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Original Article

Correlation between endothelial dysfunction and early atherosis of first degree relatives of type 2 diabetes mellitus with normal glucose tolerance

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Abstract: Objective: To discuss vascular endothelial function and early atherosis of first degree relatives (FDRs) with type 2 diabetes mellitus (T2DM) with normal glucose tolerance, and the correlation between them. Method: 48 T2DM patients with FDRs in the normal glucose tolerance range were selected as the observation group and 38 healthy people with normal glucose tolerance and without a family history of diabetes or history of abnormal glucose tolerance as the control group. The enzymic method was used to measure fasting blood-glucose (FBG), postprandial 2-hour blood glucose (P2hBG) and triglyceride (TG). Chemiluminiscence was used to measure insulin (FINS) and the ELISA method to measure the level of asymmetric diemethylarginine (ADMA) in serum and endothelin-1 (ET-1). Homeostasis model-IR (HOMA-IR), -cell function index (HOMA-IS), insulin sensitivity index (ISI) and the atherogenic index of plasma (AIP) were calculated. High-resolution ultrasound was applied to measure and calculate endothelium dependent dilatation in the brachial artery (FMD), carotid intima media thickness (IMT) and carotid artery elasticity (Ep), arterial stiffness (β) and compliance (AC). Results: Compared with control group, serum ADMA, ET-1, TG, Ln (FINS), Ln (HOMA-IR), Ln (HOMA-IS), AIP, carotid artery Ep and β significantly increased ($P<0.05$), FMD and Ln (ISI) significantly decreased ($P<0.05$); P2hBG increased and AC decreased without statistically significant difference ($P>0.05$) in observation groups. Correlation analysis showed that Ep and β were all positively related with serum ADMA and ET-1 and negatively related with FMD ($P<0.05$). According to partial correlation analysis, Ep, β and FMD were all independently related with serum ADMA and ET-1 after correcting TG level, Ln (HOMA-IR), AIP, P2hBG and SBP ($P<0.05$). Conclusions: FDRs with normal glucose tolerance in T2DM had endothelial dysfunction and arterial function changes, while the arterial function change was correlated to endothelial dysfunction and independently related to serum ADMA and ET-1 level. Serum ADMA and ET-1 levels are expected to be the biological indicator for prediction of early atherosis.

Keywords: Endothelial dysfunction, atherosis, type 2 diabetes mellitus, first degree relative

Introduction

In recent years, the morbidity of type 2 diabetes mellitus (T2DM) has gradually increased [1]. Actually, genetic factors play a very important role in the occurrence of T2DM [2], so, first degree relatives (FDRs) with T2DM, including those with normal glucose tolerance are all a high-risk population of T2DM [3]. The incidence of cardiovascular disease in patients with T2DM is 2~4 times more than those of non-diabetics [4], and the severity of vascular injury also increase significantly when the glucose tolerance is abnormal [5]. Recently, some scholars [6] thought that the change of artery function is prior to the change of structure. Indices of vascular function, including endothelium dependent dilatation in the brachial artery (FMD), carotid artery elasticity (Ep), arterial stiffness (β) and compliance (AC) have already occurred before carotid intima media thickness (IMT), so all of these indices are the early indicators of atherosis. Asymmetric dimethylarginine (ADMA) in serum and endothelin-1 (ET-1) synthesized by endothelial cells, are the marker indicators of endothelial dysfunction (ED) and play an important role in maintaining the balance of vasomotion. This work aims at discussing whether the FDRs of T2DM patients have endothelial dysfunction and early atherosis by

detecting the serum ADMA, ET-1 level and vascular function of FDRs with normal glucose tolerance in T2DM. In addition, we intended to provide evidence for the early prevention of blood vessel complications from T2DM.

