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Correlation of serum PCSK9 in CHD patients with the severity of coronary arterial lesions

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Abstract. – OBJECTIVE: The present study aims to investigate the correlation between serum level of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the severity of coronary arterial lesion in patients with coronary heart disease (CHD).

PATIENTS AND METHODS: Between August 2010 and January 2015, 126 CHD patients and 70 patients with coronary arterial stenosis < 50% (controls) were included in the present study. Serum PCSK9 level was determined using ELISA. Demographic characteristics, relevant clinical data and biochemical data were collected from all patients, and their relationship with PCSK9 was analyzed to evaluate the correlation of PCSK9 expression with the severity of coronary artery disease (CAD).

RESULTS: Concentrations of total cholesterol (TC) and fasting blood sugar (FBS) were significantly higher in CHD patients than in controls ($p < 0.05$). No significant differences were observed in gender, age, body mass index (BMI), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), family history, smoking history and history of hypertension between groups ($p > 0.05$). Serum PCSK9 levels in the CHD group were significantly higher than those in the control group [(96.4 ± 33.2) ng/mL *vs.* (81.8 ± 27.6) ng/mL, $p < 0.05$]. Compared with those of patients with single-vessel or double-vessel disease, PCSK9 levels were significantly elevated in patients with multi-vessel disease ($p < 0.05$). The Gensini score of the CHD group was significantly lower than that of the control group (11.4 ± 10.5 *vs.* 37.3 ± 10.3, $p < 0.05$). The Gensini score of patients with multi-vessel disease was significantly higher compared with patients of single-vessel or double-vessel disease ($p < 0.05$). Correlation analysis revealed that PCSK9 was positively correlated with many clinical parameters, including age, BMI, TC, TG, systolic blood pressure, FBS, Gensini score and LDL-C ($p < 0.05$). However, PCSK9 was not correlated with either gender ratio or diastolic blood pressure ($p > 0.05$).

CONCLUSIONS: Serum PCSK9 level is significantly elevated in CHD patients and its variation is correlated with the severity of CAD.

Key Words:
Coronary heart disease, Coronary artery disease, Proprotein convertase subtilisin/kexin type 9, Correlation.

Introduction

Coronary heart disease (CHD) has become the most common disorder in the elderly in China along with accelerated aging. CHD, also known as coronary artery disease (CAD), refers to the development of atherosclerotic plaque which is a leading cause of morbidity and mortality, resulting from lipid deposition in the intima of coronary arteries caused by dyslipidemia[1-3]. The accumulation and deposition of low-density lipoprotein cholesterol (LDL-C) under vascular endothelium has been reported to be the primary pathogenesis of coronary atherosclerosis. However, reduction of plasma LDL cholesterol reduces adverse cardiovascular outcomes[4,5]. Moreover, proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in the *in vivo* metabolism of LDL-C and has a certain relationship with the pathogenesis and development of CHD. Currently, most studies of PCSK9 are focused on its effect on LDL-C metabolism while few studies have been reported on the correlation between PCSK9 and the severity of the coronary arterial lesion. To this end in the present study serum PCSK9 levels in CHD patients were measured and its correlation with the severity of CAD investigated, in an effort to explore the significance of PCSK9 variation in CHD patients.

Patients and Methods

Patients

Between August 2010 and January 2015, 196 CHD patients admitted in our institution (Henan

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Provincial People's Hospital, Zhengzhou, China) were included in the present study. Inclusion criteria are listed as follows: (1) Diagnoses of all patients satisfy the diagnostic criteria of CHD[6]. (2) At least one branch of epicardial stenosis ≥ 50% was confirmed by coronary angiography. (3) Patients had neither received lipid-lowering therapy, nor history of thyroid dysfunction, autoimmune diseases and infectious diseases in recent three months. (4) Patients had no history of severe disorders of the liver, the kidney or the brain. As a result, 126 patients (male 58, female 68) with mean age of 63.4 ± 5.1 years were included in the CHD group. Of these patients, 45 were confirmed with single-vessel disease, 44 double-vessel disease and 37 multi-vessel disease. In addition, 70 patients (male 32, female 38, mean age 64.2 ± 6.1 years) with coronary artery stenosis < 50% during the same period were included in the control group. This study was approved by the medical Ethics Committee of our institution (Henan Provincial People's Hospital, Zhengzhou, China) and written informed consent was obtained from all patients.

Outcome Measures

Medical history of all patients was collected, and height as well as weight measured and documented. Coronary angiography was performed with standard Judkins technique[6] for both left and right coronary arteries in multiple positions and results documented. Fasting venous blood samples of patients were collected in the morning and analyzed by using MICRQ semi-automatic biochemistry analyzer (Ahmedabad, The Netherlands) to determine the levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), LDL-C and fasting blood sugar (FBS). In addition, anticoagulated fasting blood samples were collected from all patients for measuring plasma levels of PCSK9 by using ELISA kit (Immundiagnostik, Benshein, Germany).

