Original Article

Elevated total plasma homocysteine levels are associated with type 2 diabetes in women with hypertension

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Background and Objectives: There is only limited available evidence of a relationship between total plasma homocysteine (tHcy) levels and type 2 diabetes in hypertensive subjects. **Methods and Study Design:** A total of 5,935 Chinese essentially hypertensive subjects were recruited by cluster sampling from 60 communities. The cases had diabetes, whereas the controls did not. Anthropometric indices and biochemical parameters were assessed using standard procedures. A multivariable analysis was performed to analyze the association of tHcy and type 2 diabetes susceptibility in hypertensive subjects. **Results:** The 5,241 controls (women/men: 2,716/2,625) and 594 cases (women/men: 291/303) were recruited consecutively. The level of tHcy was dose- dependently associated with type 2 diabetes in the hypertensive women subjects. After controlling for corresponding confounding factors, a significant trend was only noted in the women subjects, with odds ratios per 5 μmol/L tHcy of 1.11 (95% confidence interval (CI), 1.07-1.16) in the crude model, 1.05 (95% CI, 1.01-1.11) in model 1, and 1.07 (95% CI, 1.02-1.13) in model 2. However, no significant result was found for levels of tHcy ≥15 μmol/L vs <15 μmol/L in the men, women and all hypertensive subjects. **Conclusions:** When the level of tHcy was divided into quartiles, tHcy was positively associated with type 2 diabetes in hypertensive women subjects. However, when the level of tHcy was separated into hyperhomocysteinemic (≥15 μmol/L) and normal (<15 μmol/L), no significant results were observed.

Key Words: tHcy, type 2 diabetes, hypertension, interaction, population-based study

INTRODUCTION

Homocysteine is a sulfur-containing non-protein amino acid and an intermediate in the metabolism of methionine.[1] Many epidemiologic studies have suggested that elevated homocysteine levels are strong and independent predictors of cardiovascular disease (CVD).[2] As hypertension and type 2 diabetes are known to be major risk factors for CVD,[3] the association of high homocysteine levels with diabetes and hypertension may confer a synergistic risk for CVD. It has also been reported that total plasma homocysteine (tHcy) levels contribute to hypertension,[4] and elevated tHcy levels have been found to be related to hypertension in patients with diabetes.[5]

Some studies have suggested a significant association between homocysteine and the pathophysiology of diabetes.[6] Homocysteinemia may play an etiologic role in the pathogenesis of type 2 diabetes by facilitating oxidative stress, systemic inflammation, and endothelial dysfunction.[7,8] However, the relationship between tHcy and type 2 diabetes remains unclear in hypertensive subjects, who may represent a high-risk group for the development of the disease. We thus hypothesized that the association of a high level of homocysteine with hypertension may confer a synergistic effect for type 2 diabetes.

The aim of this study was to assess whether tHcy could be an indicator of the development of type 2 diabetes in hypertensive subjects, and to evaluate the relative association by stratification with other conventional factors. To the best of our knowledge, this is the first comprehensive study of the relationship between tHcy and type 2 diabetes in hypertensive subjects. Such interrelations may have future therapeutic implications for preventive measures.

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MATERIALS AND METHODS

Study population

This was a retrospective study. A total of 5,935 essentially hypertensive patients were recruited consecutively from 60 community health service centers in Nanshan district, Shenzhen, from April 2010 to September 2011. All of the subjects were Chinese who had lived in Shenzhen for more than 6 months, and were recruited using a two-stage sampling method. Eight sub-districts were selected in Nanshan district, and then six to eight communities were selected from each sub-district using a simple procedure according to a random sequence of computer-generated numbers. All eligible patients were recruited, and all subjects underwent standardized anthropometric measurements and laboratory tests.

