Original Research Article

A curve model for association of serum homocysteine with carotid artery hemodynamics

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Abstract

Purpose: To investigate the correlation between carotid artery hemodynamics and serum homocysteine.

Methods: A total of 894 participants made up of 439 male (49.11 %) and 455 female (50.89 %) from Ma'anshan, China, enrolled in the cross-sectional study. Data collection included demographics, blood sample and carotid ultrasonography. Piecewise linear regression analysis was used to analyze the relationship between serum homocysteine and carotid artery hemodynamics.

Results: Homocysteine (Hcy) levels were divided into four groups by quartiles. The populations of the groups were 226, 220, 222, 226; and their mean ages were 56.52 ± 10.49, 62.27 ± 10.06, 63.42 ± 9.81 and 65.38 ± 10.56 years, respectively. After adjustment for blood biochemical and demographics factors, U-shaped and S-shaped curves were as observed between Hcy and carotid artery hemodynamics. The adjusted regression analysis showed that the threshold values of Hcy with end diastolic velocity (EDV) of right common carotid artery (CCA) were 12.50 and 19.00, while for the EDV of right internal carotid artery (ICA), the values were 11.50 and 22.00. U-shaped curves were observed between Hcy and peak systolic velocity (PSV) of left CCA, EDV of left CCA, PSV of left ICA and EDV of left ICA. The threshold values of Hcy with PSV of left CCA, EDV of left CCA, PSV of left ICA and EDV of left ICA were 14.00, 14.00, 14.00 and 13.50, respectively.

Conclusion: These results indicate that a significant correlation exists between homocysteine at different concentrations and carotid artery hemodynamics.

Keywords: Homocysteine, Hemodynamics, End diastolic velocity, Peak systolic velocity

INTRODUCTION

Cardiovascular disease (CVD) is a main cause of death across the globe, resulting in more than 17.3 million deaths in 2013, and is projected to result in the death of more than 23.6 million people by 2030 [1]. A recent study had revealed that Hcy is an independent risk and predictive factor for CVD [2]. Hcy accelerates vascular endothelial injury, low-density lipoprotein oxidation and vascular smooth muscle cells proliferation [3-5]. Carotid ultrasonic examination for evaluation of atherosclerosis (AS) mainly involves hemodynamics, intima-media thickness (IMT) and plaque size [6]. And IMT is negatively correlated with wall shear stress (WSS) in the common carotid artery (CCA) [7]. In addition, peak systolic velocity (PSV) ratio of CCA was positively associated with high-grade common carotid stenosis [8].

A recent study showed that Hcy was positively correlated with carotid intima-media thickness (IMT) in adolescent hypertension [9]. In addition, results of a cross-sectional study showed that increased Hcy was associated with advanced carotid plaque in males [10]. Despite the fact that Hcy and hemodynamics are associated with IMT and carotid plaque, their exact inter-relationship is yet to be investigated. A recent research found that pulsatility index (PI) was correlated with Hcy in patients with essential hypertension [11].

However, the relationship between Hcy and other hemodynamic parameters such as PSV and end diastolic velocity (EDV) is not yet understood. The current research is a cross-sectional study to investigate whether there is an association between serum Hcy and PSV and EDV of carotid artery.

METHODS

Patients

A total of 894 patients were recruited from December 2013 to June 2014 in Ma'anshan People's Hospital, China. Inclusion criteria were: (1) patients aged from 30 to 80; (2) patients without history of intervention therapy for carotid artery stenosis; (3) patients without history of stroke, cancer or severe infection; and (4) patients without rheumatic heart disease, hypertensive heart disease, or congenital heart disease. This study was approved by the Ethical Committee of Ma'anshan People's Hospital (approval no. 2014002) and followed the guidelines of the World Medical Association Declaration of Helsinki [12].

Survey questionnaire

The questionnaire contained social-demographic characteristics (e.g., sex, age, education level, occupation, etc); behavior characteristics (smoking status, drinking status and physical exercise), and history of disease (stroke, coronary heart disease, hypertension and diabetes). Face-to-face interview was used for the survey.

Physical examination included height, weight and blood pressure. In blood pressure measurement, all participants rested for 15 min in quiet environments and were tested with mercury sphygmomanometer (Yuyue, China). Height and weight measurements were carried out by electronic height and weight instrument (HGM-300, Tian Jinggang, China).

Hypertension was defined as either taking antihypertensive drugs or systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. Diabetes status was defined as either using anti-diabetic drugs or FPG > 7.1 mmol/L. Frequent smoker was defined as smoking more than 1 cigarette/day or never. Drinking was defined as taking more than 1 drink /week, or never, and physical exercise was defined as exercising 3-7 times/week, or never.

