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ORIGINAL RESEARCH

Elevated Circulating PCSK9 Concentrations Predict Subclinical Atherosclerotic Changes in Low Risk Obese and Non-Obese Patients

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ABSTRACT

Introduction: Many studies have highlighted the important role of PCSK9 in the development of cardiometabolic changes and its possible function as a biomarker of myocardial infarction or ischemic heart disease. This study aimed to determine the relationship between circulating PCSK9 levels and subclinical vascular changes in the group of low risk patients without manifest cardiovascular diseases.

Methods: In this study, 120 healthy patients, free of manifest cardiovascular diseases, diabetes mellitus, and without lipid-lowering therapy, were divided into three groups based on BMI: normal weight ($N = 50$), overweight ($N = 30$), and obese ($N = 40$). Biochemical parameters, including basic lipid and non-lipid ones, were analyzed. PCSK9 levels were measured by ELISA, vascular changes were quantified by carotid ultrasound (carotid artery intima-media thickness, cIMT), and arterial stiffness parameters (pulse wave velocity, PWV; augmentation index, AI; stiffness parameter, β) were measured by an echo-tracking method.

Results: Plasma levels of PCSK9 significantly increased in obese (172.78 ± 51.67 ng/mL) in comparison with overweight (120.14 ± 37.64, $p < 0.001$) and normal weight groups (114.92 ± 35.87, $p < 0.001$). Differences between the overweight and normal weight groups were not significant ($p = 0.85$). The level of PCSK9 significantly correlated with values of BMI ($p < 0.001$, $r = 0.38$). In addition to increase in laboratory parameters associated with moderate metabolic changes, significant increase in cIMT and parameters of vascular changes (β, AI, PWV) were detected in groups with elevated BMI. Significant positive linear correlation of PCSK9 concentrations and cIMT ($p < 0.001$, $r = 0.39$), PWV ($p < 0.001$, $r = 0.31$), and β ($p < 0.001$, $r = 0.3$) were found. In multivariable regression analysis after adjusting for gender, age, BMI, and LDL, the impact of PCSK9 on cIMT, β, and PWV remained significant ($p = 0.006$, 0.03, and 0.002, respectively).

Conclusion: PCSK9 plasma levels significantly correlated with subclinical vascular changes and their values were significantly elevated in obese

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subjects. We assume that PCSK9 could be used as a predictor of early vascular involvement, prior to the existence of manifest atherosclerosis. These results also highlight the role of anti-PCSK9 treatment in primary prevention.

Keywords: Atherosclerosis; Endothelium; Marker; Obesity; PCSK9

INTRODUCTION

Cardiovascular (CV) diseases are the leading cause of morbidity and mortality worldwide. Atherosclerosis is one of the main etiological factors resulting in the reduction of blood flow and ischemia in target organs. According to the World Health Organization, 80% of cases of coronary artery disease (CAD) could be avoided by some degree of primary or secondary prevention [1]. Therefore, nowadays it is important to establish methods for the detection of early vascular changes and subsequent prevention of atherosclerosis progression. Excess fat, in particular visceral, abdominal, and ectopic, is closely related to the development of metabolic syndrome, cardiovascular diseases, and diastolic dysfunction of the heart. In contrast, excess fat tissue in the periphery does not increase the risk of cardiovascular diseases [2]. Although the association of obesity with cardiovascular disease is intensely studied, the underlying mechanism is still not clearly understood. The question remains why every obese patient does not develop a cardiometabolic complication such as endothelial dysfunction and atherosclerosis. It is believed that the complication is caused by inflammatory dysregulation of visceral fat [3]. Diagnosis of early cardiometabolic changes is challenging at the initial point of the pathogenesis. Many studies have highlighted the role of increased arterial stiffness parameters in the diagnosis and prognosis of patients. However, the relationship between rigidity of blood vessels, visceral fat, and the individual parameters (age, sex) is even today highly controversial and its determination requires instrumentation and training to achieve reproducible and clinically valid results [4, 5].

