



Original Article

Evaluation of serum cathepsin D concentrations in coronary artery disease



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ABSTRACT

Background: Coronary artery disease (CAD) cannot be sufficiently explained by the presence of traditional risk factors. Cathepsin D has been proposed to serve as a surrogate marker of atherosclerosis but its alterations in CAD patients have not been studied.

Objective: To evaluate serum cathepsin D concentrations in relation to the presence and severity of CAD. **Materials and methods:** A total of 104 subjects were recruited; 71 patients with suspected CAD and 33 healthy subjects. Thirty-four patients had >50% coronary stenosis of at least one artery (CAD+); the remaining 37 patients had <50% stenosis (CAD−) based on angiography. CAD+ patients were sub-divided into three sub-groups with single (SVD; n=15), double (2VD; n=9), and triple vessel (3VD; n=10) disease. Serum soluble cathepsin D concentrations were determined using an enzyme-linked immunosorbent assay (ELISA).

Results: Serum cathepsin D concentrations were significantly higher in the CAD+ compared with healthy control (p=0.016) but not CAD− group (p=0.098). Within the CAD+ group, patients with 3VD had significantly higher serum cathepsin D concentrations compared with the SVD group (p=0.025), and also compared with the CAD− (p=0.011) and SVD (p=0.001) groups. No significant associations were found between serum cathepsin D concentrations and potential confounders including age, sex, blood pressure, smoking history and dyslipidemia.

Conclusion: Serum cathepsin D concentrations may be associated with the presence of CAD.

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1. Introduction

Ischemic heart disease is leading major cause of mortality worldwide. The global mortality rate due to coronary artery disease (CAD) will rise from 7.2 million in 2002 to 11.1 million by 2020 based on the World Health Organization (WHO) report. The Framingham Heart Study data has indicated that the lifetime risk of symptomatic CAD after age 40 is 49% and 32% in men and women, respectively. CAD is due to the obstruction of the coronary

arteries by an atheromatous plaque.¹ Atherosclerotic plaques have a complex structure composed of deposited lipids, and recruited inflammatory, immune (macrophages and T-lymphocytes) and vascular smooth muscle cells (VSMCs).² These lipid-rich core of atherosclerotic plaques is separated from the arterial lumen by a fibrous cap composed of collagen, VSMCs and extracellular matrix; and may extend and cause narrowing of the lumen, causing acute coronary syndrome.^{2–5}

Cathepsin D has been proposed to serve as a marker of atherosclerosis^{4–12}; it is an aspartic endo-protease, and is responsible for a major part of the endopeptidase lysosomal activity. Cathepsin D is released in several inflammatory conditions, including rheumatoid arthritis, Alzheimer's disease and in

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human carcinomas.^{2,6,13–15} Cathepsin D expression is increased in atherosclerotic plaques, where it can be released in the mature form to the circulation. Cathepsin D may also be released from macrophages and smooth muscle cells (SMCs) into the extracellular space, a phenomenon that is induced by cholesterol oxidation products.^{2,6,9} Cathepsin D may enhance low-density lipoprotein (LDL) modifications, leading to foam cell formation in the arterial intima.^{2,6–12,16,17} Increased cathepsin D levels may also, predispose to plaque instability and rupture resulting in acute coronary syndrome.^{2,6–12,16,18}

Existing risk calculators do not accurately identify all individuals who are at risk of CAD, and 20% of cardiovascular events occur in subjects without any of the main classic vascular risk factors. Over the past few decades, there has been a surge of interest to identify novel biomarkers of CAD risk.⁶ It has been hypothesized that serum concentration of cathepsin D may serve as a potential CAD risk factor.¹⁹ To test this hypothesis, the current study explored the association between serum concentrations of cathepsin D with the presence and severity of CAD.

2. Materials and methods

2.1. Patients

One hundred and four subjects who underwent coronary angiography in the cardiology ward between April 2012 and August 2013 were selected for this study. Exclusion criteria were diabetes mellitus, heart failure, liver dysfunction, acute myocardial infarction, stroke, malignancy, chronic inflammatory conditions, chronic renal failure, taking statins or food supplements, taking oral contraceptives or hormone replacement therapy or oral contraceptives, and pregnancy. None of the subjects had a prior history of coronary angioplasty or coronary artery bypass graft (CABG) and all subjects were negative for HBS antigen, anti-HCV and anti-HIV antibodies.

