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Positive correlation between plasma PCSK9 and tissue factors levels in patients with angiographically diagnosed coronary artery disease and diabetes mellitus

Mei WANG[1], Yan-Fang LI[1,2], Yan-Ging GUO[1], Meng-Meng CHEN[1], Zhi-Li JIANG[1], Jun-Ying SONG[1]

[1]*Emergency & Critical Care Center, Beijing Anzhen Hospital, Capital Medical University, No. 2 Anzhen Road, Chaoyang District, Beijing, China*
[2]*Beijing Institute of Heart Lung and Blood Vessel Diseases, Beijing, China*

Abstract

Background Pro-protein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein that influences plasma levels of low-density lipoprotein cholesterol (LDL-C). Both oxidized LDL and tissue factor (TF) contributed to the development of prothrombotic state. The present study aims to explore the relationship between plasma level of PCSK9 and that of TF in patient with coronary artery disease (CAD). **Methods** From July 2013 to March 2014, we enrolled 197 consecutive patients who underwent coronary angiography because of suspected CAD at Beijing Anzhen Hospital in this study. All patients had no history of using lipid-lowering medication. Of these 197 patients (131 male and 66 female, mean age 56.9 ± 11.8 years), 81 had angiographically diagnosed CAD. Clinical data were collected. Plasma PCSK9 and TF were measured using enzyme-linked immunosorbent assay (ELISA). Levels of plasma PCSK9 and TF were compared and their correlation analyzed among different patient groups. **Results** Both plasma levels of PCSK9 (279.8 ± 60.4 μg/L *vs.* 216.5 ± 45.3 μg/L, $P < 0.01$) and TF (156.4 ± 26.6 μg/mL *vs.* 112.1 ± 38.3 μg/L, $P < 0.01$) were significantly higher in patients with CAD, as compared with those without CAD. Correlation analysis showed plasma level of PCSK9 was significantly correlated with that of TF in both patients with and without CAD. However, multivariate regression analysis after adjustment for age, gender, smoking, alcohol, hypertension and hyperlipidemia showed that only in CAD patients with type 2 diabetes mellitus, there was significant positive correlation between plasma levels of PCSK9 and TF ($\beta = 0.353$, $P < 0.01$). **Conclusions** The plasma level of PCSK9 is independently and positively associated with that of TF in CAD patients with diabetes mellitus, but not in those without diabetes mellitus. Further study is needed to investigate the underlying mechanism.

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Keywords: Coronary artery disease; PCSK9; Tissue factor; Type 2 diabetes mellitus

1 Introduction

Pro-protein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein that influences plasma levels of low-density lipoprotein cholesterol (LDL-C), and high expression of PCSK9 is positively associated with plasma LDL cholesterol levels.[1] Several studies on populations with loss-of-function mutations in PCSK9 showed moderate reduction of LDL-C but much more significant decrease of coronary events,[2–4] implicating that PCSK9 may associate with cardiovascular disease (CVD) events beyond its effects on LDL-C. Tissue factor (TF) is the transmembrane receptor for factor VII/VIIa (FVII/VIIa). The TF/FVIIa complex is the major cellular initiator of the blood coagulation cascade leading to thrombosis, fibrin deposition and platelet activation, and thus, CVD events.[5] Previous studies had demonstrated that hyperlipidemia promote TF expression.[5] Therefore, we hypothesized that plasma level of PCSK9 may positively associated with that of TF in patients with coronary artery disease (CAD).

2 Methods

2.1 Study subjects

From July 2013 to March 2014, we enrolled consecutive patients who underwent coronary angiography because of suspected CAD at the Emergency Department of the Beijing Anzhen Hospital in this study. The exclusion criteria included: (1) patient who had used lipid-lowing drugs (statins,

Correspondence to: Yan-Fang LI, MD, PhD, Emergency & Critical Care Center, Beijing Anzhen Hospital, Capital Medical University; Beijing Institute of Heart Lung and Blood Vessel Diseases No. 2 Anzhen Road, Chaoyang District, Beijing 100029, China. E-mail: liyf101@sohu.com
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fibrates, nicotinic acid, and ezitimibe) within 3 months; and (2) patient with infectious or systematic inflammatory diseases, significant hematologic disorders, thyroid dysfunction, severe liver and/or renal insufficiency and malignant tumors.

