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Original Article

Serum cystatin C levels correlate with vascular endothelial function in patients with essential hypertension

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Abstract: Objective: To investigate the association between serum cystatin C and vascular endothelial function in patients with essential hypertension. Methods: A total of 434 cases (male 228, female 206) with an average age of (56.5±12.0) years were enrolled in the first hospital of Shanxi Medical University, including 343 patients with essential hypertension and 100 patients without hypertension, ranging from May 2014 to October 2015. All patients were divided into the FMD≥6.0% group (n=232) or FMD<6.0% group (n=202) according to flow mediated vasodilation (FMD) measured by ultrosound. The serum cystatin C, fasting blood glucose, serum lipid level, creatinine and urine micro albumin were measured in the morning after 12 hours fasting. The relationship between serum cystatin C and FMD was analyzed using the multiple linear regression analysis. Results: Serum cystatin C was significantly lower in the normal vascular endothelial function group than in the impaired vascular endothelial function group [(0.98±0.26) vs (1.05±0.23) mg/L, *P*<0.05]. Compared to the patients with grade 3 hypertension, FMD was significantly higher while serum cystatin C was significantly lower in the patients with grade 1 and 2 hypertension group (both *P*<0.05). FMD was negatively correlated with serum cystatin C, age and systolic blood pressure (r=-0.196, -0.347 and -0.205, all *P*<0.01). After adjusting for various confounders, muitiple linear regression analysis revealed that cystatin C was significantly associated with FMD (B=-2.310, *P*<0.01). Conclusion: Serum cystatin C was negatively correlated with vascular endothelial function in patients with essential hypertension.

Keywords: Hypertension, flow mediated vasodilation, serum cystatin C

Introduction

Hypertension is a kind of "cardiovascular syndrome" characterized by the continuous increase of arterial blood pressure. It has a high disability and mortality. Vascular endothelial dysfunction plays a key role in the development of hypertension [1], so, it is very important to detect the endothelial function earlier in prevention and treatment of hypertension. Studies have shown that Cystatin C was a stronger predictor of the risk of death and cardiovascular events in elderly persons than creatinine [2, 3]. Other studies have demonstrated that there were correlation among Cystatin C, carotid intima-media thickening, Urinary albumin excretion and left ventricular hypertrophy in the patients with hypertension [4], and the cystatin C was a sensitive indicator of organ damage in the patients with hypertension. This study investigated the relationship between serum cystatin C and vascular endothelial function in the patients with essential hypertension.

Materials and methods

Materials

434 cases (male 228, female 206) with an average age of (56.5±12.0) years were enrolled in the first hospital of Shanxi Medical University, including 343 patients with essential hypertension and 100 patients without hypertension, ranging from May 2014 to October 2015. The inclusion criteria were: all patients with essential hypertension have normal renal function. The exclusion criteria were: the presence of infection, secondary hypertension, acute cere-

Table 1. Baseline characteristics of the study participants ($\bar{x}\pm s$)

Characteristics	FMD≥6.0% (n=232)	FMD<6.0% (n=202)	t	*P*
Age, (y)[a]	54.5±12.4	59.4±10.8	3.131	0.002
Male, (n%)[c]	126 (54.3)	102 (50.5)	0.630	0.427
Smoking, (n%)[c]	64 (27.6)	43 (21.3)	2.306	0.129
Body mass index, (kg/m^2)	26.2±4.8	25.7±3.5	-0.887	0.376
Heart rate, (n/min)	70.3±11.2	68.3±9.3	-1.371	0.127
Systolic blood pressure, (mmHg)[a]	139.2±17.6	145.8±17.3	2.836	0.005
Diastolic blood pressure, (mmHg)	85.4±11.4	86.8±10.48	0.952	0.342
Fasting glucose, (mmol/L)	5.32±1.00	5.67±1.40	-1.553	0.120
Total cholesterol, (mmol/L)	4.62±1.15	4.89±1.09	1.779	0.077
Triglycerides, (mmol/L)	1.98±1.54	1.98±1.29	0.009	0.993
HDL-C, (mmol/L)	1.16±0.25	1.23±0.30	-1.685	0.097
LDL-C, (mmol/L)	2.71±0.95	2.75±0.88	0.309	0.757
Creatinine, (mmol/L)	64.5±16.3	65.8±14.1	-0.563	0.573
Cystatin C, (mg/L)[b]	0.98±0.26	1.05±0.23	2.128	0.034
Urine micro albumin	19.65±47.8	18.44±31.3	-0.161	0.872
Medication ues, No. (%)[c]				
Angiotensin-converting enzyme inhibitors	14 (6.0)	14 (6.9)	0.144	0.705
Angiotensin II receptor blockers	34 (14.7)	30 (14.9)	0.003	0.954
Calcium channel blockers	75 (32.3)	80 (39.6)	2.490	0.115
Diuretics	4 (1.7)	5 (2.5)	0.300	0.584
β-Blockers	28 (12.1)	26 (12.9)	0.064	0.801

Abbreviation: HDL-C, high-density lipoprotein chaolesterol; LDL-C, low-density lipoprotein chaolesterol; [a]P<0.01, [b]P<0.05, [c]Pearson Chi-square test was used.

bral hemorrhage and infarction, renal insufficiency.

