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Original Article

Increased levels of serum Hcy and UA as well as the thickness of carotid intima-media correlates with the severity of coronary artery lesions

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Abstract: Objective: To investigate the correlation between serum homocysteine (Hcy) and uric acid (UA) levels as well as the carotid intima-media thickness (IMT) and the severity of coronary artery lesions in elderly patients with coronary heart disease (CHD). Methods: This study recruited 156 elderly patients (56 elderly patients with CHD as the study group and 54 healthy subjects as the control group). The study group were further divided into low (≤ 20 points n = 72), middle (21-40 points, n = 56) and high (≥ 40 points, n = 28) score groups according to their SYNTAX score. Results: According to SYNTAX scores scaling, either middle or high score groups showed the significantly higher levels of serum Hcy, UA, and the IMT than the low score group, while the high score group had the highest levels. Serum Hcy, UA levels and the IMT were positively correlated with SYNTAX and Sullivan scores. Male gender, smoking, history of hypertension, systolic pressure, pulse pressure, Hcy, UA, IMT, and BMI independently correlated with the severity of CHD. Conclusion: The serum levels of Hcy and UA and the IMT in patients with CHD were significantly elevated, and positively correlated with the severity of coronary artery lesions, which may serve as diagnostic indicators.

Keywords: Coronary heart disease, homocysteine, serum uric acid, carotid intima-media thickness, correlation

Introduction

Coronary heart disease (CHD) is a common disease of the human circulatory system. It is caused by many factors including the person's lifestyle, dietary habits and heredity. Coronary atherosclerotic, the most common type of CHD, is caused by coronary atherosclerotic stenosis, which leads to necrosis of the myocardium due to the insufficient blood supply. In addition, the stenosis of the lumen caused by extensive inflammation and embolism also leads to CHD [1]. For a long time, the coronary angiography has been the "gold standard" for evaluating the severity of coronary artery lesions, but it is too traumatic to be suitable for elderly patients. However, most CHD patients are middle-aged and elderly. Therefore, it is important to identify and develop appropriate non-invasive tests and indicators for the clinical evaluation of CHD severity.

The severity of CHD coronary artery lesions have recently been shown to be rcorrelated with the levels of serum homocysteine (Hcy) and uric acid (UA) and the carotid intima-media thickness (IMT) [2]. Hcy, a sulfur-containing amino acid produced during methionine metabolism, is significantly associated with the severity of coronary artery lesions. It plays an important role in the development of cardiovascular diseases such as atherosclerosis, aneurysms, and myocardial infarction. Hcy can promote hypertrophy and the proliferation of neutrophils, which can directly damage vascular endothelial cells and impair their function, thereby, increasing the risk of cardiovascular disease.

UA is one of the most important water-soluble endogenous antioxidants in the human body and acts as an important component of the cardiovascular structure [3]. UA can aggravate atherosclerosis by promoting renal tubule reabsorption of sodium ions. It has been suspected as an independent risk factor and an independent predictor of coronary atherosclerosis [4]. UA can protect cardiovascular system from oxidative stress, not only due to its strong free

radical scavenging effect which is 10 times better than other anti-oxidants, but also good iron-chelation activity and inhibition of Fenton reaction [5]. Low level of UA may indicate better prognosis of CHD patients with acute myocardial infarction.

A previous study found that when coronary atherosclerosis occurs, the IMT increases [6]. IMT can be easily and noninvasively measured to observe atherosclerosis in patients with CHD, so it is a good indicator of the severity of coronary artery lesions.

Most importantly, CHD is an irreversible and chronic disease with high sudden death rate in late stage. Therefore, great importance has been attached to early diagnosis and treatment of CHD. What's more, the diagnosis of the severity of CHD mainly depends on the assessment of the severity of coronary artery lesions [7]. This diagnosis can help in appropriate treatment and prognosis of CHD. Hence, in the present study, we have explored the correlation between serum levels of Hcy and UA as well as the IMT, and the severity of coronary artery lesions in elderly patients with CHD, with the hope to provide more insights of the non-invasive diagnosis of CHD.