Finally, a total of 197 (131 male and 66 female, mean age 56.9 ± 11.8 years) patients were enrolled into this study. Of these patients, 81 had angiographically diagnosed CAD (Table 1.). Diabetes mellitus was diagnosed as fasting glucose levels ≥ 7.0 mmol/L in multiple determinations or was receiving at least one glucose-lowing treatment. The diagnosis of CAD was defined as the presence of coronary lesions ≥ 50% in at least one major epicardial artery segment. Hypertension was defined as repeated systolic and/or diastolic blood pressure ≥ 140 and/or ≥ 90 mmHg on at least two different occasions or currently using anti-hypertension drugs. The definition of dyslipidemia was fasting total cholesterol (TC) ≥ 200 mg/dL or triglycerides (TG) ≥ 150 mg/dL.

The study protocol was approved by the hospital ethical review board (Beijing Anzhen Hospital, Beijing, China), and written informed consent was obtained from each patient before the study.

2.2 Laboratory analyses

Blood samples were collected into EDTA-containing tubes from patients after at least 12-h fast in the morning and centrifuged 4℃ at 3,000 rpm for 10 min to separate the serum for biochemical tests. All the samples for the measurement of PCSK9 and TF levels were stored at 80 °C until analysis. The concentrations of the plasma TG, TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, lipoprotein A [LP (a)], apolipoprotein A1 (ApoA1), and ApoB, and glucose were measured by automatic biochemistry analyser (Hitachi 7150, Japan).

Serum levels of PCSK9 were measured in duplicate by enzyme-linked immunosorbent assay using a commercial kit (Eiaab, Wuhan, Hubei Province, China), and levels of TF were measured using an ELISA kit according to the manufacturer's instructions (Human TF/TFPI ELISA Kit, R&D, Minnesota, USA).

2.3 Statistical analysis

Continuous variables were given as mean ± SD and categorical variables as frequencies (percentage). The Spearman's rank correlation was used between PCSK9 and TF levels. Step-wise multivariable regression analyses were performed to determine the independent relationship between plasma PCSK9 and TF levels. Student's *t*-test and multivariable analysis were used for comparisons of PCSK9 and TF levels among patient groups. Two-sided *P*-values of < 0.05 were considered statistically significant. Statistical analysis was performed with SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Baseline characteristics of study subjects with and without coronary artery disease

The baseline characteristics of study subject were shown in Table 1. Compared with subjects without CAD, patients who had angiographically diagnosed CAD were older, more frequently to have hypertension, hyperlipidemia, with higher levels of serum glucose, HBA1c, LDL and high-sensitivity C-reactive protein (hs-CRP). Both plasma levels of PCSK9 and TF were significantly higher in patients with CAD, as compared with those without CAD. Particularly, both PCSK9 and TF levels were higher in CAD patients with diabetes than those without diabetes (289.1 ± 62. 9 μg/L *vs*. 265.3 ± 56 μg/L and 182.2 ± 32.4 μg/L *vs*.125.6 ± 20.3 μg/L, respectively). However, multivariable analyses adjusting for other clinical variables revealed no significant difference of plasma PCSK9 levels between patients with and those without CAD (data not shown).

Table 1. Baseline characteristics of study subject with and without coronary artery disease.

	Subjects with CAD (*n* = 81)	Subjects without CAD (*n* = 116)	*P*- value
Age, yrs	57.2 ± 8.8	55.0 ± 9.8	0.03
Male	54 (66.7%)	77 (66.4%)	0.84
Hypertension	60 (74.1%)	54 (45.6%)	0.03
Hyperlipidemia	70 ((86.4%)	57 (49.1%)	< 0.01
Smoking	50 (61.7%)	53 (45.7%)	0.38
Alcohol	26 (32.1%)	42 (36.2%)	0.15
Glucose (mmol/L)	6.2 ± 1.5	4.2 ± 1.2	< 0.01
HBA_1c (%)	6.4 ± 0.6	5.5 ± 0.3	< 0.01
TG (mmol/L)	1.7 ± 0.7	1.6 ± 0.3	0.27
TC (mmol/L)	5.1 ± 1.3	4.9 ± 0.9	0.32
LDL (mmol/L)	3.5 ± 1.2	2.7 ± 0.9	0.03
HDL (mmol/L)	1.1 ± 0.2	1.2 ± 0.1	0.32
Lp(a) (mg/L)	0.5 ± 0.3	0.4 ± 0.2	0.48
ApoB (g/L)	1.0 ± 0.3	0.9 ± 0.2	0.39
hs-CRP (mg/L)	6.5 ± 1.2	2.4 ± 1.1	< 0.01
TF (μg/L)	156.4 ± 26.6	112.1 ± 38.3	0.02
PCSK9 (μg/ L)	279.8 ± 60.4	216.5 ± 45.3	< 0.01

Data are presented as mean ± SD or *n* (%). ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; Lp (a): lipoprotein a; PCSK9: proprotein convertase subtilisin/kexin type 9; TC: total cholesterol; TF: tissue factor; TG: total triglycerides.

