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High Homocysteine Levels are Closely Associated with the Categories of Coronary Artery Diseases as well as Low Status of Folic Acid and Vitamin B_{12}

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Abstract

Background: Homocysteine (Hcy) has been considered as an independent risk factor for coronary artery disease (CAD). Folic acid and vitamin B_{12} are two vital regulators in Hcy metabolic process. We evaluated the associations between Hcy and the categories of CAD as well as the levels of folic acid and vitamin B_{12}.

Methods: Hcy, folic acid and vitamin B_{12} from 292 CAD patients, including 73 acute myocardial infarction (AMI), 116 unstable angina pectoris (UAP), 103 stable angina pectoris (SAP), and 100 non-CAD controls were measured, and the data were analyzed by SPSS software.

Results: Hcy concentrations of AMI patients were the highest, and UAP patients were second, and SAP patients were the third higher, which were significantly higher than controls ($p<0.01$). Compared to SAP patients, patients with AMI and UAP had higher Hcy levels with approximately average elevated (4-5) μmol/L, while SAP patients were more higher Hcy with approximately 8 μmol/L than controls. However, the levels of folic acid and vitamin B_{12} had opposite results, whose levels in AMI group had the lowest, while in controls had the highest. There were strongly moderate negative correlations between Hcy and folic acid ($r=-0.67$, $p<0.001$) and vitamin B_{12} ($r=-0.56$, $p<0.001$).

Conclusions: The present study confirms that high Hcy levels are closely associated with the categories of CAD as well as low status of folic acid and vitamin B_{12}.

Keywords: Homocysteine; Folic acid; Vitamin B_{12}; Coronary artery disease; Atherosclerosis

Introduction

Coronary artery disease (CAD) is seriously to harm people's healthy disease in borth developed and developing countries, which was predominantly caused by atherosclerosis with endothelial dysfunction [1,2]. Despite best efforts, available therapies protect only 30-40% of individuals at risk, and no therapeutic cure is anticipated for those who currently suffer from the disease [3]. The endothelium is a single layer of cells lining all blood vessels. It plays an important role in many physiological functions, including the control of blood cell trafficking, vasomotor tone, vessel permeability, and hemostatic balance. Endothelial cells produce a wide variety of substances in response to various physical and chemical stimuli, including vasodilator substances, and vasoconstrictor substances [4]. Researches have confirmed that endothelial dysfunction plays an important role in all categories of CAD from stable angina pectoris (SAP) to unstable angina pectoris (UAP), and to acute myocardial infarction (AMI) [5]. Early warning and immediate risk stratification of patients with different categories of CAD is frequently a challenging task in the current.

A large number of studies have confirmed that elevated homocysteine (Hcy) has been associated with endothelial dysfunction of atherosclerosis owing to oxidative stress [6], endoplasmic reticulum stress [7], increased level of asymmetric dimethylarginine (ADMA) [8], involved inflammation [9,10]. Genetic or nutritional deficiencies in Hcy metabolism lead to hyperhomocysteinemia (HHcy) and cause endothelial dysfunction, a hallmark of atherosclerosis [11]. Folic acid and vitamin B_{12} play an important role in regulating the metabolic process of Hcy [12]. Current studies have shown that folic acid supplementation can significantly improve endothelial dysfunction in patients with CAD [13]. Vitamin B_{12} deficiency and HHcy are related to cardiovascular risk factors in patients with coronary artery disease [14]. In present study, we evaluate the correlations between Hcy and folic acid as well as vitamin B_{12}, and explore the associations of Hcy with the categories of CAD as well as the levels of folic acid and vitamin B_{12} in patients with atherosclerotic CAD.

Materials and Methods

This study included 292 CAD patients (103 SAP, 116 UAP, 73 AMI) and 100 chest pain non-CAD patients confirmed by coronary angiography from the Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. Patients with the following diseases were excluded from this study: cancer, liver diseases, renal insufficiency, blood diseases, hyperthyroidism, thyroid dysfunction, systemic lupus erythematosus, malnutrition, pregnant woman, and supplemented folic acid and vitamin B_{12}. Participating subjects were explained their participation rights and written informed consent was

obtained, and were asked about excessive drinking (yes or no, it was defined as yes at least once a week and drinking over 45 degrees of alcohol more than 200 mL) and smoking habits (yes or no, non-smokers including never smoking and stop smoking more than one year).

