Circulating PCSK9 affects serum LDL and cholesterol levels more than SREBP-2 expression

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of article

Advances in Clinical and Experimental Medicine, ISSN 1899-5276 (print), ISSN 2451-2680 (online) *Adv Clin Exp Med*. 2017;26(4):655–659

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Funding sources
None declared

Conflict of interest
None declared

Received on November 17, 2015
Revised on December 18, 2015
Accepted on April 25, 2016

Abstract

Background. Cholesterol homeostasis is dependent upon the sterol regulatory element binding protein 2 (SREBP-2) regulatory system and the functioning of plasma proprotein convertase subtilisin/kexin type 9 (PCSK9). Many studies have also reported that low density lipoprotein receptor (LDLR) levels in cellular membranes are related to the functioning of these proteins.

Objectives. The aim of this study was to investigate the association of lipid profiles with circulating PCSK9 protein values and SREBP-2 expression levels in normal subjects.

Material and methods. The study involved 120 randomly chosen healthy subjects. Their lipid profiles were measured using routine laboratory techniques, and the plasma PCSK9 protein and SREBP-2 expression levels were determined by ELISA and real time quantitative PCR methods, respectively. A statistical analysis was carried out using a statistical software package.

Results. Linear regression analyses showed a significant correlation between total cholesterol and PCSK9 (3.54 ± 1.31 ng/mL), as well as between total cholesterol and SREBP-2 (0.1–35.38) ($p = 0.002$ and $p = 0.02$, respectively). Furthermore, multiple regression analyses showed strict correlations between PCSK9 and cholesterol-related parameters especially the total cholesterol/HDL-C ratio ($\beta = 3.53$, $p = 0.001$). There was no significant correlation between circulating PCSK9 and SREBP-2 expression levels ($r = 1.2$, $p = 0.3$).

Conclusions. The study results revealed that serum cholesterol-related parameters are strictly associated with plasma PCSK9 values, suggesting that PCSK9 function has a greater effect on serum total cholesterol levels than SREBP-2 expression does. Furthermore, the total cholesterol/HDL-C ratio was a better indicator for evaluating PCSK9 level than total cholesterol.

Key words: lipid profile, PCSK9, SREBP-2

DOI
10.17219/acem/62836

Atherosclerosis is the primary cause of coronary artery diseases (CAD) and death in the world.[1] Population studies have reported the classic factors involved in the atherosclerosis process, including age, gender, lifestyle, blood pressure, obesity, diabetes, lipid profile and genetic defects. Many studies have also confirmed that inflammatory events can trigger the development of atherosclerosis.[2]

Among the various hypotheses describing the atherosclerosis process, the development of atherosclerotic plaque and remodeling is the most widely accepted. Molecular events show that lipid accumulation, especially low-density lipoprotein (LDL) particles and its modified forms in subendothelial macrophages develop foam cells, forming the core of atherosclerotic plaques.[3] The macrophages scavenge the particles via LDL receptors (LDLR).[4] Studies have also reported that the LDLR level is dependent upon the function of factors such as sterol regulatory element binding protein-2 (SREBP-2) and proprotein convertase subtilisin/kexin type 9 (PCSK9). The reduced cellular cholesterol pool induces SREBP-2 expression, resulting in an increase in LDLR level.[5,6]

Studies have also revealed that the SREBP-2 regulatory system is involved in cholesterol homeostasis and is related to 3 main factors: SREBP-2, PCSK9 and LDLR.[7] Based on these descriptions, the authors posited that serum LDL-C levels in normal high-cholesterol cases may be related to LDL uptake and may be affected by SREBP-2 expression and PCSK9 protein levels.

Material and methods

Subjects

The study involved 120 healthy adult subjects (BMI > 20) selected by a physician. The subjects had no clinical problems (lupus, liver or kidney diseases or myocardial infarction) in their medical interviews. The ethics committee at Iran University of Medical Sciences approved the study, and informed consent was obtained from all the subjects.