The cases were hypertensive subjects with diabetes (type 2 diabetes), while the controls were hypertensive subjects without diabetes. Subjects with secondary hypertension, hemorrhagic or cardioembolic stroke, liver or renal failure (serum creatinine >1.5 mg/dL, blood urea nitrogen >30 mg/dL), cancer, pregnancy, dysthyroidism, Alzheimer's disease, a history of ischemic stroke or coronary heart disease during the previous 2 years, medical conditions or medication that could potentially influence tHcy (such as taking folic acid, vitamin B-6 or vitamin B-12), or type 1 diabetes were excluded from the study. All subjects gave their written informed consent, and the study was conducted in accordance with the principles of the declaration of Helsinki. The study was approved by the Ethical Committee of Shenzhen Nanshan Center for Chronic Disease Control (2011001).

Baseline examinations

Systolic and diastolic blood pressures were measured on the right arm in a sitting position using a mercury sphygmomanometer, after sufficient rest in the morning. Blood pressure was estimated manually and repeated twice, and the three values were averaged for the final reading. Height (no shoes, nearest 0.1 cm), weight (light indoor clothes, nearest 0.1 kg), waist and hip measurements were also recorded. The body mass index (BMI) was calculated as the weight (kg)/height squared (m^2).

Hypertension was defined as high systolic blood pressure (SBP ≥140 mmHg) and/or high diastolic blood pressure (DBP ≥90 mmHg) or the prescription of antihypertensive drugs.[9] Diabetes was diagnosed according to the American Diabetes Association 2010 criteria when the fasting plasma glucose level was 126 mg/dL or higher, the 2-h plasma glucose level was 200 mg/dL or higher, or the glycated haemoglobin concentration was 6.5% or more.[10]

Biochemical measurements

Blood samples were obtained after overnight fasting. All samples were examined for levels of tHcy, glucose (Glu), total cholesterol (TC), serum triglyceride (TG), serum creatinine (Cre), uric acid (Ua), and low-density lipoprotein cholesterol (LDL). Clinical laboratory parameters were determined using routine laboratory methods. The case-control status was blinded during the assaying of blood samples.

For the analysis of tHcy, plasma was separated within 1h of sample collection and stored at -20ºC until examination. tHcy was subsequently measured using an automatic biochemical analyzer (HITACH 7080) in an enzymatic cycling assay. Hyperhomocysteinemia was defined as tHcy ≥15 μmol/L.[11]

TC, LDL, Glu, and TG were also measured using standard enzymatic methods in the automatic biochemical analyzer HITACH 7080.

The serum levels of Ua and Cre were determined quantitatively using the uricase method and the Jaffe reaction method, respectively.

Questionnaire interview

Personal and lifestyle data were collected from the medical records of the subjects and a standardized questionnaire. Information on age, gender, smoking status, alcohol drinking, physical activities, family history of hypertension, family history of diabetes, antihypertensive medication, years of hypertension, sleep, salt intake, and psychology factors was obtained.

Both leisure and occupational physical activity levels were assessed, as in previous studies.[12,13] Physical activities were classified as no exercise, and light, moderate, and vigorous intensity. Smoking status was defined as current smoker or not (never-smoker and ex-smoker groups). Alcohol drinking was defined as absent and present. The psychology factors were assessed by the severity of depression, which was defined over 50 standard points using the Zung self-rating depression scale.[14]

Statistical analysis

Continuous variables between the cases and controls were tested by the independent t-test. The chi-squared test was used to assess the difference between categorical variables. The normality of distribution for continuous variables was detected by the Kolmogorove-Smirnov test. Differences in tHcy and TG between cases and controls were skewed, therefore the Mann-Whitney U test was used.