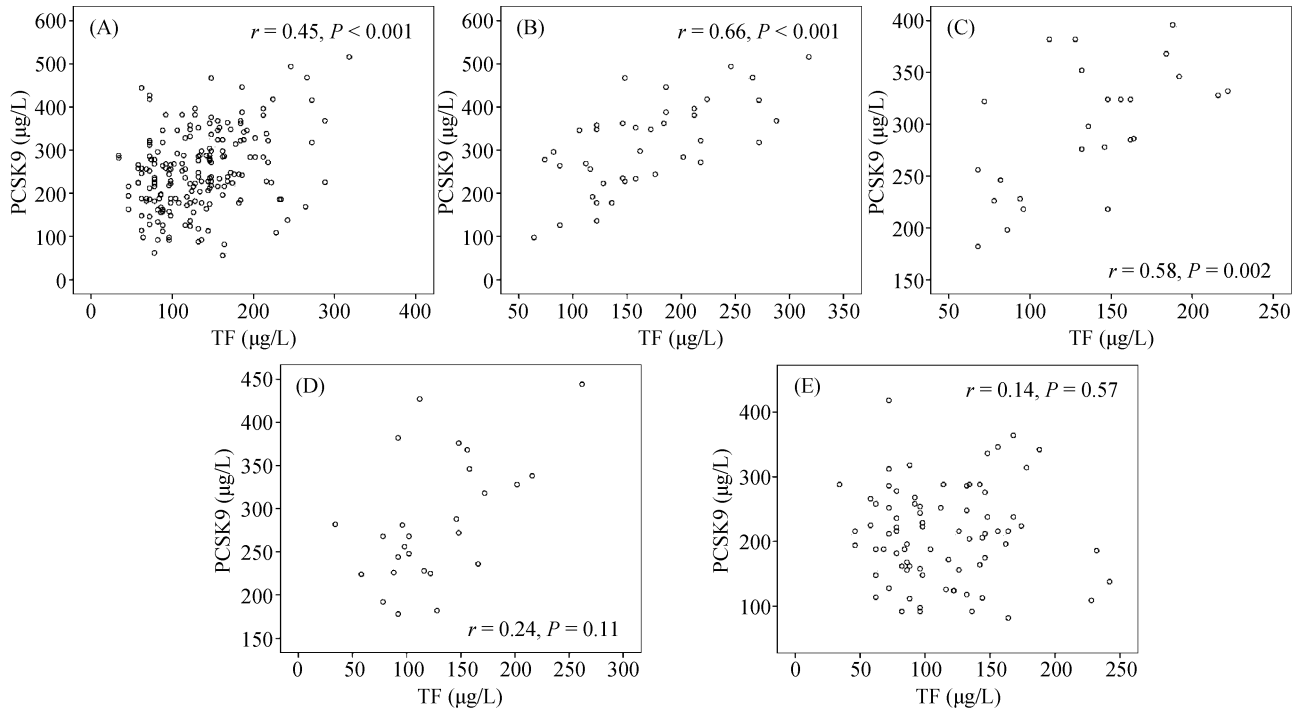

Figure 1. Correlations between plasma levels PCSK9 and TF among different patient groups. (A): All study patients; (B): patients with CAD and diabetes; (C): patients with CAD but no diabetes; (D): patients without CAD but with diabetes; (E): patients with no CAD and no diabetes. CAD: coronary artery disease; PPCSK9: proprotein convertase subtilisin/kexin type 9; TF: tussue factor.

3.2 Correlation between plasma levels of PCSK9 and TF among different patient groups

Using Spearman's correlation analysis, we observed a positive relationship between plasma levels of PCSK9 and TF in the 197 study subject, that is, both patients with and without CAD. We further examined the associations of PCSK9 and TF among different patient groups. In subgroups of patients with CAD and diabetes, patients with CAD but no diabetes, patients without CAD but with diabetes, there were also positive correlations. However, in patients with no CAD and no diabetes, we found no relationship between PCSK9 and TF (Figure 1).

