Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Running title: CAVI and Homocysteine

Abstract

Objectives: Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

Design: Descriptive Research.

Participants: 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

Methods: CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

Results: There was significant correlation between Hcy and CF-PWV, CR-PWV, and CAVI in the entire group (r=0.33, p=0.002; r=51, p<0.001; r=0.42, p<0.001; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy ⩾15umol/L group than in Hcy <15umol/L group (13.7±3.0 vs 10.8±2.5, p<0.001; 10.6±2.1 vs 9.2±1.6, p=0.001; 9.30±2.1 vs 7.79±2.1, p=0.001; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI (β=0.421, p=0.001; β= -0.309, p=0.006; β=0.297, p=0.012; respectively).

Conclusions: CAVI was positively correlated with homocysteine in vascular-related diseases.

Keywords: Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

Article summary

1. Article focus

To investigate the relationship between homocysteine (Hcy) and Cardio-ankle vascular index (CAVI) in vascular-related diseases.

2. Key messages

Homocysteine was positively correlated with CAVI in vascular-related diseases.

3. Strengths and limitations of this study

Strengths of this study: our present study firstly showed the relationship between Hcy and CAVI in vascular-related diseases.

Limitations of this study: the small simple size, cases, and controls were not perfectly matched.

1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclseosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

resulted from many factors such endothelial dysfunction, smooth muscle cells proliferation, thickening of vascular wall. Kadota et al had showed positive correlation between Hcy and CAVI in general population [12]. However, the relationship between CAVI and Hcy in vascular-related diseases such as hypertension, coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still unknown, especially in patients with one more kinds of vascular-related diseases. In the present study, we investigated the possible link between CAVI and homocysteine in vascular-related diseases such as hypertension, CAD and ASO.

2. Materials and methods

2.1 Subjects

88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from vascular medicine department of Peking University Shougang Hospital from February 2012 to April 2012 were enrolled into our study. There were 57 patients with hypertension, 43 with CAD, and 25 patients with ASO in the whole study groups.

Hypertension was defined as known cases of hypertension or blood pressure measurement ⩾140/90mmHg in three occasions at rest. CAD or ASO was defined as the narrowing or blockage of coronary artery or lower extremity artery diagnosed by angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy⩾ 15umol/L[11].

Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according

to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ⩾15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

2.2 Pulse wave velocity measurement

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically.

2.3 The assessment of CAVI

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was placed on the sternum, and cuffs were wrapped around both the arms and ankles. After automatic measurements, obtained data were analyzed by software, and the value of CAVI was obtained automatically [16].

2.4 Laboratory measurements

Blood samples were drawn from an antecubital vein in the morning after overnight fasting and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting plasma glucose, homocysteine, hs-C reactive protein were determined at the central chemistry laboratory of the Peking University Shougang Hospital.

2.5 Statistical analysis

The difference between groups were analyzed by Student' *t*-test and one-way ANOVA. Correlation coefficient was done to find linear relation between different variables using Spearman correlation coefficient. Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of the dependent variables. Values were shown as mean $\pm$ SD unless stand otherwise. $p < 0.05$ (2-tailed) was considered statistically significant.

3. Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are shown in Table 1. Among these subjects, 33 patients had only one of these three vascular-related diseases, 34

patients had two of these three vascular-related diseases, 9 patients had all of these three diseases, and 12 subjects with none of vascular-related diseases. Our results showed that with the increasing numbers of suffered vascular-related diseases, the level of Hcy was increasing. Similar results were also found in the parameters of CF-PWV and CAVI. However, we found there was significant difference about age between these four groups.

Next, we divided subjects into two groups according to the level of Hcy. As shown in Table 2, the level of CAVI was significant higher in patients with Hcy≥ 15umol/L than in group with Hcy<15umol/L. The similar result was also found in another evaluation index of arterial stiffness-PWV. However, there was significant difference about age between these two groups.

3.2 Pearson correlations between PWV , CAVI and Hcy in the entire study group

PWV is a golden evaluation of arterial stiffness of vascular diseases. There are some kinds of PWV according to different arteries, such as carotid-femoral pulse wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B).

CAVI, a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure. And CAVI was not affected by immediately blood pressure. As shown in Fig2, there was significant positive

correlation between CAVI and Hcy in all patients (r=0.42, p<0.0001).

As shown in Table 1 and Table 2, there was significant difference about age between groups. So next, we investigated the possible relationship between CAVI, PWV with Hcy after adjusting the variable of age. Our results showed that there was still significant correlation between CAVI and Hcy after adjustment for age (r=0.293, p=0.008). Also a positive correlation between PWV and Hcy was found after age adjusted (CFPWV vs Hcy, r=0.282, p=0.010; CRPWV vs Hcy, r=0.462, p<0.001; respectively).

3.3 Multiple linear regression analysis

Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of CAVI. Our results showed that Hcy, BMI, and age were independent influencing factors of CAVI (β=0.421, p=0.001; β= -0.309, p=0.006; β=0.297, p=0.012; respectively).

4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy ⩾ 15umol/L, and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter β, which is detected by carotid ultrasonic measurement [16].

CAVI is a new evaluation index of arterial stiffness independent of immediate blood pressure. Recent studies have showed the role of CAVI in the prediction of vascular events in vascular-related diseases such as metabolic syndrome (MS), diabetes, CAD, and so on. In MS patients, there was significant positive correlation between CAVI and waist circumference, and CAVI increased significantly with the number of metabolic syndrome components [17]. In another MS study, they found that CAVI was significantly decreased after 3-month period weight-reduction therapy through diet and exercise, so the determination of arterial stiffness by CAVI may be useful for evaluating and managing the CVD risks of MS patients [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD was significantly higher in the CAVI than in the brachial ankle PWV, which suggested that CAVI had increased performance over brachial ankle PWV in predicting the coronary artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of fatal and disabling diseases, some researchers fond that CAVI was significantly correlated with percentage plaque area in coronary arterial disease [21]. A lot of studies have showed that CAVI was a reliable evaluation index of vascular-related diseases. Our present study showed that with the increasing numbers of vascular-related diseases suffering, the level of CAVI was increasing (Table 1). And we also found significant correlation between PWV and CAVI in the entire group (CAVI &CF-PWV: r=0.382, $p<0.001$; CAVI &CR-PWV: r=0.225, $p=0.039$).

Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases.

Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with hypertension. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress[9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So Hcy might damage the endothelium through complex mechanisms resulting endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle cells through inflammation and so on. Endothelial dysfunction and proliferation of smooth muscle cells of arterial medium could lead to the increasing of arterial stiffness. Previous study had showed positive correlation between Hcy and CAVI in general population [12]. However, there was little research about the relationship between Hcy and CAVI in patients with one more kinds of vascular-related diseases. In the present study, we found that CAVI was positively correlated with Hcy even after adjustment of other parameters. The similar result was also found between PWV and Hcy. Hcy increases not only in hypertension patients but also in other vascular diseases. Hcy participates in the pathophysiological process of these diseases. Hyperhomocysteinemia was defined as the level of Hcy $\geqslant$15umol/L. Last, we compared the arterial stiffness between HHcy group and patients with Hcy <15umol/L. As shown in Table 2, the levels of PWV and CAVI were significantly higher in group with Hcy $\geqslant$15umol/L than that in group with Hcy <15umol/L. Finally, our research showed that Hcy was an independent influencing factor of CAVI. Our study suggested that CAVI was higher in HHcy patients, so treatment should be made to lower

homocysteine in HHcy patients in order to reduce arterial stiffness.

However, there were some limitations in the study: the small simple size, cases, and controls were not perfectly matched. Also some patients with hypertension and (or) CAD had oral medication such as amlodipine before coming to the hospital, this might affect our results to a certain extent. So thorough research should be investigated in future. However, our study suggested that CAVI was a useful evaluation index for arterial stiffness, and there was positively correlation between CAVI and Hcy.

In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Ethics approval: From the ethics committee of the Health Science Center, Peking University, China.

Contributorship

Dr. Jinbo Liu, Qi Wang, Dr. Hongwei Zhao, Dr. Hongyan Shi, Dr. Xiaolan Yu, Dr. Xiaobao Fu had contribution to the collection of information of patients. Pro. Wang designed the present study, and Pro. Wang and Dr. Liu were Equal contributors to the this paper.

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Data Sharing

No additional data available.

Competing Interests

None

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Figure legends

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	*p* values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
BMI, kg/m2	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL , mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL , mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density cholesterol. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	*p* values
Age. y	61.9±13.0	71.9±11.5	<0.01
BMI, kg/m^2	23.9±4.2	23.3±3.8	0.48
Hypertension，No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001

CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density cholesterol. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test.

