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Original Investigation

Plasma PCSK9 in Nephrotic Syndrome and in Peritoneal Dialysis: A Cross-sectional Study

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Background: Serum total and low-density lipoprotein (LDL) cholesterol levels are elevated in patients with nephrotic syndrome and those with kidney failure treated by peritoneal dialysis (PD), who are characterized by heavy losses of protein in urine and peritoneal dialysate, respectively. Hypercholesterolemia in nephrotic syndrome is associated with and largely due to acquired LDL receptor (LDLR) deficiency. Because PCSK9 (proprotein convertase subtilisin/kexin type 9) promotes degradation of LDLR, we tested the hypothesis that elevation of LDL cholesterol levels in patients with nephrotic syndrome and PD patients may be due to increased PCSK9 levels.

Study Design: Cross-sectional study.

Setting & Participants: Patients with nephrotic syndrome or treated by PD or hemodialysis and age- and sex-matched healthy Korean individuals (n = 15 in each group).

Predictor: Group and serum total and LDL cholesterol levels.

Outcomes: Plasma PCSK9 concentration.

Measurements: Concentrations of fasting serum PCSK9, lipids, and albumin, and urine protein excretion.

Results: Mean serum total and LDL cholesterol levels in patients with nephrotic syndrome (317.9 ± 104.2 [SD] and 205.9 ± 91.1 mg/dL) and PD patients (200.0 ± 27.6 and 126.7 ± 18.5 mg/dL) were significantly ($P < 0.05$) higher than in hemodialysis patients (140.9 ± 22.9 and 79.1 ± 19.5 mg/dL) and the control group (166.5 ± 26.5 and 95.9 ± 25.2 mg/dL). This was associated with significantly ($P < 0.05$) higher plasma PCSK9 levels in patients with nephrotic syndrome (15.13 ± 4.99 ng/mL) and PD patients (13.30 ± 1.40 ng/mL) than in the control (9.19 ± 0.60 ng/mL) and hemodialysis (7.30 ± 0.50 ng/mL) groups. Plasma PCSK9 level was directly related to total and LDL cholesterol concentrations in the study population ($r = 0.559$ [$P < 0.001$] and $r = 0.497$ [$P < 0.001$], respectively).

Limitations: Small number of participants may limit generalizability.

Conclusions: Nephrotic syndrome and PD are associated with higher plasma PCSK9 concentration, which can contribute to elevation of LDL levels by promoting LDLR deficiency.

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INDEX WORDS: Proteinuria; hyperlipidemia; low-density lipoprotein (LDL) receptor; low-density lipoprotein (LDL) cholesterol; cardiovascular disease; atherosclerosis; PCSK9 (proprotein convertase subtilisin/kexin type 9).

Heavy glomerular proteinuria, otherwise known as nephrotic syndrome, is associated with hypercholesterolemia and marked elevation of serum low-density lipoprotein (LDL) cholesterol levels.[1,2] Hypercholesterolemia in nephrotic syndrome is largely due to impaired clearance and catabolism of LDL and apolipoprotein B100, which is the LDL's principal apoprotein.[3,4] In an attempt to discern the mechanism of impaired LDL clearance in nephrotic syndrome, in a series of earlier studies we explored the expression of LDL receptor (LDLR) in the liver of 2 model systems, rats with puromycin aminonucleoside-induced nephrotic syndrome and Imai rats with spontaneous focal glomerulosclerosis showing heavy proteinuria and severe hypercholesterolemia. These studies showed a marked reduction in LDLR protein in hepatic tissue.[5-7] Interestingly, the severe deficiency in LDLR protein is accompanied by normal LDLR messenger RNA expression,[5,8] suggesting a post-transcriptional or post-translational cause. It should be noted that in addition to causing LDLR deficiency, alteration in the composition of LDL in nephrotic syndrome may contribute to its impaired clearance by interference with the receptor binding process.[9]

By virtue of its capacity to bind and clear LDL from the circulation, LDLR plays a pivotal role in LDL and cholesterol metabolism. When LDL binds to the LDLR on the hepatocyte surface, a complex forms and LDL undergoes endocytosis and lysosomal degradation. The LDLR then is returned to the cell membrane to repeat the cycle. Given the critical role of LDLR in

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the clearance of LDL and its cholesterol cargo, the acquired LDLR deficiency shown in the aforementioned rat studies[5-8] elucidates the principal cause of impaired LDL clearance and elevated serum LDL level in nephrotic syndrome. However, the underlying mechanism(s) by which nephrotic syndrome decreases hepatic LDLR protein expression is not known.