Samples

Both coagulated and EDTA-containing whole blood samples (5 mL each) were prepared from all the participants. Sera and buffy coat fractions were separated and preserved at -80°C.

Biochemical measurements

Each participant's serum lipid profile, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, was directly measured by routine laboratory techniques.

Plasma PCSK9

PCSK9 ELISA kits (Abnova, Taiwan) were used in accordance with the manufacturer's instructions. A standard linear curve was used to identify the plasma PCSK9 level.

RNA extraction and cDNA synthesis

Total RNA was extracted from buffy coat samples using standard kits according to the manufacturer's instructions (Total RNA Extraction Kit, Arya tous, Mashhad, Iran). The RNA quantity and quality was determined using a NanoDrop spectrophotometer (NanoDrop Products, Thermo Fisher Scientific Inc., Wilmington, USA) and agarose-gel electrophoresis (2%). cDNA was synthesized using a standard kit in accordance with the manufacturer's instructions (PrimeScript Double Strand cDNA Synthesis Kit, Takara Bio Inc., Kusatsu, Japan).

The SYBR green real-time quantitative PCR method

SREBP-2 gene expression level was determined using the QuantiFast SYBR Green PCR Kit (Qiagen, Hilden, Germany) and was normalized with reference gene (β-ACTIN, 5'-TCCCTGGAGAAGAGCTACG-3', 5'-GTAGTTTCGTGGATGCCACA-3'). The primers for SREBP-2 gene (5'-CTACGGTGCAGACAGTTGCT-3', 5'-CCAGGGTTGGTACTTGAAGGG-3') were designed with Genamics Expression Software v. 1.1 (Genamics™, New Zealand). The temperature cycles (n = 35) were performed at 95°C for 10 s and 63°C for 30 s. (E = efficiency; $\Delta CT = CT_{Ref} - CT_{Tar}$; CT = cycle threshold; Ref = reference gene; Tar = target gene) was used to compare the gene expression levels.

Statistical analysis

The statistical analysis was performed using SPSS software v. 16 (SPSS Inc., Chicago, IL, USA). The data distribution was evaluated using the Kolmogorov–Smirnov test. PCSK9 protein and SREBP-2 expression levels between genders and BMI groups (< 30 and ≥ 30) were statistically evaluated by Student's t-test and the Mann-Whitney U-test. Linear regression analyses were performed for PCSK9 protein and SREBP-2 expression levels and other biochemical (LDL-C, total cholesterol, total cholesterol/HDL-C ratio and LDL-C/HDL-C ratio) and demographic (age, gender and BMI) parameters. Multiple regression analyses were performed after the elimination of insignificant parameters obtained from the linear regression analyses. Four parameters (LDL-C, total cholesterol, total cholesterol/HDL-C ratio and LDL-C/HDL-C ratio) were applied for the PCSK9 protein expression levels, and 3 parameters (triglyceride, total cholesterol and total cholesterol/HDL-C ratio) were applied for the SREBP-2 expression levels. P values lower than 0.05 were considered statistically significant.

Results

The study population

Table 1 shows some of the biochemical and demographic characteristics in study population.

Plasma PCSK9

The results showed that the plasma PCSK9 protein levels did not differ significantly between gender and BMI subgroups ($p < 0.2$ and $p < 0.6$, respectively), although the PCSK9 level was higher in women with BMI values over 30 (Table 2). The linear regression analyses showed that circulating PCSK9 levels were significantly correlated to LDL-C ($r = 0.31$, $p = 0.001$), total cholesterol ($r = 0.29$, $p = 0.002$), total cholesterol/HDL-C ratio ($r = 0.26$, $p = 0.005$) and LDL-C/HDL-C ratio ($r = 0.32$, $p = 0.001$) (Table 3). Moreover, the multiple regression analysis for the significant parameters showed that the circulating PCSK9 level is strictly correlated with the total cholesterol/HDL-C ratio ($\beta = 3.53$, $p = 0.001$) (Table 4).