Furthermore, tHcy was stratified separately for the men and women subjects into quartile categories [Q_1 (lowest), Q_2, Q_3, and Q_4], and quartiles of tHcy for all subjects were based on the combination of the quartiles for the men and women subjects. The odds ratio (OR) and 95% confidence intervals (CI) of tHcy quartiles (using the lowest quartile as the reference category) for the men, women, and all subjects were calculated, and the OR per 5 μmol/L increment was evaluated. We assigned the respective median value for each quartile when we tested the *p* for trend. Taking the multivariable analysis of tHcy and scientific significance into account, three separate models (crude model, model 1 and model 2) were calculated to adjust for potentially confounding factors. The crude model did not adjust for any confounding factors. Model 1 adjusted for age, gender, alcohol drinking, sleep, years of hypertension, a family history of hypertension, and family history of diabetes, while model 2 further adjusted for Glu, Cre, LDL, Ua, and TG. Statistical significance was assessed using either the Wald chi-square test or by comparing the fits of the nested models with likelihood-ratio tests. Furthermore, logistic model performance was assessed using the area under curve (AUC) by re-

ceiver operating characteristic (ROC) analysis. The AUC value was calculated when tHcy was added to the model with the median of tHcy quartiles as the cut-off.

In addition, trends in the ORs of type 2 diabetes across the quartiles of tHcy were tested by modeling tHcy categories as an ordinal variable. The interactions were then calculated by involving cross-product interaction terms in the corresponding multivariable models. We examined the joint effects of age (<60, ≥60 years), gender (women, men), alcohol drinking (no/yes), sleep (good/bad), years of hypertension (<5 vs ≥5), family history of hypertension (no/yes), family history of diabetes (no/yes), and tHcy quartiles.

All analyses were performed using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). All statistical comparisons were two-tailed and considered to be significant at the $p<0.05$ level. The multiple imputation method was applied to handle the missing data in 197 patient records.

RESULTS

Basic characteristics

Data are presented as numbers of patients (%) or mean± standard deviation. Of the 6,203 patients recruited, 5,935 completed the relevant interviews and examinations and were included in this study; 268 participants were excluded according to the exclusion criteria. The clinical characteristics and the conventional risk factors for cases and controls are summarized in Table 1.

The study population comprised 5,935 hypertensive subjects (2,928 men and 3,007 women), of whom 5341 did not have type 2 diabetes (controls) and 594 did (cases). The results are presented for the men and women subjects separately.

Table 1 provides the gender-combined and gender-specific mean values of the clinical variables. Age, BMI, weight, waist, DBP, LDL, Glu, and Cre differed significantly between cases and controls for all subjects, while age, waist, DBP, LDL, Ua, TG, Glu, and Cre differed significantly in men, and age, Ua, TG, Glu, and Cre differed statistically significant in the women subjects. It should be noted that age, Glu, and Cre were higher in cases than in controls in the men, women, and all subjects (Table 1).

Statistically positive relationships were found for smoking, alcohol drinking, salt intake, sleep, years of hypertension, antihypertensive drugs, and family history of diabetes between cases and controls in all subjects (Table 1). The outcomes of conventional risk factors in the men and women subjects were similar except for smoking, psychology factors, sleep, and antihypertensive drugs. The women subjects were more likely to be non-smokers.

Levels of tHcy in cases and controls

The level of tHcy in cases was slightly higher than that in controls in all subjects (13.5 vs 12.7, $p<0.0001$) (Table 2). As shown in Table 2, tHcy was significantly higher in cases than controls in the hypertensive women subjects (12.1 vs 11.1, $p<0.0001$). Conversely, tHcy did not differ significantly in cases versus controls in the hypertensive men subjects (14.8 vs 14.6, $p=0.33$). Interestingly, tHcy was significantly higher among the men than the women subjects (14.6 vs 11.2, $p<0.0001$).

ORs (95% CI) for quartiles of tHcy

We compared the risk factors for cases and controls to elucidate potential confounding factors, and then performed a multivariable logistic analysis of all of the significant variables. The following variables were significant in multivariable analysis: age, gender, alcohol drinking, sleep, years of hypertension, a family history of hypertension, a family history of diabetes, Glu, Cre, TG, Ua, and LDL (data not shown).