PCSK9 (proprotein convertase subtilisin/kexin type 9) plays an important part in the post-translational regulation of LDLR expression and hence LDL metabolism.[10] PCSK9 is a serine protease that is produced and released in the circulation by the liver and to a lesser extent by the intestine and kidney. On the surface of hepatocytes, PCSK9 binds to the LDLR, forming a complex that is internalized and directs LDLR for intracellular degradation.[11] It should be noted that PCSK9 acts as a chaperone to facilitate intracellular degradation of LDLR, and that this role is independent of its enzymatic activity.[12,13] By promoting LDLR degradation, PCSK9 prevents recycling of LDLR to the cell membrane, leading to a post-translational reduction in LDLR expression.[10] Individuals with loss-of-function mutation of *PCSK9* exhibit a very low plasma LDL cholesterol level and a significant reduction in the risk of coronary heart disease.[14] For this reason, PCSK9 has emerged as a novel therapeutic target for the treatment of hypercholesterolemia.

Unlike the majority of hemodialysis patients, in whom serum total and LDL cholesterol levels are within or below normal limits, patients maintained on peritoneal dialysis (PD) therapy have significantly elevated levels. In this context, the lipid profile in PD patients resembles that commonly found in patients with nephrotic syndrome.[15,16] Heavy losses of protein in urine in patients with nephrotic syndrome and in PD effluent in PD patients represent a shared feature that may account for the similarity in their serum cholesterol and LDL cholesterol levels. It therefore is reasonable to assume that similar mechanisms may be involved in the pathogenesis of these lipid disorders in patients with nephrotic syndrome and PD patients.

Given the central role of PCSK9 in preventing the recycling of LDLR, the present study was undertaken to test the hypothesis that LDLR deficiency may be due in part to increased plasma PCSK9 levels. To this end, plasma PCSK9 levels were determined in a group of patients with nephrotic-range proteinuria. A group of age-, sex-, and ethnicity-matched healthy individuals served as controls. To determine the potential role of PCSK9 in the pathogenesis of hypercholesterolemia in PD patients, cohorts of patients with end-stage renal disease maintained on PD and hemodialysis therapy were included in the study as well.

METHODS

Participant Characteristics

Fifteen patients (6 men and 9 women aged 41.9 ± 17.1 [SD] years) with nephrotic-range proteinuria (urine protein excretion ≥ 3.5 g/24 h), 15 PD patients (7 men and 8 women aged 48.6 ± 7.6 years), and 15 hemodialysis patients (7 men and 8 women aged 47.4 ± 12.6 years) were recruited into the study. The underlying causes of nephrotic syndrome were as follows: immunoglobulin A nephropathy in 4; minimal change disease, membranous nephropathy, membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis, and lupus nephritis in 2 patients each; and crescentic glomerulonephritis in one patient. The underlying causes of kidney disease in the hemodialysis group were diabetes mellitus in 8, hypertension in 5, and chronic glomerulonephritis in 2. The underlying causes of kidney disease in the PD group were diabetes mellitus in 8, hypertension in 4, and chronic glomerulonephritis in 3. Fifteen apparently healthy individuals (6 men and 9 women; mean age, 45.7 ± 6.4 years) served as controls.

Individuals younger than 18 years, those with a history of malignancy or chronic liver disease, and those with a history of infection within the previous 4 weeks were excluded from the study. The study protocol was approved by the Human Subjects Institutional Review Board of the Inje University Haeundae Paik Hospital, and all participants signed the informed consent forms.