SREBP-2 expression

The results showed that SREBP-2 expression was significantly higher in men than in women ($p = 0.009$). Furthermore, the results showed significant linear correlations between SREBP-2 expression and other parameters, including triglyceride ($r = 0.34$, $p = 0.001$), total cholesterol ($r = 0.21$, $p = 0.02$) and total cholesterol/HDL-C ratio ($r = 0.16$, $p = 0.04$) (Table 5). The multiple regression analysis of triglyceride, total cholesterol and the total cholesterol/HDL-C ratio showed that the SREBP-2 expression level is more related to triglyceride ($\beta = 0.07$, $p = 0.004$) (Table 6). No significant linear correlation ($r = 0.2$, $p = 0.3$) was observed between the plasma PCSK9 protein and SRBPE-2 expression levels (Fig. 1).

Fig. 1. Linear correlation between circulating PCSK9 protein and SREBP-2 expression levels

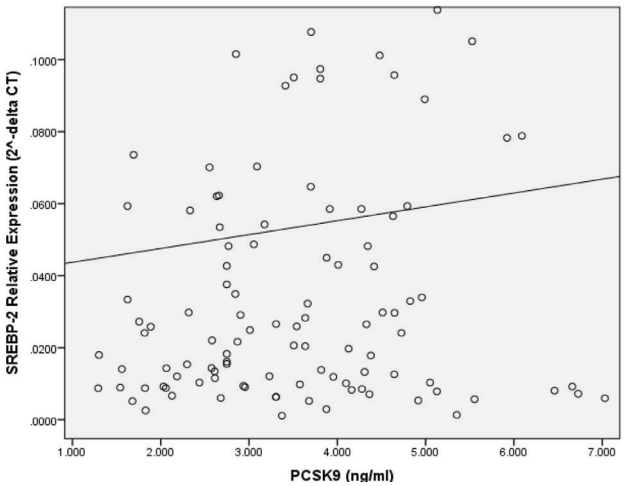

Table 1. Study population characteristics

Parameter	Mean ± SD/(n)
Age (years)	44.74 ± 11.49
Gender (male/female)	(65/55)
BMI (kg/m^2)	29.95 ± 3.64
Total Cholesterol, TC (mg/dL)	184.22 ± 59.57
LDL-C (mg/dL)	120.43 ± 26.91
HDL-C (mg/dL)	54.88 ± 10.64
Triglyceride, TG (mg/dL)	211.15 ± 124.27
LDL-C/HDL-C ratio	2.26 ± 0.68
Total Cholesterol/HDL-C ratio	3.43 ± 1.87

Table 2. PCSK9 protein and SREBP-2 expression levels

Parameter (n)	SREBP–2 ($E^{\Delta CT}$) (min–max)	PCSK9 (ng/mL) mean ± SD
Gender male (65) female (55) p-value	 0.1–35.31 0.1–27.29 0.009	 3.37 ± 1.25 3.77 ± 1.36 0.1
Total (120)	0.1–35.38	3.54 ± 1.31
BMI < 30 (77) ≥ 30 (43) p-value	 0.1–35.2 0.26–27.9 0.72	 3.49 ± 1.29 3.66 ± 1.35 0.5
Total (120)	0.1–35.38	3.54 ± 1.31

Table 3. Linear regression analyses between plasma PCSK9 level and study parameters

Parameter	Correlation coefficient (r-value)	p-value
Age (year)	0.15	0.10
Body mass index BMI (kg/m^2)	0.05	0.54
Triglyceride (mg/dL)	0.07	0.44
HDL-C (mg/dL)	-0.01	0.89
LDL-C (mg/dL)	0.31	0.001
Gender (male 1, female 2)	0.15	0.1
Total cholesterol (mg/dL)	0.29	0.002
LDL-C/HDL-C ratio	0.26	0.005
Total cholesterol/ HDL-C ratio	0.32	0.001