3.3 Multivariate analysis of the correlation between PCSK9 and TF among different patient groups

To clarify the independent relationship between PCSK9 and TF, we performed step-wise multivariable regression analyses among different patient subgroups, by adjusting for various demographic and clinical variables. The results were shown in Table 2. When all 81 patients with coronary artery disease were analyzed, adjusted for age, gender, smoking, Alcohol, hypertension and hyperlipidemia, the correlation between PCSK9 and TF became statistically insignificant (P = 0.178). However, in patients with both CAD and diabetes, the correlation remained significant after adjustment for various clinical variables.

Table 2. Multivariate regression analyses of the correlation between PCSK9 and TF among different patient groups.

	Patients with CAD (n = 81)		Patients with CAD and diabetes (n = 39)		Patients without CAD (n = 111)	
	β	P-value	β	P-value	β	P-value
Model 1[a]	0.288	0.015	0.381	< 0.01	0.107	0.184
Model 2[b]	0.281	0.016	0.379	< 0.01	0.106	0.210
Model 3[c]	0.272	0.178	0.353	< 0.01	0.102	0.244

[a]Adjusted for age and gender; [b]adjusted for age, gender, smoking and Alcohol; [c]adjusted for age, gender, smoking, Alcohol, hypertension and hyperlipidemia. CAD: coronary artery disease; TF: tissue factor.

4 Discussion

The present study aimed to test our hypothesis that circulating PCSK9 levels may positively related to TF in patients with CAD. To our knowledge, this is the first study to address the association of plasma PCSK9 concentration with TF. Interestingly, and with a little surprise, we found that only in CAD patients with diabetes, there exist independent and significant association between plasma levels of PCSK9 and TF.

Although the results do not support our primary hypothesis, the finding that PCSK9 and TF levels are independently related in CAD patients with diabetes is somewhat intriguing. Previous studies[6] have demonstrated higher TF level in

patients with diabetes, but to date, the reported relationship between type 2 diabetes mellitus and plasma levels of PCSK 9 is conflicting.[7] In our current study, both PCSK9 and TF levels were higher in CAD patients with diabetes than those without diabetes (289.1 ± 62.9 μg/L *vs*. 265.3 ± 56 μg/L and 182.2 ± 32.4 μg/L *vs*.125.6 ± 20.3 μg/L, respectively) . Our results suggest that the interactions of PCSK9 and tissue factor in the pathophysiology of CAD merit further investigation.

We also found that both plasma levels of PCSK9 and TF were significantly higher in patients with CAD than those without CAD, but these differences became insignificant after adjustment for other clinical variables. Although no previous researches had specifically compared PCSK9 levels between population with and without CAD, several studies had provided conflicting results regarding the relationship between circulating PCSK9 levels and CAD. Almontashiri, *et al*.[8] measured plasma levels of PCSK9 in angiographically defined controls (< 30% coronary stenosis) and CAD patients (> 50% stenosis in a major coronary artery) from the Ottawa Heart Genomics Study, and found that PCSK9 levels were associated with CAD in individuals taking a statin but not in those without using a statin. However, in the same study, but of those participants from the The Emory Cardiology Biobank samples who had no diabetes mellitus and not taking a statin or fibrate at the time of recruitment, they found that plasma PCSK9 levels were elevated in angiographic CAD cases (385.0 ± 146.9 ng/mL) as compared to controls (340.4 ± 125.2 ng/mL, $P < 0.001$) and Logistic regression confirmed that plasma PCSK9 level was an independent predictor of CAD. Furthermore, results of two recent studies[9,10] investigating the relationship between circulating PCSK9 and incident CVD in the general population are not in consistent. In a prospective cohort study of 4232 men and women 60 years of age at the time of recruitment, Leander K et al. found serum PCSK9 concentration is associated with future risk of CVD even after adjustments for established CVD risk factors,[9] while another study of > 28,000 initially healthy American women reported no relationship of plasma levels of PCSK9 measured at baseline and future cardiovascular events.[10] These results, together with ours, suggest the complicated relationship between PCSK9 levels and the development and progress of CAD in general population.

The most important limitation of our present study is the relatively small study sample, partly because of the difficulty in enrolling CAD patients who do not taking lipid lowering medication.

In conclusion, our study showed that plasma level of PCSK9 is independently and positively associated with that of TF in CAD patients with diabetes mellitus, but not in those without diabetes mellitus. Further study is needed to investigate the underlying mechanism.

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