CAD Severity Evaluation

Based on the results of coronary angiography, the severity of arterial lesions were evaluated using Gensini score[7,8], which grades narrowing of the lumen as 1 for 1-25 % stenosis, 2 for 26-50%, 4 for 51-75 %, 8 for 76-90 %, 16 for 91-99 % and 32 for total occlusion. This score was multiplied by a factor that accounted for the importance of a lesion's position in the coronary arterial tree: 5 for the left main coronary artery; 2.5 for the proximal left anterior descending coronary artery or proximal circumflex artery; 1.5 for the mid left anterior descending coronary artery; 1 for the proximal right coronary artery, distal left anterior descending coronary artery, obtuse marginal artery or posterior lateral artery; and 0.5 for other stenoses. The severity of disease was expressed as the sum of the scores for the individual lesions.

Statistical Analysis

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± Standard Deviation (± SD). Differences of data were analyzed using independent sample *t*-test. Logistic regression analysis was adopted to analyze the correlation between data. Categorical data were analyzed using chi-square test. $p < 0.05$ was considered statistically significant.

Results

Clinical Data

Concentrations of TC and FBS were significantly higher in CHD patients than in controls ($p < 0.05$). No significant differences were observed in gender, age, body mass index (BMI), TG, LDL-C, HDL-C, family history, smoking history and history of hypertension between groups ($p > 0.05$) (Table I).

PCSK9 Levels and Gensini Score

Plasma levels of PCSK9 in the CHD group were significantly higher than those in the control group [(96.4 ± 33.2) ng/mL *vs.* (81.8 ± 27.6) ng/mL, $p < 0.05$)]. Compared with those in patients with single-vessel or double-vessel disease, PCSK9 levels were significantly elevated in patients with multi-vessel disease ($p < 0.05$). The Gensini score of the CHD group was significantly lower than that of the control group (11.4 ± 10.5 *vs.* 37.3 ± 10.3, $p < 0.05$). The Gensini score of patients with multi-vessel disease was significantly higher compared with patients of single-vessel or double-vessel disease ($p < 0.05$) (Table II).

The Correlation of PCSK9 With Other Clinical Parameters

Correlation analysis revealed that PCSK9 was positively correlated with many clinical parame-

Table I. Clinical data of two groups ($\bar{x} \pm s$).

Group	CHD group (n = 126)	Control group (n = 70)	t	p
Gender ratio (male/female)	58/68	32/38	0.002	0.966
Mean age	63.4 ± 5.1	64.2 ± 6.1	-0.980	0.164
BMI (kg/m^2)	23.9 ± 4.7	25.1 ± 3.5	-0.934	0.176
FBS (mmol/L)	7.11 ± 1.42	6.38 ± 1.28	3.570	0.000
TC (mmol/L)	4.65 ± 0.93	4.22 ± 1.02	2.995	0.002
TG (mmol/L)	1.62 ± 0.56	1.59 ± 0.98	0.273	0.393
LDL-C (mmol/L)	2.78 ± 0.74	2.51 ± 0.82	1.482	0.048
HDL-C (mmol/L)	1.07 ± 0.41	1.13 ± 0.35	-1.033	0.151
Family history	14	6	0.317	0.574
Smoking history	18	14	1.076	0.300
History of hypertension	59	41	2.484	0.115

Table II. Serum PCSK9 levels.

Group	n	PCSK9 (ng/mL)	Gensini score
CHD group	126	94.4 ± 33.2*,#	37.3 ± 10.3*,#
Single-vessel disease	45	87.7 ± 26.3	15.7 ± 12.4
Double-vessel disease	44	93.1 ± 25.7*	23.6 ± 10.7*
Multi-vessel disease	37	108.6 ± 30.1*,#,&	56.2 ± 11.3*,#,&
Control group	70	81.8 ± 27.6#	11.4 ± 10.5#

*$vs.$ controls, $p < 0.05$; #$vs.$ single-vessel disease, $p < 0.05$; &$vs.$ double-vessel disease, $p < 0.05$.

ters, including age, BMI, TC, TG, systolic blood pressure, FBS, Gensini score and LDL-C ($p < 0.05$); however, PCSK9 was not correlated with either gender ratio or diastolic blood pressure ($p > 0.05$) (Table III).

