The relationship of plasma catestatin concentrations with metabolic and vascular parameters in untreated hypertensive patients: Influence on high-density lipoprotein cholesterol

Murtaza Emre Durakoğlugil, Teslime Ayaz, Sinan Altan Kocaman, Aynur Kırbaş**, Tuğba Durakoğlugil***, Turan Erdoğan, Mustafa Çetin, Osman Zikrullah Şahin*, Yüksel Çiçek*

Departments of Cardiology, *Internal Medicine, **Biochemistry, ***Radiology, Faculty of Medicine, Recep Tayyip Erdoğan University; Rize-*Turkey*

ABSTRACT

Objective: Catestatin has several cardiovascular actions, in addition to diminished sympatho-adrenal flow. Decreased plasma catestatin levels may reflect a predisposition for the development of hypertension and metabolic disorders. We planned to investigate the possible roles of catestatin in untreated hypertensive patients. As a secondary objective, we compared catestatin concentrations of healthy subjects with those of hypertensive patients in order to understand whether catestatin is increased reactively or diminished at onset.

Methods: Our study was cross-sectional and observational. The patient group, comprising 109 consecutive untreated hypertensive patients without additional systemic or coronary heart disease, underwent evaluations of plasma catestatin, waist circumference, lipid parameters, left ventricular mass, carotid intima-media thickness, and flow-mediated dilation of the brachial artery. Additionally, we measured catestatin concentrations of 38 apparently healthy subjects without any disease using a commercial enzyme-linked immunosorbent assay kit.

Results: We documented increased catestatin concentrations in previously untreated hypertensive patients compared to healthy controls (2.27±0.83 vs. 1.92±0.49 ng/mL, p=0.004). However, this association became insignificant after adjustments for age, gender, height, and weight. Within the patient group, catestatin levels were significantly higher in females. Among all study parameters, age, high-density lipoprotein cholesterol (HDL-C) correlated positively to plasma catestatin, whereas triglycerides, hemoglobin, and left ventricular mass correlated negatively to plasma catestatin. We could not detect an association between vascular parameters and catestatin. Catestatin levels were significantly elevated with increasing HDL-C (1.91±0.37, 2.26±0.79, and 3.1±1.23 ng/mL in patients with HDL-C <40, 40-60, and >60 mg/dL, respectively). Multiple linear regression analysis revealed age (beta: 0.201, p=0.041) and HDL-C (beta: 0.390, p<0.001) as independent correlates of plasma catestatin concentration. Additionally, male gender (beta:-0.330, p=0.001) and plasma catestatin (beta: 0.299, p=0.002) were significantly associated with HDL-C concentrations.

Conclusion: We documented that plasma catestatin is an independent predictor of high-density lipoprotein cholesterol. In addition to antihypertensive effects, catestatin appears to be related to improved lipid and metabolic profiles. Coexistence of low catestatin levels with low HDL-C may provide a probable mechanism for the predictive value of low HDL-C for increased hypertension and cardiovascular events.

(Anatol J Cardiol 2015; 15: 577-85)

Keywords: catestatin, hypertension, high-density lipoprotein cholesterol, sympatho-adrenal flow, low HDL-C

Introduction

Catestatin is a peptide formed by proteolytic cleavage of chromogranin A (CHGA) that is co-stored and co-released with catecholamines in adrenal chromaffin cells and adrenergic neurons. Catestatin has gained considerable interest due to several cardiovascular effects (1). Since its discovery in 1997, diverse actions of catestatin, including inhibition of cholinergic catecholamine secretion, stimulation of mast cells culminating in histamine release and prominent vasodilation, chemotaxis, and anti-microbial properties, have been elucidated (2). Catestatin noncompetitively antagonizes nicotine-induced cholinergic catecholamine secretion in chromaffin cells and central and peripheral neurons (3). In contrast, catestatin restores the nicotinic desensitization state due to prolonged catecholamine stimulation and thus may sustain adrenergic effects in states of increased sympathetic flow (4). In addition, three biologic variants of catestatin with varying effects on adrenergic inhibition

Address for Correspondence: Dr. Murtaza Emre Durakoğlugil, Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, İslampaşa Mah., 53100, Rize-*Türkiye*
Phone: +90 464 212 30 09 Fax: +90 464 212 30 15 E-mail: emredur@hotmail.com

Accepted Date: 03.07.2014 **Available Online Date:** 17.07.2014

DOI:10.5152/akd.2014.5536

and autonomic functions have been identified (5, 6). Decreased plasma catestatin levels have been demonstrated not only in hypertensive patients but also in normotensive offspring of hypertensive subjects (7). Therefore, catestatin may reflect a genetic predisposition for the development of hypertension.

Hypertension (HT) is one of the most important risk factors for cardiovascular diseases. Blood pressure, influenced by both genetic and environmental factors, is likely to be a complex polygenic trait (8). Hypertension is an essential part of the metabolic syndrome and is closely related to other metabolic derangements, such as diabetes mellitus (9). Since sustained sympathetic and adrenal activity may both predispose one to hypertension and lead to detrimental cardiovascular alterations, including left ventricular hypertrophy, accelerated atherosclerosis, and an impaired metabolic profile, catestatin may physiologically counteract to prevent this process. To date, the metabolic and vascular effects of catestatin have not been fully elucidated. Therefore, we intended to investigate the role of plasma catestatin concentrations in hypertension. Additionally, we planned to compare catestatin concentrations of a patient population with apparently healthy control subjects.