A questionnaire was given to all participants to obtain data on demographic information. Further information on vital signs, laboratory data, anthropometric indices, drug history, medical history, family history and cardiovascular (CV) risk factors was completed for all participants and all gave written, informed consent before entrance to the study. Blood pressure, anthropometric and biochemical measurements were performed using routine methods as described previously.²⁰

2.2. Blood sampling

Ten milliliters of whole blood was obtained from each subject after an over-night fast, and centrifuged at 3000 rpm for 10 min at room temperature to obtain serum. Serum samples were stored at -80°C until analysis.

2.3. Determination of serum cathepsin D concentration

Serum concentrations of soluble cathepsin D were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Calbiochem, UK). Each assay was calibrated using a cathepsin D standard curve using the manufacturer's instructions. The sensitivity and range of assay were 4 ng/ml and 4–100 ng/ml, respectively.

2.4. Coronary angiography

Coronary angiography was performed for all participants, except those in the control group, using standard procedures. Angiograms were analyzed by two cardiologists for evaluation of the degree and severity of coronary artery involvement. The presence of one or more stenosis causing $\geq 50\%$ narrowing of at least one major coronary artery (left main, right coronary artery, left anterior descending, circumflex) was considered as an evidence of significant CAD. Patients with $< 50\%$ stenosis were classified as CAD-. Volunteers with no history of cardiovascular disease were used as the control group. Based on the number of stenotic vessels, CAD+ patients were further classified into single-vessel (SVD), two-vessel (2VD) or three-vessel (3VD) groups.

2.5. Statistical analysis

Statistical analysis was performed using the SPSS for Windows™, version 21. All normally distributed data were presented as mean \pm SD, and for comparisons of these data two-independent sample *t*-test and One-way ANOVA followed by Tukey-Kramer post-hoc tests were used. To analyze non-normal data, Mann-Whitney and Kruskal-Wallis tests were used. Bivariate correlations between serum cathepsin D concentrations and conventional coronary risk factors were performed using Pearson's or Spearman's correlation coefficients. Multiple linear regression analysis was applied to identify the conventional risk factors could influence serum cathepsin D concentrations. A two-sided *p* value < 0.05 was considered statistically significant.

3. Results

One hundred and four patients (male/female: 57/47) were included in this study. Based on the angiogram data, subjects were classified as CAD+ and CAD-. Demographic data, laboratory findings, and traditional CV risk factors of the study groups are summarized in Table 1. The prevalence of hypertension and smoking habit was significantly greater in the CAD- compared with the CAD+ group. The prevalence of other characteristics was comparable between the study groups.

Comparison of serum cathepsin D concentrations among CAD+, CAD- and control groups showed that there was a significant

Table 1
Comparison of demographic data between the study groups.

variable	CAD+ (n = 34)	CAD- (n = 37)	Healthy (n = 33)	P ₀ (h/a-)	P ₁ (a-/a+)	P ₂ (h/a+)
Age (year)	54.79 \pm 12.29	53.97 \pm 10.84	52.21 \pm 7.53	0.762	0.941	0.571
Sex (male) %	50	43.2	42.4	0.788		
BMI (kg/m ²)	30.04 \pm 11.41	25.63 \pm 4.57	28.54 \pm 5.19	0.31	0.07	0.71
Smoking (%)	23.5	48.6	12.5	0.001	0.028	0.246
Hypertensive (%)	38.2	70.3	0	0.001	0.001	0.001
HDL-c (mg/dL)	45.26 \pm 11.29	44.56 \pm 12.4	40.64 \pm 8	0.34	0.97	0.18
LDL-c (mg/dL)	114.73 \pm 36.98	105.76 \pm 35.35	105.72 \pm 36.62	1	0.623	0.61
Total cholesterol (mg/dL)	189.54 \pm 45.14	165.3 \pm 49.44	190.7 \pm 35.85	0.08	0.1	0.99
Triglyceride (mg/dL)	147.01 \pm 76.86	141.31 \pm 91.25	107.82 \pm 58.27	0.21	0.95	0.09
Waist:hip ratio	0.96 \pm 0.06	0.94 \pm 0.08	0.95 \pm 0.08	0.91	0.71	0.91

a+: atherosclerosis a-: atherosclerosis/CAD: coronary artery disease/h: healthy two-independent sample *t*-test.

difference between CAD+ and control groups ($p=0.016$) but it was not significant between CAD+ and CAD– groups ($p=0.098$), and between CAD– and control group ($p=0.709$) (Table 2).