Fig 1

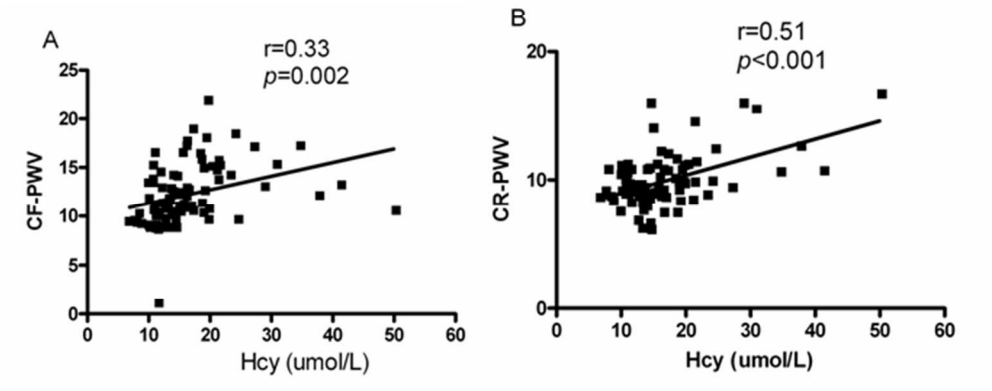

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.
58x27mm (300 x 300 DPI)

Fig 2

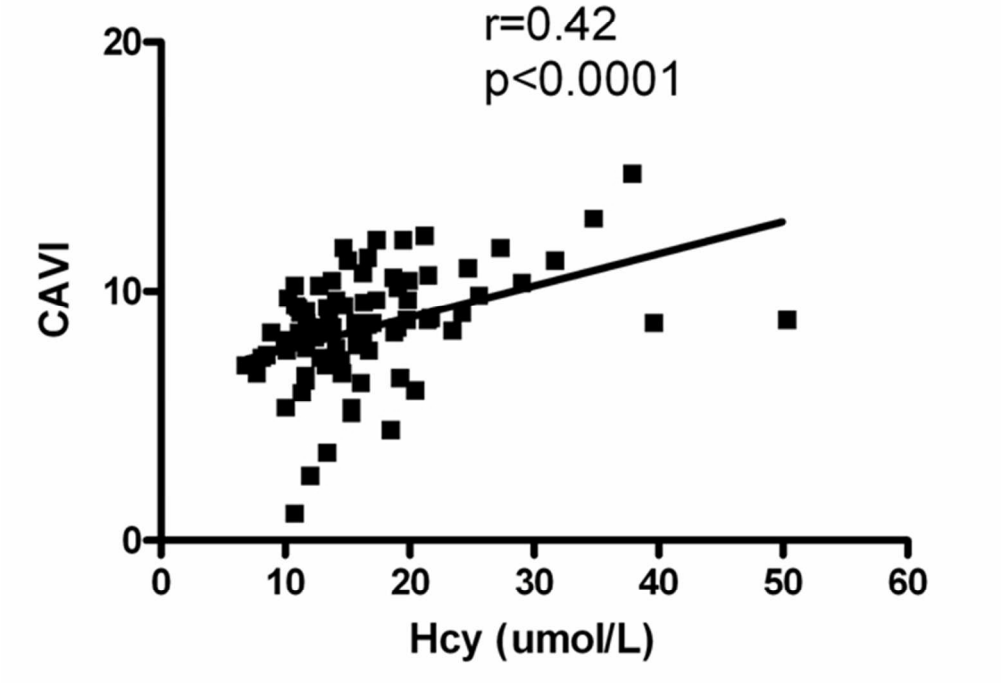

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.
57x43mm (300 x 300 DPI)

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Running title: CAVI and Homocysteine

Abstract

Objectives: Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

Design: Descriptive Research.

Participants: 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

Methods: CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

Results: There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group (r=0.33, p=0.002; r=0.51, p<0.001; r=0.42, p<0.001; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001; r=0.392, p=0.001; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy ⩾ 15umol/L group than in Hcy <15umol/L group (13.7±3.0 vs 10.8±2.5, p<0.001; 10.6±2.1 vs 9.2±1.6, p=0.001; 9.30±2.1 vs 7.79±2.1, p=0.001; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group (β=0.421, p=0.001;

β= -0.309, *p*=0.006; β=0.297, *p*=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, *p*=0.001; β= -0.331, *p*=0.009; β=0.288, *p*=0.022; respectively).

Conclusions: CAVI was positively correlated with homocysteine in vascular-related diseases.

Keywords: Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

Article summary

1. Article focus

To investigate the relationship between homocysteine (Hcy) and Cardio-ankle vascular index (CAVI) in vascular-related diseases.

2. Key messages

Homocysteine was positively correlated with CAVI in vascular-related diseases.

3. Strengths and limitations of this study

Strengths of this study: our present study firstly showed the relationship between Hcy and CAVI in vascular-related diseases.

Limitations of this study: the small sample size, cases, and controls were not perfectly matched.

1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclseosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

resulted from many factors such as endothelial dysfunction, smooth muscle cells proliferation, thickening of vascular wall. Kadota et al had showed positive correlation between Hcy and CAVI in general population [12]. However, the relationship between CAVI and Hcy in vascular-related diseases such as hypertension, coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still unknown, especially in patients with one more kinds of vascular-related diseases. In the present study, we investigated the possible link between CAVI and homocysteine in vascular-related diseases such as hypertension, CAD and ASO.

2. Materials and methods

2.1 Subjects

88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from vascular medicine department of Peking University Shougang Hospital from February 2012 to April 2012 were enrolled into our study. There were 57 patients with hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And there were 12 patients without hypertension, CAD and ASO but suffering one of these two diseases, acute upper respiratory tract infection or acute gastritis.

Hypertension was defined as known cases of hypertension or blood pressure measurement ⩾140/90mmHg in three occasions at rest. CAD or ASO was defined as the narrowing or blockage of coronary artery or lower extremity artery diagnosed by angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy⩾ 15umol/L[11].

Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ⩾15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

2.2 Pulse wave velocity measurement

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically. CFPWV and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26].And we chose the right PWV for analysis.

2.3 The assessment of CAVI

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was placed on the sternum, and cuffs were wrapped around both the arms and ankles.

After automatic measurements, obtained data were analyzed by software, and the value of CAVI was obtained automatically [16]. And we chose the right CAVI for analysis.

2.4 Laboratory measurements

Blood samples were drawn from an antecubital vein in the morning after overnight fasting and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting plasma glucose, homocysteine, hs-C reactive protein were also determined by colorimetric methods of related metabolic products using the same autoanalyzer at the central chemistry laboratory of the Peking University Shougang Hospital.

2.5 Statistical analysis

SPSS 13.0 was used as statistical software in the present study. The differences between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions were analyzed by χ^2-test. Correlation coefficient was done to find linear relation between different variables using Spearman correlation coefficient. Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of the dependent variables. Values were

shown as mean± SD unless stand otherwise. $p < 0.05$ (2-tailed) was considered statistically significant.

3. Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are shown in Table 1. Among these subjects, 33 patients had only one of these three vascular-related diseases, 34 patients had two of these three vascular-related diseases, 9 patients had all of these three diseases, and 12 subjects with none of vascular-related diseases. Our results showed that with the increasing numbers of suffered vascular-related diseases, the level of Hcy was increasing. Similar results were also found in the parameters of CF-PWV and CAVI. However, we found there was significant difference about age between these four groups.

Next, we divided subjects into two groups according to the level of Hcy. As shown in Table 2, the level of CAVI was significant higher in patients with Hcy≥ 15umol/L than in group with Hcy<15umol/L. The similar result was also found in another evaluation index of arterial stiffness-PWV. However, there was significant difference about age and sex between these two groups.

3.2 Pearson correlations between PWV , CAVI and Hcy in the entire study group

PWV is a golden evaluation of arterial stiffness of vascular diseases. There are some kinds of PWV according to different arteries, such as carotid-femoral pulse

wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CFPWV and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26]. As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B). In addition, our results showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001, respectively).

CAVI, a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure. And CAVI was not affected by immediate blood pressure. As shown in Fig2, there was significant positive correlation between CAVI and Hcy in all patients (r=0.42, p<0.0001). Also we found there was significant correlation between Hcy and CAVI in the vascular-related disease group (r=0.392, p=0.001). However, there was no significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present study (r=0.14, p=0.661; r=152, p=0.620; r=0.056, p=0.855; respectively).

As shown in Table 1 and Table 2, there was significant difference about age or sex between groups. So next, we investigated the possible relationship between CAVI, PWV and Hcy after adjusting the variable of age or sex. Our results showed that there was still significant correlation between CAVI and Hcy after adjustment for age in the entire study group(r=0.293, p=0.008). Also a positive correlation between PWV and Hcy was found after age adjusted in the entire study group (CFPWV vs Hcy, r=0.282, p=0.010; CRPWV vs Hcy, r=0.462, p<0.001; respectively). In addition, there was

significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex adjusted in the entire study group (r=0.26, p=0.022; r=0.38, p=0.001; r=0.27, p=0.014; respectively).

There were 12 patients without vascular-related diseases in the entire study group, so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients with vascular-related diseases. Our results showed that there was significant correlation between Hcy and CR-PWV, CAVI after adjustment for age in the vascular-related disease group (r=0.48, p<0.001; r=0.321, p=0.007; respectively), without significant correlation between Hcy and CFPWV (r=0.21, p=0.079). After adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI was found in the vascular-related disease group (r=0.40, p=0.001; r=0.298, p=0.013; respectively). However, there was no significant correlation between Hcy and CFPWV after age and sex adjusted (r=0.193, p=0.115).

