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Original Article

Impact of hyperhomocysteinemia on coronary microcirculation in patients referred for elective coronary angiography

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Abstract: Hyperhomocysteinemia has been identified as an independent risk factor for cardiovascular diseases. The aim of this study was to investigate the impact of hyperhomocysteinemia on coronary microcirculation in patients who have been referred for elective coronary angiography. This study included 79 consecutive participants who were referred for elective coronary angiography due to suspected myocardial ischemia at The Second Hospital of Wenzhou Medical University from May 2014 to December 2015. The index of microvascular resistance (IMR), coronary flow reserve (CFR), and fractional flow reserve (FFR) were measured in coronary arteries with intermediate stenoses. Blood samples were collected to determine homocysteine and high-sensitivity C-reactive protein (hs-CRP) levels. All participants were categorized into two groups according to the plasma homocysteine cutoff value of 10 μmol/L. Participants in the high-level homocysteine group had higher IMR and lower FFR during hyperemia compared to participants in the normal-level homocysteine group. Linear regression analysis revealed that homocysteine levels were positively correlated with IMR ($r = 0.550$, $P < 0.01$) and negatively correlated with FFR ($r = -0.173$, $P < 0.01$). Serum hs-CRP levels were positively correlated with IMR ($r = 0.699$, $P < 0.01$). Multiple regression analysis indicated that homocysteine was an independent predictor of IMR (beta coefficient, 0.50; 95% confidence interval, 0.16-0.85, $P = 0.005$). Patients with hyperhomocysteinemia were characterized by elevated hs-CRP serum levels, higher IMR, and lower FFR. Hyperhomocysteinemia may cause coronary microcirculatory dysfunction partly by promoting inflammatory responses involving hs-CRP-related mechanisms.

Keywords: Homocysteine, coronary microcirculation, high-sensitivity C-reactive protein

Introduction

Despite advances in cardiology, coronary artery disease (CAD) remains a significant health and social problem worldwide. Homocysteine, produced during methionine catabolism, is a sulfur-containing amino acid. Hyperhomocysteinemia is considered an independent modifiable risk factor for CAD [1-3]. However, the contribution of hyperhomocysteinemia to CAD is still under debate.

The coronary microcirculation plays a key role in maintaining normal cardiac physiology. Hyperhomocysteinemia may reduce coronary blood flow and coronary flow reserve (CFR) through microvasculopathic effects on coronary microcirculation [4]. Plasma homocysteine was associated with ischemia without organic stenosis in patients with slow coronary flow [5, 6]. The index of microcirculatory resistance (IMR) is a well validated invasive technique used to determine coronary microcirculation. IMR is highly reproducible and can be measured independently of hemodynamic changes [7]. IMR predicts myocardial infarct size, myocardial viability, myocardial salvage, and long-term mortality [8].

Elevated inflammatory markers have been implicated in the pathogenesis of CAD. C-reactive protein is a well-known marker of systemic inflammation. High-sensitivity C-reactive protein (hs-CRP) is a biomarker that can accurately detect low-grade inflammation. hs-CRP and homocysteine levels are commonly used as inflammatory markers [9]. Both factors contribute to the earlier stages of CAD and are associated

with family history of acute coronary syndrome [10]. hs-CRP and homocysteine have strong synergy in predicting CAD development [11].

This study was designed to investigate the impact of elevated homocysteine levels on coronary microcirculation in patients who were referred for elective coronary angiography due to suspected myocardial ischemia. This study was also designed to analyze the relationship between hyperhomocysteinemia or hs-CRP and parameters of coronary microcirculatory dysfunction.

Materials and methods

Study population

This study included 79 consecutive candidates (42 males and 37 females, mean age 56±10 years) who were referred for elective coronary angiography due to suspected myocardial ischemia at The Second Hospital of Wenzhou Medical University from May 2014 to December 2015. Suspected myocardial ischemia included chest discomfort, such as chest pain and dyspnea, and myocardial ischemic findings during noninvasive examination. The Ethics Committee of The Second Hospital of Wenzhou Medical University approved this study protocol. Written informed consent was obtained from every participant.