Biochemical assays

Venous blood specimens were collected in the morning from the patients after an overnight fast. All samples were stored at −70 ^{o}C until biochemical assay. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), fasting plasma glucose (FPG) and Hcy were determined with automated biochemical analyzer (DPP-800, Roche, Germany) in Ma'anshan People's Hospital. All data from laboratory assays and Doppler ultrasound were collected on the same day.

Carotid ultrasonography

Patients were made to take a 10-min break to stabilize blood pressure and heart rate prior to ultrasound examination in supine position with the head tilted to the contralateral side. The proximal and distal walls of the left and right CCA and internal carotid artery (ICA) were measured for PSV and EDV with 5-13 MHz linear-array probes (Aloka-a7 and Aloka-a10, Japan; Philips-IU22, America).

Statistical analysis

The study objects were segmented into four groups by quartiles (P_{25}, P_{50}, P_{75} and P_{100}) of Hcy. TG was log-transformed because its skewed distribution data, and was expressed as a median (P_{25}, P_{75}). Other continuous variables are expressed as mean ± SD. Categorical variables were presented as percentage. Chi-square test for categorical variables and ANOVA test for continuous variables were used to compare the characteristics in different Hcy levels. Generalized smoothing splines were used to evaluate relationship between serum Hcy and carotid artery hemodynamics. In order to investigate the threshold effect of Hcy on hemodynamics, piece-wise linear regression model was used. Two-side value of $p < 0.05$ was considered statistically significant. R software, version 3.0.1 (http://www.R-project.org) was used for statistical analysis.

RESULTS

Characteristics of patients

The mean age of participants was 61.89 ± 10.75 years, and the mean Hcy was 13.28 ± 7.51 µmol/L. Hcy was significantly higher in the male patients than in the female patients (14.57 ± 7.64 versus 12.04 ± 7.17 µmol/L; *p* < 0.001).

Table 1 shows baseline information by quartiles of Hcy. There was correlation between increased serum Hcy level and age, gender, SBP, smoking, drinking and HDL. Increased prevalence of carotid artery disease and hypertension were related to the raised quartiles of Hcy.

Relationship between Hcy and hemodynamics by piecewise linear regression

Figure 1 and Table 2 show that after adjustment for age, BMI, SBP, DBP, HDL, FPG, TC, TG, carotid artery disease, smoking status and drinking status, S-shaped curve was found between Hcy and EDV of right CCA, and between Hcy and EDV of right ICA by generalized smoothing splines. The threshold values of Hcy with EDV of right CCA were 12.50 and 19.00; with EDV of right ICA the threshold values were 11.50 and 22.00. U-shaped curve was observed between Hcy and PSV of left CCA; and between Hcy and EDV of left CCA. U-shaped curve was also observed between Hcy and PSV of left ICA and between Hcy and EDV of left ICA. The threshold values of Hcy with PSV of left CCA, EDV of left CCA, PSV of left ICA, and EDV of left ICA were 14.00, 14.00, 14.00 and 13.50, respectively.

DISCUSSION

The findings of this research are consistent with the results of Memisogullari *et al* [13], and demonstrate that Hcy is associated with hemodynamics. Factor such as blood pressure is reported to be related to hemodynamics [14]. After adjustment for potential confounders, the relationship between Hcy and hemodynamics was a curve pattern, and the threshold values of Hcy were diverse in different parts of the carotid artery. However, results obtained by Memisogullari *et al* [13] in a previous study on correlation between Hcy and hemodynamics in ophthalmic artery of highway toll collectors revealed a linear pattern, in which high Hcy level was related to the decreased EDV and RI. However, Memisogullari *et al* based their study on the ophthalmic artery and only 22 toll subjects, while the current study focused on the carotid artery, and used a far greater number of subjects. These differences may be responsible for the disparity in the relationships between Hcy and hemodynamics. Further studies are nonetheless needed to unravel other likely causes of the disparity.

