Original Paper

Association Between Plasma Homocysteine and Microalbuminuria in Untreated Patients with Essential Hypertension: a Case-Control Study

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Key Words

Homocysteine • Microalbuminuria • Essential hypertension • Case-control

Abstract

Background/Aims: Kidney function is critical for homocysteine (Hcy) clearance, and plasma Hcy levels are frequently increased in patients with renal failure. Microalbuminuria (MAU) is an important marker of early renal damage caused by hypertension. At present, there is insufficient evidence on the relationship between Hcy and microalbuminuria. **Methods:** This is a 1:2 matched, hospital-based case-control study. At initial visit, out of 1535 outpatients with no prior history of medication, 450 qualified subjects were selected based on inclusion and exclusion criteria. The concentration of Hcy in the serum was evaluated using a cyclic enzyme method. MAU was defined by a urine albumin/creatinine ratio (UACR) between 30 µg/mg and 300 µg/mg. **Results:** A total of 450 patients were included in this study (150 in the MAU group and 300 in the non-MAU group). The MAU group had higher mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP), heart rate (HR) and plasma Hcy levels than did the non-MAU group. The area under the receiver operating characteristics (ROC) curves was 0.772 (95% CI: 0.724–0.819, $P < 0.001$) with a cut-off value of 15.0, and the sensitivity and specificity of Hcy in predicting the MAU status in hypertensive patients were 49.3% and 92.3%, respectively. Multiple logistic regression modelling suggested that patients with a higher Hcy level (> 15 µmol/L) were more likely to have MAU (95% CI: 5.650–16.543, $P < 0.001$). The other predictive factor for MAU was 24-h mean SBP (95% CI: 0.941–0.993, $P = 0.015$). **Conclusion:** This matched case-control study indicates that Hcy may increase the susceptibility of essential hypertensive subjects to MAU.

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Introduction

The incidence and prevalence of chronic kidney disease (CKD) are increasing, resulting in poor outcomes and high cost. In particular, 147 million Chinese people are affected by CKD [1]. Early renal damage is an early stage of renal disease; hence, early detection and intervention are clinically significant. For the above reasons, our team has continued to focus on early kidney damage [2]. Microalbuminuria (MAU) is considered to be a marker of early renal damage from hypertension, and it is associated with an increased risk of cardiovascular events [3]. Plasma homocysteine (Hcy) may play a pivotal role in the development of hypertension-induced kidney damage, as plasma Hcy levels are frequently increased in patients with renal failure [4], in addition to traditional risk factors (hypertension, diabetes mellitus and hyperlipidaemia) [5, 6].

Hyperhomocysteinemia (HHcy) is commonly observed in Chinese hypertensive individuals [7]. Previous studies have demonstrated significant association of HHcy with MAU among glomerular injuries in healthy and diabetic populations. Li et al. demonstrated that the concentration of Hcy is independently associated with the occurrence of early diabetic nephropathy in Chinese patients [8]. In addition, Ye et al. found that the prevalence of both hypertension and HHcy was high and associated with target organ damage in patients with CKD [9]. Moreover, Eun-Hee Cho et al. carried out a nested case-control study in patients with type 2 diabetes and found that HHcy was associated with an increased risk of MAU [10]. However, the relationship between Hcy and MAU in hypertensive patients is not clear.

Recently, several studies have shown that Hcy is associated with endothelial dysfunction. In particular, HHcy is an independent predictor of sub-clinical carotid artery damage in subjects with hypertension [11-13]. However, the relationship between Hcy and microalbuminuria has not yet been elucidated. The aim of the present study was to evaluate the relationship between Hcy and microalbuminuria in untreated essential hypertensive patients.

Materials and Methods

Study design

This is a 1:2 age- and sex-matched, hospital-based case-control study. All of the subjects were treated at the Department of Hypertension, Anzhen Hospital of Beijing from November 2015 to October 2016. We recruited 1535 hypertensive subjects, who had not taken any antihypertensive agents or who had stopped the medications for at least a week. After screening, 150 MAU-positive patients were identified as the case group; 300 hypertensive subjects without microalbuminuria (controls) were frequency matched to the cases by age (within 2 years) and sex (Fig. 1). All of the participants provided signed written informed consent before being interviewed, and the ethical committees of Anzhen Hospital approved the study.

Case and control identification

The eligible cases had microalbuminuria, were ≥ 18 years old, were diagnosed with essential hypertension and had not taken any antihypertensive agents or stopped the medications for at least two weeks. Patients were excluded if any one of the following conditions was present: kidney disease (including a history of proteinuria, haematuria, serum creatinine greater than 1.3 mg/dl in men and 1.2 mg/dl in women and positive urine culture [14]); diabetes or abnormal glucose tolerance; congestive heart failure or a major concurrent illness, such as cancer or end-stage pulmonary or liver disease; pregnancy; secondary hypertension; and use of folic acid, vitamin B6 or vitamin B12.