Materials and methods

Clinical data were collected from first degree relatives (including children and siblings) in 52 families of T2DM patients treated in Qilu Hospital from October 2009 to June 2010. According to oral glucose tolerance tests (OGTT experiment), 48 people with normal glucose tolerance, including 23 men and 25 women aged 14~66 with an average age of (38.92±12.09) years old were selected as the observation group. 38 healthy people with normal glucose tolerance and without any family history of diabetes and history of abnormal glucose tolerance, including 18 men and 22 women aged 22~66 with an average age of (43.43±15.99) years old were selected as the control group. The diagnosis of normal glucose tolerance follows the diagnosis standard set by the WHO in 1999: fasting blood-glucose (FBG) <6.1 mmol/L and postprandial 2-hour blood glucose (P2hBG) <7.8 mmol/L. Exclusion standard: Exclude hypertension, cardiovascular and cerebrovascular diseases, family hyperlipidemia and connective tissue diseases; exclude other diseases affecting glucose metabolism, such as hypercortisolism, hypophysis auxocyte tumor and pheochromocytoma; exclude acute infection, cardiac dysfunction, severe liver dysfunction and renal dysfunction, and chronic wasting diseases. All the subjects did not take vasoactive agent, lipid-modulating and antihypertensive drugs.

Observation target

Statistics were compiled to record the age and sex of the subjects and whether they had a smoking and drinking history, and measured the height, body mass, blood pressure [systolic pressure (SBP) and diastolic blood pressure (DBP)], waist circumference (WC) and hip circumference (HC) to calculate the body mass index (BMI) and waist-hip ratio (WHR). 8 mL venous blood was taken 8~10 h after experiencing limosis, 2 mL venous blood was taken 2 h after OGTT experiment, the serum were separated and stored at -20°C for inspection. Glucose oxidase method was applied to measure FBG and P2hBG; the enzymic method was used to measure triglyceride (TG) and total cholesterol (TC); a precipitation method was used to measure high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterin (LDL-C); chemilunimiscence to measure fasting insulin (FINS); the ELISA method was used to measure serum ADMA and ET-1 level.

A steady-state model was used to evaluate and calculate the insulin resistance index (HOMA-IR) = (FBG×FIns)/22.5-cell function index (Homa-IS) = FINS×20/(FBG-3.5) insulin sensitivity index (ISI) = 1/(FBG×FINS) and atherogenic index of plasma (AIP) = Log (TG/HDL-C).

Ultrasonic testing

Vivid7 color Doppler ultrasound instrument (GE Co., Ltd., U.S.A) with a probe of 7.5~12 MHz frequency variation line matrix was used to do carotid artery and brachial artery ultrasonic testing.

Ultrasonic testing of carotid artery. Subjects lay on their backs without pillows to measure blood pressure and were connected to an electrocardiograph. The neck was completely exposed to measure the intima-media thickness of 1 cm of the carotid artery, carotid artery bifurcation and 1 cm to the internal carotid artery at the near end of the bulbus caroticus at both sides. Then, the mean value was taken as the artery IMT of subjects. Measuring the sampling line of the M-type ultrasound vertical to the long axis of the arteria carotis communis, blood vessel diameter (Dd) was measured at R peak of electrocardiogram at the end of diastole, blood vessel diameters (Ds) were measured in T wave of the electrocardiogram at the end of shrink term, and 3 cardiac cycles were measured to take the mean. Parameters were calculated as follows: carotid artery elasticity (Ep) = (Ps-Pd)/[(Ds-Dd)/Dd]; hardening parameter (β) = ln(Ps/Pd)/[(Ds-Dd)/Dd]; compliance (AC) = π (Ds×Ds-Dd×Dd)/[4(Ps-Pd)]. In the above formulas, Ps is systolic pressure (mmHg, 1 mmHg = 0.133 kPa), Pd diastolic pressure (mmHg), Ds blood vessel diameter in shrink stage (mm) and Dd blood vessel diameter in diastole (mm).