Using a plasma homocysteine cutoff value of 10 μmol/L [12], participants were categorized into two groups: a high-level homocysteine group (n = 41) or a normal-level homocysteine group (n = 38). Participants with acute and chronic myocardial infarction, uncontrolled serious heart failure, severe bradyarrhythmia, asthma or severe pulmonary dysfunction, coronary collateral circulation on angiography, impaired renal function, hepatic dysfunction, or acute and chronic infectious diseases were excluded from the study. We also excluded any participants with a history of folate, vitamin B12, or vitamin B6 intake.

Homocysteine and hs-CRP measurements

Two days prior to coronary angiography, a fasting 5 mL blood sample was collected in a tube containing ethylene diamine tetraacetic acid, which was then centrifuged at 1,500 rpm/min. Total plasma homocysteine levels (μmol/L) were determined using an enzyme-linked immunosorbent assay (ELISA) (IBL International GmbH). Serum hs-CRP was detected using a high-sensitivity particle enhanced immunoturbidimetric assay.

Quantitative coronary angiography

Coronary angiography was performed through the radial approach with a 6F Judkins catheter using a standard procedure by an experienced cardiologist. The left coronary artery and right coronary artery were visualized. All participants received the standard dose of clopidogrel (300 mg for a loading dose; 75 mg for a maintenance dose), aspirin (300 mg for a loading dose; 100 mg for a maintenance dose), and Atorvastatin (20 mg for a maintenance dose). Coronary physiology measurements were performed for all intermediate stenoses. After coronary angiography, a 0.014-inch pressure guide wire (St. Jude Medical St. Jude Medical, Inc.) was calibrated for pressure recordings and equalized to the aortic pressure in the guiding catheter. Then the wire was passed across the coronary lesion. The pressure sensor was positioned at least 3 cm beyond the lesion. An intracoronary bolus of 0.1 mg nitroglycerin was injected to ensure maximal epicardial vasodilation. Thermodilution curves were obtained by intracoronary injection of 3 mL of room-temperature normal saline. These injections were repeated twice more to measure the baseline mean transit time (bT_{mn}). Intravenous adenosine (140 μg/kg per min) was pumped into the right femoral vein to induce maximal hyperemia, followed by three more intracoronary injections of 3 mL of room-temperature normal saline. We then recorded the hyperemic mean transit time (hT_{mn}). Finally, the proximal arterial pressure (P_a) and distal pressure (P_d) of stenosis were recorded at baseline and during hyperemia.

Fractional flow reserve (FFR) was calculated as the ratio of mean P_d to mean P_a during maximal hyperemia. CFR was calculated as bT_{mn}/hT_{mn}. If patients had mild and moderate coronary artery stenosis (FFR > 0.80), IMR was calculated as: IMR = $P_d \times T_{mn}$ during maximal hyperemia. If patients had severe coronary artery stenosis (FFR < 0.80), IMR was calculated using the following, more complex formula: IMR = $P_a \times T_{mn} \times [(P_d-P_w)/(P_a-P_w)]$, where P_w is coronary artery wedge pressure (mean distal arterial pressure of the lesion when there was complete coronary stenosis or incarcerated balloon).