Materials and methods

General data

This retrospective study was approved by the Medical Ethics Committee of Affiliated Hospital of Beihua University. A total of 156 elderly patients admitted to the Affiliated Hospital of Beihua University for CHD diagnosed by coronary angiography from September 2017 to August 2018 were enrolled as the study group. According to their SYNTAX scores, the study group was further divided into three sub-groups: low score (≤ 20 points, 72 cases), middle score (21-40 points, 56 cases), and high score (≥ 40 points, 28 cases) groups [8]. All patients or their families agreed to participate in the study and signed the informed consent.

Inclusion criteria for study group: All patients: (1) met the diagnostic criteria of CHD according to the *Guidelines for Coronary Heart Disease*, and were diagnosed by coronary angiography [9]; (2) were aged from 61 to 88 years; (3) had no history of an operation, hormone use, use of immunosuppressive agents and antibiotics in the past six months; (4) had no history of gout and hyperuricemia.

Exclusion criteria: (1) those with serious diseases of liver, kidney, brain, lung, and other basic organs; (2) those with acute and chronic infections; (3) those with malignant tumors, tuberculosis, and aneurysms; (4) those with diseases of blood, endocrine, and immune systems; (5) those with lactation and pregnancy.

Another 54 healthy controls with normal coronary artery were enrolled as the control group and coronary angiography was performed at the same time.

Sample collection

First of all, patients of the control group were confirmed with no coronary artery disease according to coronary angiography operated by the imaging physicians with more than 5 years of working experience. Secondly, after all the subjects (including the study group and the control group) were admitted to the hospital, 5 mL of fasting venous blood was collected by medical technologists with more than 5 years of working experience and stayed at room temperature for 30 min. Then the serum was separated by centrifugation at a speed of 3,000 rpm for 10 min, which was further used to determine the Hcy and UA levels by circulating enzyme method with Hcy detector (Shenzhen Ausa Pharma Co., Ltd.) according to the kit instructions (Shanghai Jimian Industrial Co., Ltd.). UA enzyme colorimetry was used to measure serum UA level by automatic biochemical analyzer according to kit instructions (Shanghai Xinfan Biotechnology Co., Ltd.). Ultrasound doctors with more than 5 years working experience used VIVIVID7 ultrasound diagnostic device (Shenzhen Kaili Biomedical Science and Technology Co., Ltd.) to examine the neck vessels of the subjects in the supine position with the probe frequency ranging from 7.5 Hz to 12.0 Hz.

Observation parameters

Main parameters: (1) The levels of Hcy, UA and IMT: The levels of Hcy, UA and IMT were compared between the study group and control group as well as among the low, middle, and high score groups. IMT was measured as the mean of thickness between the anterior and

Table 1. General data ($\bar{x}$ ± sd, n, %)

Group	Low score group (n = 28)	Middle score group (n = 28)	High score group (n = 28)	F	P
Gender (male/female)	55/17	42/14	22/6	0.243	0.912
Average age (year)	71.91±13.82	71.21±14.12	73.32±14.11	0.831	0.312
BMI (kg/m^2)	24.43±2.34	24.35±2.31	24.65±2.37	0.217	0.918
Type of CHD (n, %)					
Stable type	30 (41.67)	23 (41.07)	11 (39.29)	0.047	0.977
Unstable type	19 (26.39)	15 (26.79)	8 (28.57)	0.05	0.976
Acute cardiac infarction	23 (31.94)	18 (32.14)	9 (32.14)	0.001	1.000
Co-morbidities (n, %)					
Hypertension	40 (55.56)	31 (55.36)	16 (57.14)	0.267	0.987
Diabetes	29 (40.28)	24 (42.86)	11 (39.29)	0.128	0.937
Hyperlipidaemia	43 (59.72)	34 (60.71)	17 (60.71)	0.016	0.992
Heart failure	40 (55.56)	33 (58.93)	16 (57.14)	0.011	0.912

Note: BMI: body mass index; CHD: coronary heart disease.

posterior walls of the carotid arteries, including bilateral carotid bifurcation, bilateral internal carotid artery, and the bilateral of the common carotid artery. A measurement of 0.9 mm ≤ IMT < 1.3 mm was considered as thickening, while IMT ≥ 1.3 mm was considered as atheromatous plaque formation. (2) SYNTAX score: Pearson linear correlation was used to analyze the correlation between serum Hcy, UA level, IMT and SYNTAX score. All study group patients underwent coronary angiography by Judkins method in the catheter room of Affiliated Hospital of Beihua University. They were scored according to the segmentation evaluation standard for the coronary artery image score issued by the American Heart Association [9]. SYNTAX score is calculated by computer program. The algorithm includes 12 problems and the first three problems are the dominant type of coronary artery, the number of lesions and the number of vascular segments in lesions. The maximum number of lesions was 12. Each lesion was labeled with 1, 2, 3 and so on. Each lesion may involve one or more segments, and the integral of each lesion will be calculated by the number of involved segments. The last nine problems are the adverse characteristics of lesions, according to which the integral of each lesion is obtained. The sum of the integral of each lesion is the SYNTAX score. The higher the score was, the greater the degree of coronary artery stenosis was.