Laboratory Measurements

Fasting blood samples were obtained by venipuncture from all patients and controls and 24-hour urine samples were collected in patients with nephrotic syndrome using standard containers. Urinary protein excretion and serum concentrations of albumin, total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, and urea nitrogen were measured by the central laboratory of the Inje University Haeundae Paik Hospital.

Plasma PCSK9 was measured by an enzyme-linked immunosorbent assay using the kit purchased from Cell Biolabs Inc according to the manufacturer's specifications.

Data Analysis

Mann-Whitney *U* test for continuous variables and Spearman coefficient for regression analysis were used in statistical analysis of the data, which are expressed as mean ± standard deviation. Correlations between PCSK9, total cholesterol, LDL cholesterol, and serum albumin levels initially were analyzed by univariate regression analysis followed by multivariate regression analysis. $P \leq 0.05$ was considered significant.

RESULTS

Characteristics of Study Groups

Data are summarized in Table 1. As expected, the nephrotic-syndrome group had marked proteinuria (urinary protein-creatinine ratio, 8.69 ± 7.0 [range, 3.53-29.2] g/g) and hypoalbuminemia. In contrast, serum albumin concentration was within normal limits and proteinuria was absent in the control group. Heavy proteinuria in the nephrotic-syndrome group was associated with marked elevations in serum total cholesterol, LDL cholesterol, very low-density lipoprotein cholesterol, and triglyceride concentrations. No significant difference was found in serum high-density lipoprotein cholesterol or creatinine concentrations between the nephrotic-syndrome and control groups.

Table 1. Characteristics of Nephrotic Syndrome, PD, and HD Patients and Apparently Healthy Controls

	Control (n = 15)	Nephrotic Syndrome (n = 15)	HD (n = 15)	PD (n = 15)
Age (y)	45.7 ± 6.4	41.9 ± 17.1	47.4 ± 12.6	48.6 ± 7.6
Female sex	9 (60)	9 (60)	8 (53)	8 (53)
Blood pressure				
Systolic (mm Hg)	119.3 ± 5.3	121.9 ± 16.1	137.7 ± 19.4[a]	140.5 ± 23.6[a]
Diastolic (mm Hg)	74.7 ± 6.7	74.2 ± 9.9	81.1 ± 12.1[a]	85.1 ± 15.8[a]
Cholesterol				
Total (mg/dL)	166.5 ± 26.5	317.9 ± 104.2[b]	140.9 ± 22.9[b]	200.0 ± 27.6[b,c]
LDL (mg/dL)	95.9 ± 25.2	205.9 ± 91.1[b]	79.1 ± 19.5[b]	126.7 ± 18.5[a,d]
HDL (mg/dL)	53.4 ± 12.2	56.9 ± 12.5	41.1 ± 11.2[b]	39.3 ± 8.1[a]
Triglycerides (mg/dL)	94.6 ± 51.9	273.5 ± 111.1[b]	103.8 ± 52.3	169.9 ± 104.4[b,d]
Serum urea nitrogen (mg/dL)	11.2 ± 2.8	26.7 ± 22.9[b]	73.8 ± 12.2[a]	58.3 ± 15.1[a]
Serum creatinine (mg/dL)	0.8 ± 0.1	1.5 ± 0.9	10.6 ± 2.6[a]	11.1 ± 4.6[a]
Serum albumin (mg/dL)	4.5 ± 0.2	2.6 ± 1.0[b]	3.7 ± 0.3[a]	3.3 ± 0.4[a,d]
PCSK9 (ng/mL)	9.19 ± 0.60	15.13 ± 4.99[a]	7.30 ± 0.50	13.30 ± 1.40[b,c]
Urine PCR (g/g)	NA	8.69 ± 7.0	NA	NA

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as mean ± standard deviation. Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; urea nitrogen in mg/dL to mmol/L, ×0.357; HDL, LDL, and total cholesterol in mg/dL to mmol/L, ×0.02586; triglycerides in mg/dL to mmol/L, ×0.01129.