Another study reported that endothelial dysfunction and apoptosis could result in AS [15]. Endothelial cells exert an influence on hemodynamics [16]. Hcy may enhance the oxidation of low density lipoprotein (LDL) [3], which promotes injury of endothelial cells [17]. Furthermore, Hcy increases the accumulation of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine, and is positively

Table 1: Comparison of subjects' baseline information by quartiles of Hcy

Item	Hcy Quartiles				F/x^2	P -value
	Quartile 1 (≤8.90) N=226	*Quartile 2 (8.90-11.75)* N=220	*Quartile 3 (11.75-15.40)* N=222	*Quartile 4 (>15.40)* N=226		
Age year	56.52±10.49	62.27±10.06	63.42±9.81	65.38±10.56	31.19	<0.001
Sex: Male	78 (17.77)	98 (22.32)	118 (26.88)	145 (33.03)	43.03	<0.001
Female	148 (32.53)	122 (26.81)	104 (22.86)	81 (17.80)		
BMI kg/m^2	24.23±4.21	24.45±2.96	24.18±2.97	23.97±3.15	0.70	0.553
SBP mmHg	131.85±15.52	136.49±17.05	135.23±18.12	137.14±17.91	4.10	0.007
DBP mmHg	80.62±10.81	81.65±11.14	80.43±10.17	79.82±11.43	1.00	0.393
CAD	65 (12.36)	135 (25.67)	152 (28.90)	174 (33.08)	128.25	<0.001
HTN	118 (20.24)	164 (28.13)	151 (25.90)	150 (25.73)	26.53	<0.001
Diabetes	92 (30.87)	67 (22.48)	66 (22.15)	73 (24.50)	7.48	0.058
Smoking	47 (18.15)	57 (22.01)	69 (26.64)	86 (33.02)	18.56	<0.001
Drinking	34 (18.68)	44 (24.18)	44 (24.18)	60 (32.67)	9.64	0.022
TC mmol/L	4.28±1.50	4.63±1.98	4.35±1.45	4.37±1.56	1.97	0.117
TG* mmol/L	1.24 (0.95,1.81)	1.26 (0.94,1.81)	1.31 (0.95,1.76)	1.26 (0.93,1.74)	0.86	0.462
HDL mmol/L	1.29±0.34	1.21±0.31	1.17±0.27	1.11±0.31	13.77	<0.001
FPG mmol/L	6.72±3.45	6.06±2.76	6.42±5.46	5.95±2.61	1.89	0.130

*P-values obtained by using the log transformation. Data were expressed as mean ± SD., median (P_{25}, P_{75}), or n (%), BMI =body mass index, CAD =Carotid artery disease, HTN = hypertension
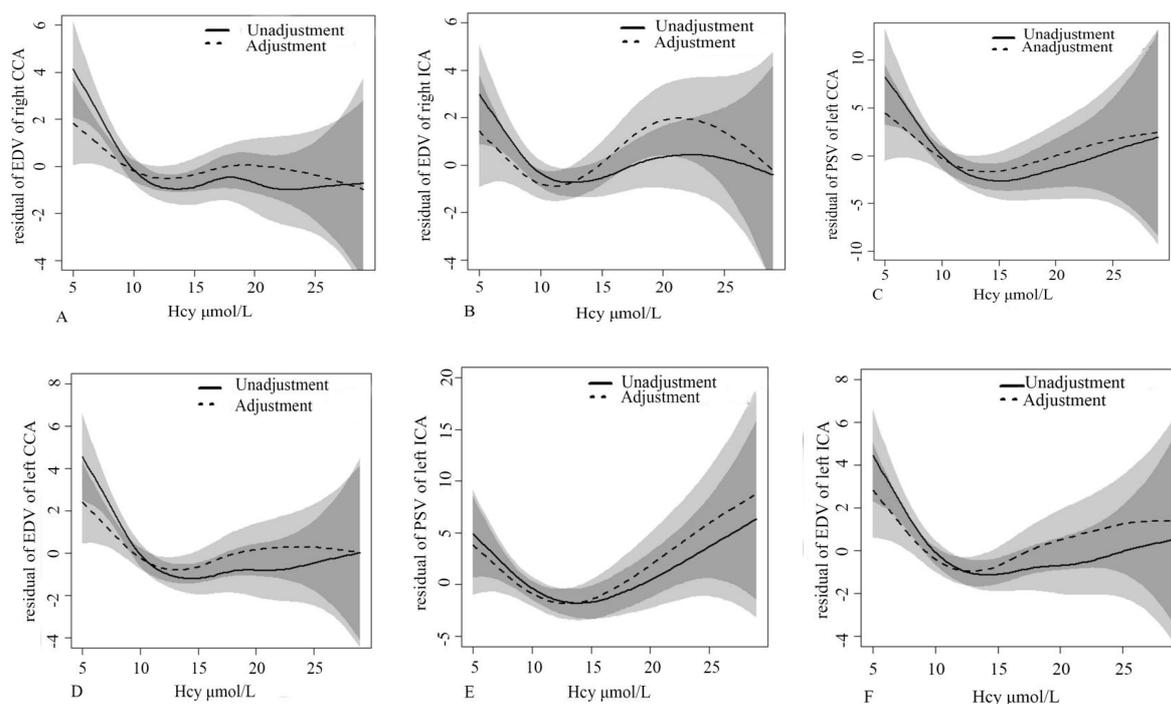
Figure 1: Relationship between Hcy and hemodynamics, using generalized smoothing splines. *Solid line: without adjustment; dotted line: adjustment for age, BMI, SBP, DBP, HDL, FPG, TC, TG, carotid artery disease, smoking and drinking status.. A: Hcy with EDV of right CCA; B: Hcy with EDV of right ICA; C: Hcy with PSV of left CCA; D: Hcy with EDV of left CCA; E: Hcy with PSV of left ICA; F: Hcy with EDV of left ICA*