Secondary parameters: (1) Sullivan score: Pearson linear correlation analysis was used to analyze the correlation between serum Hcy, UA level, IMT and Sullivan score. Sullivan score method was used to calculate the proportion of atherosclerotic plaque in the coronary artery. A high score indicates the high proportion of atherosclerotic plaque in the coronary artery. (2) Univariate analysis: The general data of study group and control group were included in the univariate analysis, including gender, body mass index (BMI), history of smoking, alcohol consumption, hypertension, diabetes and cardiovascular diseases, systolic, diastolic and pulse pressure, Hcy, UA, and IMT. (3) Logistic regression analysis: Logistic regression analysis was used to analyze the correlation between multiple factors and severity of coronary artery lesions, with the integral of coronary stenosis degree (SYNTAX score) as the dependent variable, Hcy, UA, IMT thickening as the independent variables.

Statistical methods

All data in this study were analyzed by SPSS-21.0 statistical software. The measurement data were expressed as the mean ± standard deviation ($\bar{x}$ ± sd) and analyzed using F-test. All count data were expressed as percentages (n, %) and analyzed using a χ^2 test. Pearson linear correlation analysis was used to analyze the correlation between serum Hcy, UA level as well as IMT and SYNTAX score, Sullivan score. The general data of study group and control group were included in the single factor analysis. Logistic regression analysis was used to analyze the correlation of multiple factors and severity of coronary artery lesions (SYNTAX

Table 2. Comparison of Hcy and UA levels and IMT in three sub-groups ($\bar{x}$ ± sd)

Group	Low score group (n = 28)	Middle score group (n = 28)	High score group (n = 28)
Hcy (μmol/L)	11.34±3.83	20.03±7.88***,###	28.98±8.04***
UA (μmol/L)	206.78±60.45	326.34±79.34***,###	456.67±98.34***
IMT (mm)	1.19±0.05	1.26±0.07***,###	1.33±0.08***

Note: Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness. ***P < 0.001, compared with low score group; ###P < 0.001, compared with high score group.

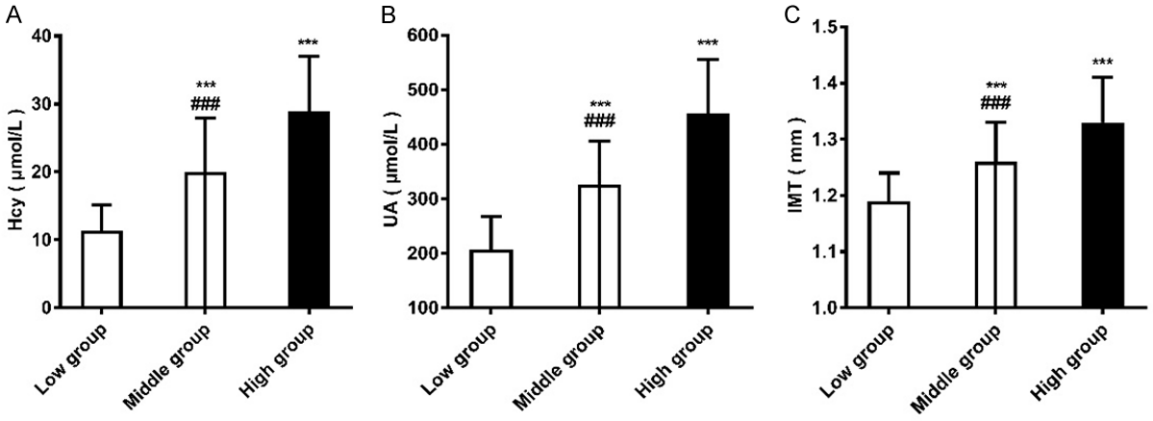

Figure 1. Comparison of Hcy (A), UA (B) and IMT (C) levels among low, middle and high groups. Compared with the low group, ***P < 0.001; compared with the high group, ###P < 0.001. Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

Table 3. Correlation of serum Hcy, UA level and IMT with SYNTAX score

Group	Correlation coefficient	Statistic	P
Hcy	0.476	10.069	0.000
UA	0.517	10.091	0.000
IMT	0.528	10.031	0.000

Note: Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

score). The integral of coronary stenosis (SYNTAX score) was taken as the dependent variable while Hcy, UA, and IMT were taken as independent variables. Differences were considered significant when P < 0.05.

