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ARTICLE

Correlation between Serum Homocysteine Level and Cystatin C Elevation in Patients with H-type Hypertension

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Abstract

Objective To investigate the correlation between serum levels of homocysteine (Hcy) and cystatin C (CysC), a sensitive marker of renal function, in patients with H-type hypertension. **Methods** A total of 857 patients with essential hypertension without moderate to severe renal impairment (estimated glomerular filtration rate 60 ml/(min-1.73 m2) by Modification of Diet in Renal Disease equation) visited the Seventh Affiliated Hospital of Sun Yat-sen University from May 2018 to May 2020 were selected as the research subjects. The observation group (n = 635) consisted of patients with H-type hypertension (essential hypertension with Hcy 10 mmol/l) and the control group (n = 222) consisted of patients with non-H-type hypertension (essential hypertension with Hcy < 10 mmol/l). Multivariate logistic regression analysis, curve fitting, and threshold analysis were used to evaluate the correlation between elevated CysC and serum Hcy levels. **Results**CysC in observation group was significantly higher than that in control group (0.96 ± 0.160 vs 0.84 ± 0.13 mmol/l; p < 0.001). Multivariate logistic regression analysis, curve fitting, and threshold analysis showed that there was a significant difference in the risk of CysC elevation between Hcy levels at 10-25 mmol/L and Hcy levels below 10 mmol/L; there was no significant difference in the risk of CysC elevation between Hcy levels above 25 mmol/L and Hcy levels below 10 mmol/L. The inflection point of Hcy was 15.23 mmol/L, and when Hcy was < 15.23 mmol/L, the probability of CysC elevation increased by 27% for each 1 mmol/L increase in Hcy (OR 1.27, 95% Cl: 1.12, 1.44; P = 0.0002). When Hcy was > 15.23 mmol/l, the probability of CysC elevation was reduced by 4% for each 1 mmol/l increase in Hcy (OR 0.96, 95% Cl: 0.92, 1.01; P = 0.1085). **Conclusions** The risk of CysC in patients with H-type hypertension is higher than that in patients with non-H-type hypertension when Hcy is between 10-25 mmol/L; and the probability of cystatin C elevation is the highest when Hcy is at 15.23 mmol/L.

Keywords: Essential hypertension; H-type hypertension; Homocysteine; Early renal function; Cystatin C

1. INTRODUCTION

The results of the National Hypertension Survey published in 2018 in China showed that the crude prevalence of hypertension in the population aged 18 years and above in China was 27.9%, and the weighted prevalence was 23.2%. According to this data, about one out of four adults has hypertension, and the total number of people suffering from hypertension is approximately 244 million [1]. Relevant research data show that the number of patients with H-type hypertension may account for about 75% of all patients with hypertension in China [2]. Hypertensive nephropathy is one of the most serious and common complications of hypertension. However, its early onset has no obvious symptoms [3], and patients with mild lesions are easily missed in routine clinical examinations. Therefore, early diagnosis of hypertensive nephropathy is of great significance to the treatment and prognosis of patients.

The estimated glomerular filtration rate (eGFR) is the "gold standard" for assessing renal function in clinical practice, but its calculation mainly relies on serum creatinine (Scr) value, which is affected by various factors such as patient age and race. However, eGFR can also be reflected by CysC. Studies have shown that CysC combined with Hcy has important clinical value in detecting the occurrence of early hypertensive nephropathy [4]. Therefore, in this study, we investigated the relationship between Hcy levels and elevated CysC, an indicator of early renal function impairment, in patients with H-type hypertension by measuring CysC levels.

2. MATERIALS AND METHODS

2.1 Research participants

A total of 857 patients diagnosed with essential hypertension in the Health Management Center of the Seventh Affiliated Hospital of Sun Yat-sen University from May 2018 to May 2020 were included in this study. Among them, 635 patients with elevated Hcy levels were included in the observation group; there were 511 males and 124 females, aged between 23 to 87 years, with a median age of 52 years. The control group consisted of 222 hypertensive patients without elevated Hcy levels, including 78 males and 144 females, aged between 27 to 76 years, with a median age of 51 years. All patients have been confirmed without diabetes, cardiovascular and cerebrovascular diseases, urinary system diseases, pregnant women, malignant tumors, acute and chronic infections, gout, autoimmune diseases, etc. The general data of the two groups were comparable. This study was approved by the Ethics Committee of the Seventh Affiliated Hospital, Sun Yat-sen University. The research subjects gave informed consent to this study and signed the informed consent form.

