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Serum cystatin C concentration as an independent marker for hypertensive left ventricular hypertrophy

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Abstract

 Background Serum cystatin C levels can be used to predict morbidity and mortality in patients with cardiovascular disease. However, the clinical relevance of serum cystatin C levels in patients with hypertensive left ventricular hypertrophy (LVH) has rarely been investigated. We designed the present study to investigate whether serum cystatin C levels are associated with cardiac structural and functional alterations in hypertensive patients. **Methods** We enrolled 823 hypertensive patients and classified them into two groups: those with LVH ($n = 287$) and those without LVH ($n = 536$). All patients underwent echocardiography and serum cystatin C testing. We analyzed the relationship between serum cystatin C levels and LVH. **Results** Serum cystatin C levels were higher in hypertensive patients with LVH than in those without LVH ($P < 0.05$). Using linear correlation analysis, we found a positive correlation between serum cystatin C levels and interventricular septal thickness ($r = 0.247$, $P < 0.01$), posterior wall thickness ($r = 0.216$, $P < 0.01$), and left ventricular weight index ($r = 0.347$, $P < 0.01$). When analyzed by multiple linear regression, the positive correlations remained between serum cystatin C and interventricular septal thickness ($\beta = 0.167$, $P < 0.05$), posterior wall thickness ($\beta = 0.187$, $P < 0.05$), and left ventricular weight index ($\beta = 0.245$, $P < 0.01$). **Conclusion** Serum cystatin C concentration is an independent marker for hypertensive LVH.

J Geriatr Cardiol 2013; 10: 286–290. doi: 10.3969/j.issn.1671-5411.2013.03.001

Keywords: Hypertension; Left ventricular hypertrophy; Cystatin C

1 Introduction

 Hypertensive target organ damage includes impaired renal function and left ventricular hypertrophy (LVH). Impaired renal function is an independent marker for LVH and a good predictor of morbidity and mortality in cardiovascular disease.[1,2] In patients with chronic kidney disease (CKD), there is a significant association between decreased estimated glomerular filtration rate (eGFR) and LVH.[3] The eGFR is widely used to evaluate renal function in clinical practice; however, eGFR is influenced by sex and body weight. Serum cystatin C is a novel and stable biomarker not influenced by sex, age, exertion, diet, or serum creatinine, that can be used to evaluate renal function. Serum cystatin C has been considered the best biomarker for early

stage renal function damage.[4] The most recent studies demonstrated that higher serum cystatin C levels were associated with increased cardiovascular mortality and morbidity.[5–8] In addition, recent studies found that cystatin C is related to left ventricular mass index (LVMI);[9] however, the association of other cardiac echocardiographic parameters such as end-diastolic interventricular septal thickness (IVS), left ventricle posterior wall thickness (PWT), PWT with LVH has not been tested.[9,10] We designed this study to investigate the association between serum cystatin C levels and cardiac structural and functional alterations in hypertensive patients.

2 Methods

2.1 Patients

 In September 2009, we recruited 823 consecutive Chinese hypertensive patients (age 45–65 years) not taking antihypertensive drugs. Hypertension was defined as systolic blood pressure (BP) $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg, according to the 2010 Chinese guidelines on hypertension. We used questionnaires to collect information on family history of premature cardiovascular disease, per-

sonal history of cardiovascular disease or peripheral artery disease, and atherosclerotic risk factors, including diabetes, hyperlipidemia, and smoking. Physical examinations included height, weight, waist circumference, and BP measurement. Body mass index (BMI) was calculated for each patient. Diabetes was defined as fasting blood glucose (FBG) ≥ 7.8 mmol/L or 2 h postprandial blood glucose (PPG) ≥ 11.1 mmol/L.

Exclusion criteria were history of stroke, diabetes, malignant tumor, secondary hypertension, valvular heart diseases, primary pulmonary hypertension, heart failure, hyperuricemia, obesity (BMI > 28 kg/m^2), potassium disturbance, liver/kidney insufficiency, metabolic disease, and immunological diseases.

2.2   Blood sample collection

After a 12 h strict fast, we collected 8 mL venous blood from each subject and deposited it into 10% Na$_2$EDTA and anticoagulant-free test tube. Blood samples were centrifuged at 3000 r/min for 10 min. Plasma and serum was used to determine biochemical markers and cystatin C concentrations, respectively.

2.3   Biochemical marker detection

All patients underwent baseline biochemical marker testing using the Hitachi 7150 Biochemistry Automatic Analyzer (Japan). We measured total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatine kinase (CK), creatine kinase-MB (CK-MB), troponin T (TNT), fasting blood glucose (FBG), blood urea nitrogen (BUN), and serum creatinine (SCr).

