

BMJ Open Association of prehypertension and hyperhomocysteinemia with subclinical atherosclerosis in asymptomatic Chinese: a cross-sectional study

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ABSTRACT

Objectives Comorbid hypertension and hyperhomocysteinemia is an important risk factor for carotid atherosclerotic plaque formation. We put forward the hypothesis that the subjects with comorbid prehypertension and hyperhomocysteinemia also had an increased risk of subclinical atherosclerosis, using carotid intima–media thickness (CIMT) as the marker of the atherosclerotic process.

Methods A total of 4102 asymptomatic Chinese subjects aged 18–60 years were divided into four groups according to blood pressure (BP) and homocysteine (HCY) level: the control group without prehypertension or hyperhomocysteinemia, isolated prehypertension group, simple hyperhomocysteinemia group and prehypertension with hyperhomocysteinemia group. Serum lipids, fasting blood glucose (FBG), HCY and CIMT were measured.

Results There was significant difference in the positive rates of increased CIMT among four groups. Compared with the controls, the subjects in the other three groups had a higher risk of increased CIMT (isolated prehypertension group, OR 2.049, 95% CI 1.525 to 2.754; simple hyperhomocysteinemia group, OR 2.145, 95% CI 1.472 to 3.125; prehypertension and hyperhomocysteinemia group, OR 3.199, 95% CI 2.362 to 4.332). However, by multiple logistic regression analysis, only comorbid prehypertension and hyperhomocysteinemia was independently associated with increased CIMT (OR 1.485, 95% CI 1.047 to 2.108, $P < 0.05$).

Conclusions Comorbid prehypertension and hyperhomocysteinemia was an independent risk factor of subclinical atherosclerosis in asymptomatic Chinese, but isolated prehypertension or hyperhomocysteinemia was not. Therefore, combined intervention for prehypertension and hyperhomocysteinemia may contribute to decrease the incident of cardiovascular disease.

INTRODUCTION

Atherosclerosis is a complex chronic disease related with a variety of risk factors, which play an important role in the generation of cardiovascular disease.¹ Many studies have identified the risk factors of atherosclerosis, including smoking, adiposity, blood pressure (BP), blood cholesterol, diabetes mellitus

Strengths and limitations of this study

- This is the first study to focus on the association of comorbid prehypertension and hyperhomocysteinemia with subclinical atherosclerosis in China.
- Many confounders including traditional risk factors, metabolism confounders, demographic factors and lifestyle habits have been taken into consideration and adjusted to control bias.
- Some definitions (hyperhomocysteinemia and increased carotid intima–media thickness) were according to Chinese guidelines which might be different from other countries.
- Because of the cross-sectional study design, the speculation regarding causality is limited and requires further research.

and hyperhomocysteinemia.² Preventive interventions according to these risk factors in subclinical stage contribute to the declines in incident cardiovascular events.³ Carotid intima–media thickness (CIMT) detected by ultrasonography is a reliable marker of subclinical atherosclerosis.⁴

Many studies were conducted to investigate the association between hyperhomocysteinemia and subclinical atherosclerosis in general population. Several studies suggested hyperhomocysteinemia was not correlated with subclinical atherosclerosis.^{5–7} A cohort of 1111 subjects survey showed inconsistent results which revealed that mild hyperhomocysteinemia was an independent risk factor for increased carotid artery wall thickness.⁸ However, it has been well established that elevated homocysteine (HCY) level was involved in progression of subclinical atherosclerosis in the patients with hypertension.^{9–11} Recent studies also suggested that comorbid hypertension and hyperhomocysteinemia showed combined effect on the generation of subclinical atherosclerosis in general population.^{12 13}

Table 1 Clinical characteristics of the normal and increased carotid intima-media thickness (CIMT) subjects