2.2 Inclusion and exclusion criteria

The inclusion criteria of the study subjects included meeting the diagnostic criteria for hypertension, normal Scr, and eGFR 60 ml/(min*1.73m2). The exclusion criteria are as follows: 1) secondary hypertension; 2) complicated with diabetes mellitus, coronary atherosclerotic heart disease, peripheral vascular disease, valvular heart disease, rheumatic heart disease, acute heart failure, congenital heart disease, etc.; 3) Combined with other urinary system diseases, such as nephritis, nephrotic syndrome, urinary tract infection and acute and chronic renal insufficiency; 4) Pregnant women, suffering from malignant tumors,

severe cardiovascular and cerebrovascular complications, acute and chronic infections, gout, autoimmunity sexually transmitted diseases [5].

2.3 CysC and Hcy detection

The patients in the study fasted for more than 12 h, and the fasting venous blood of 5 ml was drawn the next morning at 3000 r/min, centrifuged for 10 min, and the serum was separated in time. All detection tests were carried out under the condition that the instrument and reagents were in normal state and the quality control was under control, and were carried out in strict accordance with the instrument and reagent operating procedures (SOP). The correlation between CysC elevation and Hcy level was analyzed.

2.4 Statistical analysis

Statistical analysis was performed using Epowerstats and R softwares. Enumeration data such as gender were expressed as percentage (%), 2 test or Fisher exact test was used for comparison. Enumeration data conforming to normal distribution were expressed as mean ± standard deviation (SD), and analysis of variance was used for comparison. Maximum/minimum was used for non-conforming normal distribution, and Kruskal-Wallis rank sum test was used for comparison between multiple groups. The relationship between Hcy and elevated serum CysC levels in patients with H-type hypertension was analyzed by univariate and multivariate logistic regression. The screening of adjusted variables was based on whether the clinical significance or the effect on odd ratio (OR) exceeded 10%. The 2 test was used for trend test comparison. $P < 0.05$ was considered statistically significant.

3. RESULT

3.1 Comparison of metabolic parameters, CysC, and eGFR between two groups of patients

Among the included research subjects, there were 635 patients with H-type hypertension, accounting for 74%. The general information of patients with H-type hypertension and the control group were compared (Table 1). First, there was no significant difference in body mass index (BMI) and pulse pressure difference between the two groups ($P>0.05$). However, there were significant differences in gender composition, smoking, and drinking cases between the two groups ($P<0.01$). It is worth noting that the age proportion between the two groups was also different. The age of 40-60 years old and those over 60 years old between the two groups were statistically significant compared with those younger than 40 years old ($P<0.05$). There was no significant difference in the levels of metabolic parameters and early renal function impairment markers, fasting blood glucose, glycosylated hemoglobin, total cholesterol, triacylglycerol, and high-density lipoprotein cholesterol between the two groups ($P>0.05$). However, the low-density lipoprotein cholesterol in the H-type hypertension group was significantly higher than that in the control group ($P>0.05$), and the differences in uric acid, CysC, and eGFR between the two groups were also statistically significant ($P<0.01$).

Table 1. Comparison of clinical characteristics of patients with H-type hypertension (observation group) and patients with non H-type hypertension (control group)

Variables	Control group (n = 222)	Observation group(n = 635)	Standardize diff.	P value
Age (years)	51.03 ± 9.20	52.27 ± 10.77	0.21 (0.05, 0.36)	0.035
< 40	19 (8.56%)	156 (24.57%)		
> = 40, < 60	164 (73.87%)	70 (11.02%)		
> = 60	39 (17.57%)	409 (64.41%)		
Male/case (%)	78 (54.17%)	156 (24.57%)	1.03 (0.87, 1.19)	< 0.001
Smoking/case (%)	37 (16.67%)	209 (32.91%)	511/124	< 0.001
Alcohol use/case (%)	80 (36.04%)	509 (80.16%)	1.00 (0.84, 1.16)	< 0.001
Body Mass Index (kg/m2)	26.19 ± 3.62	26.39 ± 3.33	0.06 (-0.10, 0.21)	0.201
Pulse pressure difference (mmHg)	55.24 ± 12.80	54.68 ± 13.74	0.04 (-0.11, 0.19)	0.534
Fasting blood glucose (mmol/L)	5.50 ± 1.53	5.57 ± 1.96	0.04 (-0.11, 0.20)	0.605
Glycosylated hemoglobin (%)	5.89 ± 0.82	5.91 ± 1.08	0.02 (-0.14, 0.17)	0.19
Total cholesterol (mmol/L)	5.31 ± 1.04	5.35 ± 1.03	0.04 (-0.11, 0.19)	0.371
Triacylglycerol (mmol/L)	1.54 (0.45-19.14)	1.55 (0.40-29.84)	0.03 (-0.13, 0.18)	0.723
High density lipoprotein cholesterol (mmol/L)	1.17 ± 0.31	1.13 ± 0.30	0.14 (-0.01, 0.29)	0.072
Low-density lipoprotein cholesterol (mmol/L)	3.02 ± 0.90	3.18 ± 0.87	0.18 (0.02, 0.33)	0.012
Uric acid (mmol/L)	360.65 ± 94.41	417.65 ± 94.53	0.60 (0.45, 0.76)	< 0.001
CysC	0.84 ± 0.13	0.96 ± 0.16	0.84 (0.68, 1.00)	< 0.001
eGFR	115.93 ± 21.38	98.16 ± 17.77	0.90 (0.75, 1.06)	< 0.001
CysC increased/case (%)	14 (6.31%)	190 (29.92%)	0.64 (0.49, 0.80)	< 0.001