Measurement of endothelium dependent dilatation in the brachial artery. Referred to the method introduced by Celermajer [7], the measurement was conducted at room temperature

Table 1. Comparison of clinical data and blood index between the observation group and the control group ($\bar{x}\pm s$)

Clinical index	Control group (n=38)	Observation group (n=48)
Age (years)	43.43±15.9	38.92±12.09
Gender (male/female)	18/20	23/25
Smoking history cases	15	19
Drinking history cases	18	21
BMI (kg/m^2)	23.85±3.47	22.93±3.02
Waistline (*l*/cm)	77.91±9.11	80.08±10.3
Hipline (*l*/cm)	96.7±6.97	97.83±5.58
WHR	0.78±0.15	0.82±0.07
SBP (*P*/mm Hg)	111.65±14.71	112.71±12.09
DBP (*P*/mm Hg)	75.39±8.61	75.08±10.45
FBG (c/mmol·L^{-1})	5.42±0.52	5.53±0.62
P2hBG (c/mmol·L^{-1})	6.37±0.84	6.82±0.73
TC (c/mmol·L^{-1})	4.48±0.44	4.74±0.34
TG (c/mmol·L^{-1})	1.02±0.19	1.43±0.17**
HDL (c/mmol·L^{-1})	1.23±0.25	1.09±0.23*
LDL-C (c/mmol·L^{-1})	2.38±0.44	2.65±0.50
AIP	-0.11±0.45	0.028±0.64*
FINS (mU/L)	7.77±3.6	11.01±7.78*
Ln (FINS)	1.781±0.53	2.20±0.64*
Ln (HOMA-IR)	0.35±0.58	0.79±0.67*
Ln (HOMA-IS)	4.17±0.51	4.53 ±0.67*
Ln (ISI)	-3.47±0.58	-3.9±0.67*
ADMA (ρ/μmol·L^{-1})	0.60±0.22	1.00±0.31*
ET-1 (ρ/ng·L^{-1})	44.56±18.27	66.52±27.11*

*P<0.05, **P<0.01 vs. Control group.

after being in limosis for 10 h. After measuring the carotid artery elastic function, subjects rested for 15 min in clinostatasm. Then, the cuff of the sphygmomanometer was placed on the right forearm with the right upper limb extended to 15°. An ultrasonic probe was placed at 2~5 cm to upper chelidon to show the two-dimensional image of the long axis of the brachial artery. The maximum brachial artery diameter and optimal blood interface of linear arterial wall were obtained. With a distance of 1.0 mm, the pulse Doppler sampling point was placed in the centre of the vessel lumen with an incidence angle of 60°. The maximum laminar flow spectrum image was taken. The Doppler blood spectrum in the basic two-dimensional image of the brachial artery was recorded. Then, the cuff was inflated to 280~300 mmHg to completely interrupt blood flow and then rapidly deflated 5 min later. The immediate blood spectrum after deflation and two-dimensional image of the brachial artery 60~90 s later were recorded. Three cardiac cycles were taken, and artery diameter at the end of the diastole was measured at R peak of electrocardiogram. Then, the average artery diameters before and after the brachial artery (D0 and D1) were calculated. Diastolic function dependent on blood vessel endothelium (FMD) was calculated with the formula of [(D1-D0)/D0] × 100%.

Statistical treatment

SPSS16.0 software was used in statistical analysis. All the measurement data were represented with ($\bar{x}\pm s$). FINS, HOMA-IR, Homa-IS and ISI are all in skewed distribution. Natural logarithms were taken for statistical analysis after normal distribution. A non-paired *t* test was used for comparison between groups, general correlation analysis (Bivaviate process), partial correlation analysis (partial process) and multiple stepwise regression analysis were used for multiple-factor analysis. P<0.05 refers to statistically significant difference.

Results

Comparison of clinical data and blood index Serum ADMA

ET-1, TG, Ln (FINS) and Ln (HOMA-IR), Ln (HOMA-IS) and AIP of the observation group significantly increased (P<0.05), compared with the control group; HDL and Ln (ISI) significantly decreased (P<0.05); P2hBG tended to increase without statistically significant difference (P>0.05). See **Table 1**.