3.3 Multiple linear regression analysis

Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of CAVI. Our results showed that Hcy, BMI, and age were independent influencing factors of CAVI in the entire study group (β=0.421, p=0.001; β= -0.309, p=0.006; β=0.297, p=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, p=0.001; β= -0.331, p=0.009; β=0.288, p=0.022; respectively).

4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy ⩾ 15umol/L, and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CRPWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CRPWV may predict the severity of the CAD [25,26]. Our present study showed that CFPWV and

CRPWV were higher in patients with vascular-related diseases than in subjects without vascular-related diseases (12.80±2.9 vs 9.17±2.6, p<0.001; 10.00±2.2 vs 9.35±0.9, p=0.08). However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter β, which is detected by carotid ultrasonic measurement [16].

CAVI is a new evaluation index of arterial stiffness independent of immediate blood pressure. Recent studies have showed the role of CAVI in the prediction of vascular events in vascular-related diseases such as metabolic syndrome (MS), diabetes, CAD, and so on. In MS patients, there was significant positive correlation between CAVI and waist circumference, and CAVI increased significantly with the number of metabolic syndrome components [17]. In another MS study, they found that CAVI was significantly decreased after 3-month period weight-reduction therapy through diet and exercise, so the determination of arterial stiffness by CAVI may be useful for evaluating and managing the cardiovascular diseases risks of MS patients [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD was significantly higher in the CAVI than in the brachial ankle PWV, which suggested that CAVI had increased performance over brachial ankle PWV in predicting the coronary artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of fatal and disabling diseases, some researchers fond that CAVI was significantly correlated with percentage plaque area in coronary arterial disease [21].

A lot of studies have showed that CAVI was a reliable evaluation index of vascular-related diseases. Our present study showed that with the increasing numbers of vascular-related diseases suffering, the level of CAVI was increasing (Table 1). CAVI was significantly higher in patients with vascular-related diseases than in control subjects (8.73±2.3 vs 7.51±0.9, p=0.002).And we also found significant correlation between PWV and CAVI in the entire group (CAVI &CF-PWV: r=0.382, p<0.001; CAVI &CR-PWV: r=0.225, p=0.039, respectively).

Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases. Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with hypertension. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So Hcy might damage the endothelium through complex mechanisms resulting endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle cells through inflammation and so on. Endothelial dysfunction and proliferation of smooth muscle cells of arterial medium could lead to the increasing of arterial stiffness. Previous study had showed positive correlation between Hcy and CAVI in general population [12]. However, there was little research about the relationship between Hcy and CAVI in patients with one more kinds of vascular-related diseases. In the present study, we found that CAVI was positively correlated with Hcy even after adjustment of other parameters, such as age and sex. The similar result was also

found between PWV and Hcy. Hcy increases not only in hypertension patients but also in other vascular diseases. Hcy participates in the pathophysiological process of these diseases. Hyperhomocysteinemia was defined as the level of Hcy ⩾15umol/L. Next, we compared the arterial stiffness between HHcy group and patients with Hcy <15umol/L. As shown in Table 2, the levels of PWV and CAVI were significantly higher in group with Hcy ⩾15umol/L than in group with Hcy <15umol/L. Finally, our research showed that Hcy was an independent influencing factor of CAVI in vascular-related diseases. Folate administration has been consistently shown to reduce plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al found that folic acid treatment could reduce frequency of restenosis after angioplasty in patients with markedly elevated homocysteine levels [23]. Another study showed that low-dose folic acid treatment improves vascular function in CAD patients [24]. Our study suggested that CAVI was higher in HHcy patients, so treatment should be made to lower homocysteine in HHcy patients in order to reduce arterial stiffness. And thorough clinical research should be investigated in future.

However, there were some limitations in the study: the small sample size, cases, and controls were not perfectly matched. Also some patients with hypertension and (or) CAD had oral medication such as amlodipine before coming to the hospital, this might affect our results to a certain extent. So thorough research should be investigated in future. However, our study suggested that CAVI was a useful evaluation index for arterial stiffness, and there was positively correlation between CAVI and Hcy.

In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure legends

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	*p* values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m2	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL , mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL , mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by χ^2-test.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	*p* values
Age. y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m^2	23.9±4.2	23.3±3.8	0.48
Hypertension，No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by χ^2 -test.

Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Running title: CAVI and Homocysteine

Abstract

Objectives: Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

Design: Descriptive Research.

Participants: 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

Methods: CAVI, carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) were measured by VS-1000 and Complior apparatus.

Results: There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group (r=0.33, p=0.002; r=0.51, p<0.001; r=0.42, p<0.001; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001; r=0.392, p=0.001; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy $\geqslant$ 15umol/L group than in Hcy <15umol/L group (13.7±3.0 vs 10.8±2.5, p<0.001; 10.6±2.1 vs 9.2±1.6, p=0.001; 9.30±2.1 vs 7.79±2.1, p=0.001; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group (β=0.421, p=0.001;

β= -0.309, *p*=0.006; β=0.297, *p*=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, *p*=0.001; β= -0.331, *p*=0.009; β=0.288, *p*=0.022; respectively).

Conclusions: CAVI was positively correlated with homocysteine in vascular-related diseases.

Keywords: Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

Article summary

1. Article focus

To investigate the relationship between homocysteine (Hcy) and Cardio-ankle vascular index (CAVI) in vascular-related diseases.

2. Key messages

Homocysteine was positively correlated with CAVI in vascular-related diseases.

3. Strengths and limitations of this study

Strengths of this study: our present study firstly showed the relationship between Hcy and CAVI in vascular-related diseases.

Limitations of this study: the small sample size, cases, and controls were not perfectly matched.

1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclseosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

resulted from many factors such as endothelial dysfunction, smooth muscle cells proliferation, thickening of vascular wall. Kadota et al had showed positive correlation between Hcy and CAVI in general population [12]. However, the relationship between CAVI and Hcy in vascular-related diseases such as hypertension, coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still unknown, especially in patients with one more kinds of vascular-related diseases. In the present study, we investigated the possible link between CAVI and homocysteine in vascular-related diseases such as hypertension, CAD and ASO.

2. Materials and methods

2.1 Subjects

88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from vascular medicine department of Peking University Shougang Hospital from February 2012 to April 2012 were enrolled into our study. There were 57 patients with hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And there were 12 patients without hypertension, CAD and ASO but suffering one of these two diseases, acute upper respiratory tract infection or acute gastritis.

Hypertension was defined as known cases of hypertension or blood pressure measurement ⩾140/90mmHg in three occasions at rest. CAD or ASO was defined as the narrowing or blockage of coronary artery or lower extremity artery diagnosed by angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy⩾ 15umol/L[11].

Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ⩾15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

2.2 Pulse wave velocity measurement

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CFPWV) and carotid-radial PWV (CRPWV) was obtained automatically. CFPWV and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26].And we chose the right PWV for analysis.

2.3 The assessment of CAVI

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was placed on the sternum, and cuffs were wrapped around both the arms and ankles.

After automatic measurements, obtained data were analyzed by software, and the value of CAVI was obtained automatically [16]. And we chose the right CAVI for analysis.

2.4 Laboratory measurements

Blood samples were drawn from an antecubital vein in the morning after overnight fasting and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting plasma glucose, homocysteine, hs-C reactive protein were also determined by colorimetric methods of related metabolic products using the same autoanalyzer at the central chemistry laboratory of the Peking University Shougang Hospital.

2.5 Statistical analysis

SPSS 13.0 was used as statistical software in the present study. The differences between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions were analyzed by χ^2-test. Correlation coefficient was done to find linear relation between different variables using Spearman correlation coefficient. Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of the dependent variables. Values were

shown as mean± SD unless stand otherwise. $p < 0.05$ (2-tailed) was considered statistically significant.

3. Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are shown in Table 1. Among these subjects, 33 patients had only one of these three vascular-related diseases, 34 patients had two of these three vascular-related diseases, 9 patients had all of these three diseases, and 12 subjects with none of vascular-related diseases. Our results showed that with the increasing numbers of suffered vascular-related diseases, the level of Hcy was increasing. Similar results were also found in the parameters of CF-PWV and CAVI. However, we found there was significant difference about age between these four groups.

Next, we divided subjects into two groups according to the level of Hcy. As shown in Table 2, the level of CAVI was significant higher in patients with Hcy≥ 15umol/L than in group with Hcy<15umol/L. The similar result was also found in another evaluation index of arterial stiffness-PWV. However, there was significant difference about age and sex between these two groups.

3.2 Pearson correlations between PWV , CAVI and Hcy in the entire study group

PWV is a golden evaluation of arterial stiffness of vascular diseases. There are some kinds of PWV according to different arteries, such as carotid-femoral pulse

wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CFPWV and CRPWV are both reliable index for arterial stiffness of vascular diseases [2,26]. As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B). In addition, our results showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001, respectively).

CAVI, a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure. And CAVI was not affected by immediate blood pressure. As shown in Fig2, there was significant positive correlation between CAVI and Hcy in all patients (r=0.42, p<0.0001). Also we found there was significant correlation between Hcy and CAVI in the vascular-related disease group (r=0.392, p=0.001). However, there was no significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present study (r=0.14, p=0.661; r=152, p=0.620; r=0.056, p=0.855; respectively).

