The relation between serum cathepsin D level and carotid intima-media thickness in nondiabetic hypertensive patients

Nihal ÖZKAYAR[1,*], **Fatma AKYEL**[1], **Ayşe Nurdan BARÇA**[2], **Serhan Vahit PİŞKİNPAŞA**[1],
Turan TURHAN[3], **İhsan ATEŞ**[1], **Fatih DEDE**[1]
[1]Department of Nephrology, Ankara Numune Education and Research Hospital, Ankara, Turkey
[2]Department of Radiology, Ankara Numune Education and Research Hospital, Ankara, Turkey
[3]Department of Biochemistry, Ankara Numune Education and Research Hospital, Ankara, Turkey

Background/aim: We aimed to investigate the relation between carotid intima-media thickness (CIMT) and serum cathepsin D level in hypertensive patients.

Materials and methods: This was a cross-sectional study of 74 hypertensive patients (22 males and 52 females, with a mean age of 51.86 ± 11.75 years). Serum levels of cathepsin D were measured with an enzyme-linked immunosorbent assay. CIMT measurements were taken from 3 different points: right and left common carotid arteries, bifurcation, and the first 2 cm of the internal carotid artery. Mean CIMT was calculated by averaging the measurements taken 3 times from each carotid artery.

Results: Mean CIMT value was 0.76 ± 0.15 mm, and median cathepsin D level was 190.3 (12.8–2681.3) ng/mL. A marked positive correlation was found between cathepsin D levels and CIMT (r = 0.331, P = 0.04). In multivariate linear regression analysis, cathepsin D, albumin levels, and the duration of hypertension were significant predictors of CIMT (P = 0.017, P = 0.008, and P = 0.043, respectively).

Conclusions: Increased serum cathepsin D level was found to be associated with CIMT in nondiabetic hypertensive patients.

Key words: Atherosclerosis, cathepsin D, carotid intima-media thickness, hypertension

1. Introduction

Hypertension is one of the most important risk factors for cardiovascular diseases, including atherosclerotic heart disease (1,2). In an INTERHEART study conducted in 52 countries, hypertension accounted for 18% of the risk of first myocardial infarction (3). Cardiovascular diseases, such as atherosclerotic coronary artery disease, heart failure, stroke, and peripheral artery disease occurring in hypertensive patients, are closely associated with blood pressure levels (1,2).

Atherosclerosis develops from the accumulation of lipids on the vessel wall and prevents normal blood flow by obstructing the vessel lumen. Atherosclerosis starts at a young age and is multifactorial. Chronic inflammation plays a role in all stages, and each risk factor contributes to pathogenesis by accelerating the underlying inflammatory process. Traditional risk factors for the development of atherosclerosis are age, sex, hypertension, diabetes, hypercholesterolemia, obesity, and smoking (4,5). Hypertension increases the permeability of the intima layer of the artery wall. In hypertensive patients, in addition to high blood pressure, increased inflammatory mediators also contribute to the development of atherosclerosis (6).

Increase in carotid intima-media thickness (CIMT) is evaluated as an early sign of atherosclerosis and is an independent predictor of cardiovascular events, including myocardial infarction, stroke, and transient ischemic attack. CIMT is associated with cardiovascular risk factors such as hypertension, and CIMT may help to estimate the prevalence of symptomatic coronary artery disease (6–9).

Cathepsin D, a protease soluble lysosomal aspartic endopeptidase, contributes to intracellular protein degradation. Cathepsin D causes protein degradation in acidic pH in lysosomes and assists mature active peptide formation in endosomes (10). Serum cathepsin D level increases in inflammatory events. In various studies, it has been reported that increased serum cathepsin D level is associated with cardiovascular events and it may be a potential marker of atherosclerosis (11–15).