Methods

Patient population and inclusion criteria

Our study, having a cross-sectional and observational design, included 109 consecutive, previously untreated hypertensive patients who underwent echocardiography and ultrasonography to evaluate vascular status and function by measuring carotid intima-media thickness (CIMT) and flow-mediated dilation (FMD) of the brachial artery. Waist circumference, plasma catestatin concentrations, and routine blood biochemistry values, including lipid profiles, were measured in order to demonstrate the metabolic and anthropometric associations of catestatin.

Patients with previous coronary artery, peripheral vascular, and cerebrovascular disease; left ventricular systolic dysfunction; renal or hepatic failure; secondary hypertension; infectious disease; or malignancy and patients previously treated for hypertension were excluded.

Control subjects (n=38), who gave blood samples solely for catestatin, were recruited from healthy volunteers with no history of either cardiovascular disease or hypertension who were seen by their family physician for a routine annual examination and agreed to join our study for research purposes.

Blood pressure values were obtained by auscultatory method. Resting blood pressure (BP) values were obtained at the physician's office and echocardiography room by traditional auscultatory method using a sphygmomanometer. Patients were advised to refrain from smoking, consumption of coffee or tea, and physical exercise 30 minutes prior to the measurement. Before measurement, patients were seated to rest for 5 minutes. Two separate measurements were averaged to determine office blood pressure.

Baseline characteristics of the patients were recorded. Hypertension was defined as the documentation of BP of more than 140/90 mm Hg. Diabetes mellitus was defined as fasting plasma glucose levels over 126 mg/dL or a glucose level over 200 mg/dL at any measurement or active use of antidiabetic treatment. Patients who were using tobacco products on admission to our hospital and those who quit smoking within the last year were considered smokers. Body mass index (BMI) was calculated by the following formula: BMI=weight (kg)/height^2 (m). Metabolic syndrome score was calculated according to current criteria (10). Low high-density lipoprotein cholesterol (HDL-C) was accepted as HDL-C level <35 mg/dL and <40 mg/dL in men and women, respectively. Left ventricular mass (LVM) was calculated according to the formula LVM=1.04 [(LVEDD + PW + IVS)3- (LVEDD)3] -13.6 g (11).

The study was performed in accordance with the principles stated in the Declaration of Helsinki and was approved by the Local Ethics Committee. Written informed consent was obtained from all patients prior to the study.

Biochemical analyses

Blood samples were drawn by venipuncture to measure routine blood chemistry parameters after fasting for at least 8 hours. Fasting blood glucose, serum creatinine, uric acid levels, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels were recorded. Glucose, creatinine, and lipid profiles were determined by standard methods. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula. However, we used a direct method in patients with a triglyceride level >400 mg/dL. Serum C-reactive protein (CRP) was analyzed using a nephelometric technique (Beckman Coulter Image 800; Fullerton, CA, USA; normal range 0-0.8 mg/dL).

Measurement of catestatin concentrations

Fasting blood samples were drawn into lavender vacutainer tubes that contained EDTA in the morning by venipuncture. Blood samples were immediately transferred from the vacutainer tubes to centrifuge tubes containing aprotinin (0.6 TIU/mL of blood) and gently rocked several times to inhibit protease activity. Blood samples were centrifuged at 1600 g for 15 minutes at 4°C to collect the plasma. Plasma samples were frozen and stored at -80°C until examination. Plasma catestatin levels were measured using a commercial enzyme-linked immunosorbent assay kit (Phoenix Pharmaceuticals, Inc; Burlingame, USA) following the manufacturer's instructions. The interassay and intra-assay coefficients of variability for catestatin were 15% and 10%, respectively. The sensitivity was calculated to be 0.05 ng/mL.

Measurement of carotid intima-media thickness

Ultrasonography was performed on all patients using a high-resolution ultrasonography scanner (Xario, Toshiba Medical Systems, Tokyo, Japan) with a PLT-805AT linear array transducer. Measurements were performed on the right and left carotid

arteries. The patient was lying supine, with the head directed away from the side of interest and the neck slightly extended. The transducer was manipulated so that the near and far walls of the CCA were parallel, and the lumen diameter was maximized in the longitudinal plane. The region 1 cm proximal to the carotid bifurcation was identified, and the CIMT of the far wall was evaluated as the distance between the lumen-intima interface and the media-adventitia interface. The CIMT was measured on the frozen frame of a suitable longitudinal image, with the image magnified to achieve a higher resolution of detail. The CIMT measurement was obtained from 4 contiguous sites at 1-mm intervals on each carotid artery, and the average of all 8 measurements was used for analysis. The same radiologist who was blinded to the patient data performed all measurements. The intra-observer mean absolute difference in measuring the common carotid intima-media thickness was 0.026±0.043 mm (coefficient of variation: 1.6%, intra-class correlation: 0.95). CIMT thicknesses >1.2 mm were considered plaques.

Assessment of brachial flow-mediated dilation

A standard protocol was used to assess endothelial function, as previously reported (12). Briefly, for the FMD of the brachial artery, patients fasted 8 h before the study. The FMD was evaluated at the same time of the day, at 9:00 AM. The study took place in a quiet, temperature-controlled room. Caffeine intake and cigarette smoking were prohibited for at least 4-6 h before the study. The right arm was immobilized using 2 cushions supporting the elbow and the wrist. A sphygmomanometric cuff was placed on the forearm. After 10-15 min of rest, the brachial artery was visualized longitudinally with the ultrasonic scanner operating in B mode. After an optimal image of the artery was obtained, the ultrasonic transducer was fixed in this position with a custom-built probe holder. Brachial artery diameter was determined manually in end-diastole, indicated by the R wave of the electrocardiogram.