Results

General data comparison among low, middle and high score groups

There was no significant difference in the general data among the three groups (P > 0.05). See **Table 1**.

Comparison of Hcy and UA levels and IMT among low, middle and high score groups

The serum Hcy and UA levels and the IMT in the middle and high score groups were significantly higher compared to the low score group (all P < 0.001), while the same parameters in the high score group were significantly higher compared to the middle score group (all P < 0.001). See **Table 2** and **Figure 1**.

Correlation of serum Hcy, UA level and IMT with SYNTAX score

Pearson correlation analysis showed that serum Hcy and UA levels and IMT were positively correlated with SYNTAX score in the study groups (r = 0.476, 0.517, 0.528; P < 0.001), respectively. See **Table 3** and **Figure 2**.

Correlation of serum Hcy and UA levels and IMT with Sullivan score

Pearson correlation analysis showed that serum Hcy and UA levels and IMT were positively correlated with Sullivan score in the study

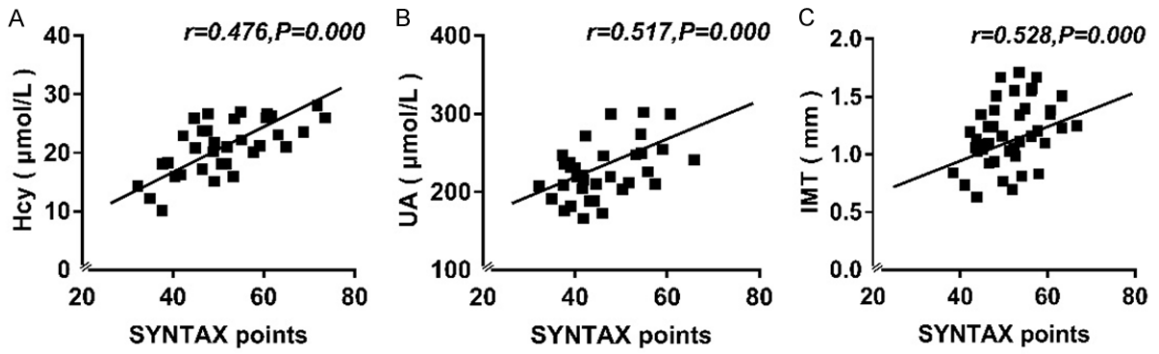

Figure 2. Correlation between serum Hcy (A), UA (B) levels and IMT (C) and SYNTAX score. Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

Table 4. Correlation of serum HCY and UA levels and IMT with Sullivan score

Group	Correlation coefficient	Statistic	P
Hcy	0.587	10.031	0.000
UA	0.563	10.043	0.000
IMT	0.617	10.034	0.000

Note: Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

groups (r = 0.587, 0.563, 0.617; P < 0.001), respectively. See **Table 4** and **Figure 3**.

Univariable analysis of general data in the experimental and control groups

Univariable analysis of the general data showed that male gender, BMI, history of smoking, history of hypertension, systolic blood pressure, pulse pressure, Hcy, UA, and IMT significantly correlated with CHD in the study groups (all P < 0.05). See **Table 5**.

Logistic regression analysis of the correlation between multivariate factors and CHD

Based on assignment table, logistic regression analysis showed that male gender, smoking, history of hypertension, systolic blood pressure, pulse pressure, Hcy, UA, IMT, and BMI independently correlated with the severity of CHD in the study groups (all P < 0.05). See **Tables 6** and **7**.