Hypercholesterolemia and hypertriglyceridemia were defined as TC ≥ 6.22 mmol/L and TG ≥ 2.26 mmol/L, respectively. According to 2007 China Adult Dyslipidemia Prevention Guide. Diabetes mellitus was diagnosed when patients' fasting plasma glucose (FPG) ≥ 7.0 mmol/L Hypertension was diagnosed when patients' systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg. Overweight and obesity were defined as BMI (24.0-27.9) kg/m^2, and ≥ 28 kg/m^2, respectively, according to 2006 Guidelines for Prevention and Control of Overweight and Obesity in Chinese Adults.

The concentrations of Hcy, TC, TG, HDL-C, LDL-C and FPG were measured, respectively, by Hitachi 7600 Automatic Biochemistry Analyzer (Hitachi High-Tech Instruments Co., Ltd., Japan). The levels of folic acid and vitamin B_{12} were quantified by ACCESS 2 Immunoassay System (Beckman Coulter, Inc., USA). The references intervals of Hcy, folic acid and vitamin B_{12} were (5-15) μmol/L, ≥ 6.8 nmol/L and (133-675) pmol/L, respectively.

Statistical Analysis

The data were analyzed by using the statistical package for social science SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). Normal distribution variables were expressed as mean ± SD, which of more than two samples were compared with the One-Way ANOVA, and the differences between two groups were compared by using the SNK-q test when $p < 0.05$. Categorical variables were expressed as percentage and compared by χ^2-test. The correlation study between Hcy and folic acid as well as vitamin B_{12} were performed on the measured data by using Pearson correlation a coefficient. A p-value < 0.05 was considered as significant.

Results

Comparison of principal characteristics between high Hcy and normal-low Hcy levels in CAD patients

Compared to normal-low Hcy group, High Hcy group were characterized by smoking, Diabetes mellitus, hypercholesterolemia and hypertriglyceridemia. There were no significant differences in the ratio of elder age, male, excessive drinking, hypertension, BMI>24 kg/m^2 between high Hcy and normal-low Hcy groups. The comparison of principal characteristics between high Hcy and normal-low Hcy levels in CAD patients (Table 1).

	High Hcy group	Normal-low Hcy group	χ^2	p value
Elder age ≥ 61 (%)	61.47	50.82	2.27	0.13
Male (%)	71.00	63.93	1.14	0.29
Smoking (%)	29.87	14.75	5.63	0.02
Excessive drinking (%)	39.39	36.07	0.23	0.64
Hypertension (%)	63.20	55.74	1.14	0.29
Diabetes mellitus (%)	30.30	14.75	5.91	0.02
Hypercholesterolemia (%)	46.32	24.59	9.37	< 0.01
Hypertriglyceridemia (%)	30.74	16.39	4.95	0.03
BMI>24 kg/m^2 (%)	40.26	44.26	0.32	0.57

Table 1: Comparison of principal characteristics between high Hcy and normal-low Hcy levels in CAD patients.

Comparison of Hcy, folic acid and vitamin B_{12} between CAD and non-CAD groups

AMI patients had the highest level of Hcy, and UAP patients were a little lower than AMI but were the second highest. Moreover, SAP patients had the third higher level of Hcy, whose were significantly higher than controls ($p<0.01$). However, the levels of folic acid and vitamin B_{12} had opposite results, whose in AMI group had the lowest, while in controlled group had the highest. The comparison of Hcy, folic acid and vitamin B_{12} between AMI, UAP, SAP groups and controls (Table 2).

Groups	n	Hcy (μmol/L)	Vitamin B_{12} (pmol/L)
Controls	100	10.81 ± 4.62	222.34 ± 62.58
Stable angina pectoris	103	18.63 ± 6.73 a	167.52 ± 56.25 b
Unstable angina pectoris	116	22.62 ± 6.37 ac	148.65 ± 62.51 bd
Acute myocardial infarction	73	23.44 ± 5.78 ac	144.57 ± 52.24 bd
p		<0.001	<0.001

Table 2: Comparison of Hcy, folic acid and vitamin B_{12} between AMI, UAP, SAP patients and controls.

Prevalence of high Hcy, and low folic acid and vitamin B_{12} in CAD patients

Approximately four-fifths of CAD patients (79.11%) had a prevalence of high Hcy. However, the levels of folic acid and vitamin B_{12} in CAD patients were reduced, the prevalence of low folic acid and vitamin B_{12} were 41.78% for folic acid, and 34.93% for vitamin B_{12}, respectively. The prevalence of high Hcy, and low folic acid and vitamin B_{12} in CAD patients (Figure 1).