3.2 The relationship between Hcy and Cys in hypertensive patients with different Hcy levels

We further investigated the relationship between Hcy level and CysC level, an early renal function impairment marker, in H-type hypertension group. As shown in Figure 1, in the generalized additive model, we found a nonlinear relationship between Hcy and the probability of CysC elevation. The red line in the middle part of the curve in Figure 1 showed a smooth curve fitting point between variables, and the upper and lower blue lines were the 95% confidence interval (Cl) of the fitting point. Logistic regression and two-part logistic regression were used to fit the association, and the best fitting model based on P was selected for log-likelihood ratio test. We chose a two-part logistic regression to fit the relationship between Hcy and CysC elevation due to the P<0.05 of the log-likelihood ratio test (Table 2). Through two-stage logistic regression and recursive algorithm, the inflection point was calculated to be 15.23. The effect size and 95% CI on the left side of the inflection point were 1.27 and 1.12-1.44, respectively. That is, when Hcy<15.23mmol/l, the probability of increasing CysC increased by 27% for every 1mmol/l increase in Hcy

(P=0.0002). The effect size and 95% CI on the right side of the inflection point were 0.96 and 0.92-1.01, respectively. That is, when CysC>15.23mmol/l, the probability of increasing CysC decreased by 4% for every 1mmol/l increase in Hcy (P=0.1085) (Table 3).

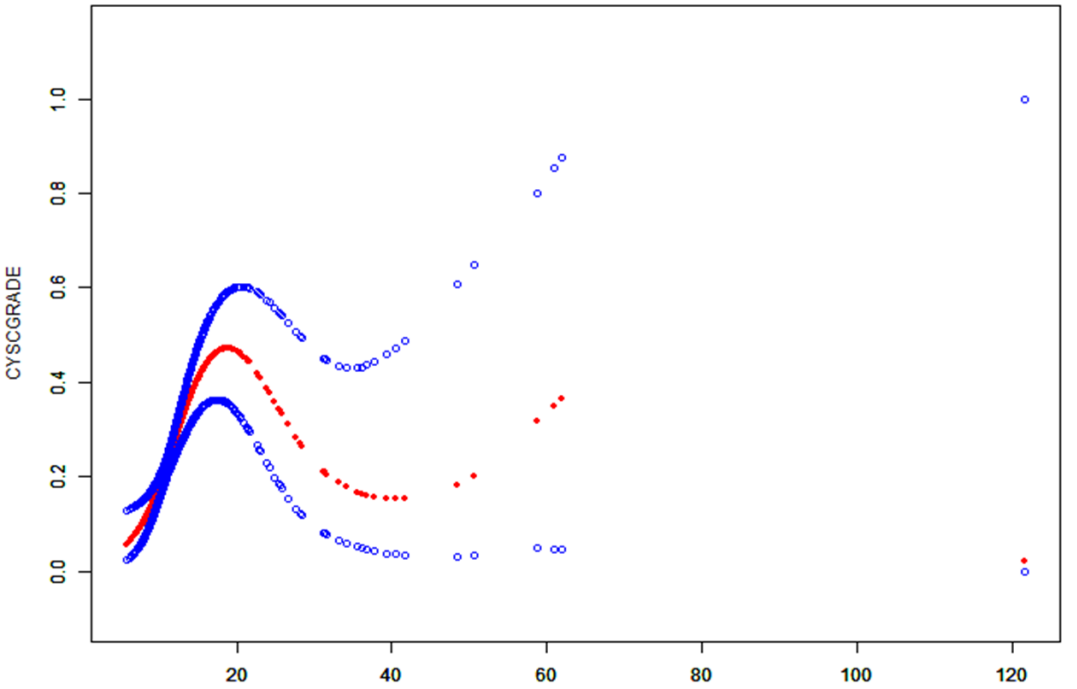

Figure 1. Smooth curve of Hcy level versus probability of CysC elevation.