Table 1. Baseline characteristics of the participants

Characteristics	High-level homocysteine (n = 41)	Normal-level homocysteine (n = 38)	*P* Value	Chi-square/ t Value
Age (years)	57±12	56±9	0.740	0.333
Gender, male (%)	23 (56%)	19 (50%)	0.587	0.294
BMI (kg/m^2)	27.19±3.20	26.77±3.79	0.603	0.523
CKD	5 (12%)	4 (11%)	0.816	0.054
Smoker	19 (46%)	16 (42%)	0.705	0.143
Hypertension	18 (44%)	14 (37%)	0.523	0.408
Diabetes mellitus	14 (34%)	12 (32%)	0.808	0.059
Dyslipidemia	18 (44%)	16 (42%)	0.872	0.026
Previous PCI	12 (29%)	15 (40%)	0.339	0.913
FBG (mmol/L)	5.84±1.14	5.80±0.93	0.871	0.163
Cre (μmol/L)	80±16	74±15	0.116	1.590
TC (mmol/L)	5.29±0.67	5.26±0.81	0.878	0.154
TG (mmol/L)	1.63±0.46	1.61±0.53	0.850	0.189
LDL-C (mmol/L)	3.03±0.53	3.01±0.61	0.832	0.213
HDL-C (mmol/L)	1.40±0.34	1.50±0.50	0.292	-1.061
SBP (mmHg)	138±13	137±13	0.697	0.391
DBP (mmHg)	78±12	75±11	0.264	1.126
Medications				
Beta-blocker	32 (78%)	29 (76%)	0.854	0.034
Calcium-blocker	22 (54%)	18 (47%)	0.576	0.312
Aspirin	41 (100%)	38 (100%)	1.000	
Clopidogrel	41 (100%)	38 (100%)	1.000	
Atorvasatin	41 (100%)	38 (100%)	1.000	
Nitrates	24 (59%)	27 (71%)	0.245	1.350
ACEI/ARB	23 (56%)	24 (63%)	0.523	0.408
Left ventricular EF,%	54±7	56±8	0.275	-1.110
LVEDd (mm)	54±5	53±6	0.360	0.921
Hs-CRP (mg/L)	4.4±1.2	2.7±1.4	0.000	5.760
Homocysteine (μmol/L)	13.81±3.10	6.57±1.67	0.000	12.780

Values are expressed as mean ± SD for quantitative variables and n (%) for qualitative variables. BMI, body mass index; CKD, chronic kidney diseases; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; EF, ejection fraction; LVEDd, left ventricular end-diastolic dimension; hs-CRP, high-sensitivity C-reactive protein.

Statistical analysis

All statistical analyses were conducted using the SPSS software package (Version 20.0, SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation (SD), and categorical variables were presented as frequencies. Continuous data between groups were compared by the Student t test. Categorical data were compared by the chi-square test.

Both unadjusted and multiple linear regression models were used to determine the association between the exposure variables and intra-coronary microcirculation. To determine the independent predictor of IMR, only variables with *P*-value < 0.1 in the unadjusted analyses were included in the stepwise multiple regression models. IMR was expressed as continuous data, and we only obtained the beta coefficient for each independent variable instead of odds ratio. Statistical significance was set at P < 0.05.

Results

Baseline characteristics

Demographic and biochemical characteristics of participants in the high-level and normal-level homocysteine groups are presented in **Table 1**. General characteristics, such as age, gender, blood glucose, systolic blood pressure, and diastolic blood pressure, were similar between the two groups (P > 0.05). Circulating levels of homocysteine and hs-CRP were significantly higher in the high-level homocysteine group compared to the normal-level homocysteine group (P < 0. 01).

Comparison of coronary angiographic findings

As shown in **Table 2**, there were no statistically significant differences in the cumulative number of coronary lesions, characteristics and distribution of lesions, or thrombolysis in myocardial infarction (TIMI) flow grading between the two groups (P > 0.05).

Comparison of parameters of coronary microcirculation during maximal hyperemia

As shown in **Table 3**, there were no statistically significant differences observed in P_a and CFR