After three baseline measurements were obtained, ischemia was induced by the inflation of the cuff to 10 mm Hg greater than the systolic arterial pressure to occlude arterial flow for 5 min. After the deflation of the cuff, diameter measurements were performed at 60 s. Following this, we used FMD at 1 min after ischemia to represent the spontaneous endothelial function. The maximal diameter obtained during ischemia-induced hyperemia was used for the calculation of the percentage of FMD (maximum diameter-baseline diameter)/baseline diameter x 100. The same radiologist performed the endothelial function study. The intra-observer reproducibility of resting arterial diameter was 0.01±0.01 mm.

Statistical analysis

Continuous variables were given as mean±SD; categorical variables were defined as percentages. Data were tested for normal distribution using the Kolmogorov-Smirnov test. The student's t-test was used for the univariate analysis of continuous variables. Pearson's and Spearman correlation coefficients were used for the correlation analysis of parametric and nonparametric variables, respectively. Mean values were compared by analysis of variance (ANOVA) among different HDL-C groups. Linear regression was used for the multivariate analysis of independent variables. All tests of significance were two-tailed. Statistical significance was defined as $p<0.05$. The Statistical Program for Social Sciences (SPSS for Windows 20, Inc., Chicago, IL, USA) was used for all statistical calculations.

Table 1. Baseline demographics and clinical characteristics of study population

Variables	Hypertensive patients (n=109)	Healthy controls (n=38)
Plasma catestatin level, ng/mL	2.27±0.83	1.92±0.49
Age, years	48±9	39±6
Gender, male	39%	47%
BMI, kg/m^2	31.7±5.3	26±3.6
Waist circumference, cm	96.5±10.2	86±9.6
Systolic BP, mm Hg	159±15	116±11
Diastolic BP, mm Hg	95±10	74±9
Pulse pressure, mm Hg	64.5±14	42±10
Diabetes mellitus	11%	0%
Smoking status	23%	0%
Dyslipidemia	18%	0%
Family history of CAD	28%	0%
Glucose, mg/dL	106±23	86±11
Creatinine, mg/dL	0.76±0.19	0.70±0.14
Uric acid, mg/dL	4.9±1.7	3.8±1.0
T. bilirubin, mg/dL	0.84±0.53	0.91±0.48
Direct bilirubin, mg/dL	0.29±0.14	0.30±0.12
Indirect bilirubin, mg/dL	0.59±0.59	0.60±0.37
Total cholesterol, mg/dL	220±37	168±38
LDL-cholesterol, mg/dL	141±32	94±40
HDL-cholesterol, mg/dL	48±13	46±8
Triglycerides, mg/dL	156±97	118±99
CRP, mg/dL	0.44±0.42	0.40±0.31
Leukocytes, /mm^3	7238±2175	6719±852
Hemoglobin, g/dL	13.8±1.8	14.6±1.3
Platelets, 10^3/mm^3	278±74	240±50
CIMT, mm, mean	0.73±0.18	-
Presence of carotid plaque	14%	-
Flow-mediated dilation %	14.8±7.2	-
Metabolic syndrome score	2.7±1.1	-
Left ventricular mass (g)	219±52	-

BMI - body mass index; BP - blood pressure; CAD - coronary artery disease; CIMT - carotid intima-media thickness; CRP - C-reactive protein; HDL - high-density lipoprotein; LDL - low-density lipoprotein; SD - standard deviation