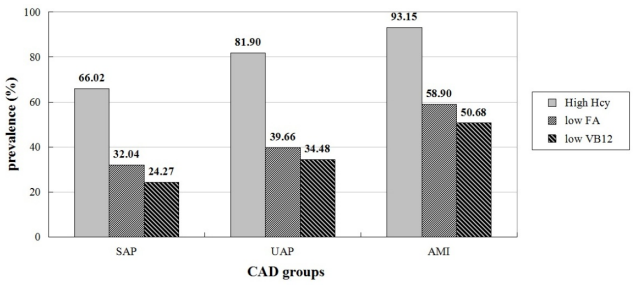

Figure 1: Prevalence of high Hcy, and low folic acid and vitamin B_{12} in 292 CAD patients aged 36-85 years.

There was a significant trend toward an increase in the prevalence of high Hcy from SAP to AMI (χ^2 = 19.93, p < 0.01). The prevalence of high Hcy progressively increased from 66.02% in SAP group to 81.90% in UAP group, and to 93.15% in AMI group. Similarly, low folic acid and vitamin B_{12} also had significant trend toward rise in the prevalence from SAP to AMI (χ^2 = 13.03 and χ^2 = 13.13, respectively, both p<0.01). The prevalence of low folic acid and vitamin B_{12} progressively increased from 32.04% and 24.27% in SAP group to 39.66% and 34.48% in UAP group, and to 58.90% and 50.68% in AMI group, respectively.

Comparison of prevalence of low folic acid and vitamin B_{12} between high Hcy group and normal-low Hcy group in CAD patient and the correlations between Hcy and folic acid and vitamin B_{12}

More than half of the CAD patients (51.08%) with high Hcy had low folic acid levels, 7 times higher than that (6.56%) in CAD patients with normal-low Hcy concentrations (χ^2=39.33, p<0.001), and 41.99% CAD patients with high Hcy had low vitamin B_{12} levels, 5 times higher than that (8.20%) in CAD patients with normal-low Hcy concentrations (χ^2=24.25, p<0.001). There were strongly moderate negative correlations between Hcy and folic acid (r=-0.67, p<0.001) and vitamin B_{12} (r=-0.56, p<0.001).

Discussion

Vascular endothelium has important regulatory functions in the cardiovascular system and a pivotal role in regulating blood flow, mediating vasodilatation, coagulation reactions, platelet activation, leukocyte adhesion, and vascular muscle function [15]. Current knowledge suggests that endothelial dysfunction, as an impairment of endothelium-dependent relaxation of blood vessels, occur as the initial event in the pathogenesis of atherosclerosis, which considered to be the initiating factor and the key point of cardiovascular disease [16,17]. A major mechanism of endothelial dysfunction appears to be related to decreased bioavailability of endothelium-derived nitric oxide (NO). NO as a most important mediator of endothelium-dependent relaxation, plays a key role in normal vascular physiology in preserving the vessel wall in a quiescent state by inhibition of inflammation, thrombosis, and cellular proliferation. This gas is a potent vasodilator to activate soluble guanylyl cyclase in vascular muscle, resulting in accumulation of cGMP and relaxation, which is produced from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of several cofactors [18].

During the last two decades, overwhelming clinical and epidemiological studies and animal experiments has been actively exploring the roles and mechanisms of Hcy in atherosclerosis with endothelial dysfunction [19,20]. HHcy may lead to increase of the level of ADMA which has been identified as a potential risk factor for atherosclerosis with endothelial dysfunction. Elevated ADMA can results in decreasing NO concentration and bioavailability [21]. Extensive experimental evidence, both in vitro and in vivo, indicates that Hcy is an independent risk factor for cardiovascular disease and elevated serum Hcy level is associated with CAD events [22-24]. Homocysteine Studies Collaboration research revealed that elevated approximately 3 μmol/L Hcy will increase about 10% risk of cardiovascular events [25]. Humphrey et al. [26] analyzed has also demonstrated that increased 5 μmol/L Hcy concentration will increase approximately 20% risk of CAD events. In present study, AMI patients had the highest level of Hcy with (23.44 ± 5.78) μmol/L, and UAP patients were a little lower than AMI but were the second highest with (22.62 ± 6.37) μmol/L, SAP patients had the third higher level of Hcy with (18.63 ± 6.73) μmol/L, which were significantly higher than that of controls with (10.81 ± 4.62) μmol/L (p < 0.01). Compared to SAP patients, patients with AMI and UAP had higher Hcy levels with approximately average elevated (4-5) μmol/L, while SAP patients were higher Hcy with approximately 8 μmol/L than controls. There was a significant trend toward an increase in the prevalence of HHcy from SAP to AMI (p<0.01). The prevalence of HHcy progressively increased from 66.02% in SAP group to 81.90% in UAP group, and to 93.15% in AMI group. The present study has provided the valuable evidence that high Hcy levels are closely associated with the categories of CAD. The more serious patients with CAD suffer, the more higher concentration their Hcy have.