Table 2. Comparison of procedural characteristics

		High-level homocysteine (n = 41)	Normal-level homocysteine (n = 38)	*P* Value	Chi-square Value
Culprit vessel number	1	23 (56%)	17 (45%)	0.313	1.018
	2	10 (24%)	11 (29%)	0.647	0.210
	3	8 (20%)	10 (26%)	0.471	0.519
Total number of lesions		68	60		
Lesion characteristics	A+B1	37 (54%)	33 (55%)	0.947	0.004
	B2+C	31 (46%)	27 (45%)	0.947	0.004
Lesion distribution	LMCA	5 (7%)	6 (10%)	0.594	0.284
	LAD	25 (37%)	21 (35%)	0.836	0.043
	Circumflex	18 (27%)	16 (27%)	0.980	0.001
	RCA	20 (29%)	17 (28%)	0.893	0.018
Lesion location of FFR and IMR	LAD	22 (54%)	20 (53%)	0.927	0.008
	Circumflex	11 (27%)	13 (34%)	0.476	0.508
	RCA	8 (20%)	5 (13%)	0.447	0.579
TIMI flow grade	0	0	0	1	
	1	0	0	1	
	2	4	2	0.451	0.567
	3	37	36	0.451	0.567

TIMI, thrombolysis in myocardial infarction; LAD, left anterior descending artery; LMCA, left main coronary artery; RCA, right coronary artery; FFR, fractional flow reserve; IMR, index of microvascular resistance.

Table 3. Hemodynamic characteristics of target and reference vessels

Characteristics	High-level homocysteine (n = 41)	Normal-level homocysteine (n = 38)	*P* Value	t Value
P_a (mmHg)	83±8	85±9	0.402	-0.843
P_d (mmHg)	65±13	71±11	0.045	-2.040
FFR	0.78±0.13	0.84±0.10	0.047	-2.021
CFR	1.87±0.38	2.09±0.55	0.039	-2.103
IMR (U)	35±8	24±5	0.000	7.640

P_a, arterial pressure; P_d, distal arterial pressure of stenosis; IMR, index of microvascular resistance; CFR, coronary flow reserve; FFR, fractional flow reserve.

between the two groups after induction of maximal hyperemia (P > 0.05). However, P_d and FFR were significantly lower and IMR was higher in the high-level homocysteine group compared to the normal-level homocysteine group (all P < 0.05).

Correlation analysis between homocysteine levels and IMR, FFR, or CFR

Linear regression analyses indicated that homocysteine levels were positively correlated with IMR (r = 0.550, P < 0.01) and negatively correlated with FFR (r = -0.173, P < 0.01). However, there was no obvious correlation between homocysteine levels and CFR (r = -0.058, P = 0.611). The relationships between homocysteine levels and the IMR or FFR are displayed in the scatter plots in **Figures 1** and **2**.

Correlation analysis between hs-CRP levels and IMR, FFR, or CFR

Linear regression analyses indicated that hs-CRP levels were positively correlated with IMR (r = 0.699, P < 0.01). However, there was no obvious correlation between hs-CRP levels and FFR (r = -0.218, P = 0.054) or CFR (r = 0.011, P = 0.926). The relationship between hs-CRP levels and the IMR is displayed in the scatter plot in **Figure 3**.

Predictors of IMR value

Multiple regression analyses of predictors of IMR are shown in **Table 4**. Homocysteine was an independent predictor of IMR (beta coefficient 0.50; 95% confidence interval, 0.16-0.85, P = 0.005). In addition, hs-CRP was also an independent predictor of IMR (beta coefficient 3.13; 95% confidence interval, 2.14-4.11, P < 0.001).