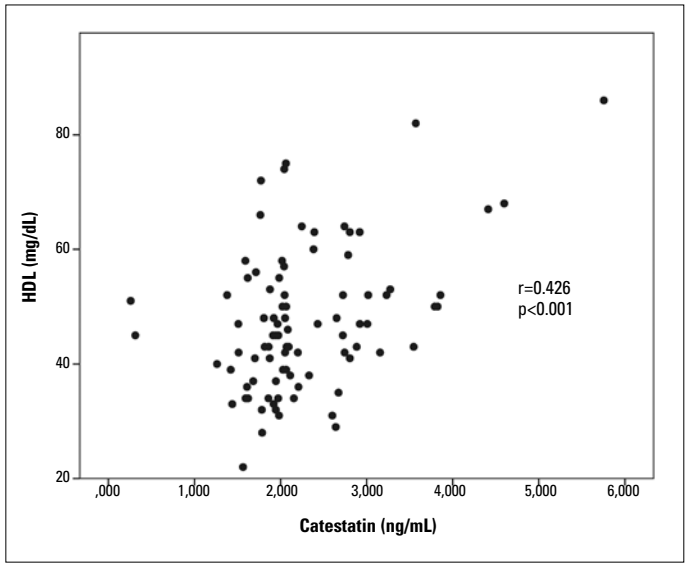

Figure 1. Relationship of plasma catestatin concentration with HDL cholesterol

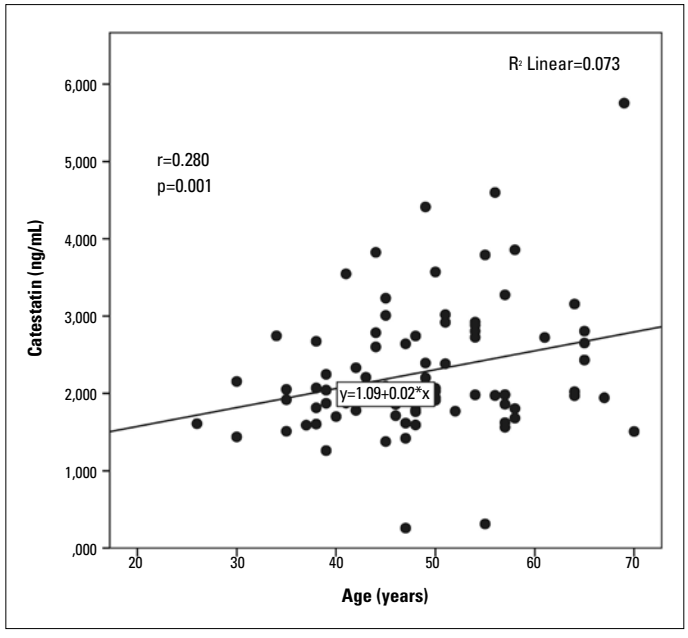

Figure 2. Relationship of plasma catestatin concentration with age

Results

Control subjects

The demographic characteristics of the patients and control group are detailed in Table 1. The control group included 38 healthy subjects (39±6 years) with no apparent risk factors. We compared catestatin concentrations of our patient group with controls and documented increased catestatin concentrations in previously untreated hypertensive patients (2.27±0.83 vs. 1.92±0.49 ng/mL, p=0.004). The difference between control subjects and hypertensive patients became insignificant after adjusting for age, gender, height, and weight. The data of the control group were not included in further analyses.

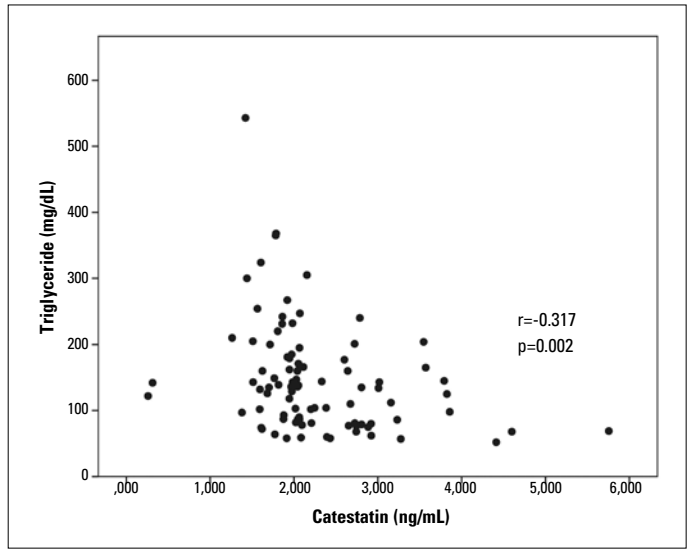

Figure 3. Relationship of plasma catestatin concentration with triglycerides

Patient characteristics

The patient population consisted of 109 middle-aged (mean age, 48±9 years) men (39%) and women with untreated hypertension. These patients were slightly obese (31.7±5.3 kg/m^2) with an enlarged waist circumference (96.5±10.2 cm). A low proportion of patients were diabetic (11%) and dyslipidemic (18%). Approximately one-fifth of the group was a current smoker.

Except for mildly increased serum glucose and cholesterol concentrations, the biochemical parameters were within normal limits. The mean carotid intima-media thickness was 0.73±0.18 mm, and 14% of study group had carotid plaques. Brachial flow-mediated dilation was 14.8±7.2. The patient group had a mean metabolic syndrome score of 2.7±1.1; therefore, the majority of our patients (53%) also had metabolic syndrome. The prevalence of low HDL-C was calculated as 23% (men: 33%, women: 17%).

Catestatin and study parameters

Catestatin levels were significantly higher in females (2.33±0.84 vs. 1.92±0.54 ng/mL, p=0.001). Among all study parameters, high-density lipoprotein cholesterol (HDL-C) (r=0.426, p<0.001) (Fig. 1) and age (r=0.280, p=0.001) (Fig. 2) correlated positively to plasma catestatin concentrations, whereas triglycerides (r=-0.317, p=0.002) (Fig. 3), hemoglobin (r=-0.273, p=0.010), and left ventricular mass (r=-0.230, p=0.034) correlated negatively to plasma catestatin concentrations (Table 2). Catestatin levels were significantly lower in patients with low HDL-C compared to patients with normal HDL-C (1.89±0.41 vs. 2.37±0.88, p=0.001).

Catestatin levels were similar in smokers compared to non-smokers (2.29±0.84 vs. 2.26±0.83, p=0.901).

The correlations between catestatin and smoking (number/day: r=-0.015, p=0.889 and pack/day: r=-0.002, p=0.982) were insignificant.

There was no significant association between plasma catestatin and carotid intima-media thickness, carotid plaques, and flow-mediated dilation of the brachial artery.