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* Correspondence: nihalozk@gmail.com

We hypothesized that serum cathepsin D level is related to CIMT and can be used as an additional marker along with CIMT to determine hypertensive patients at risk of developing cardiovascular events. Thus, this study aimed to investigate the relation between CIMT and serum cathepsin D level in hypertensive patients.

2. Materials and methods

2.1. Study design and population
This was a cross-sectional study conducted between December 2012 and May 2013 and it included 74 hypertensive patients. Patients with diabetes mellitus, coronary artery disease, heart failure, peripheral artery disease, cerebrovascular event history, acute-chronic infection, and malignancy were excluded from the study.

To exclude the presence of uncontrolled hypertension, patients were asked to take their blood pressure measurements for 5 days at the same time in the morning and evening. Patients whose mean systolic and mean diastolic blood pressures according to home measurements were >140 and >90 mmHg, respectively, were excluded from the study.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethics Research Committee. All subjects provided written informed consent prior to the study.

2.2. Laboratory procedures
Blood sampling was performed between 0800 and 0900 hours after an overnight fast. Laboratory values included complete blood count, kidney function tests, calcium and fasting plasma glucose levels, and lipid profile. For serum cathepsin D level measurement, blood samples drawn from patients were centrifuged for 15 min at 3000 rpm, and serum was separated and stored at −80 °C until analysis. Serum levels of soluble cathepsin D were measured with an enzyme-linked immunosorbent assay (ELISA) kit (QIA29 Cathepsin D, Rapid Format ELISA Kit, Calbiochem, USA). Each assay was calibrated using a cathepsin D standard curve following the manufacturer's instructions.

2.3. Carotid intima-media thickness measurements
For CIMT measurements, patients were asked to lie in the supine position with their hand bent backwards. Measurements were taken with a high-resolution B-mode ultrasound device (Logic 7, General Electric Med Inc., USA). A linear probe on the right and left common carotid arteries and an automated system were used by the same radiologist, who was unaware of the patient's characteristics. Measurements were taken from 3 different points: right and left common carotid arteries, bifurcation, and first 2 cm of the internal carotid artery. Longitudinal measurements were taken from the distances defined as vessel lumen echogenicity and media-adventitia echogenicity. Mean CIMT was calculated by averaging the measurements taken 3 times from each carotid artery (16).

2.4. Statistical analyses
All analyses were performed using SPSS 20 (IBM SPSS Inc., Armonk, NY, USA). Normal distribution of data was tested with the Kolmogorov–Smirnov test. Normally distributed numerical variables were expressed as mean ± standard deviation, and those that were not normally distributed were expressed as median. For normally and nonnormally distributed parameters, independent sample t-test and Mann–Whitney U test were used, respectively. The relations between parameters were analyzed with Pearson and Spearman correlation analysis. For determining the factors affecting CIMT, multivariate linear regression analysis was used. The variables that were found to be significant in the univariate analysis (P < 0.05) were included in a multivariate robust regression analysis. Two-tailed P values of <0.05 were considered significant.

3. Results
Of 74 patients included in the study, 22 were male (29.7%) and 52 female (70.3%). The mean age of the patients was 51.86 ± 11.75 years. The mean duration of hypertension was 4.5 years. Ten patients (13.5%) were smokers and 3 (4.1%) were ex-smokers. The results of the laboratory tests are shown in Table 1.

Table 1. Laboratory results of patients.

	Result
Glucose (mg/dL)	87.6 ± 7.9
Total cholesterol (mg/dL)	208.9 ± 4.9
Triglycerides (mg/dL)	161.6 ± 94.4
LDL cholesterol (mg/dL)	124.5 ± 36.1
HDL cholesterol (mg/dL)	52.2 ± 14.4
Creatinine (mg/dL)	0.80 ± 0.15
Calcium (mg/dL)	9.3 ± 0.5
Phosphorus (mg/dL)	3.4 ± 0.5
Total protein (g/L)	75.2 ± 3.7
Albumin (g/L)	46.38 ± 2.8
Uric acid (mg/dL)	5.61 ± 1.4
Hemoglobin (g/dL)	13.8 ± 1.6
Cathepsin D (ng/mL)	190.3 (12.8–2681.3)[a]

LDL: low-density lipoprotein, HDL: high-density lipoprotein.
Values are presented as mean ± SD or [a]median (min–max).

