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Original Article

Association of serum SPARC level with severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease

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Abstract: Objective: To investigate the association of serum SPARC level with the severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease. Methods: 120 patients with type 2 diabetic patients were the subjects. Enzyme-linked immunosorbent assay (ELISA) was used to detect levels of serum SPARC and Gensini score was used to assess extent of coronary artery lesions. The patients were divided into 4 groups: A group was the healthy control group with 40 patients. According to angiography and the World Health Organization (WHO) diagnostic criteria for diabetes the rest were divided into B, C, D group: there were 40 cases in group B (simple type 2 diabetes mellitus group), 40 cases were in group C (simple CHD group), and 40 cases were in D group (type 2 diabetes combined with coronary heart disease group). Results: Compared with that in group A, the serum SPARC level in group B, C and D increased significantly (4.22±1.19) μg/L, (3.71±1.05) μg/L and (5.96±1.40) μg/L vs (3.60±0.40) μg/L ($P<0.05$). Moreover, the serum SPARC level in group D was the highest ($P<0.05$). Serum SPARC level, insulin resistance (IR), and glycosylated hemoglobin (HbA1c) were the vital factors contributing to coronary heart disease. Serum SPARC level was positively correlated with the Gensini scores in group D ($r=0.770$, $P<0.05$), whereas it was not related to the Gensini scores in group C ($r=0.520$, $P>0.05$). Pearson correlation analysis showed that serum SPARC level was positively correlated with triglyceride, fasting insulin, Homeostasis Model Assessment for Insulin Resistance Index ($r=0.780$, 0.762 and 0.891, respectively; $P<0.05$). Conclusion: Serum SPARC level elevated in T2DM patients with coronary heart disease, which was correlated with the severity of coronary artery disease significantly.

Keywords: Type 2, diabetes, coronary heart disease, secreted protein acidic and rich in cysteine

Introduction

In recent years, the incidence of endocrine and metabolic diseases are gradually on the rise with the changes of lifestyle and diet, where coronary heart disease is the leading cause of death in patients with type 2 diabetes. Alone for the severity of coronary atherosclerosis, the type 2 diabetes patients combined with coronary heart disease was more serious than the patients with simple coronary heart disease. But the specific pathogenesis of type 2 diabetes combined with coronary heart disease is not yet entirely clear. Osteonectin (secreted protein acidic and rich in cysteine, SPARC) is a secreted protein acidic which are rich in cysteine. Previous studies have showed that it can express [1, 2] in type 2 diabetes and coronary heart disease, but the relationship between SPARC and type 2 diabetes combined with coronary heart disease has not been reported. In this study, the levels of serum SPARC and coronary angiography were investigated and explored their relationship.

Material and methods

Subjects

From October 2013 to June 2014 in our hospital 120 cases of patients were hospitalized in Endocrinology and Cardiology, including 40 cases of patients with simple type 2 diabetes (18 males, 22 females), 40 cases of patients with simple coronary heart disease (25 males and 15 females), 40 cases of patients with type

Table 1. Comparison of SPARC and biochemical parameters of subjects in each group (Mean ± standard deviation)

Group	Average Age (y)	Gender (M/F)	BMI (kg/m^2)	Duration of diabetes (y)	SPARC (ug/l)	SBP (mmHg)	DBP (mmHg)	FBG (mmol/l)	2h-FBG (mmol/l)
A	42.8±10.63	20/20	20.86±1.57	-	3.60±0.40	113.58±18.92	73.25±11.27	4.34±0.21	6.26±0.78
B	49.7±11.42	18/22	22.91±0.92	8.56±7.32	4.22±1.19[a]	126.21±18.17	74.82±11.39	11.26±1.08[a,c]	11.83±2.34[a,c]
C	41.2±12.01	25/15	21.24±1.38[c]	-	3.71±1.05[a]	128.51±13.73	79.31±11.58	4.62±0.27	4.79±0.23
D	51.3±11.79	21/19	28.32±8.29[a,b,c]	11.82±8.65	5.96±1.40[a,b,c]	134.92±14.27[a,b,c]	78.23±8.38	12.13±1.82[a,c]	16.04±2.07[a,c]
P value	>0.05		<0.05	>0.05	<0.05	<0.05	>0.05	<0.05	<0.05
F value	4.673		5.297	4.262	5.391	5.531	2.113	5.921	6.236