Table 2. Correlations of plasma catestatin level with metabolic and inflammatory parameters

Parameters	Plasma catestatin level, ng/mL	
	r	*P* value†
Age, years	0.280	0.001
BMI, kg/m^2	0.160	0.073
Waist circumference, cm	0.061	0.493
Systolic BP, mm Hg	-0.038	0.725
Diastolic BP, mm Hg	-0.021	0.847
Pulse pressure, mm Hg	-0.023	0.831
Glucose, mg/dL	-0.039	0.715
Creatinine, mg/dL	-0.083	0.441
T. bilirubin, mg/dL	-0.113	0.291
Direct bilirubin, mg/dL	-0.036	0.741
Indirect bilirubin, mg/dL	-0.138	0.196
Uric acid, mg/dL	-0.173	0.106
Total cholesterol, mg/dL	0.036	0.735
LDL-cholesterol, mg/dL	0.055	0.610
HDL-cholesterol, mg/dL	0.426	<0.001
Triglycerides, mg/dL	-0.317	0.002
CRP, mg/dL	0.045	0.680
Leukocytes, /mm^3	-0.123	0.250
Hemoglobin, g/dL	-0.273	0.010
Platelets, 10^3/mm^3	0.012	0.908
CIMT, mm, mean	0.164	0.137
Flow-mediated dilation, %	0.068	0.537
Metabolic syndrome score	-0.098	0.360
Left ventricular mass, g	-0.230	0.034

BMI - body mass index; BP - blood pressure; CRP - C-reactive protein; CIMT - carotid intima-media thickness; HDL - high-density lipoprotein; LDL - low-density lipoprotein; SD - standard deviation. †Pearson and Spearmen tests were used to analyze the relationship between catestatin and study variables where appropriate

HDL cholesterol and catestatin

We divided our patient group into three subgroups according to HDL-C concentrations: low (<40 mg/dL), normal (40-60 mg/dL), and high (>60 mg/dL) (Table 3). Catestatin levels were significantly different among the three HDL-C groups, and a gradual increase in plasma catestatin level was observed with increasing HDL-C. The plasma catestatin concentrations were 1.91±0.37, 2.26±0.79, and 3.1±1.23 ng/mL in the low, normal, and high HDL-C groups, respectively (p<0.001; statistical power for analysis was 0.95%).

Besides metabolic syndrome score, male gender, waist circumference, smoking status, serum uric acid, triglycerides, and hemoglobin concentrations differed significantly with increasing HDL-C.

Multivariate analyses

The multiple linear regression analysis, including age, gender, HDL-C, triglycerides, hemoglobin, and left ventricular mass, revealed age (beta: 0.201, p=0.041) and HDL-C (beta: 0.390, p<0.001) as independent correlates of plasma catestatin concentration (Table 4). Additionally, with a different model that included catestatin, smoking status, serum uric acid, triglycerides, waist circumference, and hemoglobin concentrations, male gender (beta:-0.330, p=0.001) and plasma catestatin (beta: 0.299, p=0.002) were identified as independent predictors of HDL-C levels.

Discussion

We documented that plasma catestatin is an independent predictor of high-density lipoprotein cholesterol (HDL-C), besides male gender and waist circumference, in untreated hypertensive patients. Moreover, among all study parameters, only age and HDL-C significantly predicted catestatin concentrations. Lastly, we found similar catestatin concentrations in previously untreated hypertensive patients and control subjects without hypertension.

Catestatin is a novel protein that regulates blood pressure by mast cell stimulation and histamine-mediated vasodilation (13), diminishing catecholamine release (3) and modulating autonomic activity (5). Since marked sympathetic and adrenal activity may both predispose one to hypertension and lead to detrimental cardiovascular alterations, including left ventricular hypertrophy, accelerated atherosclerosis, and an impaired metabolic profile-all of which lead to cardiovascular complications-catestatin may physiologically act to prevent this process. However, catestatin also restores desensitized nicotinic receptor activity and thus may increase adrenergic effects in the event of prolonged catecholamine stimulation (4). Therefore, catestatin may have dual effects on nicotinic cholinergic receptor. However, the effect that predominates under different physiological conditions has not been identified until now.

High-density lipoprotein cholesterol has diverse regulatory actions, including reversal of cholesterol transport and anti-inflammatory, anti-thrombotic, and anti-diabetic effects, all of which act to maintain both cardiovascular and metabolic health (14). Although a negative correlation between catestatin and leptin has been demonstrated in a previous study (7), the correlation between catestatin and HDL-C in our study is novel. In fact, catestatin treatment in CHGA knockout mice not only lowered circulating triglycerides, catecholamines, and leptin, it also increased fatty acid oxidation in the liver and lipolysis in adipose tissue, probably mediated through inhibition of α-adrenergic receptor and improvement in leptin receptor signaling (15). Since increased lipolysis in adipocytes is known to promote cholesterol efflux into HDL (16), we believe that this effect could form the link between catestatin and HDL-C. Interestingly, this mechanism may be disrupted in states of chronic adipose inflammation, such as diabetes mellitus (17). Since our patient group mainly included patients with metabolic syndrome, increased HDL-C concentrations with respect to higher catestatin levels may be more important. Thus, we believe that in

Table 3. The distributions of study parameters according to high-density lipoprotein cholesterol levels