Grouped data

The patients were divided into two groups according to FMD value: Patients were divided into the FMD≥6.0% group (n=232) or FMD< 6.0% group (n=202) according to flow mediated vasodilation (FMD) measured by ultrosound [5-7].

The patients were divided into the following groups according to the grade of blood pressure: There are 100 cases in the normal blood pressure group (male 41, female 59), 125 cases in the grade 1 hypertension group (male 66, female 59), 137 cases in the grade 2 hypertension group (male 77 female 60) and 72 cases in the grade 3 hypertension group (male 42, female 30).

The patients were divided into three groups according to age: Young group ≤44 years (n=69), Middle-age group 45~≤59 years (n= 202) and Elderly group ≥60 years (n=163).

Methods

Determination of serum systatin C: 3 mL elbow venous blood of all patients were collected in the morning after 12 hours fasting and saved them in -80°C refrigerator for test. The serum cystatin C levels were detected by immunoturbidimetry. The cystatin C reagent is purchased in Maccura (Sichuan, China).

Vascular endothelial function: All patients checked before a day after the 21:00, 4~6 h to avoid exercise, high fat diet, drinking coffee, vitamin C and smoking. Vascular endothelial function was measured by UNEXEF 38G ultrosound (OMRON Corporation, Kyoto, Japan). All patients were checked by the same experienced operator. Operator variation <5%, Inter operator variation <6%. FMD calculation method: $FMD=[(D_2-D_1)/D_1]\times100\%$.

Statistical analysis

Statistical analysis were performed with SPSS17.0 Package. Qualitative data was expressed as mean±standard deviation ($\bar{x}\pm s$). The

Table 2. Serum cystatin C and FMD level in different grade of blood pressure ($\bar{x}\pm s$)

Project	N (Male/ Female)	FMD (%)	Cystatin C (mg/L)
Grade 1 hypertension group	125 (66/59)	5.5±2.8[a]	0.80±0.21[a]
Grade 2 hypertension group	137 (77/60)	4.5±2.3[a]	0.86±0.30[a]
Grade 3 hypertension group	72 (42/30)	2.9±1.7[a,b,c]	1.15±0.32[a,b,c]
Normal blood pressure group	100 (41/59)	6.6±2.9	0.56±0.25

Note: compared with normal group, [a]P<0.05; compared with stage 1 hypertension group, [b]P<0.05; compared withstage 3 hypertension group, [c]P<0.05.

two groups were compared with T-test. Three groups or above were compared with the single factor ANOVA. The quantitative data were compared with Pearson Chi-square test. The relationship between cystatin C and FMD used the multiple linear regression, and the criteria for significance difference was P<0.05.

Results

Study participants and baseline characteristics

As shown in **Table 1**, There were highly significant difference in age, systolic blood pressure, cystatin C between FMD≥6.0% group and FMD<6.0% group ([a]P<0.01, [b]P<0.05). No significant difference were observed in sex, smoking, body mass index, heart rate, diastolic blood pressure, fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein chaolesterol, low-density lipoprotein chaolesterolcreatinine, creatinine, urine micro albumin, medication use between FMD≥6.0% group and FMD<6.0% group (P>0.05).

FMD values in different age groups

There were highly significant difference in FMD values among young group ≤44 years (n=69), middle-age group 45~≤59 years (n=202) and elderly group ≥60 years (n=163). The FMD values in elderly group was significantly lower than middle-aged group and young group (P<0.01), and middle-aged group was significantly lower than young group (P<0.05).

Serum cystatin C and FMD in different stage of blood pressure

As shown in **Table 2**, compared to patients with normal blood pressure group, FMD was significantly lower, while serum cystatin C was significantly higher in the patients with essential hypertension. Compared to the patients with grade 3 hypertension, FMD was significantly higher, while serum cystatin C was significantly lower in the patients with grade 1 and 2 hypertension group (both P<0.05).

Correlation analysis

FMD was significantly negatively correlated with serum cystatin C, age and systolic blood pressure (r=-0.196, -0.347 and -0.205, all P<0.01). There was no significantly correlated with FMD and sex, smoking, body mass index, heart rate diastolic blood pressure, fasting glucose, total cholesterol, triglycerides, high-density lipoprotein chaolesterol, low-density lipoprotein chaolesterolCreatinine, creatinine and urine micro albumin (P>0.05). See **Table 3**.

