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Original Article

Identification of plasma vascular endothelia-cadherin as a biomarker for coronary artery disease in Type 2 diabetes mellitus patients

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Abstract: Objects: To examine how vascular endothelia (VE)-cadherin plasma levels are correlated with parameters associated with endothelial function such as endothelin-1, nitric oxide, nitric oxide synthase and HbA_{1c} in type 2 diabetic patients with coronary artery disease. Methods: VE-cadherin levels were analyzed by enzyme-linked immunosorbent assays. Spearman's correlation and multiple stepwise regression analyses were used to examine the relationship between plasma VE-cadherin and other factors. Results: By univariate correlation analysis, plasma VE-cadherin levels were significantly associated with age, total cholesterol, triglyceride, hemoglobin A_{1c}, and endothelin-1. Multiple regression analysis (adjusted for age, total cholesterol, and triglyceride) showed that plasma VE-cadherin levels were independently and significantly associated with HbA_{1c} and ET-1. Plasma VE-cadherin levels were significantly highest in patients with diabetes mellitus and coronary artery disease. While patients with diabetes mellitus had higher levels of VE-cadherin compared with healthy subjects. Conclusions: This study found that VE-cadherin levels might be a biomarker for some endothelial dysfunction associated with coronary artery disease in type 2 diabetes mellitus.

Keywords: Vascular endothelia-cadherin, coronary artery disease, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus is associated with a markedly increased incidence of cardiovascular diseases, accounting for ~70 % of diabetic mortality [1, 2]. In the background of cardiovascular complications, disorders of microcirculation and endothelial dysfunctions precede atherosclerosis. The healthy endothelium responds to various stimuli by production of factors that regulate vascular tone, smooth muscle cell proliferation and vessel wall inflammation [3]. VE-cadherin is an endothelial-specific cell-cell adhesion protein of the adherens junction complex. VE-cadherin plays a key role in endothelial barrier function and angiogenesis, and also participates directly and indirectly in intracellular signaling pathways that control cell dynamics and cell cycle progression [4]. Elevated levels of VE-cadherin were found in cardiometabolic patients with or without diabetes mellitus [5, 6]. However, the molecular pathomechanisms of impaired endothelium-dependent in cardiovascular diseases are complicated and remains incompletely understood. In the present study, we investigated the relation of plasma levels of circulating VE-cadherin to parameters associated with endothelial function in type 2 diabetic patients with coronary artery disease (CAD). To further understanding of the functions of VE-cadherin will provide clues to their roles in cardiovascular diseases.

Methods

Patients

The study incorporated enrollment of 85 Chinese patients with Type 2 diabetes mellitus (T2DM) at our Hospital, recruited from October, 2010 to March, 2011. T2DM is defined by the criteria of World Health Organization (WHO) [7]. Coronary artery disease is defined as angio-

Table 1. Baseline characteristics of 165 study subjects

	Group A (n = 40)	Group B (n = 45)	Group C (n = 80)
Age (years)	55.28 ± 12.01	57.18 ± 7.06	52.10 ± 10.31
BMI (kg/m^2)	24.2 ± 2.11	25.47 ± 3.32	23.73 ± 1.78
Hypertension, n (%)	39 (97.5)	43 (95.6)	79 (98.8)
TC (mmol/L)	2.44 ± 1.50	2.36 ± 1.02	2.19 ± 0.39
TG (mmol/L)	1.77 ± 0.89	1.61 ± 0.71	1.57 ± 0.86
LDL-C (mmol/L)	2.87 ± 0.79	2.77 ± 1.80	2.48 ± 0.94
HDL-C (mmol/L)	1.13 ± 0.19	1.26 ± 0.25	1.19 ± 0.38
HbA_{1c} (%)	7.58 ± 1.18[a,b]	5.84 ± 0.31[a]	4.68 ± 0.24
VE-cadherin (ug/ml)	1.87 ± 0.14[a,b]	1.77 ± 0.11[a]	1.66 ± 0.17
ET (pg/ml)	49.94 ± 5.92[a,b]	46.44 ± 1.59[a]	44.37 ± 3.20
NO (umol/L)	37.56 ± 4.10[a,b]	45.13 ± 13.20[a]	53.21 ± 6.95
NOS (umol/L)	13.97 ± 3.71[a,b]	16.75 ± 3.10[a]	21.8 ± 8.18