Variables	Low HDL-C (<40 mg/dL) (n=33)	Normal HDL-C (40-60 mg/dL) (n=57)	High HDL-C (>60 mg/dL) (n=19)	*P* value*
Plasma catestatin level, ng/mL	1.91±0.37	2.26±0.79	3.1±1.23	<0.001
Age, years	47±10	48±9	50±8	0.540
Gender, male	67%	35%	0%	<0.001
BMI, kg/m^2	31.2±4.6	32.1±5.8	31.1±5.2	0.713
Waist circumference, cm	98.6±9.9	97.2±10	90.1±9.9	0.022
Systolic BP, mm Hg	159±17	161±15	152±8	0.056
Diastolic BP, mm Hg	96±12	95±9	91±6	0.274
Pulse pressure, mm Hg	64±16	66±15	61±8	0.303
Diabetes mellitus	9%	12%	11%	0.898
Smoking status	39%	19%	6%	0.014
Dyslipidemia	27%	12%	22%	0.191
Family history of CAD	73%	81%	84%	0.375
Glucose, mg/dL	108±21	106±27	101±12	0.589
Creatinine, mg/dL	0.81±0.16	0.76±0.21	0.68±0.07	0.046
Uric acid, mg/dL	5.3±1.5	4.9±1.9	4.1±0.9	0.046
T. bilirubin, mg/dL	0.98±0.77	0.80±0.38	0.72±0.31	0.160
Direct bilirubin, mg/dL	0.31±0.18	0.30±0.12	0.24±0.11	0.303
Indirect bilirubin, mg/dL	0.68±0.60	0.51±0.30	0.72±1.1	0.260
Total cholesterol, mg/dL	217±39	218±34	233±43	0.272
LDL-cholesterol, mg/dL	138±34	143±30	141±31	0.790
HDL-cholesterol, mg/dL	34.5±3.9	48.8±5.5	68.7±7.4	<0.001
Triglycerides, mg/dL	220±126	136±70	104±40	<0.001
CRP, mg/dL	0.40±0.29	0.46±0.47	0.44±0.45	0.775
Leukocytes, /mm^3	7884±2265	6951±2061	6956±2226	0.122
Hemoglobin, g/dL	14.8±1.5	13.6±1.8	12.8±1.4	<0.001
Platelets, 10^3/mm^3	270±57	281±89	282±60	0.767
CIMT, mm, mean	0.73±0.16	0.73±0.21	0.70±0.16	0.784
Presence of carotid plaque	10%	15%	12%	0.810
Flow-mediated dilation, %	14.4±7.9	14.6±7.9	15.6±5.7	0.858
Metabolic syndrome score	3.6±0.9	2.5±1.0	1.8±0.9	<0.001
Left ventricular mass, g	222±52	224±55	193±40	0.089

BMI - body mass index; CAD - coronary artery disease; CIMT - carotid intima-media thickness; CRP - C-reactive protein; HDL-C - high-density lipoprotein cholesterol; LDL - low-density lipoprotein cholesterol; SD - standard deviation. *Mean values were compared by analysis of variance (ANOVA) among different HDL-C groups

addition to its antihypertensive effects, catestatin appears to be related to improved lipid and metabolic profiles. The coexistence of low catestatin levels with low HDL-C may provide a novel mechanism for the increased risk of hypertension and cardiovascular events.

Catestatin deficiency in CHGA-knockout mice produced elevated blood pressure, diminished baroreflex sensitivity, caused loss of diurnal blood pressure variation, increased plasma catecholamines, and increased left ventricular mass and dimensions, whereas external catestatin supplementation or replacement of CHGA loci both restored baroreflex sensitivity and lowered blood pressure and catecholamines (18, 19). In addition to diminished catecholamine secretion through a negative feedback mechanism, catestatin also has potent vasodilator effects in vivo, mediated by released histamine that acts on H1 receptors (20). Interestingly, Fung et al. (21) reported that catestatin induces vasodilation prominently in female subjects. Moreover, they found higher catestatin concentrations and lower CHGA levels in healthy females, reflecting higher conversion from CHGA to catestatin (21). Similarly, we observed higher

Table 4. Linear regression analyses for prediction of plasma catestatin and HDL-C levels

Linear regression analysis	**Dependent variable: Plasma catestatin level**			
Independent variables	***P* value**	**Beta±SE (Unstandardized Coefficients)**	**†*P* value**	**Beta±SE (Unstandardized Coefficients)**
Age, years	0.006	0.024±0.008	0.041	0.018±0.009
Gender, male	0.381	-0.220±0.250		
HDL-C, mg/dL	0.089	0.013±0.008	<0.001	0.026±0.006
Triglycerides, mg/dL	0.118	-0.002±0.001		
Hemoglobin (g/dL)	0.837	-0.012±0.060		
Left ventricular mass (g)	0.189	-0.003±0.002		
Constant	0.055	1.775±0.913	0.737	0.162±0.482
Adjusted R^2	0.268	0.202		
Linear regression analysis	**Dependent variable: HDL-C level**			
Independent variables	***P* value**	**Beta±SE (Unstandardized Coefficients)**	**†*P* value**	**Beta±SE (Unstandardized Coefficients)**
Gender, male	0.038	-7.1±3.35	0.001	-8.39±2.46
Waist circumference, cm	0.023	-0.280±0.121	0.071	-0.204±0.111
Systolic BP, mm Hg	0.512	0.053±0.081		
Catestatin level, ng/mL	0.001	4.8±1.45	0.002	4.49±1.43
Smoking status	0.570	-1.66±2.91		
Creatinine, mg/dL	0.115	-10.26±6.44		
Uric acid, mg/dL	0.114	1.23±0.77		
Hemoglobin, g/dL	0.734	-0.306±0.90		
Constant	<0.001	72±18	<0.001	68±12
Adjusted R^2	0.304	0.301		

*Linear regression analysis with enter method was used for all relevant independent variables, which were included if they were significantly different in the univariate analyses.
†In addition, the analysis was repeated after a pre-elimination with the stepwise method for independent variables.
BP - blood pressure; HDL-C - high-density lipoprotein cholesterol; SE - standard error

catestatin concentrations in female patients, revealing another difference in cardiovascular physiology (22).