Group	f-INS (mUl/l)	2h-INS (mUl/l)	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	HbA1c (%)	HOMA-IR
A	3.7±1.3	32.6±4.9	1.87±0.93	1.21±0.68	1.32±0.33	2.18±0.32	5.2±0.5	1.79±0.45
B	8.4±3.1	20.8±5.2[a,c]	5.86±0.71[a]	2.72±0.71	1.29±0.37[a]	3.14±1.58	7.8±1.3	3.11±0.82
C	3.2±1.5	29.2±3.6	5.37±0.86[a,b]	3.38±1.12[a,b]	1.39±0.28[a,b]	3.82±0.18[a,b]	4.7±0.6[a,c]	2.03±0.27[a]
D	11.6±2.7[a,c]	12.4±4.1[a,b,c]	5.41±1.05	4.18±1.21[a,b,c]	1.02±0.26[a,b,c]	4.63±1.68[a,b,c]	8.8±1.5[b]	3.62±1.13[a,b]
P value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
F value	6.634	4.864	5.671	6.525	6.871	5.153	5.632	6.398

Note: Group A: healthy control, group B : type 2 diabetes mellitus, group C: coronary heart disease, group D: type 2 diabetes combined with coronary heart disease; BMI: Body mass index; SPARC: Secreted protein acidic and rich in cysteine; SBP: Systolic blood pressure; I mmHg = 0.133 kPa; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; f-INS: Fasting serum insulin; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein. Cholesterol; TG: Triglyceride; HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment for insulin resistance index; a: Compared with group A, P<0.05; b: Compared with group B, P<0.05; c: Compared with group C, P<0.05.

2 diabetes combined with coronary heart disease (21 males, 19 females), Alternatively 40 cases of healthy volunteers (20 males, 20 females) were involved. The above patient were named group A (healthy), group B (simplex type 2 diabetes), group C (simple CHD patients), D group (type 2 diabetes patients with coronary heart disease). Inclusion criteria: 1999 WHO diagnostic criteria was used for diagnosis of diabetes: the symptoms of diabetes combined with plasma glucose ≥11.1 mmol/l (200 mg/dl) at any time, or FPG ≥7.0 mmol/l (126 mg/dl), or OGTT2Hpg ≥11.1 mmol/l (200 mg/dl), and it is need to be repeated to confirm with diabetes. Type 2 diabetes need to eliminate type 1 diabetes, gestational diabetes, and special types of diabetes; Diagnosis criteria of coronary atherosclerotic heart disease: according coronal angiography results showed that at least one diameter of the left main, left anterior descending artery, circumflex artery and the right coronary artery stenosis was more than 50% that can be diagnosed. Exclusion criteria: acute complications of diabetes: diabetic ketoacidosis, lactic acidosis, hyperosmolar hyperglycemic state; chronic complications of diabetes: diabetic nephropathy, diabetic retinopathy, diabetic peripheral neuropathy; recent stressful events: surgery, trauma, infection, etc.; severe liver and kidney dysfunction, cancer, hyperlipidemia, arthritis; the recent history of hormone use, previous old myocardial infarction.

Methods

SPARC determination: Serum specimens were collected from patients after 8-10 h fasting and fasting in the morning from upper extremity venous. They stand for 30 min and then 2500 r/min centrifugation was performed after 4°C for 25 min. Take the supernatant, and the SPARC was tested by enzyme-linked immunosorbent assay (ELISA). The procedure of the test was in accordance with the kit instructions (kit was provided by Xiamen Jia Hui biotechnology Co., Ltd.).

Determination of biochemical indicators: 3 ml fasting venous blood were collected from all the subjects and used for measuremets in chemical and biological laboratory and endocrine laboratory. 75 g glucose load insulin, C-peptide release test were performed on some patients. Blood pressure was measured when patients were admitted to hospital; homeostasis model was used to assess insulin resistance index: (HOMA-IR) = FINS (mu/l) × FPG (mmol/l)/22.5.

Quantitative analysis of coronary stenosis by Gensini: No stenos recorded 0 point. 1% to 25% recorded 1 point. 26%-50% recorded 2 points. 51% to 75% recorded 4 points. 76% to 90% recorded 6 points. 91% to 99% recorded 16 points. 100 percent recorded 32 points. Left main disease recorded five points. Left anterior descending artery or circumflex artery proximal lesion recorded 2.5 points. The middle of the left anterior descending artery lesion recorded 1.5 points. The left anterior descending artery distal lesions recorded 1 point. Lesions from middle and far sections of left circumflex artery recorded 1 score. Right coronary artery lesions recorded 1 score. Small branch lesions recorded 0.5. Total score of coronary artery lesions were the sum of each segment.

Statistical methods

SPSS13.0 statistical software was used for analysis; measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$); t test was used to compare the biochemical indices and serum SPARC level in each group; Pearson correlation analysis was used for correlation analysis; Logistic regression analysis was used to assess the relevance between SPARC and type 2 diabetes combined with coronary heart disease; $P<0.05$ was considered statistically significant.