Table 3 shows the association between the quartiles of tHcy and type 2 diabetes separately for the men and women subjects. The multivariable analysis revealed a dose-dependent relation between tHcy and type 2 diabetes in the women subjects. The ORs (95% CI) across Q2, Q3, and Q4 for crude model, model 1 and model 2 are shown in Table 3, and the multivariable ORs (95% CI) for type 2 diabetes comparing the highest with the lowest quartile of tHcy were 2.04 (1.42-2.93, p trend<0.0001) in the crude model, 1.83 (1.26-2.43, p trend<0.0001) in model 1 and 1.85 (1.28-2.51, p trend<0.0001) in model 2. The OR per 5 μmol/L tHcy (95% CI) in women was 1.11 (1.07-1.16, $p<0.0001$) in the crude model, 1.05 (1.01-1.11, $p<0.0001$) in model 1, and 1.07 (1.02-1.13, $p<0.0001$) model 2 (Table 3). Multivariable analysis did not show significant associations between tHcy and type 2 diabetes in the men and all hypertensive subjects (Table 3). However, there were no significant associations in the men, women and all hypertensive subjects for tHcy ≥15 vs <15 μmol/L.

The addition of tHcy substantially improved the logistic performance (AUC of ROC) in model 1 (from 0.71 to 0.76) and model 2 (from 0.71 to 0.76) to predict the occurrence of type 2 diabetes in the hypertensive women subjects.

Joint effects of tHcy and other risk factors on type 2 diabetes

We analysed interaction between tHcy quartile and other risk factors (age, gender, alcohol drinking, sleep, years of hypertension, family history of hypertension, and family history of diabetes) on the risk for type 2 diabetes.

After multivariable adjustment, the trend across categories of tHcy was statistically significant for gender (p trend=0.02); the multivariable OR (95% CI) when the highest and the lowest quartile of tHcy were compared in the women subjects was 1.52 (1.03-2.26, p trend=0.01). No significant differences in the strength of the associations were observed among the subgroups stratified by age (<60 vs ≥60 y), alcohol drinking (no/yes), sleep (good/bad), years of hypertension (<5 vs ≥5 y), family history of hypertension (no/yes), and family history of diabetes (yes/no).

DISCUSSION

Until now, it has not been known whether tHcy is a predictor for the occurrence of type 2 diabetes in hypertensive subjects. In the present study, a multivariable analysis showed that tHcy was positively, gender-specifically associated with type 2 diabetes in the hypertensive women subjects independently of known confounding factors,