Characteristics	Normal CIMT (<0.9 mm)	Increased CIMT (≥0.9 mm)	P
Age (Year)	43±8	51±6	<0.001
SBP (mm Hg)	121±10	124±9	<0.001
DBP (mm Hg)	74±8	76±8	<0.001
BMI (kg/m ²)	23.7±3.0	24.7±2.7	<0.001
CHO (mmol/L)	4.94±0.93	5.30±0.98	<0.001
HDL (mmol/L)	1.46±0.34	1.40±0.33	0.003
LDL (mmol/L)	3.06±0.78	3.43±0.80	<0.001
TG (mmol/L)	1.68±1.11	1.88±0.97	0.001
FBG (mmol/L)	5.30±0.99	5.68±1.35	<0.001
HCY (μmol/L)	9.62±4.58	10.56±4.38	<0.001
Gender			<0.001
Male	2137	292	
Female	1557	116	
Smoking status			<0.001
Non-smoker	2784	267	
Smoker	910	141	

Continued variables were expressed as mean±SD and compared using Student's t-test; the categorical variables were expressed as number and analysed by χ^2 test.

BMI, body mass index; CHO, total cholesterol; DBP, diastolic blood pressure; FBG, fasting blood glucose; HCY, homocysteine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglycerides.

Prehypertension is prevalent in asymptomatic population. A survey based on 17 584 subjects in China suggested that the prevalence of prehypertension was about 36.0% in general population.¹⁴ It is meant that prehypertension has not progressed to hypertension in many general populations. However, few studies devoted to the probable association of comorbid prehypertension and hyperhomocysteinemia with subclinical atherosclerosis. Therefore, we investigated this association in asymptomatic Chinese, using CIMT as the marker of the atherosclerotic process.

METHODS

Subjects

The survey used a random sample of 5822 subjects. All participants were admitted to the Second Affiliated Hospital of Zhejiang University School of Medicine to have a general health examination from September 2014 to December 2015. All the subjects were asked to complete a questionnaire by similar investigators trained in inquiry methods, including gender, date of birth, occupation, marital status, smoking status, alcohol intake history, medical history and family history. After exclusion of participants with cardiovascular and cerebrovascular disease, atherosclerotic disease, cancer, hypotension, hypertension, diabetes, renal disease, hepatic disease,

thyroid disease, infectious diseases, heavy drinkers¹⁵ or any drug therapy, the final sample size was 4102 (2429 men and 1673 women: aged 18–60).

The subjects were divided into four groups according to BP and HCY level: the control group without prehypertension or hyperhomocysteinemia, isolated prehypertension group, simple hyperhomocysteinemia group and prehypertension with hyperhomocysteinemia group. An HCY level ≥ 10 μmol/L was defined as hyperhomocysteinemia.¹⁶ Occasional or smoke ≥ 1 cig/day was defined as smoker.¹⁷ Prehypertension was defined as BP between 120 and 139 mm Hg systolic or 80 and 89 mm Hg diastolic.¹⁸ Normotension was defined as BP values <120/80 mm Hg.

Physical examination and laboratory methods

The body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m²). The BP was measured in right arm, using an automated device (Omron 711, USA), with the patient in a seated position. The participant had refrained from caffeinated beverages, smoking and exercise for 30 min and had rested quietly for at least 10 min. The means of twice BP measurements (5 min between each) were recorded. In cases of a difference between the two measurements greater than 10 mm Hg, a third measurement followed.

All the subjects fasted overnight (at least 12 hours). All the blood samples were collected into 5 mL gel separator tubes (BD, USA) between 8 AM and 10 AM. The samples were centrifuged at 3000 revolutions/min immediately. Serum was subsequently isolated from the whole blood. All of the analyses including fasting blood glucose (FBG), total cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and HCY were performed with a Beckman AU5800 automatic analyser (Beckman Coulter, Tokyo, Japan) within 1 hour. The HCY was measured by an enzymatic cycling method. Axis-Shield HCY Control Kit was used as the daily quality control (intra-assay coefficient of variation <5%, interassay coefficient of variation <6%).