Abbreviations: HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable; PCR, protein-creatinine ratio; PCSK9, proprotein convertase subtilisin/kexin type 9; PD, peritoneal dialysis.

[a] $P < 0.001$ versus control group.
[b] $P < 0.05$ versus control group.
[c] $P < 0.001$ versus HD group.
[d] $P < 0.05$ versus HD group.

Likewise, arterial blood pressure was similar in the 2 groups. However, serum urea nitrogen concentration was significantly higher in the nephrotic-syndrome group compared with controls. As in the group with nephrotic syndrome, serum total and LDL cholesterol concentrations were elevated in the PD group, but were within or below normal values in the hemodialysis group.

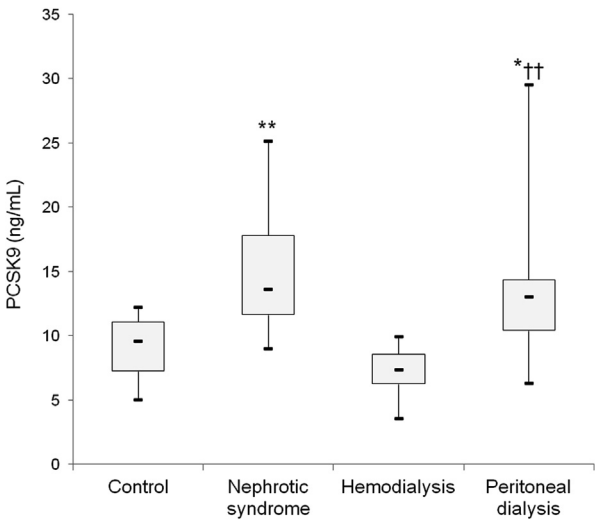

Figure 1. Box plots depicting fasting plasma PCSK9 (proprotein convertase subtilisin/kexin type 9) concentrations in the nephrotic-syndrome, hemodialysis, peritoneal dialysis, and control groups. *$P < 0.05$, **$P < 0.001$ versus control group. †$P < 0.05$, ††$P < 0.001$ versus hemodialysis group.

Plasma PCSK9 Data

Plasma PCSK9 concentration in the group with nephrotic syndrome (15.13 ± 4.99 ng/mL) was significantly ($P < 0.05$) higher than that in the control group (9.19 ± 0.60 ng/mL; Fig 1). Plasma PCSK9 concentration correlated positively with total cholesterol and LDL cholesterol concentrations and negatively with serum albumin concentration. As in the group with nephrotic syndrome, plasma PCSK9 level was elevated in PD but not hemodialysis patients. Multiple regression analysis revealed a significant direct correlation between plasma PCKS9 and serum total cholesterol and LDL cholesterol concentrations in the nephrotic-syndrome and PD groups (Fig 2; Table 2).

DISCUSSION

The present study demonstrated the association of hypercholesterolemia and elevated LDL cholesterol concentration, which are the hallmarks of nephrotic syndrome, with a significant increase in plasma PCSK9 levels in patients with nephrotic syndrome. Our finding of elevated plasma PCSK9 levels in patients with nephrotic syndrome is consistent with a study conducted in rats with nephrotic syndrome,[17] which showed marked elevation of serum total and LDL cholesterol levels and a significant reduction in hepatic LDLR, accompanied by marked upregulation of hepatic tissue PCSK9 expression and heightened liver X receptor activation. In addition, rats with