Table 1. The clinical characteristics of cases and controls

	Total hypertensive subjects					Men					Women				
	Hypertensive subjects without T2DM (controls, n=5341)		Hypertensive subjects with T2DM (cases, n=594)		*p*	Hypertensive subjects without T2DM (controls, n=2625)		Hypertensive subjects with T2DM (cases, n=303)		*p*	Hypertensive subjects without T2DM (controls, n=2716)		Hypertensive subjects with T2DM (cases, n=291)		*p*
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Age (year)	58.5	12.2	63.2	10.5	<0.0001	57.2	13.1	62.3	11.3	<0.0001	59.8	11.1	64.1	9.63	<0.0001
BMI (kg/m^2)	24.3	3.04	24.6	2.93	0.01	24.7	2.93	25	2.95	0.09	23.9	3.1	24.2	2.86	0.13
Height (cm)	162	7.86	162	7.94	0.86	168	5.86	168	6.1	0.62	157	5.16	157	5.16	0.70
Weight (kg)	64.6	10.5	65.5	10.6	0.04	70.2	9.61	70.9	9.86	0.22	59.2	8.23	59.9	8.24	0.17
Waist (cm)	86.6	9.63	87.5	9.66	0.01	88.8	9.30	89.9	9.47	0.04	84.4	9.44	85.1	9.24	0.25
Hip (cm)	95.9	8.83	96.0	9.13	0.67	96.9	8.84	97.2	8.54	0.67	94.9	8.7	94.9	9.59	0.97
SBP (mmHg)	133	15.1	133	15.0	0.17	133.7	14.7	132	14	0.13	133	15.4	133	15.9	0.62
DBP (mmHg)	83.7	10.4	82.4	11.9	0.002	85.0	10.5	83.3	11.5	0.007	82.5	10.1	81.4	12.2	0.07
TC (mmol/L)	5.1	1.02	5.03	1.11	0.08	4.91	0.95	4.84	1.11	0.21	5.29	1.06	5.22	1.08	0.29
LDL (mmol/L)	3.01	0.8	2.91	0.82	0.005	2.95	0.76	2.8	0.77	0.002	3.07	0.83	3.02	0.85	0.37
Ua (umol/L)	342	95.4	350	93.1	0.09	380	91.9	366	96.3	0.01	307	84.4	333	86.5	<0.0001
TG† (mmol/L)	1.55	1.12	1.58	1.13	0.44	1.61	1.2	1.49	1.12	0.01	1.51	1.05	1.68	1.25	0.0003
Glu (mmol/L)	5.5	1.08	6.91	2.11	<0.0001	5.53	1.15	7	2.38	<0.0001	5.47	1.02	6.81	1.8	<0.0001
Crea (umol/L)	78.0	17.7	84.3	28.3	<0.0001	88.7	15.6	95.3	28.4	<0.0001	67.7	12.7	72.8	23.2	<0.0001
Gender						2928 (total number of men)					3007 (total number of women)				0.38
Smoke	779 (14.6)		69 (11.6)		0.04	736 (28.0)		66 (21.8)		0.02	43 (1.58)		3 (1.03)		0.46
Alcohol drinking	1418 (26.6)		115 (19.4)		<0.001	1111 (42.3)		94 (31.0)		<0.001	307 (11.3)		21 (7.22)		0.03
Salt intake (g/d)					<0.001					0.01					0.02
<6	966 (18.6)		147 (24.8)			448 (17.1)		73 (24.1)			518 (19.1)		74 (25.4)		
6-13	3848 (72.6)		395 (66.5)			1894 (72.2)		199 (65.7)			1954 (71.9)		196 (67.4)		
>13	527 (9.87)		52 (8.75)			283 (10.8)		31 (10.2)			244 (8.98)		21 (7.22)		
Physical activity					0.28					0.29					0.45
No exercise	2046 (38.3)		229 (38.6)			998 (38.0)		109 (36.0)			1048 (38.6)		120 (41.2)		
Light intensity	1410 (26.4)		175 (29.5)			716 (27.3)		98 (32.3)			694 (25.6)		77 (26.5)		
Moderate intensity	1083 (20.3)		113 (19.0)			550 (21.0)		56 (18.5)			533 (19.6)		57 (19.6)		
Vigorous intensity	802 (15.0)		77 (13.0)			361 (13.8)		40 (13.2)			441 (16.2)		37 (12.7)		
Psychology factors					0.08					0.08					0.03
Depression	1494 (28.0)		186 (31.3)			724 (27.6)		86 (28.4)			770 (28.4)		100 (34.4)		
Sleep					0.004					0.13					0.02
Well	1816 (34)		167 (28.1)			953 (36.3)		95 (31.4)			863 (31.8)		72 (24.7)		
General	2556 (47.9)		294 (49.5)			1244 (47.4)		148 (48.8)			1312 (48.3)		146 (50.2)		
Bad	969 (18.1)		133 (22.4)			428 (16.3)		60 (19.8)			541 (19.9)		73 (25.1)		

Continuous variables were described by mean±SD, Categorical variables are described by numbers (%) and evaluated by Chi-square tests.
SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL: low-density lipoprotein; Ua: Uric Acid; TG: Triglyceride; Glu: Glucose; Crea: Creatinine.
†This variable was skewed, the results were given as median (Q-range), and the statistics was analyzed using nonparametric tests.

Table 1. The clinical characteristics of cases and controls (cont.)