2.4   Evaluation of eGFR

Jaffe's kinetic equations[11] were used to adjust SCr as follows:

SCr (mg/dL) = 0.795 × [SCr (mg/dL)] + 0.29

We used the C-MDRD equation to calculate eGFR:

eGFR (mL/min per 1.73m^2) = 175 × standardized SCr − 1.154 × age − 0.203 × 0.742 (if female)

2.5   Echocardiography

We measured left ventricular end-systolic dimension (LVESD), left ventricular end-diastolic dimension (LVEDD), end-diastolic interventricular septal thickness (IVST), and left ventricle PWT using ultrasonic Doppler echocardiography (Acuson Sequoia 512, Siemens Medical Solutions, Mountain View, CA). Three successive cardiac cycles were studied and left ventricle ejection fraction (LVEF, %) and fractional shortening (FS, %) were obtained based on the data.

LV mass = 0.8 × 1.04 [(IVS + LVIDD + PWT)^3 − LVIDD^3] + 0.6

LVMI = LVM/height$^{2.7}$

Left ventricular hypertrophy was defined as LVMI > 49.2 g/m$^{2.7}$ in men and LVMI > 46.7 g/m$^{2.7}$ in women.[12] Repeat measurements of IVS, PWT, LVESD, and LVEDD were obtained in 10% of the subjects. The within-run and between-run variations for these measurements were 4% and 3.9% for IVS, 5.0% and 3.7% for PWT, 7.1% and 6.3% for LVESD, and 6.6% and 7.0% for LVEDD.

2.6   Cystatin C assay

Scattering immunoturbidimetry was used to measure serum cystatin C concentration.

2.7   Statistical analysis

All data were collected in an EpiData database (EpiData, Odense, Denmark) and were analyzed using SPSS 13.0 (Chicago, IL). Measurement data were expressed as mean ± SD and *t* test was used to analyze between-group variance. Enumeration data were expressed as *n* and χ^2 test was used to analyze between-group variance. Linear correlation analysis was used to analyze the correlation between serum levels of cystatin C and IVST, PWT, LVEDD, and LVESD. Multiple linear regression was used to analyze the correlation between serum cystatin C levels and LVH, after adjusting for cardiovascular risk factors such as age, sex, BMI, systolic BP, diastolic BP, BG, TC, TG, LDL-C, and HDL-C. Statistical significance was established at *P* < 0.05. Multivariant logistic regression was used to analyze the correlation between serum cystatin C levels and LVH after adjusting for the above-mentioned cardiovascular risk factors.

3   Results

3.1   General data

Among 823 patients, 287 were classified as hypertensive with LVH and 536 as hypertensive without LVH, according to the echocardiographic guidelines of Devereux *et al*.[12] There were no statistically significant differences between the two groups in age, sex, BMI, systolic BP, diastolic BP, BG, TC, TG, LDL-C, HDL-C, or eGFR (*P* > 0.05; Table 1).

3.2   Serum level of cystatin C

Hypertensive patients with LVH had higher serum cystatin C levels than hypertensive patients without LVH (1.33 ± 0.18 mg/dL *vs*. 1.02 ± 0.09 mg/L; *P* < 0.05; Figure 1).

Table 1. Baseline characteristics of hypertensive patients with and without LVH.

	Hypertension with LVH ($n = 287$)	Hypertension with-out LVH ($n = 537$)
Age, yrs	54.9 ± 14.9	53.5 ± 15.2
Sex, male/female	166/121	323/214
BMI, kg/m²	26.3 ± 3.88	25.1 ± 3.81
Smoking, %	27.3	26.5
Systolic BP, mmHg	163.9 ± 25	161.1 ± 26
Diastolic BP, mmHg	106 ± 15	102 ± 17
eGFR, mL/min per 1.73 m²	84 ± 12	88 ± 17
FBG, mmol/L	5.19 ± 0.92	4.78 ± 0.98
TC, mmol/L	4.65 ± 0.85	4.21 ± 0.77
TG, mmol/L	1.59 ± 0.51	1.34 ± 0.64
HDL-C, mmol/L	0.88 ± 0.31	1.02 ± 0.26
LDL-C, mmol/L	3.19 ± 0.69	2.61 ± 0.79

BMI: body mass index; BP: blood pressure; eGFR: estimated glomerular filtration rate; FBG: fasting blood glucose; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; LVH: left ventricular hypertrophy; TC: total cholesterol; TG: total triglyceride.

Table 2. Association between serum cystatin C and hypertensive left ventricular hypertrophy.