Protein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease synthesized in the liver. PCSK9 plays a key role in the regulation of lipid metabolism by the stimulation of endosomal and lysosomal degradation of hepatic LDL receptor (LDLR) [6]. This results in quantitative reduction of LDLR on the cell membrane and increased plasma levels of LDL-C [7]. Many studies show that PCSK9 may accelerate atherosclerosis and CAD by several mechanisms that are independent of elevated liver LDLR degradation [8]. Inhibition of this protein show promising results in the treatment of atherosclerosis and dyslipidemias.

Previous epidemiological studies have reported a significant relation between plasma PCSK9 concentrations and glucose and insulin levels, suggesting a role for PCSK9 in development of diabetes mellitus [9, 10]. Clinical studies have also shown a significant relation between the increased PCSK9 concentrations and the risk of first cardiovascular event [11] or generally increased cardiovascular risk [12, 13] mainly in the groups of high risk patients.

This study aimed to investigate the relationship between the circulating PCSK9 levels and early subclinical vascular changes in obese and non-obese patients with a low cardiovascular risk.

METHODS

Study Design

This study was designed as a prospective trial with the aim of determining the relation between the circulating levels of PCSK9 and subclinical vascular changes. From November 2015 until May 2016, general practitioners randomly selected 350 patients without manifest CV diseases, who were examined on the Cardiology and General Medicine outpatient clinics of the Faculty of Medicine, UPJŠ Košice. From these patients, 120 eligible patients were selected according to the inclusion and exclusion criteria (see below). All patients that volunteered for this research study agreed by their own free will and signed the informed consent approved by the local ethics committee of the

Medical Faculty of Pavol Jozef Šafárik University (7/14/2015). This study was not registered on ClinicalTrials.gov because of its prospective and non-invasive character. The study was registered under the UPJŠ Košice projects list. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013.

Inclusion Criteria

- Patients with a BMI in the range of normal weight, overweight, and obesity
- Low cardiovascular risk (SCORE ≤ 1%)
- Total cholesterol level below 8 mmol/L
- TG levels below 2.3 mmol/L
- Age between 18 and 54 years
- Signed informed consent to participate in the study

Exclusion Criteria

- Patients receiving lipid-lowering therapy (statins, ezetimibe, etc.) or patients who do not meet the minimum period of 3 months of discontinuation of therapy
- The lipid profile outside the inclusion criteria
- Diabetes mellitus
- SCORE > 1%
- Proven secondary causes of dyslipidemia
- Presence of manifest cardiovascular system disease in the form of coronary artery disease, past stroke, TIA, MI, etc.
- Presence of acute diseases, chronic deterioration, or presence of infection, which may distort the laboratory parameters
- Significant lifestyle changes, mainly of dietary habits and physical activity in the period shorter than 6 months

By meeting the inclusion and exclusion criteria, the selected group of 120 patients underwent basic history-taking, physical examination, and blood sampling for laboratory examination. Subsequently, they were divided into three groups based on BMI (kg/m^2): BMI_{20-25} ($N = 50$), BMI_{25-30} ($N = 30$), and $BMI_{>30}$ ($N = 40$).

Laboratory Analysis

Lipid parameters (LDL, HDL, TG, total cholesterol) and non-lipid parameters (AST, ALT, ALP, etc.) were quantified by standard laboratory tests on a Daytona automatic analyzer (RANDOX). Circulating PCSK9 levels in plasma were detected by ELISA (Abcam, Human PCSK9 ELISA Kit®) using a spectrophotometric method at 450 nm.