4. DISCUSSION

In China, H-type hypertension accounts for about 75% of all adult hypertensive patients. Compared with non-H-type hypertension, H-type hypertension is more likely to cause atherosclerosis and stenosis of the blood vessels, resulting in a gradual increase in blood pressure, which poses a serious threat to the life and health of patients [6]. The prevalence of renal injury caused by hypertension is increasing year by year, and has become the second leading cause of end-stage renal disease [7]. The only way to prevent the deterioration of renal disease is early diagnosis and early treatment to reverse the damaged renal function [8]. Therefore, early diagnosis of hypertensive nephropathy is of great significance to the treatment and prognosis of patients.

CysC is a low molecular weight protein in the body. Its expression in the human body is not affected by factors such as age, diet, and drugs, and it can freely pass through the glomerular filtration membrane without being reabsorbed and degraded by the proximal convoluted tubule, and will not return to the blood circulation. The kidney is the only organ that clears CysC [9]. Because renal tubular epithelial cells do not secrete CysC, mild glomerular damage can also lead to markedly elevated serum CysC levels, which can lead to disease progression. Therefore, CysC is considered to be a more sensitive and specific

Table 2. Regression analysis of the correlation between serum Hcy and CysC elevation

Variable	Uncorrected		After correction	
	OR (95% Cl)	P value	OR (95% Cl)	P value
Hcy	1.05 (1.02, 1.07)	0.0007	1.01 (0.99, 1.04)	0.3206
Hcy subgroup				
<10	1.0		1.0	
>=10	6.34 (3.60, 11.19)	<0.0001	3.66 (1.88, 7.15)	0.0001
Hcy subgroup				
<10	1.0		1.0	
>=10, <15	5.18 (2.91, 9.23)	<0.0001	3.36 (1.71, 6.61)	0.0004
>=15, <20	14.24 (7.25, 27.98)	<0.0001	7.17 (3.14, 16.34)	<0.0001
>=20, <25	18.16 (6.46, 51.06)	<0.0001	4.87 (1.41, 16.83)	0.0122
>=25	3.54 (1.16, 10.79)	0.0264	2.07 (0.57, 7.48)	0.2691

Note: Adjusted for confounders, including gender, age, smoking, alcohol consumption, uric acid, fasting blood glucose, glycosylated hemoglobin, triacylglycerol, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

Table 3. Threshold effect analysis of Hcy and CysC elevation

Item	OR (95% Cl)	P value
Model I		
Linear effect	1.00 (0.98, 1.03)	0.9348
Model II		
Breakpoint	15.23	
< K-segment effect1	1.27 (1.12, 1.44)	0.0002
> K-segment effect2	0.96 (0.92, 1.01)	0.1085
Log-likelihood ratio test		0.0002

endogenous substance for evaluating GFR [10]. However, when the renal function is severely damaged, CysC cannot be used as an index reflecting renal function.

Hcy is a sulfur-containing amino acid formed by the metabolism of methionine. Previous studies have confirmed that its elevation is related to the pathogenesis of cardiovascular and cerebrovascular diseases, chronic kidney disease, diabetes and many other diseases [11]. In addition, Hcy is also a reactive vascular injury amino acid [12]; it can cause damage to the glomerular microvascular endothelium, leading to glomerular microcirculation disturbance, thereby causing renal damage.

In this study, patients with essential hypertension without moderate or severe renal impairment (with normal Scr and eGFR calculated by the modified MDRD formula) were selected. We found that the CysC level and increased proportion in H-type hypertension patients were significantly higher than those in non-H-type hypertension group. The level of eGFR in the H-type hypertension group was lower than that in the non-H-type

hypertension group. Moreover, when Hcy10mmol/L, it can be further divided into several subgroups: 10-15mmol/L, 15-20mmol/L, 20-25mmol/L, and more than 25mmol/L. The increased risk of CysC in each group was higher than that of normal Hcy (the 15-20mmol/L group had the highest increased risk). In addition, through two-stage logistic regression and recursive algorithm, we calculated the inflection point to be 15.23 mmol/. When Hcy<15.23mmol/l, the probability of increasing CysC increased by 27% for every 1mmol/l increase in Hcy (P=0.0002). At 15.23 mmol/l, every 1 mmol/l increase of Hcy decreased the probability of CysC increase by 4% (P=0.1085). To sum up, the risk of early renal function impairment in the H-type hypertension group was significantly higher than that in the non-H-type hypertension group, which is consistent with the results of many studies [5, 13-15]. Therefore, CysC is of great significance for the early diagnosis and treatment monitoring of H-type hypertensive nephropathy.

In conclusion, our data suggest that the level of Hcy in patients with H-type hypertension is associated with the risk of hypertensive renal injury. When the Hcy level of patients with H-type hypertension is 10-25mmol/L, the risk of CysC elevation is higher than that of non-H-type hypertension patients. When Hcy is 15.23mmol/L, the probability of CysC elevation is the highest. Therefore, controlling Hcy to an appropriate level in H-type hypertension patients may slow down the occurrence of hypertensive kidney damage.

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