Folic acid and vitamin B_{12} as two vital regulators play an important role in regulating the metabolic process of Hcy [27,28]. Folic acid and vitamin B_{12} deficiency would result in elevated level of Hcy [29,30]. In biological cells, Hcy is derived from methionine after its utilization as a methyl group donor in biological methylation reactions. However, approximately 50% Hcy is produced to remethylate back to methionine by the transmethylation of methionine, while 50% Hcy metabolize via transsulfuration to cystathionine [31]. In this cycle, methionine is activated by condensation with ATP to give the methyl donor, S-adenosylmethionine (SAM). SAM is transformed into S-adenosylhomocysteine (SAH) by donating its methyl group to the substrates of methylation reactions. Subsequently, SAH gives rise to Hcy in a reversible reaction that favors SAH over Hcy production [32]. Methyl-tetra-hydrofolic acid (MTHF) which derivate of folic acid provide methyl to remethylated of Hcy. Vitamin B_{12} is agon of methionine synthetase that catalyzed this reaction and participate transfusion of methyl [19]. Folic acid deficiency will prevent remethylation of Hcy because of raw material deficiency. Moreover, Folic acid deficiency will also influence the production of MTHF through to affect activity of methylene tetrahydrofolate reductase (MTHFR) [33,34]. Vitamin B_{12} deficiency will also result in HHcy because methyl of MTHF can't transfer, which influence production of methionine and regeneration of tetrahydro-folic acid (THF) [35].

Guo et al. [36] measured plasma levels of Hcy, folic acid and vitamin B_{12} levels in 152 patients with > or = 3 risk factors of CAD and 136 patients with 1-2 risk factors and 48 individuals with no risk factors, found that folic acid supplementation may be useful in reducing plasma Hcy level in high risk patients with hyperhomocysteinaemia. Cheng et al. [37] investigated also demonstrated that supplement folic acid, vitamin B_{12} in patients with HHcy could reduce Hcy levels. Our study revealed that the levels of folic acid and vitamin B_{12} in AMI and in UAP patients were obviously lower compared to those in SAP and non-CAD patients (p<0.05). The prevalence of low folic acid and vitamin B_{12} progressively increased from 32.04% and 24.27% in SAP group to 39.66% and 34.48% in UAP group, and to 58.90% and 50.68% in AMI group, respectively. 51.08% CAD patients with HHcy had low folic acid levels, 7 times higher than that (6.56%) in CAD patients with normal-low Hcy concentrations (p<0.001), and 41.99% CAD patients with HHcy had low vitamin B_{12} levels, 5 times higher than that (8.20%) in CAD patients with normal-low Hcy concentrations (p<0.001). There were strongly moderate negative correlations between Hcy and folic acid (r=-0.67, p<0.001) and vitamin B_{12} (r=-0.56, p<0.001). Our results confirmed that serum folic acid and vitamin B_{12} influence Hcy metabolism as cosubstrate and cofactor, respectively,

and low status of folic acid and vitamin B_{12} are closely associated with High Hcy levels.

Limitations

Since present study was just an investigation that the association between Hcy levels and the categories of CAD, and the correlation of Hcy with folic acid and vitamin B_{12}. The larger sample number of multicenter study and longer prospective investigation are necessary to further observe serum Hcy changes and incidence of adverse cardiovascular events by supplementation of folic acid and vitamin B_{12} in CAD patients.

Conclusion

The present study confirmed that HHcy and traditional cardiovascular risk factors may be synergistically prompt the formation and development of atherosclerosis in CAD patients. High Hcy levels are closely associated with the categories of CAD as well as low status of folic acid and vitamin B_{12}.

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