Quantification of Vascular Changes

Subclinical vascular changes were detected by ultrasound measurement of carotid artery intima-media thickness (cIMT) and by an echo-tracking method. Measurement of cIMT was performed in B mode in the lateral projection by linear duplex probe with a frequency of 10 MHz using an Aloka ProSound Alpha 10 device (ALOKA Europe). The measurements were made using the standardized protocol on the distal 2 cm of the common carotid artery (CCA). Images were captured in end-diastolic phase (confirmed by ECG). Measurement of cIMT was established on the opposite wall of the right distal CCA, as an average thickness of locating the segment of 10 mm proximal to the bifurcation. Edges and distance were detected and calculated using semi-automatic software and given as cIMTmean [14].

Echo-tracking measurement was based on changes in diameter of the artery. They are evaluated by measuring the distance between the two gates based on the borders of the intraluminal part of the vessel wall. The measurement was performed in the same localization as cIMT quantification. Following parameters were further analyzed: β (stiffness parameter), AI (augmentation index), and PWV (pulse wave velocity).

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0 for Windows (SPSS, version 20.0. ARMONK, NY: IBM Corp.). The values of each parameter were expressed as the mean ± SD. Univariate and multivariate linear regression

analyses were performed to assess the correlations between plasma levels of PCSK9 and subclinical vascular changes, biochemical parameters, and BMI. Changes in quantitative results (PCSK9, vascular changes) in study groups were determined by one-way ANOVA with a multiple comparison Tukey–Kramer post hoc test. The value of $p < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the entire study population and subsequent groups are listed in Table 1. The average age of the study population was 34.87 ± 12.95 years, 60 were women (50%), and 60 were men (50%). The average ages of men and women were not significantly different (36.2 ± 33.5 vs. 13.36 ± 12.54 years). The average weight in the group BMI_{20-25} was 59.32 ± 13.34 kg and average BMI was 22.61 ± 1.48 kg/m^2. In the group BMI_{25-30}, average weight was 72.79 ± 9.85 kg and average BMI was 27.33 ± 1.46 kg/m^2. The group of patients with obesity (group $BMI_{>30}$) had an average weight of 97.16 ± 13.20 kg and average BMI of 37.02 ± 5.03 kg/m^2. In the study groups, no significant differences in basic parameters, such as age or proportion of men and women, were detected. Differences that have been identified were particularly in biochemical parameters (lipid and non-lipid), clinical, or in blood pressure, i.e., resulting from a basic research focus.

Significantly higher concentrations of PCSK9 (Table 2) were detected in the obese in comparison with the normal weight ($p < 0.001$) and overweight groups ($p < 0.001$). Significant difference between the overweight and normal weight groups was not detected ($p = 0.85$). Values of cIMT (Table 2) were significantly increased in both groups with elevated BMI (BMI_{25-30}, $p = 0.03$; $BMI_{>30}$, $p < 0.001$) in comparison with the normal weight group. Augmentation index was significantly increased in the BMI_{25-30} ($p = 0.03$) and $BMI_{>30}$ groups ($p = 0.001$) in comparison with the normal weight group. Vascular stiffness index β was significantly increased in the overweight and

obese groups in comparison with the normal weight group ($p = 0.01$, $p < 0.001$). Parameter PWV was significantly increased in the group BMI_{25-30} ($p < 0.001$) and $BMI_{>30}$ ($p < 0.001$) in comparison with the normal weight group. Significant differences between overweight and obese patient groups in cIMT, AI, β, and PWV were not detected (Table 2).

Correlations between concentrations of PCSK9, vascular changes, and BMI are shown in Table 3. Univariate analyses indicated that concentrations of PCSK9 positively correlated with subclinical vascular changes. Moreover, multivariate regression analysis (Table 4) showed that PCSK9 concentrations were a significant positive predictor of increased cIMT, β, and PWV.

Multivariate regression analysis of the data only in the group of obese patients (Table 5) showed that PCSK9 concentrations were again a significant positive predictor of increased cIMT and PWV. Univariate analysis showed significant correlation of BMI with parameters of subclinical vascular changes; however, after consideration of other risk factors in the multivariable analysis, the significance of BMI as a positive predictor of vascular changes was not proved.