Carotid ultrasound measurement

The distal segment, stigma compartments of the cephalic artery and the proximal segment of the internal carotid artery were measured on both sides.¹⁹ Each blood vessel was measured in three sections within a range of 1 cm in the proximal wall and distant from the side walls. The subject was defined with increased CIMT when the CIMT ≥ 0.9 mm.²⁰

Statistics

The statistical analysis was performed using the IBM SPSS V.20.0 software package. Continued variables were expressed as the mean±SD and compared using Student's t-test. The categorical variables were analysed by χ^2 test. The OR and 95% CI were calculated by logistic regression analysis. Multivariate logistic regression

Table 2 The ORs for increased carotid intima–media thickness (CIMT) in different blood pressure and homocysteine (HCY) groups

Groups	CIMT positive (N)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Normotension with normal HCY	69 (5.4%)	1.00 (ref)	–	–	–	–	–
Normotension with hyperhomocysteinemia	52 (10.8%)	2.145 (1.472 to 3.125)	<0.001	1.00 (ref)	–	–	–
Prehypertension with normal HCY	150 (10.4%)	2.049 (1.525 to 2.754)	<0.001	0.956 (0.684 to 1.334)	>0.05	1.00 (ref)	–
Prehypertension with hyperhomocysteinemia	137 (15.3%)	3.199 (2.362 to 4.332)	<0.001	1.492 (1.061 to 2.097)	0.021	1.561 (1.218 to 2.001)	<0.001

The ORs and 95% CIs were calculated by binary logistic regression analysis.

analysis was applied to adjust the possible confounders. A value of $P < 0.05$ was considered statistically significant.

RESULTS

In 4102 health examination subjects, there were 408 (9.9%) ones detected with increased CIMT. Clinical characteristics were shown in [table 1](#). All characteristics were significantly different between subjects with and without increased CIMT. Age, systolic blood pressure, diastolic blood pressure, BMI, CHO, LDL, TG, fasting blood glucose (FBG) and HCY increased in subjects with increased CIMT ($P < 0.05$), whereas the levels of HDL showed the reverse trend ($P < 0.05$).

The subjects were divided into four groups to investigate the association of prehypertension and hyperhomocysteinemia with increased CIMT. The increased CIMT positive rate among four groups was significantly different ([table 2](#)). There were 5.4% (69/1218), 10.8% (52/428), 10.4% (150/1292) and 15.3% (137/756) in the control group without prehypertension or hyperhomocysteinemia, simple hyperhomocysteinemia group, isolated prehypertension group and comorbid prehypertension and

hyperhomocysteinemia group, respectively. The patients with comorbid prehypertension and hyperhomocysteinemia had a higher risk of increased CIMT, than isolated prehypertensive ones, with an OR of 1.561 (95% CI 1.218 to 2.001, $P < 0.05$). Compared with the controls, the OR in prehypertensive patients with hyperhomocysteinemia was 3.199 (95% CI 2.362 to 4.332, $P < 0.05$).

Based on these results, we further explored the potential independent risk factor of increased CIMT by multivariate logistic regression analysis ([table 3](#)). Only comorbid prehypertension and hyperhomocysteinemia was independently associated with increased CIMT (OR 1.485, 95% CI 1.047 to 2.108, $P < 0.05$) after adjusting for age, sex, BMI, CHO, HDL-C, LDL-C, TG, FBG and smoking; however, isolated prehypertension or hyperhomocysteinemia was not.

DISCUSSION

Our study suggested that comorbid prehypertension and hyperhomocysteinemia was an independent risk factor of subclinical atherosclerosis, but simple hyperhomocysteinemia or isolated prehypertension was not, based on the cross-sectional analysis of asymptomatic Chinese subjects. This result was consistent with the former study which demonstrated that prehypertension with hyperhomocysteinemia manifested adverse cardiometabolic risk factors, such as TG, high hs-C reactive protein and uric acid.²¹ Aggressive approach such as screening and early management of asymptomatic population with comorbid prehypertension and hyperhomocysteinemia may contribute to delay the progression to cardiovascular disease.