	Total hypertensive subjects			Men			Women		
	Hypertensive subjects without T2DM (controls, n=5341)	Hypertensive subjects with T2DM (cases, n=594)	*p*	Hypertensive subjects without T2DM (controls, n=2625)	Hypertensive subjects with T2DM (cases, n=303)	*p*	Hypertensive subjects without T2DM (controls, n=2716)	Hypertensive subjects with T2DM (cases, n=291)	*p*
Years of hypertension, year			<0.0001			<0.0001			<0.0001
<2	1095 (20.5)	65 (10.9)		506 (19.3)	36 (11.9)		589 (21.7)	29 (9.97)	
2-5	1506 (28.2)	129 (21.7)		798 (30.4)	67 (22.1)		708 (26.1)	62 (21.3)	
5-10	1259 (23.6)	146 (24.6)		625 (23.8)	68 (22.4)		634 (23.3)	78 (26.8)	
>10	1481 (27.7)	254 (42.8)		696 (26.5)	132 (43.6)		785 (28.9)	122 (41.9)	
Antihypertensive drugs	4268 (79.9)	502 (84.5)	0.007	2123 (80.9)	257 (84.8)	0.09	2145 (79.0)	245 (84.2)	0.03
Family history of hypertension	2437 (45.6)	254 (42.8)	0.18	1230 (46.9)	131 (43.2)	0.23	1207 (44.4)	123 (42.3)	0.47
Family history of diabetes	333 (6.23)	139 (23.4)	<0.0001	159 (6.06)	71 (23.4)	<0.0001	174 (6.41)	68 (23.4)	<0.0001

Continuous variables were described by mean±SD, Categorical variables are described by numbers (%) and evaluated by Chi-square tests.
SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL: low-density lipoprotein; Ua: Uric Acid; TG: Triglyceride; Glu: Glucose; Crea: Creatinine.
†This variable was skewed, the results were given as median (Q-range), and the statistics was analyzed using nonparametric tests.

Table 2. Total plasma homocysteine levels and the prevalence of hyperhomocysteinemia (≥15 μmol/L) by gender in cases and controls

	Men					Women					Total				
	N	Homocysteine, μmol/L		Hyperhomocy-steinemia		*N*	Homocysteine, μmol/L		Hyperhomocy-steinemia		*N*	Homocysteine, μmol/L		Hyperhomocy-steinemia	
		Median (quartile range)	*p*	N (%)	*p*		Median (quartile range)	*p*	N (%)	*p*		Median (quartile range)	*p*	N (%)	*p*
Homocysteine, (μmol/L)	2928	14.6 (6.7)		1360 (46.5)		3007	11.2 (4.2)		503 (16.7)		5935	12.7 (5.6)	<0.0001**	1863 (31.4)	<0.0001***
Hypertensive subjects without T2DM (controls)	2625	14.6 (6.8)	0.33*	1210 (46.1)	0.26*	2716	11.1 (4.05)	<0.0001*	447 (16.5)	0.23	5341	12.7 (5.7)	<0.0001	1657 (31.0)	0.07
Hypertensive subjects with T2DM (cases)	303	14.8 (15.5)		150 (49.5)		291	12.1 (4.1)		56 (19.2)		594	13.5 (5.4)		206 (34.7)	

tHcy was skewed, the results were given as median (quartile range), and the statistics was analyzed using nonparametric tests.
*: the differences of tHcy or hyperhomocysteinemia between cases (hypertensive subjects with T2DM) and controls (hypertensive subjects without T2DM) in men, women and whole subjects.
**: the median of tHcy in men vs the median of tHcy in women.
***: the number of hyperhomocysteinemia in men vs the number of hyperhomocysteinemia in women.