DISCUSSION

In this study, we set out to find the relationship between circulating levels of PCSK9 and subclinical vascular changes and to find out the relation between overweight, obesity, and PCSK9. Selection of patients with low cardiovascular risk according to the SCORE system allowed us to eliminate the effect of various other risk factors and proves the role of PCSK9 as a marker of subclinical changes as well as a mediating factor of these changes. Many studies have assumed that for the formation of cardiovascular changes and its complications, the interplay of various risk factors such as obesity, dyslipidemia, hypertension, insulin resistance, etc. is needed. Those factors increase the progression of vascular changes and the cardiovascular risk as well [15]. It was found that even fatty tissue overgrowth (especially visceral) on

Table 1 Baseline characteristics of subjects

	BMI_{20-25}	$BMI_{<25}$ vs. BMI_{25-30}	BMI_{25-30}	BMI_{25-30} vs. $BMI_{>30}$	$BMI_{>30}$	$BMI_{<25}$ vs. $BMI_{>30}$
Sample	50	NS	30	NS	40	NS
Male/female	27/23	NS	13/17	NS	20/20	NS
Age	32.42 ± 11.8	NS	35.96 ± 13.18	NS	37.11 ± 14.2	NS
Weight (kg)	59.32 ± 13.34	<0.001	72.79 ± 9.85	<0.001	97.16 ± 13.2	<0.001
BMI (kg/m^2)	22.61 ± 1.48	<0.001	27.33 ± 1.46	<0.001	37.02 ± 5.03	<0.001
WC (cm)	77.1 ± 6.9 (F)/ 84.2 ± 6.5 (M)	0.015/<0.001	84.1 ± 7.8/ 95.3 ± 7.9	<0.001/0.01	98.6 ± 7.1/ 103.2 ± 9.5	<0.001/ <0.001
Total cholesterol (mmol/L)	4.60 ± 1.13	0.007	5.47 ± 1.01	0.88	5.61 ± 1.43	<0.001
LDL-C (mmol/L)	3.33 ± 0.92	0.09	3.75 ± 0.94	0.21	3.92 ± 1.28	0.03
HDL-C (mmol/L)	1.42 ± 0.23	0.13	1.3 ± 0.25	0.12	1.21 ± 0.18	<0.001
TG (mmol/L)	0.92 ± 0.51	0.002	1.27 ± 0.37	0.65	1.36 ± 0.34	<0.001
AST (μkat/L)	0.44 ± 0.12	0.24	0.37 ± 0.16	0.02	0.47 ± 0.19	0.24
ALT (μkat/L)	0.40 ± 0.19	0.19	0.44 ± 0.29	0.1	0.53 ± 0.33	0.08
ALP (μkat/L)	1.12 ± 0.23	0.29	1.11 ± 0.19	0.009	1.28 ± 0.34	0.01
AMS(μkat/L)	0.97 ± 0.37	0.03	0.64 ± 0.32	0.05	0.96 ± 0.83	0.23
sTK (mmHg)	110.27 ± 19.33	<0.001	129.11 ± 15.98	0.19	135.11 ± 18.23	<0.001
dTK (mmHg)	69.73 ± 12.21	<0.001	82.11 ± 14.72	0.16	83.98 ± 14.13	<0.001

NS not significant

its own acts as an independent risk factor of cardiometabolic disorders, and obesity itself interferes with dyslipidemia pathogenesis, insulin resistance, and hepatic steatosis. The precise pathomechanisms and relationship between obesity and cardiovascular risk are still not fully understood [16].