Prehypertension is associated with subclinical atherosclerosis, including increased coronary atherosclerosis,²² carotid brachial intima–media thickness.²³ A Swedish study demonstrated that prehypertension was associated with thickening of the intimal layer of the radial artery.²⁴ Manios *et al* found that prehypertensive patients had higher common carotid artery intima–media thickness (CCA-IMT) and left ventricular mass than their normotensive counterparts. Prehypertension status seemed to cross sectionally associated with subclinical atherosclerosis

Table 3 Multivariate logistic regression analysis for increased carotid intima–media thickness (CIMT) among four subject groups

	β	OR* (95% CI)	P
Normotension with normal HCY	–	1.000 (ref)	–
Normotension with hyperhomocysteinemia	0.211	1.235 (0.815 to 1.870)	0.319
Prehypertension with normal HCY	0.177	1.193 (0.864 to 1.647)	0.283
Prehypertension with hyperhomocysteinemia	0.391	1.479 (1.042 to 2.099)	0.028

*Adjusted for age, sex, BMI, CHO, HDL-C, LDL-C, TG, FBG and smoking.
 BMI, body mass index; CHO, total cholesterol; FBG, fasting blood glucose; HCY, homocysteine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

and target-organ damage.²⁵ However, a recent study suggested that actual prehypertensive and normotensive subjects did not differ significantly in terms of CCA-IMT values.²⁶ In our study, there was no significant difference in the increased CIMT positive rate between prehypertensives subjects and normotensive ones after adjustment of confounders.

HCY is an acceptable risk factor for cardiovascular disease. Several studies suggested that elevated plasma HCY levels were related to increased CIMT.^{27,28} However, another study of 187 patients with cardiovascular disease in Germany did not find a positive association between HCY levels and CIMT, which was consistent with our results.²⁹ In our study, hyperhomocysteinemia was not an independently risk factor of subclinical atherosclerosis. There was no significant difference of increased CIMT positive rate between simple hyperhomocysteinemia group and control group.

Cardiovascular diseases have a complex aetiology. Age, dyslipidaemia and diabetes are established traditional risk factors for cardiovascular diseases. These risk factors interact and make progression from subclinical atherosclerosis to cardiovascular diseases. In our study, we found that isolated prehypertension or simple hyperhomocysteinemia was not independent risk factors for subclinical atherosclerosis; comorbid prehypertension and hyperhomocysteinemia seemed to synergistically aggravate the development of subclinical atherosclerosis. That might be related to endothelium dysfunction and inflammation. It is well known that hyperhomocysteinemia plays an important role in the pathogenesis of atherosclerosis, which could induce oxidative stress, endothelium dysfunction and inflammation.³⁰ Hu *et al* also found that structure and function impairments of carotid artery were more serious and inflammatory factors were significantly higher in the patients with H-type hypertension (comorbid hypertension and hyperhomocysteinemia) than non-H-type hypertensive ones.³¹ However, the clear mechanisms need further study. Early identification of apparently healthy individuals with comorbid prehypertension and hyperhomocysteinemia and control of related factors may help delay the development of subclinical atherosclerosis to clinical atherosclerosis and cardiovascular events.

There are some limitations to our study. Some definitions (hyperhomocysteinemia and increased CIMT) were according to Chinese guidelines which might be different among institutions and countries. Other confounders, such as former smoker, which we did not take them into consideration, might be a limitation. Because of the cross-sectional study design, the speculation regarding causality is limited and requires further research.

CONCLUSION

Our study suggested that comorbid prehypertension and hyperhomocysteinemia was an independent risk factor of subclinical atherosclerosis in asymptomatic Chinese,

but isolated prehypertension or hyperhomocysteinemia was not. Therefore, combined intervention for prehypertension and hyperhomocysteinemia may contribute to decrease the incident of cardiovascular disease.

Contributors BL participated in the data collection and drafted the manuscript. XD participated in the data collection. GQ and ZC participated in the design of the study and undertook statistical analyses. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Competing interests None declared.

Patient consent Obtained.

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