For each subject with MAU, we selected two essential hypertensive subjects as matched controls according to age (within 2 years) and sex. Hypertension was defined as ambulatory BP displayed as 24-h SBP ≥ 130 mm Hg or 24-h DBP ≥ 80 mm Hg [15]. This study used the urine microalbumin/urine creatinine ratio (UACR), which is commonly used in clinical studies and is easy to detect. Microalbuminuria was defined by a UACR between 30 $\mu\text{g}/\text{mg}$ and 300 $\mu\text{g}/\text{mg}$ [16].

Fig. 1. Flowchart representing patient selection.

24h ambulatory blood pressure

ABPM was performed during the participants' normal daily routine with a validated and calibrated Space Labs 90207 ABPM monitor (Space Labs Inc., Issaquah, Washington). Measurements were performed between 8 and 12 AM, and the same device was used at each visit. The BP cuff was adjusted to arm circumference and placed on the nondominant arm. Systolic and diastolic BP were automatically measured every 20 minutes between 06:00 and 21:59 and every 30 minutes at night (22:00 to 05:59) for 24 consecutive hours, with the screen turned off to blind the subjects to the BP readings. Bed and awakening times were recorded in a diary. ABPM was considered valid if the recording over 24 h was at least 70% of the expected values; had at least 20 valid awake and seven valid asleep measurements; and had at least two valid daytime and one valid night-time measurement per hour [17].

Laboratory analyses

After a 12-h fasting period, venous blood samples were collected from all patients and placed into tubes containing clot activator (to obtain serum). Clinical and standard biochemical analyses were performed immediately (within 30 minutes) after blood collection. Serum creatinine (Scr) level was measured on a Roche/Hitachi Modular System P (Roche Diagnostics GmbH, Mannheim, Germany) using the compensated rate-blanked Jaffe assay. The concentration of Hcy was determined by the cyclic enzyme method using a Hcy assay kit (DiaSys Diagnostic Systems, Shanghai, China). S-adenosine Hcy levels were measured using a small molecule capture technology (SMT). Hcy is converted to its free form by the reaction with a covalent substrate and cycling to generate adenosine. Adenosine is immediately hydrolysed to ammonia and hypoxanthine, and ammonia is converted to NAD by glutamate dehydrogenase to reduced beta-nicotinamide adenine dinucleotide (NADH). The concentration Hcy in the sample is proportional to the conversion of NADH to

NADH. The kit uses a cyclic enzyme method that does not interfere with cystathionase and has a detection range of 1–50 $\mu\text{mol/L}$. In addition, fasting blood glucose level, uric acid level and fasting serum lipid status including total cholesterol (TC), triglyceride, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were also recorded for each participant.

For calculating the UACR, urine samples were collected from study subjects at the first urine void in the morning. Urine albumin concentration was measured using the immunoturbidimetric method, and urinary creatinine was estimated by automated enzymatic assay. Urinary albumin excretion was represented by the urinary albumin creatinine ratio (UACR).

Statistical analysis

Data are presented as the mean values and standard deviations for continuous variables and as numbers (percentage) for the categorical variables. Hcy was measured as a continuous variable. Differences between quantitative variables were analysed using Student's *t* test. Differences between qualitative variables were analysed using the Chi-squared test. Pearson's correlation coefficients were calculated if appropriate. Multiple logistic regression analyses were used to calculate the odds ratio of independent variables. The receiver operating characteristics (ROC) curves demonstrated the relationship between Hcy and MAU.

All of the probability values for the statistical significance tests were 2-tailed, and all of the CIs were estimated at 95%. All analyses were performed with R (<http://www.R-project.org>) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc. Boston, MA) [18].

Results

General characteristics of the study subjects

The total sample size was 450, with 150 hypertensive subjects with MAU (cases) and 300 hypertensive subjects without MAU (controls). Cases and controls were matched by age and sex. There were no differences in age (case, 43.3 ± 10.7 vs. controls, 43.8 ± 10.2) and sex between the two groups. The baseline characteristics are shown in Table 1. Compared with the controls, the cases were more likely to have a higher 24-h mean SBP (139.8 ± 12.7 vs 133.6 ± 10.3 , $P < 0.001$) and 24-h mean DBP (91.4 ± 10.6 vs 87.2 ± 7.7 , $P < 0.001$). Notably, cases had higher Hcy levels than did the controls (17.9 ± 8.7 vs 12.6 ± 5.0 , $P < 0.001$) (Table 1).