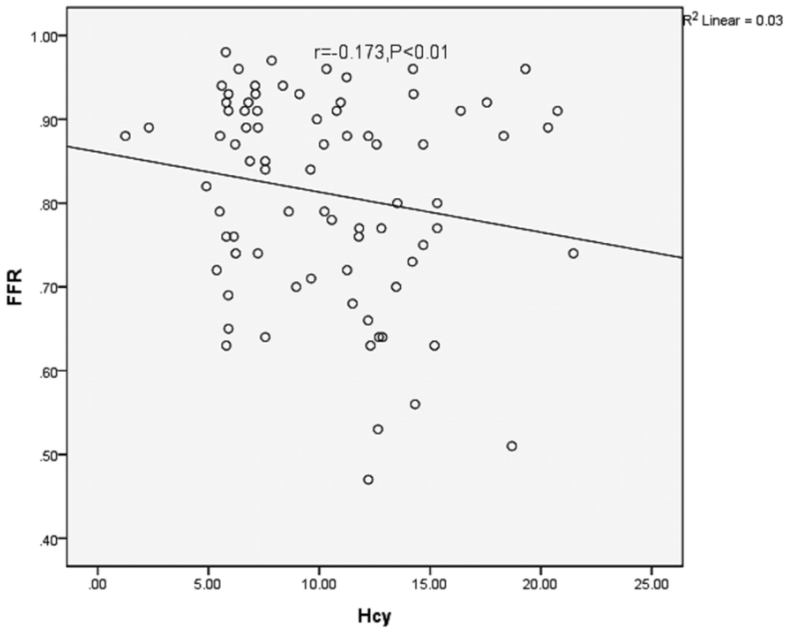

Figure 1. Correlation of homocysteine levels and FFR. Hcy = homocysteine; FFR = fractional flow reserve.

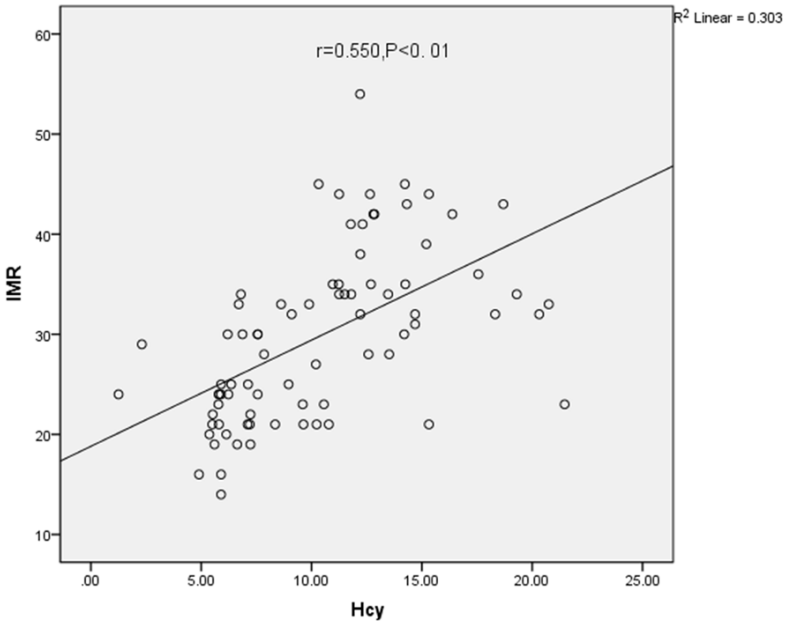

Figure 2. Correlation of homocysteine levels and IMR. Hcy = homocysteine; IMR = index of microvascular resistance.

Discussion

The current study showed that patients with high levels of homocysteine had elevated hs-CRP serum levels, higher IMR, and lower FFR compared to patients with normal levels of homocysteine. In contrast, there were no differences in CFR between the two groups. Linear regression analyses indicated that homocysteine levels were positively correlated with IMR and negatively correlated with FFR. Multiple regression analyses revealed that homocysteine was an independent predictor of IMR. Moreover, serum hs-CRP levels were positively correlated with IMR. These findings suggest that hs-CRP-related inflammatory mechanisms may be involved in homocysteine related coronary microcirculatory dysfunction. From this point of view, management of inflammation associated with hyperhomocysteinemia might improve coronary microcirculation.