In the current medical literature, there are only two studies that have reported catestatin levels in the hypertensive population so far. Although statistically insignificant, O'Connor et al. (7) revealed a trend toward lower catestatin concentrations in hypertensive subjects compared with controls. Moreover, normotensive offspring of hypertensive patients had lower catestatin values than normotensive subjects without a family history (7). Subjects with low plasma catestatin displayed higher blood pressure responses to cold stress, which implicates a predisposition to the development of hypertension (20). These results suggest that decreased catestatin concentrations may occur at the initial stages in the pathogenesis of hypertension. In contrast, Meng et al. (23) revealed increased catestatin in patients with hypertension compared to controls in a recent study. Moreover, they showed a lower catestatin-to-norepinephrine ratio in hypertensive patients, which might indicate inadequate inhibition of sympathetic activity by catestatin (23). We revealed similar catestatin concentrations in hypertensive patients compared to healthy individuals. There might be a few possible explanations for this inconsistency among studies. First of all, three studies, including ours, reflect different stages of hypertension. Our patient group, including patients with never-treated hypertension, remains in the middle of previous studies, with pre-hypertensive patients at an earlier phase and those with established hypertension at a later phase. Therefore, it is quite logical that low catestatin concentrations in the very early stage may result in increased blood pressure due to loss of vasodilator and anti-sympathetic effects. However, at later stages, as in patients with longstanding hypertension, catestatin levels may increase in order to limit catecholamine excess (24). Additionally, since catestatin may have dual effects depending on the sensitization state of the cholinergic receptor, catestatin with preexisting excess catecholamines might paradoxically increase blood pressure, especially at the later stages of hypertension. Lastly, racial differences and the small sample size may have affected our results. Thus, it seems that catestatin concentrations, which may be low in the early stages facilitating the progression of hypertension, may increase with increasing duration of hypertension. According to our results, we speculate that there might be a point where catestatin values increase and get

comparable to controls, as in new-onset hypertension, which implicates the failure or inadequacy of endogenous defense mechanisms through catestatin.

Another interesting and novel finding of our study is that plasma catestatin was independently related to age. Sympathetic over-activity is very important in all stages of hypertension, including the early phase (25). Evidence suggests that sympathetic activity increases with age (26). Since catestatin not only inhibits catecholamine secretion but also modulates autonomic activity (3, 5), this association might reflect a secondary increase in plasma catestatin due to higher sympathetic activity with aging. However, since increased catestatin levels may not completely inhibit sympathetic influence, the decreased effectiveness of catestatin with aging may partially explain why elderly patients have increased blood pressure levels. Catestatin concentrations did not differ by age in the control population. Therefore, we speculate either that group size may be small or that sympathetic activity may not be as prominent as in hypertensive patients to make a difference in control subjects. On the other hand, the association between HDL and catestatin was age-independent.

Approximately one-fourth of our study group was smokers. Since catestatin non-competitively antagonizes nicotine actions at various locations and may also potentiate nicotine activity in the case of sympathetic over-activity, the interaction between smoking and catestatin is complex and currently unpredictable. Moreover, smoking also has a negative influence on lipid profiles. We checked catestatin levels in different subgroups of our study data (smoking, DM, dyslipidemia) and found no relationship between these factors and catestatin.

We could not demonstrate any relationship between catestatin and carotid intima-media thickness, presence of carotid plaque, and flow-mediated dilation. Since atherosclerosis is a progressive disorder, our patients may have less vascular involvement, and the association between catestatin and atherosclerosis may be weaker than that of catestatin and hypertension. Accordingly, the vascular impairment tests of our patients were relatively normal [FMD: 14.8±7.2% (abnormal cut-off: <12%) and CIMT: 0.73±0.18 mm (abnormal cut-off: >9 mm)]; thus, we were not able to demonstrate the possible correlations between catestatin and vascular tests.

Study limitations

Our study has several limitations. The main limitation is the small sample size. Another limitation is that our study included previously untreated patients with earlier hypertensive processes. These patients may have less vascular involvement than in longstanding hypertension, which would reduce the statistical power. Other metabolic mediators, including leptin and catecholamines, could have been analyzed. Moreover, our study is cross-sectional in nature; therefore, our results can not implicate causality. However, in order to decrease the variability in measuring atherosclerotic parameters, we utilized validated endpoints, which is the stronger aspect of our study.

Conclusion

We documented that plasma catestatin is higher in patients with previously untreated hypertension. Moreover, plasma catestatin was an independent predictor of high-density lipoprotein cholesterol (HDL-C). We believe that in addition to its antihypertensive effects, catestatin appears to be related to improved lipid and metabolic profiles. Coexistence of low catestatin levels with low HDL-C may provide a novel mechanism for the increased risk of hypertension and cardiovascular events. The physiology and clinical significance of this association remain unclear and require further studies to be identified.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - M.E.D., S.A.K.; Design - M.E.D., S.A.K.; Supervision - Y.Ç., T.E.; Resource - T.A., O.Z.Ş.; Material - A.K., O.Z.Ş.; Data collection and/or processing - A.K., T.D.; Analysis and/or Interpretation - S.A.K., T.A.; Literature search - T.D., M.Ç.; Writing - M.E.D.; Critical review - M.E.D., T.A., S.A.K., A.K., T.D., T.E., M.Ç., O.Z.Ş., Y.Ç.; Other - Y.Ç., T.E., M.Ç.

