane. Doppler measurements were calculated from an average of five consecutive cardiac cycles. The following parameters were obtained: peak E and late A transmitral filling velocities, E/A and DT. IVRT, defined as the time from aortic valve closure to mitral valve opening, was assessed by simultaneously measuring the flow into the LV outflow tract and mitral inflow by Doppler echocardiography *(34,35)*. LV diameters and thicknesses were measured with two-dimensional targeted M-mode echocardiography, using the criteria of the American Society of Echocardiography.

Color M-Mode Flow Propagation Velocity

Apical four-chamber view, the color sector map of the mitral inflow, was adjusted to obtain the longest column of color flow from the mitral annulus to apex. An M-mode cursor was placed through the center of this flow, avoiding boundary regions. Vp was measured as the slope of the first aliasing velocity during early filling from the mitral valve plane to 4 cm distally into the LV cavity *(36)*.

Pulsed-Wave Doppler Tissue Imaging

The pulsed-wave DTI was performed using the same apparatus. A variable-frequency, phased-array transducer (2.5 MHz) was used. To display tissue velocities, the high-pass filter was bypassed. Gains were minimized to allow for a clear tissue signal with minimal background noise. From the apical four-chamber view, the Doppler sample volume (20-mm axial length) was placed at the lateral corner of the mitral annulus. The Nyquist limit was adjusted to a velocity range of –15 cm/s to 15 cm/s. The monitor sweep speed was set at 50 to 100 mm/s to optimize the spectral display of myocardial velocities. The following measurements were made from the pulsed-wave DTI recordings: Sm, Em, Am velocities, Em/Am, E/Em, and E/Vp.

Flow-Mediated Dilatation

A standard protocol was used to assess endothelial function, as previously reported, according to recommendation *(37,38)*. For the FMD of brachial artery, patients fasted ≥ 8 h before the study. Patients were studied in a quiet, temperature-controlled room. Caffeine intake and cigarette smoking were prohibited for at least 4–6 h before the study. The right arm was immobilized using two cushions supporting the elbow and the wrist. A sphygmomanometric cuff was placed on the forearm. After 10–15 min of rest, the brachial artery was visualized longitudinally with the ultrasonic scanner operating B mode. After an optimal image of the artery was obtained, the ultrasonic transducer was fixed in this position with a custom-built probe holder. Brachial artery diameter was determined in end-diastole, indicated by the R wave of the electrocardiogram. After three baseline measurements were obtained, ischemia was induced by the inflation of the cuff to 100 mmHg greater than the systolic arterial pressure to occlude arterial flow for 5 min. After the deflation of the cuff, diameter measurements were performed 30 s, 1 min, 2 min, 3 min, and 4 min, consecutively. Because the arterial dilatation most likely relating to nitric oxide release occurs at 1 min after ischemia, we used FMD at 1 min postischemia to represent the spontaneous endothelial function. Maximal obtained diameter during ischemia-induced hyperemia was used for the calculation of the percentage FMD, [(maximum diameter-baseline diameter / baseline diameter] × 100.

Biochemical Parameters

Urine albumin content was measured in a 24-h urine sample by immunonephelometric technique (Dade Behring, Marburg, Germany). Microalbuminuria was defined as an albumin excretion rate of 30–300 mg/d.

Statistical Analysis

All the results are expressed as mean ± standard deviations. Baseline and echocardiographic variables were compared by chi-square test for categoric variables and Student's *t* test for continuous variables. Pearson and Spearman correlation coefficients were used for calculation. A value of $p < 0.05$ was considered statistically significant. A stepwise multiple regression analysis was performed to determine the independent predictors of microalbuminuria.

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