Table 2: A piecewise linear regression model of the relationship between Hcy and hemodynamics (adjustment for age, BMI, SBP, DBP, HDL, FPG, TC, TG, carotid artery disease, smoking and drinking status)

Ultrasound parameter	Hcy (µmol/)	Unadjusted			Adjusted		
		β	SE	P	β	SE	P
EDV of RCCA	≤12.50	-0.75	0.16	<0.001	-0.39	0.18	0.036
	12.50~	0.24	0.21	0.253	0.10	0.23	0.669
	>19.00	0.20	0.30	0.513	-0.39	0.32	0.220
EDV of RICA	≤11.5	-0.93	0.22	<0.001	-0.51	0.25	0.042
	11.50~	0.04	0.16	0.813	0.29	0.18	0.110
	>22.00	-0.31	0.60	0.612	-0.20	0.70	0.781
PSV of LCCA	≤14.00	-1.21	0.36	0.001	-0.81	0.40	0.044
	>14.00	0.47	0.36	0.188	0.43	0.41	0.301
EDV of LCCA	≤14.00	-0.61	0.12	<0.001	-0.35	0.13	0.007
	>14.00	0.11	0.13	0.372	0.10	0.13	0.673
PSV of LICA	≤14.00	-0.84	0.30	0.006	-0.76	0.35	0.031
	>14.00	0.64	0.36	0.077	0.98	0.38	0.008
EDV of LICA	≤13.50	-0.61	0.14	<0.001	-0.40	0.15	0.005
	>13.50	0.10	0.13	0.446	0.21	0.14	0.141

RCCA = right common carotid artery, RICA = right internal carotid artery, LCCA = left common carotid artery, LICA = left internal carotid artery

related to flow-mediated vasodilatation [18]. Coronary hemodynamics are affected by nitric oxide (NO) due to vasodilation and inhibition of platelet adhesion and aggregation [19]. It has been reported that Hcy level was related to polymorphism of endothelial NO gene intron [20].

Although the exact mechanism involved in the correlation between Hcy and hemodynamics is not clear, several studies showed that Hcy had a positive correlation with IMT [9]. An inverse relationship has been demonstrated between

IMT and mean cerebral blood flow velocity (mCBFV), with decreased CBFV corresponding to elevated IMT [21]. In addition, Mohan *et al* [22] suggested that exposure to Hcy could induce platelet activation and hypercoagulability. Since platelet activating factor can reduce reducing coronary blood flow [23], these results indicate that Hcy may affect hemodynamics.

Interestingly, a curve relationship was found between Hcy and hemodynamics in the present study. The reason for the curve model is not

clear, but it might be related to hyper-homocysteinaemia (HHcy). HHcy is defined as Hcy > 15 µmol/L [24]. The results obtained in this study showed that at Hcy levels below 15 µmol/L, increased Hcy was related to decreased hemodynamics, while Hcy levels higher than 15 µmol/L were positively correlated with hemodynamics. This is due to the fact that Hcy is responsible for the hypercoagulability status of blood [22], and so it can reduce blood flow velocity. Hemodynamics is positively associated with carotid stenosis and carotid stenosis could result from HHcy [25]. Therefore, higher Hcy levels might promote increases in hemodynamics. Our results further showed that at Hcy level of about 14µmol/L, the effect on hemodynamics was different. Based on this, it can be hypothesized that the threshold value of HHcy should be close to 14µmol/L.

Limitations of the study

This study has two limitations. First, the mechanism involved in the effect of Hcy on hemodynamics is still not clear; further research is needed to establish this in an animal model or cell culture. Second, blood viscosity was not measured in this study.

CONCLUSION

The findings obtained in this study demonstrate a significant correlation between homocysteine (at different concentrations) and carotid artery hemodynamics. They also confirm the role of Hcy and hemodynamics in atherogenesis as well as provide evidence for the prevention of atherosclerosis.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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