Data are expressed as mean ± S.D. Abbreiations: BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA_{1c}, hemoglobin A_{1c}; ET-1, endothelin; NO, nitric oxide; NOS, nitric oxide synthase. [a]Compared with group C, $P < 0.05$; [b]Compared with group B, $P < 0.05$. Group A: T2DM patients with coronary artery disease, Group B: T2DM patients without coronary artery disease or other complications, Group C: Chinese healthy subjects.

graphic evidence of stenosis in more than 30% of the major coronary arteries and more than 50% of branching coronary arteries. Two well matched groups were identified for comparison. Group A (n = 40) consisted of T2DM patients with coronary artery disease. Group B (n = 45) consisted of T2DM patients without coronary artery disease or other complications. Another 80 Chinese healthy subjects (non-diabetic, non-cardiovascular volunteers) were recruited as healthy control group C. Patients with acute phase and unstable conditions including severe valvular disease, active infection, untreated malignant diseases, active autoimmune disease, and severe congestive heart failure were excluded.

Written informed consent was obtained from all the patients before enrollment in the study. This study was performed and approved by our hospital.

Data collection and laboratory measurements

Height (cm) and weight (kg) were measured. BMI was calculated as weight in kilograms divided by the square of height in meter. Hypertension was recorded if the patient was taking antihypertensive drugs or had two separated measured blood pressures ≥ 140/90 mmHg. Blood were sampled after overnight fasting by venipuncture to check for total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hemoglobin A_{1c} (HbA_{1c}), VE-cadherin, endothelin (ET)-1, nitric oxide (NO), and nitric oxide synthase (NOS). TC, TG, LDL-C, and HDL-C were measured by standard enzymatic methods. HbA_{1c} was measured by an automated ion-exchange chromatographic method (Bio-Rad, Her cules, CA, USA) VE-cadherin, ET-1, nitric oxide, and nitric oxide synthase concentrations were determined by a quantitative sandwich ELISA using commercially available kits (R&D Systems, Inc., Minneapolis, USA).

Statistical analysis

Variables are presented in this study as mean ± SD. Statistical significance was tested using unpaired Student test. Spearman rank correlation matrix and multiple stepwise regression analysis were used to examine the relationship between VE-cadherin and other factors. Factors that were significantly correlated withplasma VE-cadherin were selected for multiple stepwise regression analysis. Statistical significance was defined as $P < 0.05$ using two-tailed test. Statistical analyses were performed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of the patients can be seen in **Table 1**. According to this table, levels of HbA_{1c}, VE-cadherin, and ET-1 in group A and B patients were significantly higher than group C, in group A patients were significantly higher than group B. Meanwhile, levels of NO and NOS in group A and B patients were significantly lower than group C, in group A patients were lower than group B. However, there were no significant differences among three group patients in terms of age, BMI, hypertension, TC, TG, LDL-C, and HDL-C.

Using Spearman rank correlation analysis, plasma VE-cadherin levels were most signifi-

Table 2. Factors associated with plasma VE-cadherin levels

Factor	VE-cadherin (n = 165)	
	r	P
Age	0.005	0.007
BMI	0.071	0.089
Hypertension	0.008	2.35
TC	0.047	0.015
TG	0.045	0.02
LDL-C	0.112	0.213
HDL-C	0.209	1.67
HbA_{1c}	0.962	< 0.001
ET-1	0.084	< 0.001
NO	-0.086	0.579
NOS	-0.221	0.453

Abbreiations: BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA_{1c}, hemoglobin A_{1c}; ET-1, endothelin; NO, nitric oxide; NOS, nitric oxide synthase.