An additional analysis was performed to compare different subgroups of CAD+ patients according to the number of stenotic vessels (1VD, 2VD and 3VD), with CAD– and control groups (Table 2). The results revealed a significant difference between subgroup of CAD+ patients with 3VD compared with CAD– ($p=0.011$), control ($p=0.001$) and SVD groups ($p=0.025$).

In this study, hypertension, dyslipidemia, smoking and age above 55 years for women and 45 years for men were considered as CVD risk factors. None of these traditional risk factors had a significant effect on serum cathepsin D concentration. Comparisons of cathepsin D concentrations among these subgroups are shown in Table 3.

Patients were classified into four different body mass index (BMI) groups. Comparison of serum cathepsin D concentration among the BMI subgroups did not reveal any significant difference ($p>0.05$). Moreover, in this study no significant difference in serum cathepsin D concentration was found between males and females ($p>0.05$), nor between subgroups with and without peripheral artery disease ($p=0.677$).

We performed a multiple linear regression test, considering cathepsin D as a dependent factor, and age, sex, weight, BMI, HDL-c, LDL-c, triglycerides (TG), systolic blood pressure (SBP), diastolic blood pressure (DBP) and smoking as independent factors. Results showed that none of these factors had an effect on cathepsin D levels.

4. Discussion

In this study, we found no significant difference in serum cathepsin D levels between subgroups of patients with and without traditional CV risk factors. This finding may indicate the potential role of cathepsin D as an independent risk factor of CAD; however, this notion needs to be verified in large scale studies. In some of previous studies an association between cathepsin D serum levels and some traditional cardiovascular risk factors has been reported. In our previous study in hemodialysis patients, cathepsin D was correlated with patients' age and TG levels,² which is consistent with some other studies.^{19,21} Based on the results of our earlier study,² a significant correlation was reported between serum concentration of cathepsin D and carotid intima media thickness (CIMT), therefore, it can be concluded that cathepsin D might have a specific function in the atherosclerotic plaques development.²

In spite of major advances in the field of CV risk prediction, still a considerable proportion of CV events occur in subjects who are at a low-risk based on traditional risk factors and the Framingham risk scoring system.^{2,22,23} This highlights the limitations of purely relying on traditional risk prediction models, and also necessitates

further attempts to identify novel CV risk factors.²⁴ Owing to the emerging role of inflammation in the pathogenesis of CV disease, inflammatory factors, e.g. hsCRP, homocysteine and pro-inflammatory cytokines have been the subject of increasing research interest to be used as diagnostic and prognostic markers. Cathepsin D is another inflammatory factor that has been shown to be increased in hemodialysis patients in correlation with CIMT.²

Previous studies have shown that cysteinyl cathepsins are implicated in initiation and progression of CAD via promotion of arterial wall matrix protein degradation, vascular cell migration, invasion, proliferation and apoptosis, foam cell formation, hypoxia-induced angiogenesis, and cytokine, chemokine and growth factor activation, liberation, and modifications.²⁵ Moreover, cathepsins regulate vascular homeostasis and tissue remodeling through protease-activated receptors (PARs)-dependent and PARs-independent mechanisms, and also serve as mediators of the inflammatory response.^{26,27} However, most of the previous studies were focused on cathepsins B, F, K, L, S and V, and data regarding the role of cathepsin D in CVD has been scarce despite the reported increase in serum cathepsin D levels in patients with endothelial dysfunction, which may reflect the cardiovascular importance of this protein.²⁸ Under hypersensitivity conditions such as Kounis syndrome, activation of mast cells occurs and a number of different substances, including the enzymes cathepsins, chymase and tryptase, cytokines, histamine and heparin are released from their granules.^{29,30} Cathepsin-D and chymase are considered as proteases with the activity of converting angiotensin I to angiotensin II which is a key vasoconstricting mediator.³¹ The protease tryptase has thrombotic and fibrinolytic activities.³¹ Alteration of plasma albumin, induction of histamine-releasing peptides, activation of mast cells and inflammation are some effects triggered by proteases. In addition, proteases promote degradation of endothelial basement membrane and also weaken the adhesion of endothelial cells to the atherosclerotic plaque, thus contributing to plaque rupture.³²