Comparison of the brachial artery and carotid artery ultrasound

Compared with the control group, carotid artery Ep and β of the observation group significantly increased (P<0.01), FMD significantly decreased (P<0.01), AC tended to decrease without statistically significant difference (P> 0.05). The change of IMT was not obvious. See **Table 2**.

2.3 Multiple linear correlation analysis among various factors that is, Bivaviate process showed that Ep and β were all positively related to serum ADMA, ET-1, TG level, Ln (HOMA-IR),

Table 2. Comparison of brachial artery and carotid artery ultrasound in the observation group and control group ($\bar{x}\pm s$)

Group	FMD (%)	Ep (*P*/mmHg)	AC (mm^2/mmHg)	β	IMT (δ/mm)
Control group	12.58±6.12	1103.2±204.78	220±116	3.83±0.27	0.65±0.36
Observation group	7.67±4.62*	1579.3±455.14**	168±79	4.12±0.32**	0.66±0.2

*P<0.05, **P<0.01 vs. Control group.

Table 3. Ep, β and level of serum ADMA, ET-1, TG, Ln(HOMA-IR), AIP, P2hBG, SBP and FMD correlation

Item	Ep		β	
	Correlation coefficient *r*	*P*	Correlation coefficient *r*	*P*
ADMA	0.325	0.023	0.292	0.037
ET-1	0.368	0.009	0.352	0.013
level of TG	0.315	0.031	0.291	0.038
Ln (HOMA-IR)	0.341	0.017	0.327	0.022
AIP	0.296	0.034	0.316	0.0
P2hBG	0.352	0.013	0.340	0.017
SBP	0.34	0.017	0.367	0.10
FMD	-0.268	0.043	-0.251	0.048

Table 4. TG level correction, Ln(HOMA-IR), AIP, P2hBG, SBP, Ep, β, FMD, AC and correlation between serum ADMA and ET-I

Item	ADMA		ET-1	
	Correlation coefficient *r*	*P*	Correlation coefficient *r*	*P*
FMD	-0.325	0.023	-0.317	0.03
Ep	0.312	0.032	0.358	0.012
β	0.279	0.041	0.298	0.033
AC	-0.294	0.034	-0.352	0.013

AIP, P2hBG and SBP (P<0.05) and negatively related to FMD (P<0.05). See in **Table 3**. FMD was negatively related to ADMA, ET-1, TG level, Ln (HOMA-IR), AIP, P2hBG and SBP (with values of r -0.372, -0.368, -0.342, -0.384, -0.357, -0.287 and -0.316, respectively, and P<0.05). Partial process showed that Ep, β, AC and FMD were all positively related to serum ADMA and ET-1 after correcting TG level, Ln (HOMA-IR), AIP, P2hBG and SBP, while FMD was independently and negatively related to serum ADMA and ET-1 (P<0.05). See **Table 4**.

Linear stepwise regression

With ADMA, ET-1, TG level, Ln (HOMA-IR), AIP, P2hBG and SBP as an independent variable and β as a dependent variable, β was positively related with Ln (HOMA-IR) and P2hBG the multiple correlation coefficients R2 were 0.467 and 0.353, P<0.01). With AC as a dependent variable, AC was negatively related to Ln (HOMA-IR) and P2hBG (with multiple correlation coefficients R2 were -0.414 and -0.331, respectively, P<0.05). With Ep as an independent variable, Ln (HOMA-IR) was the independent risk factor of Ep (with multiple correlation coefficient R2 was -0.449, P<0.01).

Discussions

T2DM is a polygenetic disease with familial aggregation, so FDRs of T2DM patients belong to a high-risk group. Atherosis is the primary cause for death and disability of T2DM patients [8]. At present, the diagnosis standard of diabetes is the diabetic microangiopathy, especially retinopathy. However, serious vascular disease has already occurred before the diagnosis of diabetes. Therefore, screening and prevention of early atherosis among high-risk group of T2DM are the hot spots for current research.

