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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 groups ($p = 0.025$), 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.

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 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 ≥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 ± 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 ± 12.29	53.97 ± 10.84	52.21 ± 7.53	0.762	0.941	0.571
Sex (male) %	50	43.2	42.4	0.788		
BMI (kg/m^2)	30.04 ± 11.41	25.63 ± 4.57	28.54 ± 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 ± 11.29	44.56 ± 12.4	40.64 ± 8	0.34	0.97	0.18
LDL-c (mg/dL)	114.73 ± 36.98	105.76 ± 35.35	105.72 ± 36.62	1	0.623	0.61
Total cholesterol (mg/dL)	189.54 ± 45.14	165.3 ± 49.44	190.7 ± 35.85	0.08	0.1	0.99
Triglyceride (mg/dL)	147.01 ± 76.86	141.31 ± 91.25	107.82 ± 58.27	0.21	0.95	0.09
Waist:hip ratio	0.96 ± 0.06	0.94 ± 0.08	0.95 ± 0.08	0.91	0.71	0.91

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

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