Potent cathepsin inhibitors have been suggested as potential drugs for CVD.^{33,34} Moreover, long-term use of statins and angiotensin antagonists has been shown to prevent cardiovascular damage in animal models through reducing plasma and tissues cathepsin K levels.³⁵ Cathepsin D is also regarded as another potential therapeutic target owing to its role in plaque instability. It has been reported that in patients with atorvastatin treatment, the expression of cathepsin D was lower than non-treated patients.⁴

Cheng et al reported cathepsin K as a potential CAD biomarker. Cathepsin K is a potent collagenase in mammals and its deficiency could reduce diet-induced atherosclerosis. It was shown that cathepsin K expression is increased in the atherosclerotic plaques of the human aorta, and higher levels of this protein in patients with CAD can result in plaque destabilization and acute coronary syndrome (ACS). A positive correlation between cathepsin K and hsCRP levels has also been demonstrated. Similar reports also exist for cathepsins S and L.³⁵ With respect to cathepsin D, although there are reports on the upregulation of this protein in unstable atherosclerotic lesions and in patients with ACS,^{2,11,12} There has been no previous study evaluating its role in atherosclerosis plaque development and its role in predicting future CV events.

Although the present study is the first to indicate increased serum cathepsin D concentrations in patients with CAD, it was limited by a small population size, which made subgroup analyses difficult. Hence, future studies with larger populations are required to ascertain the association between serum cathepsin D concentrations with the severity of disease and also other cardiovascular risk factors. Furthermore, comparison of serum cathepsin D levels between CAD+ and CAD– subjects needs to be re-evaluated in a larger population.

Table 2

Serum concentrations of Cathepsin D among CAD+, CAD– and control groups.

	Number of subjects	Concentration of cathepsin D ^a
CAD+	34	14.07 ± 7.89 [*]
CAD+ sub-groups	SVD	10.50 ± 2.95
	2VD	14.58 ± 6.40
	3VD	18.96 ± 11.40 [*]
CAD–	37	10.54 ± 8.40
Control	33	9.20 ± 3.97

^{*}Significant difference between CAD+ and Control group ($P=0.016$; 95%CI: 0.74–9.00).

^{*}Significant difference between 3VD and SVD group, but not the others ($P=0.025$; 95%CI: 0.72–16.2).

^{*}One-way ANOVA.

^a Mean ± SD.

Table 3

Comparison of serum cathepsin D concentrations between patients with and without cardiovascular risk factors.

	Serum cathepsin D concentration (ng/ml) in patients with n = 71	Serum cathepsin D concentration (ng/ml) in patients without n = 33	P(95%CI)
Hypertension 120 mmHg/80 mmHg	12.51 ± 6.14	12.45 ± 9.43	0.98(−4.33,4.22)
Dyslipidemia High LDL, high TGs and low HDL	12.29 ± 6.14	12.69 ± 9.29	0.85(−4.13,4.95)
Age >55 women and >45 men	11.45 ± 6.73	10.89 ± 8.52	0.72(−3.61,2.49)
Smoking	12.00 ± 8.84	12.67 ± 8.16	0.75(−3.52,4.87)

5. Conclusion

In conclusion, the present results suggested that serum cathepsin D concentrations are associated with CAD and maybe its severity (based on the number of stenotic vessels) but the latter claim needs further confirmation. Further studies are required to clarify the association between elevated cathepsin D levels and CV outcomes, and also the impact of conventional and novel lipid-modifying agents.^{36–40} on serum levels of cathepsin D.

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Conflict of interests

The Authors declare that there is no conflict of interests.

Compliance with ethical standards

Procedures were performed in accordance with the principles outlined in the Declaration of Helsinki.

Ethical approval

The study protocol was approved by the Ethics Committee at the Mashhad University of Medical Sciences, Mashhad, Iran.

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