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Correlations of High-frequency Ultrasound Evaluation of Brachial Artery Endothelial Dilatation and Carotid Atherosclerosis with Glucose and Lipid Metabolism, Inflammatory Cytokines, Severity of Coronary Artery Disease and Vascular Endothelial Function in Elderly Patients

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ABSTRACT

The study aimed to explore the correlations of the results of the high-frequency ultrasound evaluation of the brachial artery endothelial dilatation and carotid atherosclerosis with glucose and lipid metabolism, inflammatory cytokines, the severity of coronary artery disease (CAD) and vascular endothelial function in elderly patients. 78 elderly patients with CAD in Beijing Anzhen Hospital were selected. The high-frequency ultrasonography was carried out to observe the flow-mediated dilatation (FMD) and intima-media thickness (IMT) and to analyze their correlations with inflammatory cytokines [C-reactive protein (CRP) and plasminogen activator inhibitor (PAI-1)], endothelial function [nitric oxide (NO) and endothelin-1 (ET-1)], glycolipid metabolism [high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG) and fasting blood glucose (FBG)] and the severity of CAD. FMD, NO and HDL-C: patients with single-vessel CAD> those with double-vessel CAD>those with multi-vessel CAD. IMT, CRP, PAI-1, FBG, ET-1, TC and TG: patients with single-vesselCAD< those with double-vessel CAD <those with multi-vessel CAD (p<0.05). The FMD had negative correlations with CRP, PAI-1, FBG, ET-1, TC and TG and positive correlations with NO and HDL-C (p<0.05). The IMT was positively associated with CRP, PAI-1, FBG, ET-1, TC and TG and negatively related to NO and HDL-C (p<0.05). As the disease becomes severe, the endothelial dilatation of brachial arteries in elderly patients with CAD becomes weaker, and the risk of carotid atherosclerosis increases.

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Introduction

As a common heart disease in clinical practice, coronary artery disease (CAD), also known as coronary atherosclerotic heart disease, is often caused by myocardial ischemia and hypoxia due to atherosclerosis in the coronary arteries and narrowing of the vessel lumen. The primary clinical manifestations of the disease include discomfort of the precordial area, palpitation and lacking strength, angina pectoris, fever and even heart failure and shock (1, 2). CAD patients take a very high proportion of patients with type 2 diabetes mellitus, a chronic epidemic around the world. As the economy develops and the people's living standards are improved in China, the incidence rate of CAD combined with type 2 diabetes mellitus exhibits an increasing uptrend and it belongs to inflammatory vascular disease (3). The involvement of vascular endothelium in the regulation of vascular function exerts crucial effects on the maintenance of smooth muscle proliferation and vasodilatation as well as in the development of arteriosclerosis (4). In clinical practice, the brachial artery is often regarded as a target vessel for studying vascular endothelial function (5). Ultrasonography is non-invasive, easy to operate and repeatable (6). Examining the brachial artery endothelial dilatation and carotid atherosclerosis via high-frequency ultrasonography contributes to a good understanding of the condition of CAD with type 2 Diabetes Mellitus and the guidance of the treatment for the disease.

Materials and methods

General data

A total of 78 elderly patients with CAD combined with diabetes mellitus admitted to Beijing Anzhen Hospital from May 2017 to June 2018 were randomly

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selected as the study subjects. Inclusion criteria: 1) patients who met the diagnostic criteria for CAD and type 2 diabetes mellitus (7), 2) patients aged ≥ 60 years old who underwent high-frequency ultrasonography and 3) patients who signed the informed consent. Exclusion criteria: 1) patients who took lipid-lowering and anti-oxidation drugs in the past three months, 2) patients with the pericardial disease, congenital heart disease and myocardial disease or 3) patients with CAD combined with rheumatoid arthritis, systemic lupus erythematosus, arthritis or other diseases that might affect the determination of carotid atherosclerosis. General data of patients are shown in Table 1.