Hyperhomocysteinemia can be caused by a methionine-rich diet, genetic abnormality, folate deficiency, vitamin B6, vitamin B12, and impaired kidney disease. Hyperhomocysteinemia can be improved by statin therapy [13, 14]. Moreover, this class of lipid-lowering agents can also decrease C-reactive protein and other inflammatory markers [15]. In our study, all patients simultaneously received Atorvasatin therapy; however, there was no statistically significant difference between the two groups ($P > 0.05$). Therefore, the observed plasma homocysteine and hs-CRP levels may be lower than the actual values. However, this factor does not likely affect the results of our study. A well-designed meta-analysis [16] indicated that each 5 μmol/L increase in homocysteine levels confers a 20% greater risk of coronary heart disease events, independently of traditional risk factors. The increased levels of plasma

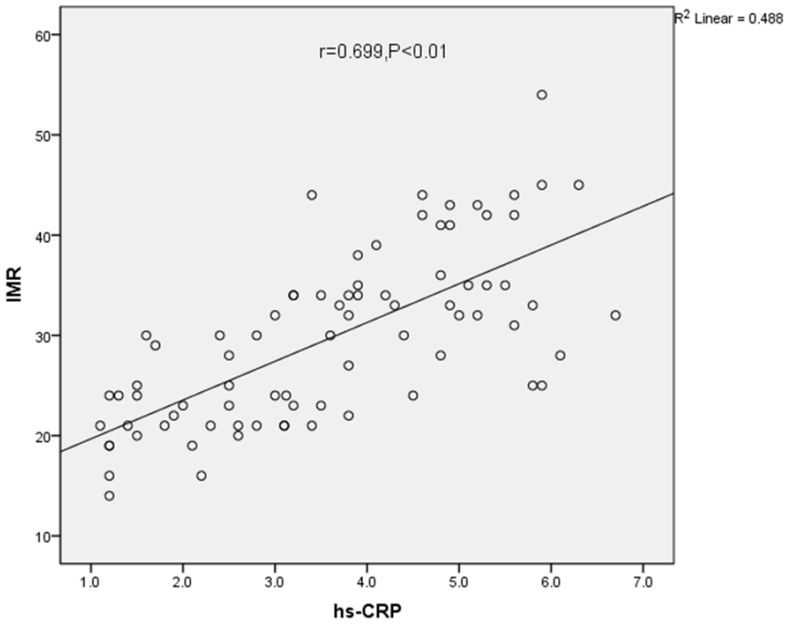

Figure 3. Correlation of hs-CRP levels with IMR. hs-CRP = high-sensitivity C-reactive protein; IMR = index of microvascular resistance.

homocysteine were associated with the degree of ischemic findings without organic stenosis in patients with slow coronary flow [6].

Coronary microcirculation contributes substantially to the pathophysiology of cardiovascular disease. Coronary microcirculatory dysfunction can impair coronary blood flow in individuals with the presence or absence of detectable coronary lesions. IMR is a relatively simple invasive index for evaluating microcirculatory function of the coronary artery. IMR correlated with true microvascular resistance independent of epicardial arteries [17, 18]. In our study, the IMR value was higher in the high-level homocysteine group compared to the normal-level homocysteine group ($P < 0.05$), indicating that there is impaired coronary microcirculatory function in hyperhomocysteinemic individuals. Furthermore, homocysteine levels were positively correlated with IMR ($r = 0.550$).

CFR is a commonly used index of coronary microvascular function and reflects the regulatory ability of the coronary microcirculation to increase blood flow. Several studies [19-22] indicated a significant negative correlation between homocysteine levels and CFR. In contrast, despite a trend towards a decrease in CFR values in the high-level homocysteine group, we did not observe a statistically significant difference in the current study. The cutoff value of hyperhomocysteinemia was relatively low (> 10 μmol/L) which might partly explain why the correlations between homocysteine levels and CFR were not stronger. In addition, CFR is not specific to the microcirculation, as both epicardial and microvessels can contribute to alterations in blood flow [23].