Discussion

CHD is recognized as a multi-factorial disease generated by multiple genetic factors and environmental factors. Blood lipid level is one the most important risk factors for the pathogenesis of atherosclerosis and CHD[9,10]. Dyslipidemia is caused by a complex pathogenic mechanism resulted from the interactions between multiple genes or between multiple genes and environmental factors[11,12]. Along with the continuous advancing in the research on hypercholesterolemia and after the discovery of the genes of LDL receptor and apolipoproteins, the PCSK9 gene has been discovered. PCSK9 is able to degrade cell surface LDL receptor (LDLR) and affect the clearance of LDL, leading to the elevation of

Table III. Correlation of PCSK9 with other parameters.

Group	r	p	Significance
Gender ratio (male/female)	0.094	0.388	> 0.05
Mean age (years)	0.342	0.012	< 0.05
BMI (kg/m^2)	0.180	0.025	< 0.05
TC (mmol/L)	0.431	0.000	< 0.05
TG (mmol/L)	0.424	0.001	< 0.05
Systolic blood pressure (mmHg)	0.159	0.044	< 0.05
Diastolic blood pressure (mmHg)	0.066	0.685	> 0.05
FBS (mmol/L)	0.397	0.008	< 0.05
Gensini Score	0.441	0.000	< 0.05
LDL-C (mmol/L)	0.162	0.038	< 0.05

LDL level. PCSK9 may have a direct relationship with the development of atherosclerosis. PCSK9 plays an important role in the development of CHD through regulation of LDL level[13,14]. Based on these findings, the present study was designed to measure PCSK9 levels in CHD patients and investigate its correlation with the severity of coronary artery lesion, in an effort to explore the significance of PCSK9 variation in CHD population.

PCSK9 is a type of proprotein convertase newly found in recent years. Loss-of-function mutation of its gene can induce familial hypercholesterolemia and increase the incidence of cardiovascular event. Some study has shown that the expression of PCSK9 is directly associated with and involved in atherosclerosis and regulation of cholesterol, in that the lack of PCSK9 can mitigate the development of atherosclerosis whereas its overexpression can aggravate the progression of atherosclerosis[15,16]. Moreover, PCSK9 expression can induce degradation of LDLR on the liver cell surface and, hence, increase circulating LDL-C level. In addition, PCSK9 is also involved in the development of atherosclerosis by inducing endothelial injury, cell apoptosis and several other pathways as well. More importantly, suppression of serum PCSK9 level can downregulate LDL-C level in both healthy population and patients with hypercholesterolemia, reducing the morbidity and mortality of cardiovascular diseases[17,18].

In the present study, plasma PCSK9 levels of 126 CHD patients were evaluated and analyzed. The results showed that serum PCSK9 levels were significantly higher in CHD patients than in controls. Therefore, high level of plasma PCSK9 may be associated with the development of CHD. In CHD group, plasma PCSK9 levels were significantly higher in patients with multi-vessel disease than in those with single-vessel and double-vessel diseases ($p < 0.05$). Serum PCSK9 levels in patients with severe coronary stenosis were significantly higher than those with mild stenosis ($p < 0.05$), indicating that the higher the PCSK9 expression, the more severe the severity of coronary atherosclerosis. Gensini score is an effective method to evaluate coronary arterial lesion. More severe is the atherosclerosis, higher is the Gensini score. The present study demonstrated that plasma PCSK9 expression was positively correlated with Gensini score and LDL-C level in CHD patients ($p < 0.05$), suggesting that PCSK9 can reflect the severity of CHD.

Conclusions

The impact of plasma PCSK9 level on CHD is presented as follows: (1) PCSK9 can influence blood lipid level, particularly LDL-C level and promote the progression of coronary atherosclerosis. LDL-C is an important risk factor for coronary atherosclerosis. Oxidized LDL (ox-LDL) accumulates in the vascular endothelium, induce endothelial injury and promote the generation and rupture of the atherosclerotic plaque, leading to a severe injury of cerebrovascular system[19,20]. In addition, 70% of LDL-C *in vivo* are metabolized through LDLR. Circulating LDL-C is reduced through degradation and absorption in lysosomes, thereby effectively stabilizing cholesterol level. PCSK9 has been shown to mediate the degradation LDLR in lysosomes. Dysregulation of LDL-C by LDLR can inhibit the clearance of circulating LDL-C[21-23]. The results of the present study also revealed that PCSK9 was positively correlated with LDL-C level and Gensini score. (2) PCSK9 is also involved in the inflammation of vascular wall and promotes the apoptosis of endothelial cells as well as monocytic macrophages.

In brief, plasma PCSK9 level is significantly elevated in CHD patients and the variation in plasma PCSK9 level is associated with the severity of the coronary arterial lesion. However, the underlying mechanism needs to be further studied.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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