3.1. Carotid intima-media thickness and serum cathepsin D level

Mean CIMT value was 0.76 ± 0.15 mm, and median serum cathepsin D level was 190.3 (12.8–2681.3) ng/mL. A marked positive correlation was found between serum cathepsin D level and CIMT (r = 0.331, P = 0.04; Figure).

3.2. Other laboratory parameters affecting carotid intima-media thickness

When the correlation between other laboratory parameters and CIMT was evaluated, a positive correlation was found between serum creatinine, uric acid levels, and CIMT (r = 0.293, P = 0.011 and r = 0.282, P = 0.015, respectively). A negative correlation was found between serum albumin level and CIMT (r = –0.321, P = 0.005), and a positive correlation was found between the duration of hypertension and CIMT (r = 0.292, P = 0.012).

With multivariate linear regression analysis, it was determined that serum creatinine and uric acid levels had no effect on CIMT (P > 0.05); however, serum cathepsin D, albumin levels, and the duration of hypertension remained significant (P = 0.017, P = 0.008, and P = 0.043, respectively) (Table 2).

4. Discussion

In this study, we showed that serum cathepsin D level is positively associated with CIMT testing results in hypertensive patients. Patients at risk of cardiovascular diseases should be accurately diagnosed to start preventive therapies. Hypertension is a well-known risk for cardiovascular diseases. Although the risk of developing cardiovascular disease is higher in hypertensive patients than in normotensive subjects, this risk varies among hypertensive patients depending on the presence of other risk factors, such as atherosclerosis, diabetes, and obesity. Accurate risk analyses for the development of cardiovascular diseases in hypertensive patients are necessary for planning appropriate and timely preventive measures. CIMT testing is a safe, simple, and inexpensive method for evaluating cardiovascular risk in hypertensive patients. CIMT shows the combined thickness of the intimal and medial layers of the arterial wall, and increased CIMT indicates plaques or atheromas that are associated with accelerated atherosclerotic disease and increased risk of coronary artery disease, myocardial infarction, and stroke (17). We evaluated the relation between serum cathepsin D level and CIMT in hypertensive patients without cardiovascular symptoms. CIMT is widely used as a marker for atherosclerotic disease and is directly associated with increased risk of cardiovascular disease. We took CIMT measurements with a high-resolution B-mode ultrasound device, which is a reliable method for assessing CIMT in hypertensive patients (18).

Cathepsin D, a soluble lysosomal aspartic endopeptidase, is involved in nonspecific protein degradation in acidic medium of lysosomes. A previous study demonstrated the regulatory role of cathepsin D in apoptosis and the importance of preprocathepsin D and cathepsin D in pathological processes such as cancer, Alzheimer disease, and atherosclerosis (10). Cathepsin D was recently suggested as a potential marker of
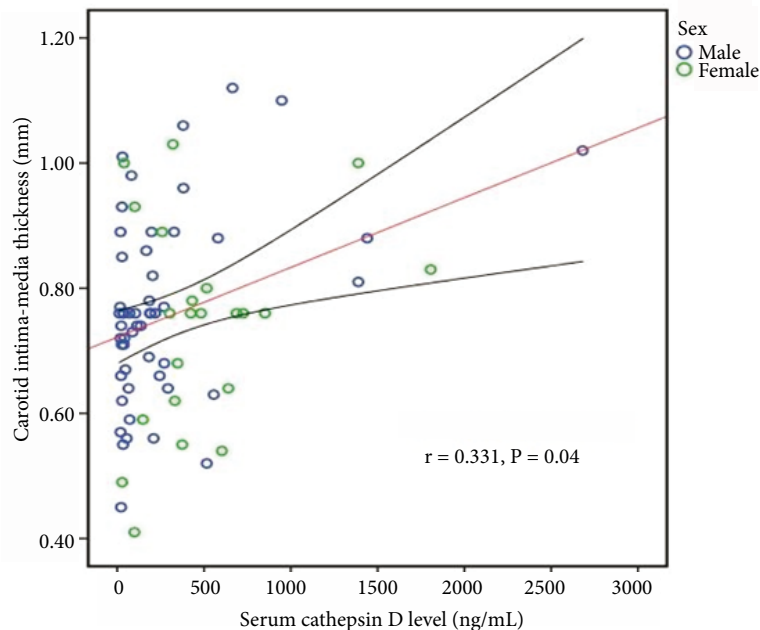