Table 1. General data of study subjects

Item	Subject (n=78)
Average age (years old)	68.78±5.54
Gender (male/female)	46/32
Body mass index (Kg/m^2)	24.78±3.58
History of hypertension [n (%)]	35 (44.87)
History of diabetes mellitus [n (%)]	14 (17.95)
Severity of CAD [n (%)]	
Single-vessel CAD	19 (24.36)
Double-vessel CAD	30 (38.46)
Multiple-vessel CAD	29 (37.18)

Detection of brachial artery endothelial dilatation

GE Vivid 7 color ultrasound diagnosis equipment was adopted. Related parameters: probe frequency: line array type: 7.5-10 MHz and phased array type: 1.7-3.4 MHz, respectively. 15 minutes before the examination, patients were instructed to take a supine position for a rest, and the palm of the right upper limb was kept up. The doctor placed the high-frequency ultrasound probe above the cross grain of the patient's right elbow (about 10 cm) to explore the brachial artery. Horizontal segmentation and longitudinal segmentation were conducted successively, and images were magnified. Finally, the longitudinal section of the brachial artery was selected, and the magnification and depth were gradually adjusted until the image became the clearest. Firstly, the inner diameter of the brachial artery before the reactive hyperemia in patients was measured, andthe sphygmomanometer cuff was bundled. Secondly, the equipment was restarted, and the pressure was added to about 290 mmHg for about 5 minutes. Finally, the air was released suddenly and quickly, and the inner diameter of the brachial artery after reactive hyperemia was measured within 1 minute. The average was taken after the measurement 3 times. The doctor should keep the probe position unchanged during the whole examination process.

Carotid atherosclerosis examination

Patients were instructed to take a supine position for a 5-minute rest. Then the thin pillow was placed under the neck of the patient, and the head was kept biased to one side. The frequency of the high-frequency line array probe was set as 7-12MHz, and the intima-media thickness (IMT) in the patient's common carotid artery, the sphere of the carotid artery and internal carotid artery on both sides were measured and averaged. It should be noted that the pressure during the examination should not be high, so as to avoid artificial vascular stenosis.

Evaluation criteria

Determination of the flow-mediated dilatation (FMD)

Values obtained in 3 tests were averaged and used to calculate FMD, i.e., FMD = [(D1-D0) / D0] × 100%, in which D0 represented the inner diameter of the brachial artery before reactive hyperemia, and D1 stood for the inner diameter of the brachial artery after reactive hyperemia. The IMT of the carotid artery was applied as a quantitative indicator for atherosclerosis (8,9).

Laboratory indicators

A total of 2-5mL fasting venous blood was collected from patients in the morning. The serum C-reactive protein (CRP), plasminogen activator inhibitor (PAI-1), endothelin-1 (ET-1) and nitric oxide (NO) were detected via the enzyme-linked immunosorbent assay. Related kits were provided by R&B (USA), and operations were conducted in strict accordance with the kit instructions. The fasting blood glucose (FBG) was measured using the glucose oxidase assay, the triglyceride (TG) and total cholesterol (TC) were detected via the oxidase assay, and the high-density lipoprotein cholesterol (HDL-C)was determined by the homogeneous enzyme colorimetry.

Statistical processing

Data were processed using Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS Inc.,

Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and detected using the *t*-test. Count data were expressed as a percentage and detected via the χ^2 test.The correlation was analyzed using Pearson's correlation coefficient.p<0.05 represented that the difference was statistically significant.

Results and discussion

Changes in the diameter of the brachial artery of patients

Detection by the same doctor using the same device revealed that the endothelial dilatation became weakened (Figure 1).

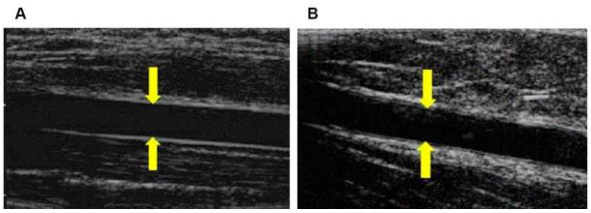

Figure 1. A male patient aged 67 years old with CAD combined with type 2 diabetes mellitus. A: the inner diameter of the brachial artery before reactive hyperemia (D0) is 3.6 mm, B: the inner diameter of the brachial artery after reactive hyperemia (D1) is 3.7 mm. The FMD is 2.78%.

FMD and the IMT of the carotid artery in patients with different degrees of CAD

FMD: patients with single-vesselCAD> those with double-vessel CAD>those with multi-vessel CAD. IMT: patients with single-vesselCAD< those with double-vessel CAD <those with multi-vessel CAD (p<0.05) (Figure 2).