Discussion

There are many risk factors for developing CHD in the elderly, among which age is the most significant factor, followed by carotid plaque formation. In addition, vitamin B, gender, obesity, smoking, and alcohol can affect the severity of coronary artery disease by influencing the level of serum Hcy and UA [10]. In recent years, the improvement of therapeutic efficacy has been the hot spot, with the increasing incidence and mortality of CHD in the elderly. Under the circumstances of slow progression and high risk of late stage, the key to the effective management of CHD is timely and effective diagnosis. Since the severity evaluation of coronary artery lesions can mainly reflect the severity of CHD in patients, the key points are therefore, to find suitable non-invasive, low-cost markers [11].

Our study showed that the serum Hcy level in the CHD group was significantly higher than that in the control group, indicating that the serum Hcy level may be an independent risk factor for CHD. We also showed that the level of serum Hcy in high and middle score groups was significantly higher than that in the low score group, and the high score group obtained the highest scores. This indicated that the level of serum Hcy was positively correlated with SYNTAX score, namely, higher level of serum Hcy indicates more serious coronary lesions. Hcy is produced in the liver and other organs by the methionine metabolic cycle and convert to methionine and cysteine. Normally, Hcy has a lower serum concentration of about 8 μmol/L [12]. Hcy induces coronary artery disease by damaging proteins through oxidative stress, resulting in the decrease or inactivation of enzymes and enzyme receptors, along with the decrease in nitric oxide synthesis, and subsequently impairs endothelial function, ultimately hindering vascular expansion. Besides, Hcy is an important factor in promoting thrombo-

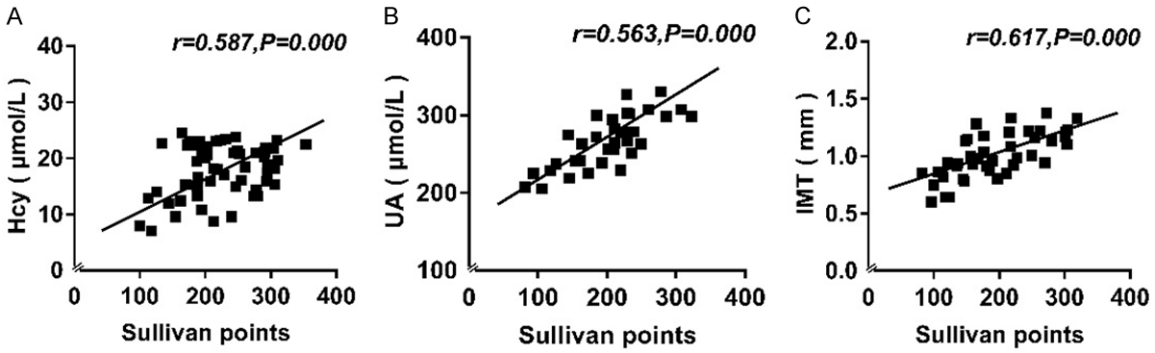

Figure 3. Correlation between serum Hcy (A), UA (B) levels and IMT (C) and Sullivan scores. Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

Table 5. Univariable analysis of general data

Group	Control group (n = 54)	Study group (n = 150)	P	χ^2
Male (n, %)	23 (42.59)	119 (76.28)	0.000	24.254
BMI (kg/m^2)	30.21±4.13	24.32±2.12	0.000	10.033
Smoking (n, %)	12 (22.22)	60 (38.46)	0.030	4.695
Drinking (n, %)	10 (18.52)	21 (13.46)	0.367	0.815
Hypertension history (n, %)	10 (18.52)	105 (67.31)	0.000	38.545
Diabetes history (n, %)	8 (14.81)	16 (10.26)	0.364	0.823
CVD history (n, %)	12 (22.22)	35 (22.44)	0.974	0.001
Systolic pressure (mmHg)	122.21±8.32	135.32±11.34	0.000	9.033
Diastolic pressure (mmHg)	76.34±5.43	75.34±8.32	0.317	1.005
Pulse pressure (mmHg)	45.43±10.32	60.12±12.32	0.000	8.560
Hcy (μmol/L)	7.59±0.46	18.45±5.66	0.000	23.740
UA (μmol/L)	155.33±47.79	294.18±85.31	0.000	14.722
IMT (mm)	0.81±0.05	1.23±0.07	0.000	47.645

Note: BMI: body mass index; CVD: cardiovascular disease; Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

sis. Under high Hcy level, the hypercoagulable blood causes a large number of platelets to adhere and aggregate together. At the same time, Hcy induces lumen stenosis by stimulating the proliferation of vascular smooth muscle cells, and also results in an imbalance in the lipid, carbohydrate and protein metabolism, leading to the formation of lipid deposition plaques [13, 14]. Multiple studies have shown that there is a significant correlation between high serum Hcy level and CHD [15]. Wang et al. also found the significant correlation between serum Hcy level and severity of coronary artery disease [16]. These previous results are consistent with the observations in this study [17, 18].