As shown in Table 1 and Table 2, there was significant difference about age or sex between groups. So next, we investigated the possible relationship between CAVI, PWV and Hcy after adjusting the variable of age or sex. Our results showed that there was still significant correlation between CAVI and Hcy after adjustment for age in the entire study group(r=0.293, p=0.008). Also a positive correlation between PWV and Hcy was found after age adjusted in the entire study group (CFPWV vs Hcy, r=0.282, p=0.010; CRPWV vs Hcy, r=0.462, p<0.001; respectively). In addition, there was

significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex adjusted in the entire study group (r=0.26, p=0.022; r=0.38, p=0.001; r=0.27, p=0.014; respectively).

There were 12 patients without vascular-related diseases in the entire study group, so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients with vascular-related diseases. Our results showed that there was significant correlation between Hcy and CR-PWV, CAVI after adjustment for age in the vascular-related disease group (r=0.48, p<0.001; r=0.321, p=0.007; respectively), without significant correlation between Hcy and CFPWV (r=0.21, p=0.079). After adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI was found in the vascular-related disease group (r=0.40, p=0.001; r=0.298, p=0.013; respectively). However, there was no significant correlation between Hcy and CFPWV after age and sex adjusted (r=0.193, p=0.115).

3.3 Multiple linear regression analysis

Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of CAVI. Our results showed that Hcy, BMI, and age were independent influencing factors of CAVI in the entire study group (β=0.421, p=0.001; β= -0.309, p=0.006; β=0.297, p=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, p=0.001; β= -0.331, p=0.009; β=0.288, p=0.022; respectively).

4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy ⩾ 15umol/L, and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CRPWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CRPWV may predict the severity of the CAD [25,26]. Our present study showed that CFPWV and

CRPWV were higher in patients with vascular-related diseases than in subjects without vascular-related diseases (12.80±2.9 vs 9.17±2.6, p<0.001; 10.00±2.2 vs 9.35±0.9, p=0.08). However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter β, which is detected by carotid ultrasonic measurement [16].

CAVI is a new evaluation index of arterial stiffness independent of immediate blood pressure. Recent studies have showed the role of CAVI in the prediction of vascular events in vascular-related diseases such as metabolic syndrome (MS), diabetes, CAD, and so on. In MS patients, there was significant positive correlation between CAVI and waist circumference, and CAVI increased significantly with the number of metabolic syndrome components [17]. In another MS study, they found that CAVI was significantly decreased after 3-month period weight-reduction therapy through diet and exercise, so the determination of arterial stiffness by CAVI may be useful for evaluating and managing the cardiovascular diseases risks of MS patients [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD was significantly higher in the CAVI than in the brachial ankle PWV, which suggested that CAVI had increased performance over brachial ankle PWV in predicting the coronary artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of fatal and disabling diseases, some researchers fond that CAVI was significantly correlated with percentage plaque area in coronary arterial disease [21].

A lot of studies have showed that CAVI was a reliable evaluation index of vascular-related diseases. Our present study showed that with the increasing numbers of vascular-related diseases suffering, the level of CAVI was increasing (Table 1). CAVI was significantly higher in patients with vascular-related diseases than in control subjects (8.73±2.3 vs 7.51±0.9, p=0.002).And we also found significant correlation between PWV and CAVI in the entire group (CAVI &CF-PWV: r=0.382, p<0.001; CAVI &CR-PWV: r=0.225, p=0.039, respectively).

Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases. Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with hypertension. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So Hcy might damage the endothelium through complex mechanisms resulting endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle cells through inflammation and so on. Endothelial dysfunction and proliferation of smooth muscle cells of arterial medium could lead to the increasing of arterial stiffness. Previous study had showed positive correlation between Hcy and CAVI in general population [12]. However, there was little research about the relationship between Hcy and CAVI in patients with one more kinds of vascular-related diseases. In the present study, we found that CAVI was positively correlated with Hcy even after adjustment of other parameters, such as age and sex. The similar result was also

found between PWV and Hcy. Hcy increases not only in hypertension patients but also in other vascular diseases. Hcy participates in the pathophysiological process of these diseases. Hyperhomocysteinemia was defined as the level of Hcy ⩾15umol/L. Next, we compared the arterial stiffness between HHcy group and patients with Hcy <15umol/L. As shown in Table 2, the levels of PWV and CAVI were significantly higher in group with Hcy ⩾15umol/L than in group with Hcy <15umol/L. Finally, our research showed that Hcy was an independent influencing factor of CAVI in vascular-related diseases. Folate administration has been consistently shown to reduce plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al found that folic acid treatment could reduce frequency of restenosis after angioplasty in patients with markedly elevated homocysteine levels [23]. Another study showed that low-dose folic acid treatment improves vascular function in CAD patients [24]. Our study suggested that CAVI was higher in HHcy patients, so treatment should be made to lower homocysteine in HHcy patients in order to reduce arterial stiffness. And thorough clinical research should be investigated in future.

However, there were some limitations in the study: the small sample size, cases, and controls were not perfectly matched. Also some patients with hypertension and (or) CAD had oral medication such as amlodipine before coming to the hospital, this might affect our results to a certain extent. So thorough research should be investigated in future. However, our study suggested that CAVI was a useful evaluation index for arterial stiffness, and there was positively correlation between CAVI and Hcy.

In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure legends

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	*p* values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m2	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL , mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL , mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by χ^2-test.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	*p* values
Age. y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m^2	23.9±4.2	23.3±3.8	0.48
Hypertension，No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by χ^2 -test.

Fig 1

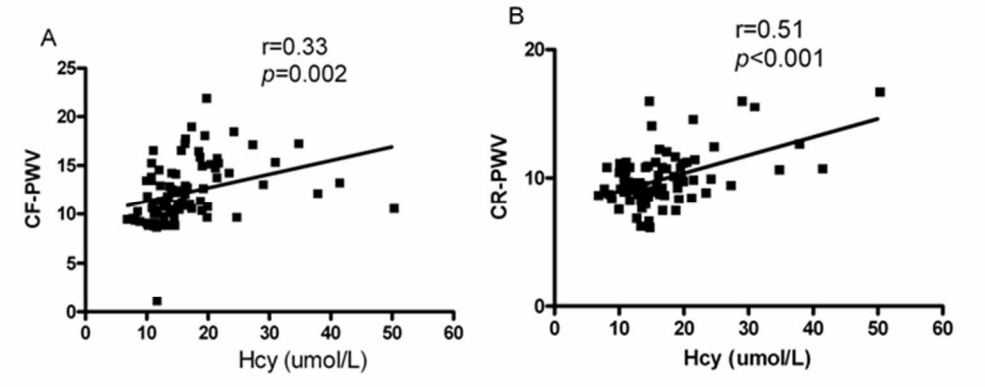

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.
58x27mm (300 x 300 DPI)

Fig 2

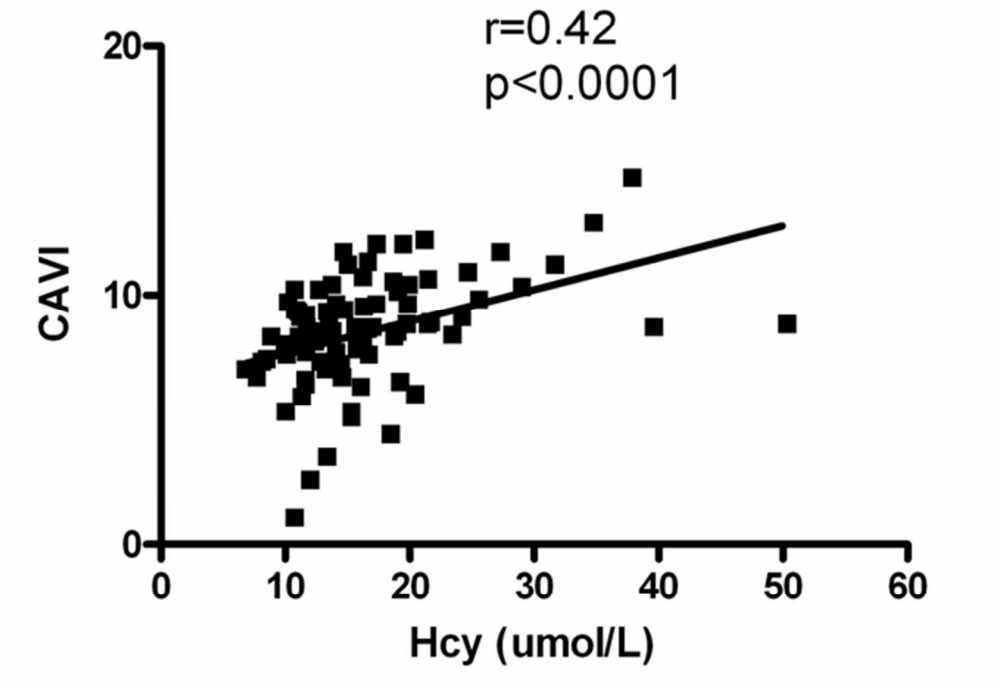

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.
57x43mm (300 x 300 DPI)

Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Running title: CAVI and Homocysteine

Abstract

Objectives: Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

Design: Descriptive Research.

Participants: 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

Methods: CAVI, carotid-femoral pulse wave velocity (CF-PWV) and carotid-radial pulse wave velocity (CR-PWV) were measured by VS-1000 and Complior apparatus.