References

1. Helle KB. The chromogranin A-derived peptides vasostatin-I and catestatin as regulatory peptides for cardiovascular functions. Cardiovasc Res 2010; 85: 9-16. [CrossRef]
2. Mahata SK, Mahata M, Fung MM, O'Connor DT. Catestatin: a multifunctional peptide from chromogranin A. Regul Pept 2010; 162: 33-43. [CrossRef]
3. Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, et al. Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. J Clin Invest 1997; 100: 1623-33. [CrossRef]
4. Mahata SK, Mahata M, Parmer RJ, O'Connor DT. Desensitization of catecholamine release. The novel catecholamine release-inhibitory peptide catestatin (chromogranin a344-364) acts at the receptor to prevent nicotinic cholinergic tolerance. J Biol Chem 1999; 274: 2920-8. [CrossRef]
5. Rao F, Wen G, Gayen JR, Das M, Vaingankar SM, Rana BK, et al. Catecholamine release-inhibitory peptide catestatin (chromogranin A(352-372)): naturally occurring amino acid variant Gly364Ser causes profound changes in human autonomic activity and alters risk for hypertension. Circulation 2007; 115: 2271-81. [CrossRef]
6. Mahata SK, Mahata M, Wen G, Wong WB, Mahapatra NR, Hamilton BA, et al. The catecholamine release-inhibitory "catestatin" fragment of chromogranin a: naturally occurring human variants with different potencies for multiple chromaffin cell nicotinic cholinergic responses. Mol Pharmacol 2004; 66: 1180-91. [CrossRef]
7. O'Connor DT, Kailasam MT, Kennedy BP, Ziegler MG, Yanaihara N, Parmer RJ. Early decline in the catecholamine release-inhibitory peptide catestatin in humans at genetic risk of hypertension. J Hypertens 2002; 20: 1335-45. [CrossRef]

8. Delles C, Padmanabhan S. Genetics and hypertension: is it time to change my practice? Can J Cardiol 2012; 28: 296-304. [CrossRef]
9. Cheung BM, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep 2012; 14: 160-6. [CrossRef]
10. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112: 2735-52. [CrossRef]
11. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 1977; 55: 613-8. [CrossRef]
12. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257-65. [CrossRef]
13. Kruger PG, Mahata SK, Helle KB. Catestatin (CgA344-364) stimulates rat mast cell release of histamine in a manner comparable to mastoparan and other cationic charged neuropeptides. Regul Pept 2003; 114: 29-35. [CrossRef]
14. Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. Circ Res 2012; 111: 1079-90. [CrossRef]
15. Bandyopadhyay GK, Vu CU, Gentile S, Lee H, Biswas N, Chi NW, et al. Catestatin (chromogranin A(352-372)) and novel effects on mobilization of fat from adipose tissue through regulation of adrenergic and leptin signaling. J Biol Chem 2012; 287: 23141-51. [CrossRef]
16. Verghese PB, Arrese EL, Soulages JL. Stimulation of lipolysis enhances the rate of cholesterol efflux to HDL in adipocytes. Mol Cell Biochem 2007; 302: 241-8. [CrossRef]
17. Zhang Y, McGillicuddy FC, Hinkle CC, O'Neill S, Glick JM, Rothblat GH, et al. Adipocyte modulation of high-density lipoprotein cholesterol. Circulation 2010; 121: 1347-55. [CrossRef]
18. Mahapatra NR, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, et al. Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. J Clin Invest 2005; 115: 1942-52. [CrossRef]
19. Gayen JR, Gu Y, O'Connor DT, Mahata SK. Global disturbances in autonomic function yield cardiovascular instability and hypertension in the chromogranin a null mouse. Endocrinology 2009; 150: 5027-35. [CrossRef]
20. Kennedy BP, Mahata SK, O'Connor DT, Ziegler MG. Mechanism of cardiovascular actions of the chromogranin A fragment catestatin in vivo. Peptides 1998; 19: 1241-8. [CrossRef]
21. Fung MM, Salem RM, Mehtani P, Thomas B, Lu CF, Perez B, et al. Direct vasoactive effects of the chromogranin A (CHGA) peptide catestatin in humans in vivo. Clin Exp Hypertens 2010; 32: 278-87. [CrossRef]
22. Papakonstantinou NA, Stamou MI, Baikoussis NG, Goudevenos J, Apostolakis E. Sex differentiation with regard to coronary artery disease. J Cardiol 2013; 62: 4-11. [CrossRef]
23. Meng L, Ye XJ, Ding WH, Yang Y, Di BB, Liu L, et al. Plasma catecholamine release-inhibitory peptide catestatin in patients with essential hypertension. J Cardiovasc Med (Hagerstown) 2011; 12: 643-7. [CrossRef]
24. Schillaci G, De Vuono S, Pucci G. An endogenous brake on the sympathetic nervous system: the emerging role of catestatin in hypertension. J Cardiovasc Med 2011; 12: 609-12. [CrossRef]
25. Gamboa A, Okamoto LE, Diedrich A, Choi L, Robertson D, Farley G, et al. Sympathetic activation and nitric oxide function in early hypertension. Am J Physiol Heart Circ Physiol 2012; 302: H1438-43. [CrossRef]
26. Seals DR, Esler MD. Human ageing and the sympathoadrenal system. J Physiol 2000; 528: 407-17. [CrossRef]