Correlations between Hcy and other variables in hypertensive patients

Pearson's correlation analysis showed that serum Hcy level was positively correlated with sex ($r = 0.1990$, $P < 0.001$, Table 2), 24-h mean SBP ($r = 0.2234$, $P < 0.001$), 24-h mean DBP ($r = 0.2348$, $P < 0.001$) and Scr level ($r = 0.1819$, $P < 0.001$). Negative relationship was observed between Hcy level and age ($r = -0.1549$, $P = 0.001$). The other variables demonstrated no association with Hcy (Table 2).

Multiple logistic regression analysis for MAU

Receiver operating characteristics (ROC) curves were used to analyse the relationship between MAU status and Hcy in hypertensive patients. The area under the curve was 0.772 (95% CI: 0.724–0.819, $P < 0.001$) with a cut-off value of 15.0 $\mu\text{mol/L}$, which suggested that the MAU status could be predicted by the Hcy level in hypertensive patients with a specificity of 92.3% and a sensitivity of 49.3% (Fig. 2). Based on the cut-off value of Hcy, the subjects were divided into two groups: group 1 (Hcy ≤ 15.0 $\mu\text{mol/L}$) and group 2 (Hcy > 15.0 $\mu\text{mol/L}$). A multivariable logistic regression model was constructed to assess the relationship between MAU and Hcy, after controlling for serum creatinine level, SBP, and DBP. The results suggested that patients with a higher Hcy level (> 15.0 $\mu\text{mol/L}$) had a higher risk of MAU [(group 2 OR = 9.667, 95% CI: 5.650–16.543, $P < 0.001$)] than the patients with a lower Hcy. The other predictive factor for MAU was the 24-h mean SBP (OR = 0.967, 95% CI: 0.941–0.993, $P = 0.015$) (Table 3).

Table 1. Clinical characteristics of hypertensive patients with and without MAU. SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; LDL: low density lipoprotein; ALT: alanine aminotransferase; AST: aspartate transaminase

	microalbuminuria		
Parameters	Controls (n=300)	Cases (n=150)	p Value
Demographic			
Age, y	43.8 ± 10.2	43.3 ± 10.7	0.647
Sex			1.000
Male	184 (61.3%)	92 (61.3%)	
Female	116 (38.7%)	58 (38.7%)	
Clinical Indexes			
24h Mean SBP, mmHg	133.6 ± 10.3	139.8 ± 12.7	<0.001
24h Mean DBP, mmHg	87.2 ± 7.7	91.4 ± 10.6	<0.001
24h Mean HR, bpm	74.3 ± 8.4	75.4 ± 8.6	0.170
Triglyceride, mmol/dL	1.8 ± 1.2	1.9 ± 1.1	0.269
LDL-cholesterol, mmol/dL	3.1 ± 0.8	3.1 ± 0.7	0.791
Total cholesterol, mmol/dL	5.0 ± 1.0	5.0 ± 0.9	0.904
Fasting glucose, mmol/dL	5.7 ± 1.0	5.8 ± 1.3	0.281
ALT, mmol/L	31.0 ± 23.3	30.7 ± 22.5	0.888
AST, mmol/L	25.4 ± 11.4	25.9 ± 11.8	0.660
Homocysteine, μmol/L	10.6 ± 3.2	16.8 ± 9.2	<0.001
Renal function index			
Urea, mmol/L	4.7 ± 1.2	4.8 ± 1.1	0.349
Uric acid, μmol/L	346.8 ± 83.5	361.6 ± 78.2	0.071
Serum Creatinine,μmol/L	69.1 ± 13.9	70.4 ± 14.2	0.343
Microalbuminuria, μg /mg	12.4±8.2	73.3±58.4	<0.001

Table 2. Correlation coefficients of relation between Hcy and some parameters. SBP: systolic blood pressure; DBP: diastolic blood pressure

Parameters	Correlation coefficients	p
Age	-0.1549	0.001
Sex	0.1990	< 0.001
24h Mean SBP	0.2234	< 0.001
24h Mean DBP	0.2348	< 0.001
Serum Creatinine	0.1819	< 0.001
Fasting glucose	0.0174	0.7128
Triglyceride	0.0265	0.576
Total cholesterol	-0.0397	0.4021
Microalbuminuria	0.372	< 0.001

Fig. 2. Receiver operating characteristic curve for the prediction of microalbuminuria (MAU) based on plasma homocysteine levels in untreated essential hypertensive patients.