Results: There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group (r=0.33, p=0.002; r=0.51, p<0.001; r=0.42, p<0.001; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001; r=0.392, p=0.001; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy ⩾ 15umol/L group than in Hcy <15umol/L group (13.7±3.0 vs 10.8±2.5, p<0.001; 10.6±2.1 vs 9.2±1.6, p=0.001; 9.30±2.1 vs 7.79±2.1, p=0.001; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group (β=0.421, p=0.001;

β= -0.309, *p*=0.006; β=0.297, *p*=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, *p*=0.001; β= -0.331, *p*=0.009; β=0.288, *p*=0.022; respectively).

Conclusions: CAVI was positively correlated with homocysteine in vascular-related diseases.

Keywords: Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

Article summary

1. Article focus

To investigate the relationship between homocysteine (Hcy) and Cardio-ankle vascular index (CAVI) in vascular-related diseases.

2. Key messages

Homocysteine was positively correlated with CAVI in vascular-related diseases.

3. Strengths and limitations of this study

Strengths of this study: our present study firstly showed the relationship between Hcy and CAVI in vascular-related diseases.

Limitations of this study: the small sample size, cases, and controls were not perfectly matched.

1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclseosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

resulted from many factors such as endothelial dysfunction, smooth muscle cells proliferation, thickening of vascular wall. Kadota et al had showed positive correlation between Hcy and CAVI in general population [12]. However, the relationship between CAVI and Hcy in vascular-related diseases such as hypertension, coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still unknown, especially in patients with one more kinds of vascular-related diseases. In the present study, we investigated the possible link between CAVI and homocysteine in vascular-related diseases such as hypertension, CAD and ASO.

2. Materials and methods

2.1 Subjects

88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from vascular medicine department of Peking University Shougang Hospital from February 2012 to April 2012 were enrolled into our study. There were 57 patients with hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And there were 12 patients without hypertension, CAD and ASO but suffering one of these two diseases, acute upper respiratory tract infection or acute gastritis.

Hypertension was defined as blood pressure measurement ⩾140/90mmHg in three occasions at rest or subjects with known cases of diagnosed hypertension before and taking antihypertensive drugs at present. CAD or ASO was defined as the narrowing or blockage of coronary artery or lower extremity artery diagnosed by angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy⩾

15umol/L[11].

Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ⩾15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

2.2 Pulse wave velocity measurement

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CF-PWV) and carotid-radial PWV (CR-PWV) was obtained automatically. CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular diseases [2,26].And we chose the right PWV for analysis.

2.3 The assessment of CAVI

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was

placed on the sternum, and cuffs were wrapped around both the arms and ankles. After automatic measurements, obtained data were analyzed by software, and the value of CAVI was obtained automatically [16]. And we chose the right CAVI for analysis.

2.4 Laboratory measurements

Blood samples were drawn from an antecubital vein in the morning after overnight fasting and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting plasma glucose, homocysteine, hs-C reactive protein were also determined by colorimetric methods of related metabolic products using the same autoanalyzer at the central chemistry laboratory of the Peking University Shougang Hospital.

2.5 Statistical analysis

SPSS 13.0 was used as statistical software in the present study. The differences between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions were analyzed by χ^2-test. Correlation coefficient was done to find linear relation between different variables using Spearman correlation coefficient. Multiple linear regressions were used to estimate the coefficients of the linear equation, involving

independent variables that affected the value of the dependent variables. Values were shown as mean± SD unless stand otherwise. $p < 0.05$ (2-tailed) was considered statistically significant.

3. Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are shown in Table 1. Among these subjects, 33 patients had only one of these three vascular-related diseases, 34 patients had two of these three vascular-related diseases, 9 patients had all of these three diseases, and 12 subjects with none of vascular-related diseases. Our results showed that with the increasing numbers of suffered vascular-related diseases, the level of Hcy was increasing. Similar results were also found in the parameters of CF-PWV and CAVI. However, we found there was significant difference about age between these four groups.

Next, we divided subjects into two groups according to the level of Hcy. As shown in Table 2, the level of CAVI was significant higher in patients with Hcy≥ 15umol/L than in group with Hcy<15umol/L. The similar result was also found in another evaluation index of arterial stiffness-PWV. However, there was significant difference about age and sex between these two groups.

3.2 Pearson correlations between PWV , CAVI and Hcy

PWV is a golden evaluation of arterial stiffness of vascular diseases. There are

some kinds of PWV according to different arteries, such as carotid-femoral pulse wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular diseases [2,26]. In the present study, patients with ASO had bilateral vascular lesions. And there was no significant difference between right side ankle-brachial index (ABI) and left side ABI in the entire study group (1.08±0.13 vs 1.07±0.15, p=0.612). In addition, there was no significant difference between right side ABI and left side ABI in subjects with ASO(1.01±0.18 vs 1.05±0.14, p=0.376). And we chose the right PWV and CAVI for analysis.

As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B). In addition, our results showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001, respectively).

CAVI, a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure. And CAVI was not affected by immediate blood pressure. As shown in Fig2, there was significant positive correlation between CAVI and Hcy in all patients (r=0.42, p<0.0001). Also we found there was significant correlation between Hcy and CAVI in the vascular-related disease group (r=0.392, p=0.001). However, there was no significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present study (r=0.14, p=0.661; r=152, p=0.620; r=0.056, p=0.855; respectively).

As shown in Table 1 and Table 2, there was significant difference about age or sex between groups. So next, we investigated the possible relationship between CAVI, PWV and Hcy after adjusting the variable of age or sex. Our results showed that there was still significant correlation between CAVI and Hcy after adjustment for age in the entire study group(r=0.293, p=0.008). Also a positive correlation between PWV and Hcy was found after age adjusted in the entire study group (CF-PWV vs Hcy, r=0.282, p=0.010; CR-PWV vs Hcy, r=0.462, p<0.001; respectively). In addition, there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex adjusted in the entire study group (r=0.26, p=0.022; r=0.38, p=0.001; r=0.27, p=0.014; respectively).

There were 12 patients without vascular-related diseases in the entire study group, so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients with vascular-related diseases. Our results showed that there was significant correlation between Hcy and CR-PWV, CAVI after adjustment for age in the vascular-related disease group (r=0.48, p<0.001; r=0.321, p=0.007; respectively), without significant correlation between Hcy and CF-PWV (r=0.21, p=0.079). After adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI was found in the vascular-related disease group (r=0.40, p=0.001; r=0.298, p=0.013; respectively). However, there was no significant correlation between Hcy and CF-PWV after age and sex adjusted (r=0.193, p=0.115).

3.3 Multiple linear regression analysis

Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of CAVI. Our results showed that Hcy, BMI, and age were independent influencing factors of CAVI in the entire study group (β=0.421, p=0.001; β= -0.309, p=0.006; β=0.297, p=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, p=0.001; β= -0.331, p=0.009; β=0.288, p=0.022; respectively).

4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy ⩾ 15umol/L, and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CR-PWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CR-PWV may predict the severity of the CAD [25,26]. Our present study showed that CF-PWV and

CR-PWV were higher in patients with vascular-related diseases than in subjects without vascular-related diseases (12.80±2.9 vs 9.17±2.6, $p<0.001$; 10.00±2.2 vs 9.34±0.92, $p=0.08$, respectively). However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter β, which is detected by carotid ultrasonic measurement [16].

CAVI is a new evaluation index of arterial stiffness independent of immediate blood pressure. Recent studies have showed the role of CAVI in the prediction of vascular events in vascular-related diseases such as metabolic syndrome (MS), diabetes, CAD, and so on. In MS patients, there was significant positive correlation between CAVI and waist circumference, and CAVI increased significantly with the number of metabolic syndrome components [17]. In another MS study, they found that CAVI was significantly decreased after 3-month period weight-reduction therapy through diet and exercise, so the determination of arterial stiffness by CAVI may be useful for evaluating and managing the cardiovascular diseases risks of MS patients [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD was significantly higher in the CAVI than in the brachial ankle PWV, which suggested that CAVI had increased performance over brachial ankle PWV in predicting the coronary artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of fatal and disabling diseases, some researchers fond that CAVI was significantly correlated with percentage plaque area in coronary arterial disease [21].

A lot of studies have showed that CAVI was a reliable evaluation index of vascular-related diseases. Our present study showed that with the increasing numbers of vascular-related diseases suffering, the level of CAVI was increasing (Table 1). CAVI was significantly higher in patients with vascular-related diseases than in control subjects (8.73±2.3 vs 7.51±0.9, p=0.002).And we also found significant correlation between PWV and CAVI in the entire group (CAVI &CF-PWV: r=0.382, p<0.001; CAVI &CR-PWV: r=0.225, p=0.039, respectively).

Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases. Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with hypertension. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So Hcy might damage the endothelium through complex mechanisms resulting endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle cells through inflammation and so on. Endothelial dysfunction and proliferation of smooth muscle cells of arterial medium could lead to the increasing of arterial stiffness. Previous study had showed positive correlation between Hcy and CAVI in general population [12]. However, there was little research about the relationship between Hcy and CAVI in patients with one more kinds of vascular-related diseases. In the present study, we found that CAVI was positively correlated with Hcy even after adjustment of other parameters, such as age and sex. The similar result was also

found between PWV and Hcy. Hcy increases not only in hypertension patients but also in other vascular diseases. Hcy participates in the pathophysiological process of these diseases. Hyperhomocysteinemia was defined as the level of Hcy ⩾15umol/L. Next, we compared the arterial stiffness between HHcy group and patients with Hcy <15umol/L. As shown in Table 2, the levels of PWV and CAVI were significantly higher in group with Hcy ⩾15umol/L than in group with Hcy <15umol/L. Finally, our research showed that Hcy was an independent influencing factor of CAVI in vascular-related diseases. Folate administration has been consistently shown to reduce plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al found that folic acid treatment could reduce frequency of restenosis after angioplasty in patients with markedly elevated homocysteine levels [23]. Another study showed that low-dose folic acid treatment improves vascular function in CAD patients [24]. Our study suggested that CAVI was higher in HHcy patients, so treatment should be made to lower homocysteine in HHcy patients in order to reduce arterial stiffness. And thorough clinical research should be investigated in future.

However, there were some limitations in the study: the small sample size, cases, and controls were not perfectly matched. Also some patients with hypertension and (or) CAD had oral medication such as amlodipine before coming to the hospital, this might affect our results to a certain extent. So thorough research should be investigated in future. However, our study suggested that CAVI was a useful evaluation index for arterial stiffness, and there was positively correlation between CAVI and Hcy.

In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Ethics approval: From the ethics committee of the Health Science Center, Peking University, China.

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Figure legends

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	*p* values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m2	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL , mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL , mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by χ^2-test.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	*p* values
Age. y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m^2	23.9±4.2	23.3±3.8	0.48
Hypertension，No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by χ^2 -test.

Descriptive study of possible link between cardio-ankle vascular index and homocysteine in vascular-related diseases

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Running title: CAVI and Homocysteine

Abstract

Objectives: Cardio-ankle vascular index (CAVI) is a new index of arterial stiffness independent of immediate blood pressure. Homocysteine (Hcy) is an independent risk factor for vascular diseases. The aim of this study was to investigate the relationship between Hcy and CAVI in vascular-related diseases.

Design: Descriptive Research.

Participants: 88 patients (M/F 46/42) with or without hypertension, coronary artery disease or arteriosclerosis obliterans were enrolled into our study. They were divided into two groups according to the level of Hcy.

Methods: CAVI, carotid-femoral pulse wave velocity (CF-PWV) and carotid-radial pulse wave velocity (CR-PWV) were measured by VS-1000 and Complior apparatus.

Results: There was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the entire group (r=0.33, p=0.002; r=0.51, p<0.001; r=0.42, p<0.001; respectively). And there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001; r=0.392, p=0.001; respectively). The level of Hcy was significantly higher in patients with one or more vascular diseases than in patients without vascular diseases. The levels of CF-PWV, CR-PWV, and CAVI were significantly higher in Hcy ⩾ 15umol/L group than in Hcy <15umol/L group (13.7±3.0 vs 10.8±2.5, p<0.001; 10.6±2.1 vs 9.2±1.6, p=0.001; 9.30±2.1 vs 7.79±2.1, p=0.001; respectively). Multiple linear regression showed that Hcy, body mass index (BMI), and age were independent associating factors of CAVI in the entire study group (β=0.421, p=0.001;

β= -0.309, *p*=0.006; β=0.297, *p*=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, *p*=0.001; β= -0.331, *p*=0.009; β=0.288, *p*=0.022; respectively).

Conclusions: CAVI was positively correlated with homocysteine in vascular-related diseases.

Keywords: Cardio-ankle vascular index; Homocysteine; Vascular-related diseases

Article summary

1. Article focus

To investigate the relationship between homocysteine (Hcy) and Cardio-ankle vascular index (CAVI) in vascular-related diseases.

2. Key messages

Homocysteine was positively correlated with CAVI in vascular-related diseases.

3. Strengths and limitations of this study

Strengths of this study: our present study firstly showed the relationship between Hcy and CAVI in vascular-related diseases.

Limitations of this study: the small sample size, cases, and controls were not perfectly matched.

1. Introduction

Arterial stiffness is a strong predictor of future cardiovascular events and all-cause mortality. And it is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall [1]. Arterial stiffness can be measured by pulse wave velocity (PWV), which is considered as the gold standard method suggested by European Society of Hypertension/European Society of Cardiology guidelines [2]. And our previous studies also showed that PWV was positively correlated with pulse pressure and it was increased in hypertension patients with left ventricular hypertrophy [3, 4]. However, PWV itself is essentially dependent on blood pressure especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure [5]. Recent studies have showed that CAVI was a reliable index of arterial stiffness in many vascular-related diseases [6,7].

Homocysteine has been considered as an independent risk factor for atherosclseosis [8]. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress. Recent studies showed that homocysteine caused endothelial dysfunction through inhibiting the reactions between endothelial nitric oxide synthase (eNOS) and tetrahydrobiopterin (BH4) [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. Increased arterial stiffness

resulted from many factors such as endothelial dysfunction, smooth muscle cells proliferation, thickening of vascular wall. Kadota et al had showed positive correlation between Hcy and CAVI in general population [12]. However, the relationship between CAVI and Hcy in vascular-related diseases such as hypertension, coronary artery disease (CAD), and arteriosclerosis obliterans (ASO) was still unknown, especially in patients with one more kinds of vascular-related diseases. In the present study, we investigated the possible link between CAVI and homocysteine in vascular-related diseases such as hypertension, CAD and ASO.

2. Materials and methods

2.1 Subjects

88 patients (M/F: 46/42) with or without hypertension, CAD or ASO from vascular medicine department of Peking University Shougang Hospital from February 2012 to April 2012 were enrolled into our study. There were 57 patients with hypertension, 43 with CAD, and 25 patients with ASO in the whole study group. And there were 12 patients without hypertension, CAD and ASO but suffering one of these two diseases, acute upper respiratory tract infection or acute gastritis.

Hypertension was defined as blood pressure measurement ⩾140/90mmHg in three occasions at rest or subjects with known cases of diagnosed hypertension before and taking antihypertensive drugs at present. CAD or ASO was defined as the narrowing or blockage of coronary artery or lower extremity artery diagnosed by angiography. Hyperhomocysteinemia was defined as the level of plasma Hcy⩾

15umol/L[11].

Enrolled patients were divided into four groups according to numbers of suffering vascular-related diseases. Also they were divided into two groups according to level of Hcy (Hcy <15umol/L group, N=43, and Hcy ⩾15umol/L group, N=45). All participants gave their written informed consent. This study was approved by the ethics committee of the Health Science Center, Peking University.

2.2 Pulse wave velocity measurement

Arterial stiffness was evaluated by measuring automatic PWV using the Complior apparatus. The basic principle of PWV assessment is that pressure pulse generated by ventricular ejection is propagated along the arterial system at a speed determined by elasticity of the arterial wall. Knowing the distance and pulse transit time, the velocity can be calculated. Patients were placed in recumbent position and, after a 10-minute rest, underwent PWV measurement and carotid-femoral PWV (CF-PWV) and carotid-radial PWV (CR-PWV) was obtained automatically. CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular diseases [2,26].And we chose the right PWV for analysis.

2.3 The assessment of CAVI

CAVI was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) with the participant resting in a supine position. ECG electrodes were placed on both wrists, a microphone for detecting heart sounds was

placed on the sternum, and cuffs were wrapped around both the arms and ankles. After automatic measurements, obtained data were analyzed by software, and the value of CAVI was obtained automatically [16]. And we chose the right CAVI for analysis.

2.4 Laboratory measurements

Blood samples were drawn from an antecubital vein in the morning after overnight fasting and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were analyzed by colorimetric enzymatic assays with the use of an autoanalyzer (HITACHI-7170, Hitachi, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated. Fasting plasma glucose, homocysteine, hs-C reactive protein were also determined by colorimetric methods of related metabolic products using the same autoanalyzer at the central chemistry laboratory of the Peking University Shougang Hospital.

2.5 Statistical analysis

SPSS 13.0 was used as statistical software in the present study. The differences between groups were analyzed by Student' *t*-test and one-way ANOVA. Proportions were analyzed by χ^2 -test. Correlation coefficient was done to find linear relation between different variables using Spearman correlation coefficient. Multiple linear regressions were used to estimate the coefficients of the linear equation, involving

independent variables that affected the value of the dependent variables. Values were shown as mean± SD unless stand otherwise. $p < 0.05$ (2-tailed) was considered statistically significant.

3. Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are shown in Table 1. Among these subjects, 33 patients had only one of these three vascular-related diseases, 34 patients had two of these three vascular-related diseases, 9 patients had all of these three diseases, and 12 subjects with none of vascular-related diseases. Our results showed that with the increasing numbers of suffered vascular-related diseases, the level of Hcy was increasing. Similar results were also found in the parameters of CF-PWV and CAVI. However, we found there was significant difference about age between these four groups.

Next, we divided subjects into two groups according to the level of Hcy. As shown in Table 2, the level of CAVI was significant higher in patients with Hcy≥ 15umol/L than in group with Hcy<15umol/L. The similar result was also found in another evaluation index of arterial stiffness-PWV. However, there was significant difference about age and sex between these two groups.