	β	R^2	Adjusted P Value
IVS, mm	0.167	0.39	0.007
PWT, mm	0.187	0.41	0.01
LVEDD, mm	0.089	0.10	0.21
LVESD, mm	0.077	0.09	0.11
LVMI, g/m²·⁷	0.245	0.44	0.002

IVS: end-diastolic interventricular septal thickness; LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; LVMI: left ventricular mass index; PWT: left ventricle posterior wall thickness.
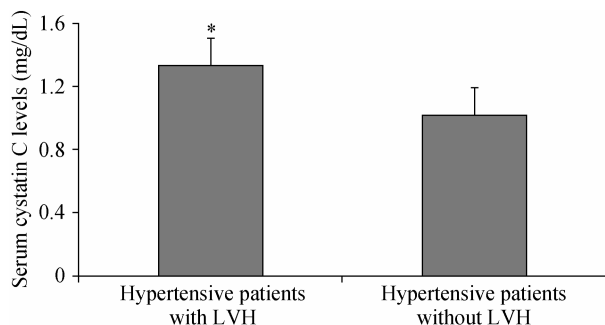

Figure 1. Serum cystatin C levels in hypertensive patient with or without LVH. *$P < 0.05$ compared with hypertensive patient without LVH. LVH: left ventricular hypertrophy.

3.3 Association analysis of serum cystatin C and LVH

There was a positive correlation between serum cystatin C and IVST ($r = 0.247$, $P < 0.01$), PWT ($r = 0.216$, $P < 0.01$), and LVMI ($r = 0.347$, $P < 0.01$) in linear correlation analysis. The positive correlation between serum cystatin C and IVST ($β = 0.167$, $P < 0.05$), PWT ($β = 0.187$, $P < 0.05$), and LVMI ($β = 0.245$, $P < 0.01$) remained when analyzed by multiple linear regression after adjusting for such cardiovascular risk factors as age, sex, BMI, systolic BP, diastolic BP, BG, TC, TG, LDL-C, and HDL-C. Multivariant logistic regression suggested that serum cystatin C was an independent marker for hypertensive LVH (OR = 2.01; 95% CI: 1.21–3.06), Table 2.

4 Discussion

Cystatin C was first identified by Clausen in 1961 in human cerebrospinal fluid. It is a low molecular weight, non-glycosylated cationic basic protein and belongs to the cystatin superfamily of endogenous cysteine proteinase inhibitors. Cystatin C is a typical secretory protein and can be found in high concentrations in cerebrospinal fluid, blood, saliva, and semen. It is constantly transcribed and expressed in all nucleated cells.[13] Cystatin C participates in intracellular and extracellular proteolytic modulation, and prevents cellular hydrolysis from endogenous and exogenous proteases. Cystatin C has diverse bioactivity not only in tumor invasion, metastasis, degradation of ossein, and anti-infection neutrophil migration, but also in cardiovascular diseases.[14–17] The clearance of circulatory cystatin C uniquely depends on the kidney. Because of its low molecular weight (13.3 kDa) and positive charge at physiologic pH levels, cystatin C is freely filtered by the kidney and cannot be reabsorbed or re-secreted from the kidney tubules.[18] Cystatin C is relatively stable and its serum concentration is independent of age, sex, race, and nutrition.[19] Therefore, cystatin C is an ideal endogenous marker that is more sensitive and accurate than creatinine in reflecting renal function.[20,21]

Left ventricular hypertrophy is an important form of target organ damage in essential hypertension. A meta-analysis found that 30%–50% of hypertensive patients have concurrent LVH.[22] Furthermore, LVH is an independent risk factor for cardiac death, arrhythmia, and heart failure. LVH is a sensitive marker of hypertension and confers a five-fold increased risk of mortality in hypertensive patients.[23] Among patients with hypertension, the prognosis is best for those without LVH, followed by those with eccentric hypertrophy, and is worse for those with concentric hypertrophy. Due to the high mortality and morbidity associated

with hypertensive LVH, clinicians and geneticists have tried to determine its underlying mechanism and strategies for early intervention.

Chen *et al*.[24] found that cystatin C mRNA was significantly increased in the LV myocardium of hypertension-induced LV hypertrophy rats and in hypertension-induced LV hypertrophy patients. The same increase was not found in the myocardium of rats or humans with hypertension-induced heart failure, indicating that cystatin C may play an important role in cardiac structural and functional alterations. Our study demonstrated a positive correlation between serum cystatin C levels and IVS, PWT, and LVMI, and that the serum level of cystatin C is an independent marker for hypertensive LVH. All data suggest that cystatin C is associated with LVH. We propose three possible mechanisms to explain this association. First, LVH results from the increased production and decreased degradation of cardiomyocyte extracellular matrix. Cystatin C, as an important endogenous cysteine proteinase inhibitor, participates in the balance of production and degradation of extracellular matrix.[25,26] Second, the main component of cardiomyocyte extracellular matrix is collagen protein. Cystatin C inhibits the bioactivity of cathepsin B and promotes the accumulation of fibrin and type I/III collagen.[27,28] Finally, cysteine proteinase and cysteine proteinase inhibitors participate in the degradation of cardiac collagen; imbalances in these levels result in cardiac structural and functional alterations.[24]

In conclusion, serum cystatin C levels are associated with LVH in hypertensive patients and serum cystatin C might play a role in the development of LVH. Further studies should focus on the mechanism of LVH development in hypertensive patients and should provide new insights into possible interventions in patients with hypertensive LVH.

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