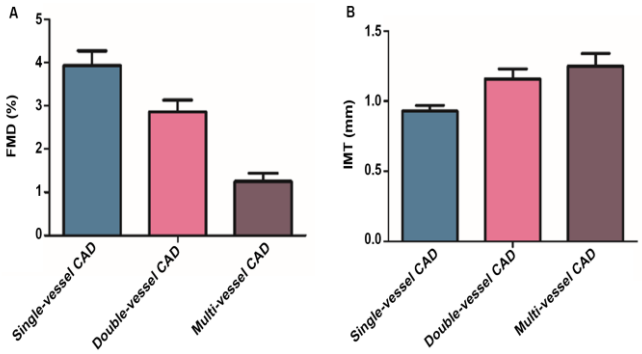

Figure 2. A: the FMD of patients with different degrees of CAD, B: the IMT of patients with different degrees of CAD. p<0.05 in pairwise comparison.

Levels of inflammatory cytokines (CRP and PAI-1) and endothelial function indexes (NO and ET-1) in patients with different degrees of CAD.

The levels of CRP, PAI-1 and ET-1 in patients with single-vessel CAD were significantly lower than those with double-vessel and multiple-vessel CAD. Still, the level of NO in the former was remarkably higher than that in the latter (p<0.05) (Table I2).

Table 2. Inflammatory cytokines and endothelial function indexes in patients with different degrees

Group	n	CRP (mg/L)	PAI-1 (AU/mL)	NO (μmol/L)	ET-1 (ng/L)
Single-vessel CAD group	19	3.92±1.01	8.07±1.17	62.76±3.38	63.15±3.42
Double-vessel CAD group	30	5.48±1.16*	10.24±1.23*	53.65±3.27*	68.74±3.56*
Multiple-vessel CAD group	29	8.69±1.23*#	12.93±1.24*#	45.43±3.48*#	73.92±3.73*#

Note: *#p<0.05 vs. single-vessel CAD group, and #p<0.05 vs. double-vessel CAD group

Glucose and lipid metabolism in patients with different degrees of CAD

The TC, TG and FBG in patients with single-vessel CAD were lower than those in patients with double-vessel and multi-vessel CAD, while the HDL-C in the former was markedly higher than that in the latter (p<0.05) (Table 3).

Table 3. Glucose and lipid metabolism in patients with different degrees of CAD

Group	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	FBG (mmol/L)
Single-vessel CAD group	19	3.85±0.12	1.47±0.13	1.86±0.16	7.17±1.07
Double-vessel CAD group	30	4.17±0.16*	1.63±0.15*	1.74±0.13*	8.23±1.16*
Multiple-vessel CAD group	29	4.39±0.18*#	1.79±0.16*#	1.62±0.15*#	9.18±1.18*#

Note: *#p<0.05 vs. single-vessel CAD group, and #p<0.05 vs. double-vessel CAD group.

Analyses of correlations of the FMD and IMT with inflammatory cytokines, endothelial function and glucose and lipid metabolism

The FMD had negative correlations with CRP, PAI-1, FBG, ET-1, TC and TG and positive

correlations with NO and HDL-C (p<0.05). The IMT was positively associated with CRP, PAI-1, FBG, ET-1, TC and TG and negatively correlated with NO and HDL-C (p<0.05) (Table 4).

Table 4. Analyses of correlations of the disease activity score and the delay enhancement-magnetic resonance imaging score of the coronary artery with various inflammatory indexes

Item	Correlation with the FMD		Correlation with the IMT	
	r	*p*	*r*	*p*
CRP	-0.405	0.023	0.426	0.012
PAI-1	-0.413	0.008	0.437	0.009
NO	0.438	0.012	-0.446	0.014
ET-1	-0.435	0.024	0.445	0.013
TC	-0.401	0.007	0.403	0.008
TG	-0.406	0.004	0.402	0.003
HDL-C	0.439	0.006	-0.435	0.004
FBG	-0.401	0.013	0.405	0.022

CAD is a disease with the damaged integrity of vascular endothelial cells. Under the influence of various factors, the damaged vascular endothelial cells and vascular smooth muscle cells in the body will cause inflammatory responses and fibrosis, thus resulting in CAD (10). A large number of studies have confirmed that the major cause of CAD is coronary atherosclerosis since over 95% of CAD is caused by atherosclerosis. A large amount of plaques produced during atherosclerosis will cause stenosis or occlusion of the arterial lumen, which affects the normal blood oxygen supply of the myocardium. As the disease progresses, these plaques will rupture, release numerous inflammatory cytokines, promote the formation of the thrombus and ultimately trigger myocardial infarction, thereby posing a serious threat to the health of patients (11,12). The endothelial function of patients with CAD in the early stage usually changes. The damage to the vascular endothelial function is an early marker of the formation of arteriosclerosis in CAD patients. Therefore, it is of great significance to evaluate the endothelial function early in CAD patients for the diagnosis and treatment of the disease.