An important finding in this study is that PCSK9 concentrations were significantly elevated in overweight and obese individuals in comparison with the normal weight group. It is well known that the progression of atherosclerotic vascular changes depends on the metabolism of lipids, mainly of LDL particles. When an imbalance in lipid serum levels persists together with other risk factors, endothelial dysfunction

followed by subclinical and clinical atherosclerotic changes arise [17]. PCSK9 protein plays a key regulatory role in the metabolism of lipoproteins, but nowadays many non-lipid effects are studied such as effect on inflammation, glycemia, liver regeneration, and steatosis. Many studies have shown that PCSK9 may accelerate atherosclerosis and CAD by mechanisms independent from increased hepatic LDLR degradation [8]. In our study, concentrations of PCSK9 significantly correlated with increased values of BMI. Increased weight and further increase of BMI increased the circulating levels of PCSK9 as well. Moreover, in overweight and obese patients, significant increase of cIMT in comparison with the normal weight group

Table 2 Vascular changes and PCSK9 concentrations in study groups

	BMI_{20-25}	p values $BMI_{<25}$ vs. BMI_{25-30}	BMI_{25-30}	p values BMI_{25-30} vs. $BMI_{>30}$	$BMI_{>30}$	p values $BMI_{<25}$ vs. $BMI_{>30}$
PCSK9 (ng/mL)	114.92 ± 35.87	0.85	120.14 ± 37.64	<0.001	172.78 ± 51.67	<0.001
cIMT (mm)	0.46 ± 0.09	0.03	0.56 ± 0.14	0.22	0.63 ± 0.26	<0.001
AI (%)	4.13 ± 9.22	0.03	10.17 ± 12.22	0.736	12.06 ± 10.60	0.001
β (stiffness)	5.19 ± 1.61	0.56	6.67 ± 2.26	0.01	6.94 ± 2.71	<0.001
PWV (m/s)	4.93 ± 0.84	<0.001	5.85 ± 0.96	0.59	5.88 ± 1.12	<0.001

Table 3 Correlations between subclinical vascular changes, PCSK9 concentrations, and BMI

	BMI		PCSK9	
	R	p value	R	p value
cIMT	0.39	<0.001	0.31	0.003
AI	0.36	<0.001	0.29	<0.001
β	0.37	<0.001	0.30	<0.001
PWV	0.43	<0.001	0.31	<0.001
BMI	–	–	0.38	<0.001

was observed. Values of cIMT significantly correlated with PCSK9 concentrations. Significant correlation of PCSK9 with cIMT as well as BMI suggests that circulating levels of PCSK9 could be a mediator of obesity-induced cardiometabolic changes. Many studies have shown that increased cIMT is an important prognostic marker for subsequent cardiovascular events such as MI and stroke, and a predictive factor of cardiovascular morbidity and mortality [18–21]. Similar increase in cIMT values or of the degree of atherosclerosis in obese in comparison with normal weight population has been observed in other studies [22, 23]. Ranges of cIMT physiological values have side (left or right side of internal carotid artery), age, and sex specificity. They increase with age and have slightly higher values in male subjects. Therefore, multivariate analysis was performed to analyze the effect of PCSK9, age, sex, BMI, and LDL on the values of cIMT. Significant effects of age, LDL, and PCSK9 were proved, according to which we suggest that PCSK9 could act as a significant mediator of vascular changes alongside LDL cholesterol and could also be an early marker of these changes. In this analysis the effect of BMI was not significant. We assume that significant elevation of PCSK9 could be regulated by paracrine function of the adipose tissue and this could be the mediator of metabolic changes, not the obesity itself. Similar results were found in other studies

Table 4 Multivariable regression analysis of the effects of various parameters on vascular changes

	CIMT		Beta (β)		AI		PWV	
	β	p value	β	p value	β	p value	β	p value
Sex	0.033	0.106	-0.235	0.53	-3.4	0.02	0.08	0.67
Age	0.005	<0.001	0.13	<0.001	0.38	<0.001	0.06	<0.001
BMI	0.002	0.381	0.009	0.78	0.11	0.36	0.02	0.35
PCSK9	7.05×10^{-4}	0.006	0.01	0.03	0.02	0.29	0.006	0.002
LDL	0.045	0.002	0.16	0.49	4.52	<0.001	0.09	0.41