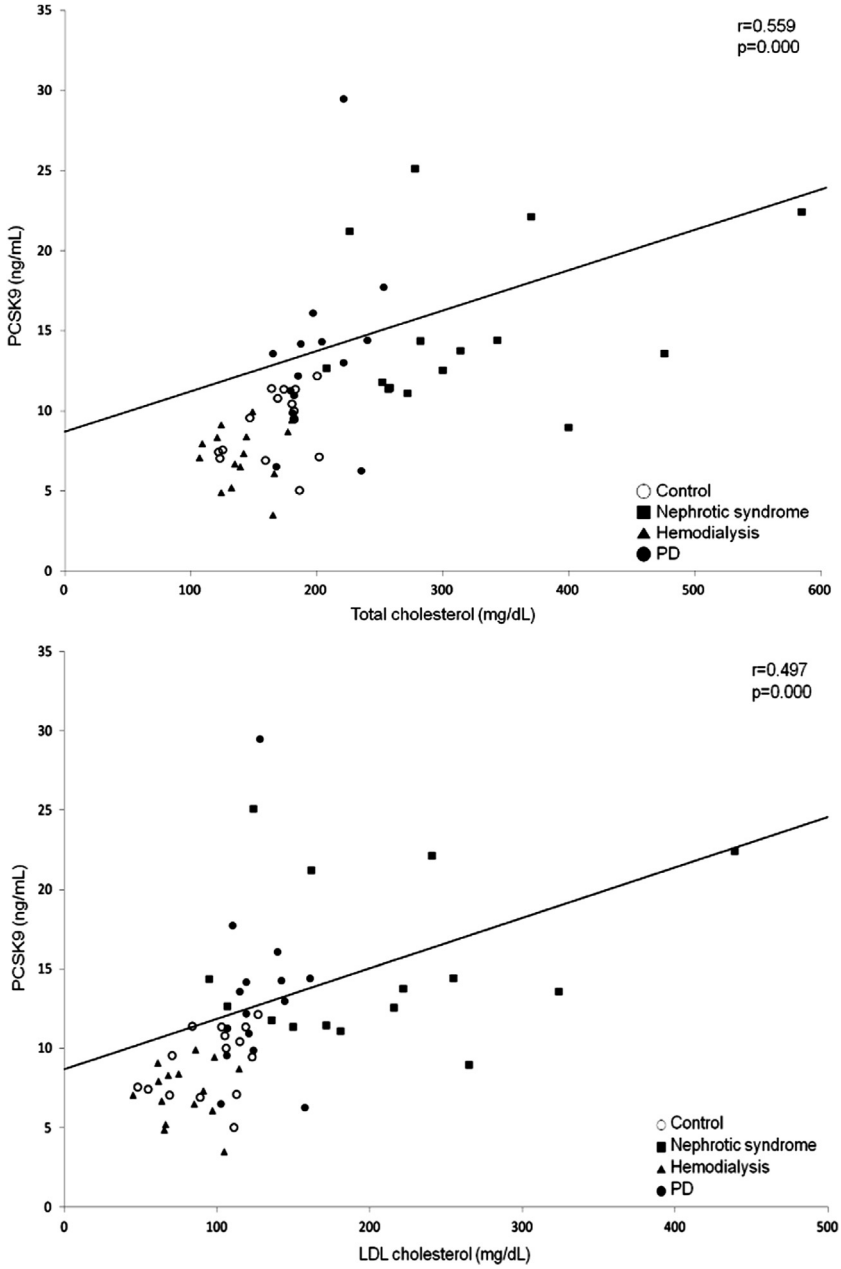

Figure 2. Correlations of serum total and low-density lipoprotein (LDL) cholesterol concentrations with plasma PCSK9 (proprotein convertase subtilisin/kexin type 9) levels in the study population. The *r* value represents the nonparametric Spearman correlation coefficient. Abbreviation: PD, peritoneal dialysis.

nephrotic syndrome showed a significant increase in expression of inducible degrader of LDLR, which is the intracellular counterpart of PCSK9. Together, upregulation of plasma PCSK9 and hepatocyte-inducible degrader of LDLR work in concert to cause LDLR deficiency in nephrotic syndrome. Previously, we had shown in animal models of spontaneous and puromycin aminonucleoside–induced nephrotic syndrome that hepatic LDLR gene expression level is normal; these new findings help explain why the receptor nevertheless is depleted in nephrotic syndrome.[5-8]