Results

Comparison of SPARC and biochemical parameters of subjects in each group (**Table 1**).

SPARC comparison

SPARC levels in Group B, group C and group D was significantly higher than that in A group ($P<0.01$), which was the highest in D group (all $P<0.05$), but no significant difference had been found between group B and group C.

Biochemical data were compared

In B, C, D groups, TG, TC, LDL-C levels were higher than those in group A ($P<0.05$); compared with group B, FINS, HbA1c and IR in D group were higher, and the difference was statistically significant ($P<0.05$).

Table 2. Multivariate Logistic regression analysis of all relevant factors for type 2 diabetes combined with coronary heart disease

Variable	B	SE	Wald	df	*P* value	OR value	95%	CI
SPARC (ug/l)	0.905	0.193	13.721	1	0.004	1.056	0.879	1.183
HOMA-IR	0.645	0.121	10.336	1	0.002	1.457	1.356	1.676
HbA1c	0.641	0.032	15.334	1	0.003	1.194	1.053	1.335
2h-INS	0.667	0.332	0.832	1	0.075	1.142	0.731	1.552
TG	0.826	0.021	0.756	1	0.673	1.083	0.812	1.353
TC	0.015	0.167	1.741	1	0.921	1.109	0.776	1.441

Note: SPARC, HOMA-IR, HbA1c, 2h-INS, TG, TC were used as the independent variables to performed Logistic regression analysis.

Table 3. Correlation between SPARC and TG, FINS, HOMA-IR and Gensini scores

Correlation		TG	FINS	HOMA-IR	Gensini scores			
					Group A	Group B	Group C	Group D
SPARC	r	0.780	0.762	0.891	-	-	0.52	0.77
	P	<0.05	<0.05	<0.05			>0.05	<0.05

Logistic regression analysis

In patients with type 2 diabetes, the complication of coronary heart disease was used as the dependent variable and SPARC, HOMA-IR, HbA1c, 2h-INS, TG, TC were used as the independent variables to performed Logistic regression analysis; the results showed that: serum SPARC level, IR and HbA1c were the influencing factors for coronary heart disease, P<0.05, the regression coefficient >1, OR value >1, indicating the positive correlation with the incidence and development of type 2 diabetes combined with coronary heart disease (**Table 2**).

Relationship between SPARC levels and Gensini integration

In D group, SPARC levels were positively correlated with Gensini scores (r=0.77, P<0.05). In group C, SPARC levels had no significant correlation with Gensini scores (r=0.52, P>0.05, **Table 3**).

Pearson correlation analysis showed that SPARC were significantly positively correlated with TG, FINS and HOMA-IR (r=0.780, 0.762, 0.891, all P<0.05).

Discussion

SPARC is an extracellular matrix-related glycoprotein with small molecule; it can be secreted by heart, brain, kidney, pancreas and skeletal muscle, but the SPARC in human circulating blood is mainly from differentiated subcutaneous adipose tissue [3]. Based on their participation in angiogenesis and repair of damaged tissues [4], clinical research pay more attention to the relationship of SPARC with tumor [5]; but recent research has shown that SPARC is involved in the pathophysiological processes of obesity [3], insulin resistance [6], type 2 diabetes [7, 8] and its complications, such as: diabetic nephropathy [9], diabetic retinopathy [10] as well as gestational diabetes [11]. Type 2 diabetes not only is an independent risk factor for cardiovascular disease, but also has many common risk factors with cardiovascular disease [12], so the close relationship between SPARC and type 2 diabetes and its complications suggests that there is a certain relevance between SPARC and the incidence of coronary heart disease.

The results of this study confirmed that SPARC level in simplex type 2 diabetes group, coronary heart disease alone group and type 2 diabetes combined with coronary heart disease group were higher than that in the normal control group, and it was the highest in type 2 diabetes patients with coronary heart disease; there were no significant differences between simple type 2 diabetes group and coronary heart disease alone group, which was consistent with the conclusion of previous studies that SPARC may be associated with the onset of diabetes and its associated complications; it also prompted that SPARC may be involved in the development of coronary heart disease, and due to diabetes has the same risk as coronary heart disease, the severity of coronary atherosclerosis of patients with coronary heart disease combined with diabetes can increase. The biochemical data showed that: in simple type 2 diabetes group, coronary heart disease alone group and type 2 diabetes combined with coronary heart disease group, TG, TC and LDL-C levels were higher than those in the normal con-