3.2 Pearson correlations between PWV , CAVI and Hcy

PWV is a golden evaluation of arterial stiffness of vascular diseases. There are

some kinds of PWV according to different arteries, such as carotid-femoral pulse wave velocity (CF-PWV), and carotid-radial pulse wave velocity (CR-PWV). CF-PWV and CR-PWV are both reliable index for arterial stiffness of vascular diseases [2,26]. In the present study, patients with ASO had bilateral vascular lesions. And there was no significant difference between right side ankle-brachial index (ABI) and left side ABI in the entire study group (1.08±0.13 vs 1.07±0.15, p=0.612). In addition, there was no significant difference between right side ABI and left side ABI in subjects with ASO(1.01±0.18 vs 1.05±0.14, p=0.376). And we chose the right PWV and CAVI for analysis.

As shown in Fig 1. CF-PWV was positively correlated with Hcy in entire group (r=0.33, p=0.002, Fig 1A). There was also significant positive correlation between CR-PWV and Hcy in all patients (r=0.51, p<0.001, Fig 1B). In addition, our results showed that there was significant correlation between Hcy and CF-PWV, CR-PWV in the vascular-related disease group (r=0.23, p=0.048; r=0.51, p<0.001, respectively).

CAVI, a new index of arterial stiffness independent of blood pressure, is recently developed by measuring of PWV and blood pressure. And CAVI was not affected by immediate blood pressure. As shown in Fig2, there was significant positive correlation between CAVI and Hcy in all patients (r=0.42, p<0.0001). Also we found there was significant correlation between Hcy and CAVI in the vascular-related disease group (r=0.392, p=0.001). However, there was no significant correlation between Hcy and CF-PWV, CR-PWV, CAVI in patients without vascular-related diseases in the present study (r=0.14, p=0.661; r=152, p=0.620; r=0.056, p=0.855; respectively).

As shown in Table 1 and Table 2, there was significant difference about age or sex between groups. So next, we investigated the possible relationship between CAVI, PWV and Hcy after adjusting the variable of age or sex. Our results showed that there was still significant correlation between CAVI and Hcy after adjustment for age in the entire study group(r=0.293, p=0.008). Also a positive correlation between PWV and Hcy was found after age adjusted in the entire study group (CF-PWV vs Hcy, r=0.282, p=0.010; CR-PWV vs Hcy, r=0.462, p<0.001; respectively). In addition, there was significant correlation between Hcy and CF-PWV, CR-PWV, CAVI after age and sex adjusted in the entire study group (r=0.26, p=0.022; r=0.38, p=0.001; r=0.27, p=0.014; respectively).

There were 12 patients without vascular-related diseases in the entire study group, so in next step, we analyzed relationship between PWV, CAVI and Hcy in patients with vascular-related diseases. Our results showed that there was significant correlation between Hcy and CR-PWV, CAVI after adjustment for age in the vascular-related disease group (r=0.48, p<0.001; r=0.321, p=0.007; respectively), without significant correlation between Hcy and CF-PWV (r=0.21, p=0.079). After adjustment for age and sex, significant correlation between Hcy and CR-PWV, CAVI was found in the vascular-related disease group (r=0.40, p=0.001; r=0.298, p=0.013; respectively). However, there was no significant correlation between Hcy and CF-PWV after age and sex adjusted (r=0.193, p=0.115).

3.3 Multiple linear regression analysis

Multiple linear regressions were used to estimate the coefficients of the linear equation, involving independent variables that affected the value of CAVI. Our results showed that Hcy, BMI, and age were independent influencing factors of CAVI in the entire study group (β=0.421, *p*=0.001; β= -0.309, *p*=0.006; β=0.297, *p*=0.012; respectively). And Hcy, BMI, and age were independent influencing factors of CAVI in vascular-related disease group (β=0.434, *p*=0.001; β= -0.331, *p*=0.009; β=0.288, *p*=0.022; respectively).

4. Discussion

In the present study, we found that there was positive correlation between Hcy and CAVI in vascular-related diseases. CAVI and PWV were higher in patients with Hcy ⩾ 15umol/L, and Hcy was an independent influencing factor of CAVI in vascular-related diseases.

An increase in arterial stiffness is not only a pathological status of hypertension, diabetes and coronary artery disease but also a strong predictor for the cardiovascular morbidity and mortality caused by these diseases. With the increasing of arterial stiffness, the incidence of hypertension, coronary heart disease increases. And arterial stiffness can be measured by pulse wave velocity (PWV) suggested by European Society of Hypertension/European Society of Cardiology guidelines. A lot of studies have showed the effect of PWV in the evaluation of arterial stiffness of vascular diseases. Aortic PWV was increasing in patients with diabetes mellitus or end-stage renal disease, indicating a higher arterial stiffness compared with health persons [13]. A research of 710 hypertension patients revealed that aortic PWV is a useful marker and predictor of cardiovascular risk in these subjects [14]. Recently, in a prospective study of general Danish population, the investigator found that aortic PWV was a useful predictor for cardiovascular outcomes above and beyond traditional cardiovascular risk factors such as 24-hour mean blood pressure [15]. Recent study showed that CR-PWV was a discriminator of intrinsic wall alterations during evaluation of endothelial function by flow-mediated dilatation and CR-PWV may predict the severity of the CAD [25,26]. Our present study showed that CF-PWV and

CR-PWV were higher in patients with vascular-related diseases than in subjects without vascular-related diseases (12.80±2.9 vs 9.17±2.6, p<0.001; 10.00±2.2 vs 9.34±0.92, p=0.08, respectively). However, PWV itself is essentially dependent on blood pressure, especially immediate blood pressure. Cardio-ankle vascular index (CAVI), a new index of arterial stiffness, is derived from stiffness parameter β, which is detected by carotid ultrasonic measurement [16].

CAVI is a new evaluation index of arterial stiffness independent of immediate blood pressure. Recent studies have showed the role of CAVI in the prediction of vascular events in vascular-related diseases such as metabolic syndrome (MS), diabetes, CAD, and so on. In MS patients, there was significant positive correlation between CAVI and waist circumference, and CAVI increased significantly with the number of metabolic syndrome components [17]. In another MS study, they found that CAVI was significantly decreased after 3-month period weight-reduction therapy through diet and exercise, so the determination of arterial stiffness by CAVI may be useful for evaluating and managing the cardiovascular diseases risks of MS patients [18]. In a comparative study, researchers showed that the diagnostic accuracy of CAD was significantly higher in the CAVI than in the brachial ankle PWV, which suggested that CAVI had increased performance over brachial ankle PWV in predicting the coronary artery disease [16, 17]. Namekata showed that the CAVI method was a useful tool to screen persons with moderate to advanced levels of arteriosclerosis. CAD is one of fatal and disabling diseases, some researchers fond that CAVI was significantly correlated with percentage plaque area in coronary arterial disease [21].

A lot of studies have showed that CAVI was a reliable evaluation index of vascular-related diseases. Our present study showed that with the increasing numbers of vascular-related diseases suffering, the level of CAVI was increasing (Table 1). CAVI was significantly higher in patients with vascular-related diseases than in control subjects (8.73±2.3 vs 7.51±0.9, p=0.002).And we also found significant correlation between PWV and CAVI in the entire group (CAVI &CF-PWV: r=0.382, p<0.001; CAVI &CR-PWV: r=0.225, p=0.039, respectively).

Homocysteine (Hcy) is an independent risk factor of cardiovascular diseases. Hyperhomocysteinemia (HHcy) has been found in more than one half of patients with hypertension. The possible mechanism of this process includes endothelial cell damage, vascular endothelial dysfunction and enhanced oxidative stress [9,10]. Our previous study showed that chronic hyperhomocysteinemia contributed to coronary artery disease by inhibiting dysfunction of the coronary artery endothelium [11]. So Hcy might damage the endothelium through complex mechanisms resulting endothelial dysfunction. Also Hcy could promote the proliferation of smooth muscle cells through inflammation and so on. Endothelial dysfunction and proliferation of smooth muscle cells of arterial medium could lead to the increasing of arterial stiffness. Previous study had showed positive correlation between Hcy and CAVI in general population [12]. However, there was little research about the relationship between Hcy and CAVI in patients with one more kinds of vascular-related diseases. In the present study, we found that CAVI was positively correlated with Hcy even after adjustment of other parameters, such as age and sex. The similar result was also

found between PWV and Hcy. Hcy increases not only in hypertension patients but also in other vascular diseases. Hcy participates in the pathophysiological process of these diseases. Hyperhomocysteinemia was defined as the level of Hcy ⩾15umol/L. Next, we compared the arterial stiffness between HHcy group and patients with Hcy <15umol/L. As shown in Table 2, the levels of PWV and CAVI were significantly higher in group with Hcy ⩾15umol/L than in group with Hcy <15umol/L. Finally, our research showed that Hcy was an independent influencing factor of CAVI in vascular-related diseases. Folate administration has been consistently shown to reduce plasma Hcy even in healthy individuals without elevated Hcy levels [22]. Lange et al found that folic acid treatment could reduce frequency of restenosis after angioplasty in patients with markedly elevated homocysteine levels [23]. Another study showed that low-dose folic acid treatment improves vascular function in CAD patients [24]. Our study suggested that CAVI was higher in HHcy patients, so treatment should be made to lower homocysteine in HHcy patients in order to reduce arterial stiffness. And thorough clinical research should be investigated in future.