By mediating degradation and limiting recycling of LDLR, upregulation of PCSK9 must play a major role in the pathogenesis of hypercholesterolemia and the associated risk of cardiovascular disease, as we have shown in both humans and animals with nephrotic syndrome. Several studies have demonstrated significant direct correlations between plasma PCSK9 and LDL cholesterol levels in the general population.[18-20] Moreover, elevated plasma PCSK9 level has been shown to be predictive of recurrent clinical events in patients with stable cardiovascular disease treated with low-dose atorvastatin.[21]

PCSK9 expression is regulated primarily by the transcription factor sterol regulatory element-binding protein 2 (SREBP-2),[22,23] which is activated when intracellular free cholesterol levels decline and is

Table 2. Spearman Correlation of Serum Cholesterol Concentrations With Plasma PCSK9 Levels

	r	*P*
Control		
Total cholesterol	0.348	0.2
LDL cholesterol	0.396	0.1
Nephrotic syndrome		
Total cholesterol	0.509	0.001
LDL cholesterol	0.414	0.003
Hemodialysis		
Total cholesterol	0.389	0.03
LDL cholesterol	0.361	0.04
Peritoneal dialysis		
Total cholesterol	0.499	0.005
LDL cholesterol	0.384	0.04

Abbreviations: LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

inhibited when they increase. Several factors tend to reduce hepatocyte free cholesterol concentration and thereby promote activation of SREBP-2 and upregulation of PCSK9 expression. Chief among them is acyl-coenzyme A cholesterol acyltransferase 2 (ACAT-2; encoded by the *SOAT2* gene), which catalyzes esterification of free cholesterol. Earlier studies by our group have shown marked upregulation of hepatic ACAT-2 in nephrotic syndrome.[7,24] The potential role of upregulation of ACAT-2 is evidenced by the dramatic amelioration of hypercholesterolemia and marked reduction in plasma LDL cholesterol level with administration of ACAT inhibitor in animals with nephrotic syndrome.[7] In addition, by lowering the uptake of LDL and its cholesterol cargo from the circulation, depletion of hepatic LDLR in nephrotic liver contributes to the reduction in hepatocyte cholesterol, which can increase PCSK9 expression by activation of SREBP-2. Thus, upregulation of PCSK9 and depletion of LDLR participate in a vicious circuit in which each begets and intensifies the other.

Because of the small number of participants in this single-center study, generalizability of the findings may be limited. However confirmation of these findings in our carefully conducted study in animals with nephrotic syndrome[17] strongly supports the validity of the results presented here.

Unlike hemodialysis patients, in whom serum total and LDL cholesterol levels usually are within or below normal limits, PD patients frequently exhibit increased serum cholesterol and LDL cholesterol levels. This is associated with and most likely due to losses of proteins in PD effluent, which simulates nephrotic syndrome in people who are functionally anephric. The elevation in plasma PCSK9 level found in PD patients but not hemodialysis patients supports this contention.

It should be noted that plasma PCSK9 level in humans has a marked diurnal rhythm, which parallels that of cholesterol biosynthesis. Hepatic PCSK9 expression and its plasma levels dramatically decline during fasting.[25-27] For this reason, plasma PCSK9 levels should be measured at a defined period of the day, such as in the morning after an overnight fast, as was done here, to achieve accurate comparisons between and within groups.

In conclusion, hypercholesterolemia and elevation of plasma LDL cholesterol levels in patients with nephrotic syndrome and PD patients are associated with increased plasma PCSK9 levels. Results from a separate animal study suggest that increases in plasma PCSK9 levels can cause LDLR deficiency by promoting degradation and limiting recycling of the receptor. The resulting LDLR deficiency in turn can be expected to heighten the risk of cardiovascular events by increasing the concentration and prolonging the residence time of LDL in the circulation. Therefore, strategies aimed at lowering plasma PCSK9 levels may be effective in attenuating hypercholesterolemia and the associated risk of cardiovascular events in patients with nephrotic syndrome and PD patients.

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