trol group, indicating that lipid was related with the incidence of diabetes and coronary heart disease. Therefore, lipid control can delay the progression of diabetes and coronary heart disease. Compared with the simple type 2 diabetes group, FINS, HbA1c and IR in Type 2 diabetes complicated with coronary heart disease group were higher, indicating that the higher the blood glucose, the higher the risk of coronary heart disease in type 2 diabetes patients. Multivariate analysis showed that SPARC, IR and HbA1c were independent factors of type 2 diabetes complicated with coronary heart disease, and SPARC was positively correlated with HbA1c and IR, which was consistent with the previous findings [13]; research has shown that: every 1% increase in HbA1c, the risk of coronary heart disease increased by 10%, which probably due to that: HbA1c increase represented the hyperglycemia state in nearly 2-3 months in the body, which can cause high coagulation, inflammation and oxidative stress, so as to promote cells to secrete SPARC. SPARC level and Gensini integral analysis showed that: in type 2 diabetes combined with coronary heart disease group, SPARC level was positively correlated with Gensini score, indicating that SPARC level was related to the degree of coronary artery stenosis; while in simple CHD group, SPARC level was not correlated with Gensini score, suggesting that SPARC level was positively correlated with coronary artery atherosclerosis extent, which may related with that SPARC damaged the vascular barrier and thus participated in the formation of coronary atherosclerosis [14-16]; so SPARC levels can be used as an predictor of coronary sclerosis; If we can reduce SPARC content, the onset of type 2 diabetes combined with coronary heart disease would be delayed. Pearson correlation analysis results suggest that SPARC had significantly positive correlation with TG, FINS and HOMA-IR, which is consistent with the finding of previous studies that SPARC was involved in insulin resistance.

SPARC-induced type 2 diabetes combined with coronary heart disease may be mainly caused by: SPARC is involved in the occurrence and development of insulin resistance: Insulin resistance is an important characteristic of type 2 diabetes. Since SPARC can cause fatty fibrosis, increase the excessive lipid in circulation and re-locate it in liver, pancreas, blood vessels and other non-adipose tissues, which not only causes TG increase in the circulating blood, but also causes insulin resistance; in addition, SPARC can promote GLUT4 (glucose transporter 4) to uptake glucose by increasing AMPK (AMP-activated protein kinase) expression, leading to glucose and lipid metabolism disorders in muscle [17]; moreover, SPARC is also involved in insulin resistance by activating PI3K/AKT pathway, which is the main insulin transduction pathway; SPARC and inflammatory factors: coronary heart disease was the lesion caused by severe vascular stress reactions; SPARC can lead to changes in the structure of extracellular matrix (ECM) by affecting the deposition of fibronectin and laminin [18], regulate cell migration, and has anti-cell adhesive effect; when body oxidative stress occurs, SPARC secretion increases. Studies have shown that and SPARC is closely related to inflammatory response factors and fat factors [19]: adiponectin can relieve the inflammation of endothelial cells, protect vascular endothelial and increase insulin sensitivity; SPARC was positively correlated with adiponectin; leptin can activate the tyrosine kinase (JAK) signaling system-related factors to be involved in the formation of atherosclerosis, and SPARC was negatively correlated with leptin; PAI-1 can inhibit the activity of fibrinolysis enzyme which can destroy the basement membrane growth of fat cells, thus promoting the thrombosis; SPARC can contribute to the increase in PAI-1mRNA levels [20]; SPARC can increase the activity and content of matrix metalloproteinase (MMP3) to increase endothelial cell permeability and destroy the barrier, thus promoting cell migration and affecting damage repair; furthermore SPARC also has some relevance with tumor necrosis factor-α (TNF-α) and macrophage migration inhibitory factor (MMIF) Since inflammatory cytokine itself can cause atherosclerosis, and it is also closely related to the formation of insulin resistance, thus SPARC can promote the occurrence of coronary artery disease through this synergistic effect. In addition, SPARC can dissolve the cell adhesion plaques to mediate anti-adhesion effect, and ultimately affect the vascular repair; and when tissue is damaged, a variety of pro-growth-repair factors are associated with SPARC; related research has shown that SPARC can inhibit the proliferation and differentiation of VEGF, PDGF and FGF-stimulated fibroblasts, smooth muscle cells

and endothelial cells to impede vascular repair process, damage the blood vessel barrier to induce atherosclerosis, and promote the deposition of smooth muscle cells in the intima to speed vascular endothelial atherosclerosis.

In summary, the present study has found that in type 2 diabetic patients combined with coronary heart disease, SPARC level was significantly increased and SPARC level was positively correlated with the degree of coronary artery stenosis; therefore SPARC may be related with the incidence of coronary heart disease combined with type 2 diabetes. The specific mechanism needs to be confirmed by large-sample clinical trials.

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Disclosure of conflict of interest

None.

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