Figure. Scatter plot for CIMT versus cathepsin D levels of 74 patients.

Table 2. Risk factors affecting CIMT determined by multivariate robust regression analysis.

Variable	β + SE	β	T	P	95% confidence interval	
					Lower	Upper
Duration of HT	0.009 ± 0.005	0.232	2.061	0.043	0.710	1.828
Albumin	−0.016 ± 0.006	−0.289	−2.714	0.008	−0.027	−0.004
Uric acid	0.001 ± 0.015	0.011	0.091	0.928	−0.031	0.028
Creatinine	0.189 ± 0.010	0.194	1.556	0.124	−0.053	0.430
Cathepsin D	0.021 ± 0.001	0.351	2.941	0.017	0.058	0.210

HT: hypertension.
$P < 0.05$ is considered significant for statistical analyses; β ± SE, regression coefficient ± standard error obtained in linear regression analysis.

atherosclerosis. Moallem et al. (15) reported a positive correlation between serum cathepsin D level and CIMT in 31 hemodialysis patients. Similarly, we found marked positive correlation between serum cathepsin D level and CIMT ($r = 0.331$, $P = 0.04$).

In addition to the relation between serum cathepsin D level and CIMT, we also studied other laboratory parameters and the duration of hypertension in terms of their relation with CIMT. We found that serum creatinine, uric acid levels, and the duration of hypertension positively correlated with CIMT, although serum albumin level correlated negatively. However, in multivariate analysis, serum creatinine and uric acid levels had no effect on CIMT, yet serum cathepsin D, albumin levels, and the duration of hypertension remained significant. The role of serum uric acid as an independent risk factor for cardiovascular disease in hypertension is controversial (19–22). Tavil et al. (19) analyzed the relation between uric acid levels and CIMT in 30 hypertensive patients and found that serum uric acid levels independently, but modestly, correlated with CIMT (beta = 0.42, $P = 0.002$). However, Zand et al. (21) suggested that serum uric acid is not a risk factor for coronary artery disease. The absence of a relationship between serum uric acid levels and CIMT, found by multivariate analysis in this study, might be due to the low-level atherosclerotic burden of the study population.

The duration of hypertension is known as a major determinant for CIMT, because long-term durations of elevated blood pressure are crucial in carotid atherosclerosis pathogenesis (23). Based on this knowledge, our finding of a positive relation between the duration of hypertension and CIMT is expected.

Negative correlation between serum albumin level and CIMT in the present study can be explained by the fact that low serum albumin level correlated with most traditional cardiovascular risk factors, and it may be a marker of renal loss of albumin (24). Thus, low serum albumin levels can predict the extent of atherosclerosis.

The main limitations of the study were the cross-sectional, relatively small sample size and study population, including patients from a wide range of hypertension severity. As a result, although a relationship was found between cathepsin D levels and CIMT in hypertensive patients, it is difficult to say that increased cathepsin D levels could predict atherosclerosis, since our study was cross-sectional. To determine whether cathepsin D is a marker for atherosclerosis, prospective studies are required.

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