However, there were some limitations in the study: the small sample size, cases, and controls were not perfectly matched. Also some patients with hypertension and (or) CAD had oral medication such as amlodipine before coming to the hospital, this might affect our results to a certain extent. So thorough research should be investigated in future. However, our study suggested that CAVI was a useful evaluation index for arterial stiffness, and there was positively correlation between CAVI and Hcy.

In conclusion, our study showed that CAVI and Hcy are closely associated among vascular-related diseases. More studies should be made to investigate the role of Hcy in the development of arterial stiffness.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure legends

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.

Table 1 Clinical characteristics in different groups according to the numbers of vascular-related diseases.

Characteristics	Group 0 N=12	Group 1 N=33	Group 2 N=34	Group 3 N=9	*p* values
Age, y	54.4±9.5	63.5±13.1	73.2±10.0	76.1±10.3	<0.01
Sex(Male/Female)	4/8	20/13	16/18	6/3	0.283
BMI, kg/m2	22.4±3.3	23.9±3.9	23.3±4.4	25.7±2.6	0.32
LDL , mmol/L	1.82±0.4	1.83±0.6	1.71±0.4	1.84±0.2	0.759
HDL , mmol/L	1.90±2.9	0.95±0.2	1.01±0.3	1.00±0.25	0.078
Hs-CRP, mg/L	4.73±10.8	8.14±15.3	10.79±15.3	17.2±35.8	0.496
HbA1c %	5.78±0.3	5.92±0.5	5.86±0.3	5.98±1.7	0.917
Hcy (umol/L)	11.0±2.8	19.0±9.1	16.7±6.4	21.1±8.5	0.006
Urinary Microalbumin	3.66±4.4	16.69±39.0	7.50±10.9	13.90±22.4	0.522
Heartrate	75.5±9.6	72.4±13.3	74.7±13.4	69.4±8.5	0.621
ABI	1.10±0.13	1.10±0.09	1.06±0.14	1.02±0.21	0.284
SBP, mmHg	126.0±13.8	138.6±19.8	145.3±23.1	154.3±23.4	0.012
DBP, mmHg	79.5±7.2	81.7±9.5	82.7±9.7	86.1±9.9	0.479
CF-PWV	9.17±2.6	12.33±3.0	13.03±2.8	13.85±1.86	<0.001
CR-PWV	9.34±0.92	10.12±1.98	9.8±2.21	10.29±2.96	0.648
CAVI	7.51±0.9	8.23±2.4	9.09±2.3	9.34±2.0	0.08

CAD: coronary artery disease. ASO: arteriosclerosis obliterans. Group 0: without diseases of hypertension, CAD, ASO; group 1: with one of diseases of hypertension, CAD, ASO; group 2: with two of diseases of hypertension, CAD, ASO; group 3: with all diseases of hypertension, CAD, ASO; BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The differences between groups were analyzed by one-way ANOVA. Proportions were analyzed by χ^2-test.

Table 2 Clinical characteristics in patients with Hcy<15umol/ and Hcy≥15umol/.

Characteristics	Hcy<15umol/L (n=43)	Hcy≥15umol/L (n=45)	*p* values
Age. y	61.9±13.0	71.9±11.5	<0.01
Sex(Male/Female)	14/29	32/13	<0.001
BMI, kg/m^2	23.9±4.2	23.3±3.8	0.48
Hypertension，No(%)	26(60.5)	31(68.9)	0.41
CAD, No(%)	18(41.2)	25(55.6)	0.2
ASO, No(%)	8(18.6)	17(37.8)	0.03
LDL , mmol/L	1.72±0.4	1.84±0.6	0.31
HDL , mmol/L	1.19±1.5	1.04±0.3	0.53
Hs-CRP, mg/L	7.70±0.17	11.34±19.5	0.36
HbA1c %	5.80±0.3	5.96±0.8	0.34
Hcy umol/L	11.89±2.0	22.19±8.0	<0.001
Urinary Microalbumin	4.35±4.3	17.58±35.0	0.057
Heartrate	72.0±9.7	74.9±14.5	0.28
ABI	1.09±0.12	1.06±0.13	0.23
SBP, mmHg	134.1±18.2	147.0±23.2	0.005
DBP, mmHg	81.2±8.4	83.1±10.0	0.36
CF-PWV	10.8±2.5	13.7±3.0	<0.001
CR-PWV	9.2±1.6	10.6±2.1	0.001
CAVI	7.79±2.1	9.30±2.1	0.001

Results were shown as mean ±SD unless stand otherwise. CAD: coronary artery disease. ASO: arteriosclerosis obliterans. BMI: body mass index. LDL: low-density lipoprotein. HDL: high-density lipoprotein. CRP: C-reactive protein. Hcy: homocysteine. ABI: ankle-brachial index. SBP: systolic blood pressure; DBP: diastolic blood pressure. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity. CAVI: cardio-ankle vascular index. The difference between groups were analyzed by Student' *t*-test. Proportions were analyzed by χ^2 -test.

Fig 1

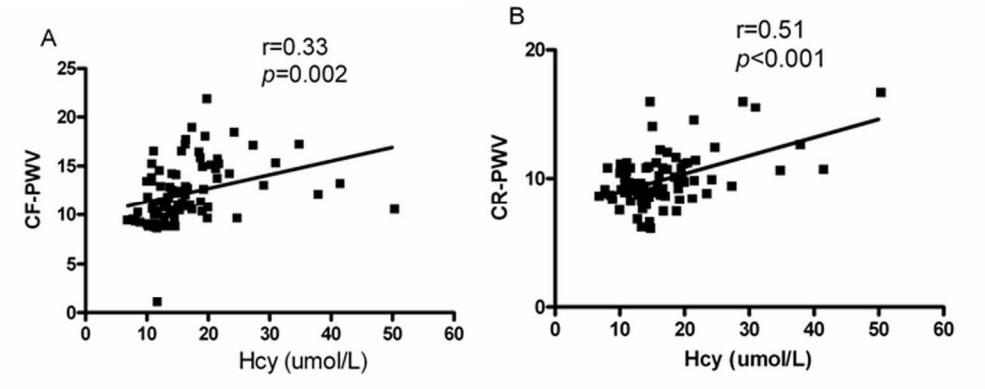

Figure 1 Relationship between CF-PWV and Hcy (Fig 1A) , CR-PWV and Hcy (Fig 1B) in the entire study group. Hcy: homocysteine. CF-PWV: carotid-femoral pulse wave velocity. CR-PWV: carotid-radial pulse wave velocity.
189x90mm (300 x 300 DPI)

Fig 2

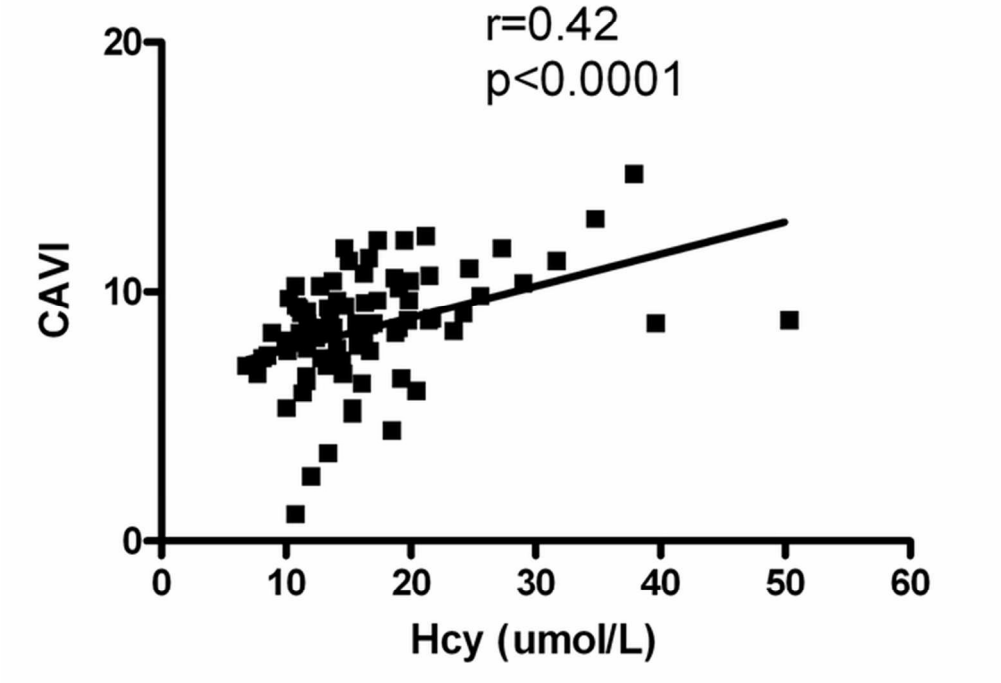

Figure 2 Relationship between CAVI and Hcy in the entire study group. Hcy: homocysteine. CAVI: cardio-ankle vascular index.
118x90mm (300 x 300 DPI)