Table 4. Multiple regression analysis for plasma PCSK9 level

Parameter	β	SE	p-value
LDL-C (mg/dL)	0.104	0.025	0.007
Total cholesterol (mg/dL)	0.059	0.015	0.001
LDL-C/HDL-C ratio	-5.079	1.299	0.005
Total cholesterol/ HDL-C ratio	3.533	0.853	0.001

Table 5. Linear regression analyses between SREBP-2 expression level and study parameters

Parameter	Correlation coefficient (r-value)	p-value
Age (year)	0.08	0.36
Body mass index, BMI (kg/m^2)	0.006	0.95
Triglyceride (mg/dL)	0.34	0.001
HDL-C (mg/dL)	0.033	0.72
LDL-C (mg/dL)	0.14	0.12
Gender (male 1, female 2)	-0.11	0.22
Total cholesterol (mg/dL)	0.21	0.02
LDL-C/HDL-C ratio	0.07	0.45
Total cholesterol/ HDL-C ratio	0.16	0.04

Table 6. Multiple regression analysis for SREBP-2 expression

Parameter	β	SE	p-value
Total cholesterol (mg/dL)	0.01	0.001	0.05
Total cholesterol / HDL-C ratio	-0.009	0.008	0.366
Triglyceride (mg/dL)	0.07	0.001	0.004

Discussion

Experimental studies on the role of PCSK9 in cholesterol homeostasis have revealed that PCSK9 acts as a plasma chaperone for LDLR internalization and LDLR lysosomal degradation.[8] Furthermore, finding the sterol regulatory element (SRE) within the PCSK9 promoter showed that PCSK9 expression level is related to the SREBP-2 transcription factor.[9] In the present study, the participants' lipid profiles, PCSK9 protein levels and SREBP-2 expression levels were evaluated. Some studies have suggested that the effect of PCSK9 on cellular membrane LDLR levels might be different in extra-hepatic tissues producing steroids[10,11] In the present study, the circulating PCSK9 level was significantly correlated with LDL-C and total cholesterol levels, confirming other studies[12,13] The results also revealed that the PCSK9 level is strictly correlated with the total cholesterol/HDL-C ratio, which might be a better indicator for assessing PCSK9. The data also showed a linear correlation, but not a significant one, between the plasma PCSK9 protein and SREBP-2 expression levels. Since liver tissue is the primary site for PCSK9 expression[14], it might be due to physiological characteristics of extra-hepatic tissues that excess SREBP-2 increases LDL uptake due to LDLR over-expression.[15,16] There is a controversy over the function of PCSK9 and SREBP-2, since the former internalizes LDLR while the latter induces it. Furthermore, regression analyses showed that circulating PCSK9 is more closely associated with serum cholesterol-related parameters than SREBP-2 expression levels are. The authors suggested that cholesterol homeostasis could be due to degradation of LDLR rather than SREBP-2 induction. In agreement with this hypothesis, Dong et al. suggested that PCSK9 plays a significant role in LDLR degradation in hamsters.[17]

Moreover, many studies have reported the increase of SREBP-2 in response to reduced intracellular cholesterol levels.[18] The results of the present study show a weak correlation between total cholesterol and SREBP-2 expression levels. The authors suggested that the induction of intracellular cholesterol synthesis by SREBP-2 reduces the need of extracellular cholesterol source in accordance with PCSK9 function.

Conclusions

In conclusion, the results of the current study revealed that serum cholesterol-related parameters are associated with circulating PCSK9 protein. Furthermore, total cholesterol/HDL-C ratio was strictly correlated with PCSK9 function. Many studies have reported that SREBP-2 is able to increase PCSK9 and LDLR expression levels so that they can inversely affect serum LDL-C levels. The present authors suggest that PCSK9 function has a greater effect on serum total cholesterol levels than SREBP-2 expression.

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