Atherosis is a systemic disease. Carotid artery IMT is treated as the morphology index of early atherosis [9] and the indication of atherosis. This work showed that FDRs of T2DM patients with normal glucose tolerance already have increased Ep and β in the carotid artery (P<0.05). This result is consistent with the conclusion of Furumoto, et al. [6] that the change of vascular function is prior to the change of structure. Therefore, the parameter of carotid atherosclerosis Ep, β and AC, which are great vessel functional parameters can be treated as indices of early arthrosis. It can be seen that FDRs of T2DM patients with normal glucose tolerance have already had early atherosis.

Atherosis starts at ED [10]. ED with major performance of FMD is treated as the early manifestation before AS anatomy evidence occurs [11] and the starting link and central link of atherosis. Endothelial cells are treated as the biggest metabolic organs of internal secretion,

paracrine and autocrine with active function. This organ can generate and secrete dozens of bioactivators, including nitric oxide (NO) and endothelin to adjust angiokinesis and maintain vessel tension. ADMA is the endogenous competitive antagonist of nitric oxide synthase (eNOs) which can affect the diastolic function of a vessel by inhibiting eNOs activity and reducing NO synthesis. ADMA is a new predictive factor of incomplete endothelium function [12]. ET-1 is the strongest vasoconstrictor, the contraction function of which is ten times that of angiotensin II. It plays a role in adjusting vessel elasticity, tissue reorganization, inducing the proliferation and chemotaxis of macrophage and activating and differentiating smooth muscle cells [13]. As the biomarker of ED, ET-1 participates in the occurrence and development of atherosis. When endothelial function was damaged, the secretion of ET-1 and ADMA r rose, vasoconstrictive substances increased, and vascular dilation substance decreased, which led to the disequilibrium of angiokinesis and the increasing of stiffness, as a result, the occurrence and development of atherosis are triggered and accelerated. This work showed that compared with the control group, ADMA and ET-1 level significantly increased and FMB significantly decreased in the observation group. It indicated that FDRs of T2DM patients with normal glucose tolerance have endothelial dysfunction. Ep and β are all positively related to serum ADMA and ET-1, and negatively related to FMD ($P<0.05$). It showed that early atherosis was closely related to endothelial dysfunction and played an important role in the occurrence and development of arthrosis. Therefore, serum ADMA and ET-1 can be treated as the biological indicators for prediction of early atherosis.

This work showed that Ep, β and FMD were all significantly related to serum TG level, Ln (HOMA-IR), AIP, P2hBG and SBP ($P<0.05$). It indicated that the change of arterial function was related to TG, IR, AIP, P2hBG and SBP, while the regression results showed that Ep, β and AC were independently related to IR. This result is consistent with the conclusion of Suzuki et al. [14] that IR is one of the determinants of endothelial function, and ED and early vessel change are all attributed to IR. Blood vessel endothelium is the target organ for insulin action. In physiological condition, insulin will combine with the insulin receptor in endothelium cells to promote endothelium synthesis, No release, and expansion of vascular smooth muscle cell by the insulin receptor substrate, INS-PI-3 kinase pathway. Endothelial injury may cause the abnormality of structure and distribution and affect the combination of insulin and receptor, so as to generate IR, and affect the NO release and further affect endothelial function. Endothelial injury participates in the occurrence of IR [15] which forms a vicious circle in tandem with endothelial injury. T2DM is caused by IR with insufficient insulin secretion or insufficient insulin secretion with IR, in which IR plays a very important role. T2DM aggravates metabolic disorder, promotes ED, and further affects atherosis. Therefore, ED promotes the occurrence and development of T2DM and atherosis.

In conclusion, FDRs of T2DM patients with normal glucose tolerance have ED and early atherosis, while early atherosis has significant positive relation to endothelial dysfunction and independent relation with serum ADMA and ET-1. Therefore, serum ADMA and ET-1 are expected to become the biological predictive index of early atherosis. The positive intervention treatment for FDRs of T2DM patients with normal glucose tolerance can delay and even interdict the occurrence and development of T2DM and its great vessel complications.

Disclosure of conflict of interest

None.

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