In clinical practice, the vascular endothelial function can be evaluated using many methods, among which the ultrasound diagnosis, as a novel non-invasive examination, is favored by patients and doctors in clinical practice (13). Arteriosclerosis affects the aorta, coronary arteries and carotid arteries. Clinically, the detection of cervical artery vascular lesions is commonly used to reflect whether arteriosclerosis occurs in coronary arteries (14). Prior to the appearance of high-resolution ultrasonography, arteriosclerosis is often assessed using pathological examination and angiography. They were invasive examination methods and can only reflect the stenosis of patients. However, vascular stenosis usually occurs in the late stage of CAD, so the examination for early arteriosclerosis is largely limited (15). Previous studies on CAD show a causal relationship between endothelial function and vascular elasticity (16). In this study, the high-frequency ultrasonography revealed that the higher the degree of CAD in patients, the lower the FMD value would be, indicating weakened blood vessel elasticity of patients and obvious endothelial dysfunction. In the development of arteriosclerosis, the first part affected is the intima of the arterial wall. Clinically, whether the IMT becomes larger through observation is an early marker of arteriosclerosis. The high-frequency ultrasound diagnosis in this study manifested that the severer CAD represented the larger IMT.

Atherosclerosis is the primary pathological change in CAD. Based on related studies, the most important risk factor and predictor of CAD is lipid metabolism disorder, and its independent risk factor is the HDL-C level (17). Vascular endothelial injury is an important mechanism for the development of CAD. Through reverse cholesterol transport, anti-inflammation, increased activity of NO synthases and enhanced NO secretion, HDL-C can improve vascular endothelial function. If the HDL-C level declines, the vascular endothelial function in patients cannot be effectively improved, thus leading to the aggravation of CAD (18). It was found from the results of this study that the FMD had negative correlations with TC and TG and a positive correlation with HDL-C. The IMT was positively related to TC and TG, and negatively related to HDL-C, indicating that the lipid metabolism disorder has close associations with internal dilatation and cervical arteriosclerosis of patients.

Inflammatory responses are always accompanied by the occurrence and development of CAD. As a commonly used marker of inflammatory responses in clinical practice, CRP can directly reflect the inflammatory state and stability of atherosclerotic plaques in patients, and it has a certain predictive value for the patient's prognosis (19). PAI-1 is an

inflammatory factor of single-chain glycoproteins mainly derived from vascular endothelial cells, which has a close relationship to the insulin signal transduction and can reflect vascular endothelial dysfunction to some extent (20). The results of this study demonstrated that the levels of CRP and PAI-1 in serum were obviously increased in patients with severe CAD. The increased CRP level damaged the vascular endothelial function so as to increase the expression of endothelial cell adhesion molecules, promote the formation of arteriosclerotic plaques and induce the production of a large amount of PAI-1. Meanwhile, a large quantity of PAI-1 would be activated under the condition of hyperglycemia, which decreased the degradation ability of local fibrous proteins, thus stimulating the formation of arteriosclerosis. Therefore, FMD was negatively correlated with CRP and PAI-1, but IMT was positively associated with CRP and PAI-1.

As the patient's disease develops, the endothelial dilatation will be evidently decreased. It was found in the results of this study that in patients with severe CAD, the secretion of ET-1 was obviously increased, while the secretion of NO was remarkably reduced, indicating that the endothelial function is more severely damaged in these patients, which leads to the weakened vascular dilatation and lower pressure of blood flow to the vascular wall, thus triggering a proliferative change in the vascular wall and eventually resulting the reduced elasticity of blood vessels. Therefore, FMD is negatively correlated with ET-1 and positively correlated with NO.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YC wrote the manuscript. YC and TS were responsible for the detection of brachial artery endothelial dilatation. CY and SW helped with carotid atherosclerosis examination. ZL and YT worked on the determination of the flow-mediated dilatation. ZZL and HZ analyzed and interpreted laboratory indicators. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the ethics committee of Beijing Anzhen Hospital and written informed consents were signed by the patients and/or guardians.

Consent for publication

Not applicable.

Interest conflic

The authors declare that they have no competing interests

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