Table 5 Multivariable regression analysis of the effects of various parameters on vascular changes in the group of obese patients

	CIMT		Beta (β)		AI		PWV	
	β	p value	β	p value	β	p value	β	p value
Sex	0.1	0.04	−0.384	0.7	−1.1	0.68	0.27	0.54
Age	0.009	<0.001	0.147	0.007	0.52	<0.001	0.07	0.002
PCSK9	8.8×10^{-4}	0.05	0.008	0.42	0.01	0.72	0.007	0.04
LDL	0.065	0.04	−0.6	0.35	4.57	0.01	−0.33	0.24

which observed a significant increase of PCSK9 levels in patients with metabolic syndrome and type 2 diabetes. In those studies, levels of PCSK9 correlated with proatherogenic phenotype and degree of insulin resistance [23].

Increase of the arterial stiffness represents the precursor of subclinical and manifest atherosclerotic vascular changes, whose quantification allows us to easily detect early changes. Arterial stiffness is strongly associated with the prevalence of cardiovascular diseases and cardiovascular mortality [4]. Results of previous studies have shown that the parameters β and Ep correlate with the PWV and the elevations of these parameters have been associated with increased arterial stiffness and increased cardiovascular risk. Nevertheless, use of Ep in clinical practice may be limited, since the value can be affected by the pulse pressure. In contrast, the parameter β is less affected and is relatively independent of blood pressure [4]. In a clinical study, Hirai et al. [24] found that the value of β does not change significantly with repeated measurements before and after injection of sodium nitroprusside, confirming that the value of β is relatively independent of blood pressure. Therefore, in our study, only the PWV, β, and AI were included for the measurement of arterial stiffness. Significant increase in all the aforementioned parameters was observed in overweight and obese groups, which confirms the impact of obesity on the artery wall. Significant linear positive correlation of PCSK9 and PWV, β, and AI was detected. After consideration of previously mentioned factors such as sex, age, BMI, and LDL, the significant effect of PCSK9 in multivariable regression persisted for PWV and β. Several previous studies have shown a correlation of increased levels of

PCSK9 with ongoing acute coronary syndrome and polytrauma. A recent study showed that measuring circulating levels of PCSK9 in elderly patients could help determine the increase in cardiovascular risk and future risk of cardiovascular events. It was found that increased levels of PCSK9 were linked to more frequent cardiovascular events during a subsequent period of 15 years of study. Even after adjusting the levels of LDL cholesterol and the risk factors such as the diabetes mellitus, obesity, and the use of statins, this elevated risk persisted [12]. In pathological conditions such as acute coronary syndrome, massive atherosclerosis, and multiple trauma, significant elevations in PCSK9 levels are present. Our study has shown that PCSK9 quantification in low risk patients could help us to identify patients who could benefit from statin, anti-PCSK9, or other dyslipidemia treatment prior to the development of plaque or manifest atherosclerosis. Our results also suggest that the mediator of obesity-induced cardiovascular risk can be elevated PCSK9 levels on its own.

CONCLUSION

Significant increases in PCSK9 levels were detected in patients with increased weight as well as significant positive correlation between PCSK9 concentrations and BMI. Accordingly, it has suggested that fat tissue has an effect on PCSK9 production. Significant positive correlation of PCSK9 with subclinical vascular changes was found as well. Therefore, it is assumed that quantification of PCSK9 can have significant value in primary prevention and life modification in those with high levels of PCSK9, but still

low cardiovascular risk, according to widely used quantification methods.

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Compliance with Ethics Guidelines. All patients that volunteered for this research study agreed by their own free will and signed the informed consent approved by the local ethics committee of the Medical Faculty of Pavol Jozef Šafárik University (7/14/2015). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013.

Data Availability. The datasets analyzed during the current study are available from the corresponding author on reasonable request after the end of the study.

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