Multiple linear regression

When corrected for age and sex, multiple linear regression analysis showed that cystatin C was one of influencing factors of FMD (P<0.05). After adjusting various confounders, including age, sex, smoking, body mass index, heart rate, diastolic blood pressure, fasting glucose, total cholesterol, triglycerides, high-density lipoprotein chaolesterol, low-density lipoprotein chaolesterol, creatinine and urine micro albumin, multiple linear regression analysis revealed that cystatin C was significantly associated with FMD (B=-2.310, P<0.01).

Discussion

Vascular endothelial disfunction is a key step in the development of hypertension. This study shows that FMD was significantly lower in the patients with essential hypertension compared to the patients with normal blood pressure. Compared to the patients with grade 3 hypertension, FMD was significantly higher in the patients with grade 1 and 2 hypertension group (both P<0.05). Therefore, it has an important clinical significance that the endothelial function was detected earlier in the prevention and treatment of hypertension.

Cystatin C is one of the members of cysteine protease inhibitors family expressed in all mononuclear cells and most plants, animals and the parasites, involving in the protease hydrolysis inside and outside of the cells [8]. Cystatin C has been considered as an accurate marker

Table 3. The influence factors of FMD

Independent variable	Model one					Model two				
	B	SE	β	t	*P*	B	SE	β	t	*P*
Cys C	-2.523	1.135	-0.217	-2.222	0.029	-2.310	0.877	-0.196	-2.635	0.009
Age	-0.090	0.023	-0.362	-3.956	0.000	-0.091	0.018	-0.347	-4.945	0.000
SBP	-0.004	0.013	-0.234	-3.207	0.002	-0.036	0.013	-0.205	-2.803	0.006

Abbreviation: Cys C, Cystatin C; FMD, Flow mediated vasodilation; SBP, Systolic blood pressure. Model one have corrected age and gender; Model two have corrected age, gender, smoking, body mass index, systolic blood pressure, fasting glucose and blood lipid.

of renal function all along, because of its constant production which is not influenced by sex, age, body mass and inflammatory factors. Recent studies have shown that serum cystatin C levels predict the risk of cardiovascular events. Researches had proved that serum cystatin C were closely related to intima-media thick-ness of carotid artery, left ventricular hypertrophy and urinary albumin excretion in the patients with hypertension [4, 9], and it is a sensitive indicator of target-organ injury in hypertension. This study showed serum cystatin C in hypertension group is significantly higher than the normal blood pressure group and the cystatin C in grade 3 hypertension group is higher than grade 1 and 2 hypertension group (both $P<0.05$), this showed that cystatin C may be an important marker for the diagnosis and prognosis of hypertension. The result was consistent with Mena C's research that Cystatin C was closely related to systolic blood pressure and pulse pressure [10].

As a cysteine protease inhibitor, Cystatin C not only involved in the regulation of intracellular and extracellular proteolysis but also protected cells from inappropriate endogenous or exogenous protease hydrolysis [11]. Galina K et al. have reported that cystatin C decreased in the arterial atherosclerosis compared with the normal artery by the study of the model of LDL receptor deficient mice [12]. Bengtsson E et al. found that the lack of cystatin C could lead to the increase of plaque by the study of tissue from the anterior-lateral aneurysm wall [13]. It is assumed that the impaired endothelial cell activated the macrophages and smooth muscle cells, making them release active cathepsin to degrade extracellular matrix, and cystatin C is consumed by inhibiting the overexperssion of cathepsin. So the cystatin C within the section of plaques decreases. The body will synthesize more cystatin C to regulate the degradation of cathepsin inside and outside to achieve balance. Eventually cystatin C levels in serum rises [14, 15]. The process suggests that cystatin C serum levels can indirectly reflect the state of vascular endothelium.

In addition, cystatin C has protective effects against various oxidative stresses that induce cell death [16]. This study demonstrated that FMD was significantly negatively correlated with cystatin C, age and systolic blood pressure ($r=-0.196$, -0.347 and -0.205, all $P<0.01$). After adjusting various confounders, muitiple linear regression analysis revealed that cystatin C significantly associated with FMD ($B=-2.523$, $P<0.05$). We speculated that cystatin C may be a predictor of impaired vascular endothelial function in the patients with essential hypertension and its mechanism and pathway needs further study.

This study is a cross-sectional study and the sample size is relatively small. Thus, the clinical significance of serum cystatin C in hypertension patients with vascular endothelial injury remains to be large-scale prospective studies to be confirmed.

In conclusion, this study demonstrates that the serum cystatin C was negatively correlated with vascular endothelial function in the patients with essential hypertension.

Disclosure of conflict of interest

None.

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