This study showed that the serum UA levels in the study group weresignificantly higher compared to the control group. Also, the serum UA level in high and middle score groups was significantly higher compared to the low score group, while high score group had the highest serum UA level. This indicated that serum UA may also be an independent risk factor for CHD and was positively correlated with the severity of coronary artery disease. UA is a water-soluble antioxidant distributed in the human cardiovascular system and is normally stable in the serum. The purine metabolism produced hypoxanthine, which was then oxidized to xanthine under the action of enzymes and finally oxidized to UA [19]. Some studies have pointed out that serum UA levels in the elderly diagnosed with CHD show significant changes, suggesting that it has a certain correlation with coronary artery disease [20]. In elderly coronary artery disease, UA level increases as a result of increasing production but slow metabolism. Studies have

Table 6. Index assignment

Index	Assignment	
	0	1
Gender	Female	Male
Body mass index (kg/m^2)	≥ 26	< 26
Smoking	No	Yes
Hypertension history	No	Yes
Systolic pressure (mmHg)	≤ 128	> 128
Pulse pressure (mmHg)	≤ 52	> 52
Hcy (μmol/L)	≤ 11	> 11
UA (μmol/L)	≤ 190	> 190
IMT (mm)	≤ 1	> 1

Note: Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

Table 7. Logistic regression analysis

Index	OR	95% CI	P
Male	10.22	1.23-70.32	0.000
Body mass index	9.21	2.21-61.24	0.000
Smoking	10.23	2.21-31.22	0.000
Hypertension history	9.32	1.12-33.42	0.000
Systolic pressure	11.33	2.21-32.33	0.000
Pulse pressure	11.35	2.22-32.22	0.000
Hcy	10.88	1.38-71.43	0.000
UA	11.21	2.13-61.13	0.000
IMT	12.35	5.08-31.02	0.000

Note: CHD: coronary heart disease; OR: odds ratio; CI: confidence interval; Hcy: homocysteine; UA: uric acid; IMT: intima-media thickness.

shown that elevated serum UA level can lead to sympathetic excitation and renal hemodynamic changes. Specifically, UA promotes the reabsorption of sodium in the proximal convoluted tubules of the glomeruli and accelerates the production of endothelin in blood, thus inducing CHD and also aggravating the severity of coronary artery disease, which was consistent with our research [21, 22].

In our study, IMT in the study group was significantly higher compared to the control group. IMT in middle and high score groups was significantly higher compared to the low score group whereas IMT in the high score group was significantly higher compared to the middle score group, indicating that IMT is significantly correlated with the severity of coronary artery disease. IMT is an important visual index for evaluating the severity of coronary artery disease. A study has shown that IMT value positively correlates with the severity of coronary artery disease [23]. To explain the pathological progression of CHD in the elderly, serum lipid was deposited down to the intima of the vessel as a result of endothelial damage, which promotes inflammation and plaque formation, and eventually thickens smooth muscle and hardens the vessel wall, leading to the increased IMT [24]. Our results were consistent with those of the above studies [25].

Pearson correlation analysis showed that serum Hcy, UA levels and IMT were positively correlated with SYNTAX and Sullivan score, respectively. Logistic regression analysis showed that male gender, smoking, history of hypertension, systolic blood pressure, pulse pressure, Hcy, UA, IMT, and BMI were independently associated with the severity of CHD (SYNTAX score). The innovation of this study lies in the use of serum detection indicators in the diagnosis of CHD, which is non-invasive, easy to operate and low-cost. It is expected to be widely used in clinical practice with a promising future. However, the greatest disadvantage of this study is the small sample size.

The levels of serum Hcy and UA and IMT in patients with CHD were significantly elevated, and their increases positively correlated with the severity of coronary artery lesions. Therefore, the levels of serum Hcy and UA and IMT can also be used as diagnostic indices for evaluating the severity of CHD in clinic.

Disclosure of conflict of interest

None.

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