Table 3. Multiple stepwise regression analysis adjusted for age, TC, TG, HbA_{1c}, and ET

Factors	VE-cadherin	
	β	P
HbA_{1c}	0.28	< 0.001
ET-1	0.115	< 0.001

β is a standardized coefficient and a p alue less than 0.05 was considered statistically significant. Abbreiations: BMI, body mass index; TC, total cholesterol; TG, triglyceride; HbA_{1c}, hemoglobin A_{1c}; ET-1, endothelin.

cantly associated with HbA_{1c} ($r = 0.962$, $P < 0.001$). The second most significant association was observed between plasma VE-cadherin and ET ($r = 0.084$, $P < 0.001$). VE-cadherin levels were also associated with age ($r = 0.05$, $P = 0.007$), TC ($r = 0.047$, $P = 0.015$), TG ($r = 0.045$, $P = 0.02$). No significant correlation was seen between VE-cadherin levels and BMI, hypertension, LDL-C, or HDL-C (**Table 2**). We conducted multiple regression analysis adjusted for age, TC, TG, HbA_{1c}, and ET-1. Plasma VE-cadherin levels were significantly associated with HbA_{1c} and ET-1 (**Table 3**).

Discussion

Endothelial dysfunction is thought to be a crucial cause of diabetic cardiovascular complications. And it is well established that ET-1, NO, and NOS are key factors of normal function of endothelium [8]. In the present study, we found that plasma levels of ET-1 was elevated, while NO and NOS were declined significantly in patients with DM compared with non-diabetic and non-cardiovascular control patients. Furthermore, similar statistical significances existed between diabetic patients with CAD and diabetic patients without CAD. These results predicted the presence of endothelial dysfunction in diabetic patients, and more serious endothelial dysfunction in diabetic patients with CAD. These conclusions were in line with previous findings in other studies [9, 10].

VE-cadherin is expressed specifically by only endothelial cell to maintain stability of the endothelial cell adherens junction and also suppresses apoptosis of endothelial cell [11, 12]. The levels of VE-cadherin associated with the degree of in vivo endothelial cell injury [5]. In this study we obtained the similar association between VE-cadherin and endothelial injury. In additional, endothelial dysfunction is associated with cardiovascular diseases. These results suggested that plasma VE-cadherin level could be a promising biomarker for CAD in patients with T2DM.

The plasma level of VE-cadherin was independently and significantly associated with HbA_{1c} and ET-1 (**Table 3**). Subjects with high levels of VE-cadherin exhibited higher levels of HbA_{1c} and ET-1. Hyperglycaemia has been shown to have a direct effect on endothelial cell, acting as a pro-inflammatory [13] and pro-permeability [14] agent. The proximity of the transmembrane junctional molecules to surface receptors of pro-angiogenic and pro-permeability growth factors, such as vascular endothelial growth factor (VEGF), allows the junctional molecules to act as receptors for external signaling [15, 16]. VEGF has been shown to induce the phosphorylation of VE-cadherin, β-catenin [15]. In diabetes, endothelial cell produces VEGF faster [17, 18]. As a consequence of phosphorylation, VE-cadherin release from junctional regions resulting in increased quantities of plasma VE-cadherin and paracellular permeability.

Hyperglycaemia also leads to an increase ET-1 production, and ET-1 then feeds back to increased inflammation [19]. ET-1 not only constricts vessels but also recruits neutrocytes on the surface of endothelial cells. The more neu-

trocytes aggregate, the more endothelium damage it accumulates through produce toxic products such as proteases, oxygen free radicals [20]. Injury endothelial cells release VE-cadherin into blood, and the level of plasma VE-cadherin is elevated consequently.

Several studies had reported that the production of NO and NOS were inhibited in DM subjects [21, 22]. The similar results were observed in this study. Decreased NO and NOS also play a part in glucose-induced endothelium injury [23-26]. However, may be due to small sample size, we had not found association between VE-cadherin and NO and/or NOS in this study.

Limitation of our study was that the results were based on relatively small study samples. More large well-designed studies, involving more relevant variables, should be warranted in future.

In conclusion, we demonstrated the VE-cadherin increased in plasma of patients with T2DM, especially in those individuals with CAD. Therefore, VE-cadherin may be a useful surrogate marker for evaluating endothelial dysfunction and/or injury. Treatment of CAD patients that focuses on the integrity and condition of ECs based on assessments of plasma VE-cadherin levels may provide potential therapeutic strategies for preventing cardiovascular complications.

Disclosure of conflict of interest

None.

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