Increased CRP levels have been observed in hyperhomocysteinemic individuals [24]. Plasma homocysteine levels were associated with hs-CRP levels in the Physician's Health Study [25]. Elevated levels of homocysteine and hs-CRP were associated with severity of CAD [26, 27]. These findings suggested that a pro-inflammatory state is associated with hyperhomocysteinemia. Elevated homocysteine contributes to the initiation and development of atherosclerosis *via* activating monocytes, resulting in the secretion of cytokines that amplify the inflammatory response [28, 29]. We demonstrated that hs-CRP levels were higher in the hyperhomocysteinemic group compared to the normal-level homocysteine group ($P < 0.05$). Serum hs-CRP levels were strongly positively correlated with IMR ($r = 0.699$) as determined by linear regression analysis. These findings suggest that both homocysteine and hs-CRP are implicated in coronary microcirculatory dysfunction.

However, mechanisms underlying hyperhomocysteinemia induced coronary microcirculatory dysfunction are largely unknown. The coronary endothelium may be the link between elevated homocysteine levels and microcirculatory function [30]. Experimentally induced hyperhomocysteinemia by oral methionine load has been reported to impair microvascular dilator function through decreasing nitric oxide bioavailability [19]. High homocysteine levels directly increase oxidative injury to the endothelium, leading to endothelial dysfunction [31]. In addition, high homocysteine levels can induce a pro-coagulant state [32] and inflammatory

Table 4. Multiple regression analysis of predictors of IMR

	Univariate regression analysis			Multivariate regression analysis		
	beta coefficient	95% CI	P	beta coefficent	95% CI	P
Hcy	1.1	(0.7, 1.4)	< 0.001	0.50	(0.16, 0.85)	0.005
Male	1.2	(-2.5, 5.0)	0.519			
Hs-CRP	3.9	(3.0, 4.8)	< 0.001	3.13	(2.14, 4.11)	< 0.001
LVEDd	0.2	(-0.2, 0.5)	0.338			
EF	-0.1	(-0.3, 0.2)	0.473			
ACEI/ARB	0.8	(-3.0, 4.7)	0.675			
Nitrates	1.4	(-2.5, 5.3)	0.480			
Calcium-blocker	1.5	(-2.3, 5.3)	0.433			
Beta-blocker	0.7	(-3.8, 5.1)	0.767			
Previous PCI	0.1	(-3.8, 4.1)	0.948			
Dyslipidemia	0.7	(-3.1, 4.5)	0.716			
Diabetes mellitus	2.9	(-1.1, 6.8)	0.162			
Hypertension	2.2	(-1.6, 6.0)	0.258			
Smoker	-0.8	(-4.6, 3.0)	0.692			
CKD	-2.4	(-8.3, 3.5)	0.434			
BMI	-0.2	(-0.8, 0.3)	0.410			
Age	-0.1	(-0.3, 0.0)	0.143			

CI, confidence interval; Hcy, homocysteine; Hs-CRP, high-sensitivity C-reactive protein; LVEDd, left ventricular end-diastolic dimension; EF, ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; PCI, percutaneous coronary intervention; CKD, chronic kidney diseases; BMI, body mass index.

response [33]. All of these factors may contribute to microcirculatory dysfunction.

Several limitations of this study should be noted. First, the main limitation is the small sample size of participants, who were all from a single-center. Second, participants in this current study were all undergoing elective coronary angiography, so the normal-level homocysteine group may not be representative of the general population. Third, one of the major causes of hyperhomocysteinemia is folate and vitamin B12 deficiency; however, we did not simultaneously measure folic acid and vitamin B12 levels to determine the possible disorder of homocysteine metabolism. Finally, plasma homocysteine genotypes are also risk factors for coronary artery disease in patients undergoing coronary angiography [34, 35] and the genotypes of homocysteine might affect the current findings.

Conclusions

Hyperhomocysteinemia is characterized by elevated hs-CRP serum levels, higher IMR, and lower FFR. Individuals with elevated plasma homocysteine levels are more prone to coronary microcirculatory dysfunction and inflammation. Hyperhomocysteinemia may cause coronary microcirculatory dysfunction partly by promoting inflammatory responses involving hs-CRP-related mechanisms.

Disclosure of conflict of interest

None.

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