Table 3. Multivariable logistic regression analysis of prediction of MAU. SBP: systolic blood pressure; DBP: diastolic blood pressure; Hcy: homocysteine

Variable	Odds ratio	95% confidence interval	P
Serum creatine	1.007	0.989 to 1.026	0.440
24h mean SBP	0.967	0.941 to 0.993	0.015
Hcy group 1	1.00	—	
Hcy group 2	9.667	5.650 to 16.543	< 0.001
Constant	138.613	-	0.002

Discussion

In this age- and sex-matched, hospital-based case-control study, we evaluated the relationship between Hcy level and MAU in untreated hypertensive patients. We found that serum Hcy > 15 μmol/L was positively associated with MAU. These findings highlight the value of using HHcy for predicting MAU in essential hypertensive subjects.

In terms of the general characteristics of hypertensive patients, the case group had higher levels of Hcy, 24-h mean SBP and 24-h mean DBP. The present findings indicated that moderately elevated levels of Hcy were significantly associated with MAU among Chinese essential hypertensive subjects. This relationship did not change after adjusting for serum creatinine levels, 24-h mean SBP and 24-h mean DBP. Jager A et al [19] conducted a population-based cohort study and found that a 5 μmol/L increase in the Hcy level was associated with an increased risk of microalbuminuria (OR 1.38, 95% CI: 0.97 to 1.95) after a mean follow-up of 6.1 years. However, the study was conducted in subjects without diabetes and in non-insulin-dependent patients with diabetes mellitus. In our study, the same results were obtained in Chinese patients with essential hypertension. Recently, a prospective cohort study demonstrated that plasma Hcy levels were associated with a rapid decline in renal function and incidence of CKD in a hypertensive population. These results indicated that an elevated plasma Hcy level is a risk factor for renal dysfunction and CKD incidence in a hypertensive population [20].

In addition, in the present study, we found that the cut-off value of Hcy is 15.0 $\mu\text{mol/L}$ based on ROC curves, with the specificity and sensitivity of Hcy levels (when Hcy > 15 $\mu\text{mol/L}$) in predicting MAU at 92.3% and 49.3%, respectively. These results are consistent with the CSPPT study, which suggested that the average Hcy level in hypertensive patients in China is approximately 15 $\mu\text{mol/L}$, and this value is significantly higher than that in healthy individuals [21]. MAU has been shown to be a marker of early renal damage in patients with hypertension. Although urine collection over 24 h is still considered a gold standard to assess urine albumin excretion, it is inconvenient to execute. In recent reports, MAU was determined based on the albumin to creatinine ratio in the first urine void in the morning, preferably from two non-consecutive urine collections. Due to the low cost and convenience, the determination of MAU using this method has been shown to be sufficiently accurate and has been widely adopted in clinical practice [22]. The prevalence of microalbuminuria has been reported to vary from 5% to 30% in patients with primary hypertension [23], with higher values in the presence of diabetes [24]. Similarly, previous reports have shown that Hcy and microalbuminuria are closely related in patients with diabetes [25]. The above evidence may explain how HHcy is associated with increased MAU susceptibility.

In recent years, there have been many studies on the function of Hcy in cardiovascular and cerebrovascular diseases. The precise mechanism by which Hcy affects the susceptibility to MAU may be explained as follows: Hcy exerts toxic effects on vascular endothelial cells, leading to vascular endothelial dysfunction [1]. In addition, Hcy can be easily oxidized into free radicals in the blood, which can damage vascular endothelial cells. This can affect the functions of the kidney endothelial and glomerular basement membrane cells; therefore, changes in charge selectivity and pore size in the glomerular filtration membrane can result in the accumulation of proteins in urine. Microalbuminuria is considered a marker of endothelial dysfunction, and it usually results from damage to glomerular endothelial cells. There is also increasing evidence that epigenetic dysregulation (including DNA methylation) plays a significant role in the development of MAU.

The present study has several limitations that should be addressed. First, because it is a case-control study, we cannot establish causal relation between plasma Hcy level and microalbuminuria. Second, there may be a selection biases because of the small sample size and the case-control study design. Finally, the traditional risk factors that we evaluated were not comprehensive and did not include factors such as age of onset of hypertension, smoking, and family history, all of which are associated with Hcy level or microalbuminuria. The main strength of this study is that patients undergoing antihypertensive treatment were not included, which eliminated the influence of antihypertensive treatment on MAU. Thus, for the first time, this study demonstrates the relationship between Hcy and microalbuminuria in patients diagnosed with essential hypertension.

Conclusion

Our findings suggested that plasma Hcy is associated with MAU in Chinese untreated patients with essential hypertension. However, this is not a prospective study, and thus we cannot demonstrate a causative relationship between Hcy levels and MAU susceptibility. More studies in different populations with sufficient sample sizes are needed to shed light into the relationship between Hcy and early kidney damage.

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Disclosure Statement

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