Table 3. Odds ratios (ORs) (95% confidence intervals [CI]) for the quartiles of plasma homocysteine (per 5 μmol/L)

	Quartiles of tHcy (μmol/L)					OR per 5 μmol/L tHcy	OR tHcy (≥15 vs <15 μmol/L)
	Q_1	Q_2	Q_3	Q_4 (high)	*p* for trend		
Men							
Median of tHcy (μmol/L)	10.6	13.3	16.2	24.1			
Controls/cases	645/57	666/85	648/94	666/67			
Crude	1	1.44 (1.02-2.05)	1.64 (1.16-2.32)	1.14 (0.79-1.65)	0.40	0.95 (0.9-1.01)	1.15 (0.90-1.45)
Model 1	1	1.25 (0.86-1.81)	1.37 (0.95-1.98)	0.88 (0.59-1.3)	0.53	0.94 (0.88-1.01)	0.97 (0.75-1.25)
Model 2	1	1.24 (0.86-1.82)	1.38 (0.94-1.99)	0.87 (0.63-1.31)	0.55	0.95 (0.88-1.02)	1.03 (0.95-1.11)
Women							
Median of tHcy (μmol/L)	8.4	10.35	12.1	15.9			
Controls /cases	676/48	687/55	689/92	664/96			
Crude	1	1.13 (0.76-1.68)	1.88 (1.31-2.71)	2.04 (1.42-2.93)	<0.0001	1.11 (1.07-1.16)	1.21 (0.88-1.65)
Model 1	1	1.07 (0.7 -1.63)	1.51 (1.02-2.23)	1.83 (1.26-2.43)	<0.0001	1.05 (1.01-1.11)	0.90 (0.65-1.25)
Model 2	1	1.09 (0.71-1.68)	1.59 (1.06-2.37)	1.85 (1.28-2.51)	<0.0001	1.07 (1.02-1.13)	0.89 (0.64-1.24)
Total							
Controls/cases	1321/105	1353/140	1337/186	1330/163			
Crude	1	1.3 (1-1.7)	1.75 (1.36-2.25)	1.54 (1.19-1.99)	0.0001	0.99 (0.95-1.04)	1.18 (0.99-1.41)
Model 1	1	1.16 (0.88-1.52)	1.42 (1.09-1.86)	1.33 (0.96-1.69)	0.16	0.98 (0.92-1.05)	0.94 (0.77-1.15)
Model 2	1	1.16 (0.88-1.53)	1.45 (1.11-1.90)	1.15 (0.87-1.52)	0.14	0.98 (0.94-1.08)	0.95 (0.77-1.60)

Model 1: adjusted for age, gender, alcohol drinking, sleep, years of hypertension, family history of hypertension, family history of diabetes (all of these variables were statistically significant in the multivariable analysis).
Model 2: additional adjustment for Glu, Crea, LDL, TG.

whereas tHcy was not a determinant of type 2 diabetes in the hypertensive men subjects. An interaction test was thus performed to determine whether gender and other risk factors modified the relationship between tHcy and type 2 diabetes; a significant interaction was observed for gender, especially in the women subjects, whereas the trend across tHcy quartiles was not significant for the other risk factors. Therefore, the putative association between tHcy and type 2 diabetes in the hypertensive subjects might be modified by gender, suggesting that gender hormones may play a mediating role, because estrogen has been shown to influence the levels of plasma homocysteine in women.[15] However, when we separated the levels of tHcy into hyperhomocysteinemia (≥15 μmol/L) and normal (<15 μmol/L), no significant results were observed.

We also found that the levels of tHcy were significantly higher in the men subjects than in the women subjects (p<0.0001), which was consistent with the findings of some previous studies.[16,17] The reason for the gender difference in tHcy may be primarily the differences in muscle mass and concentrations of gender hormones.[18] As Wouters reported, lack of estrogen may result in an elevation of tHcy in men.[19]

Levels of tHcy have been shown to increase with age,[20] and aging is accompanied by elevated levels of homocysteine. Because of the differences in the ages of all subjects, of men and of women, we performed a multivariate-adjusted analysis (model 1) and an interaction analysis to test whether age modified the association between tHcy and type 2 diabetes. However, our findings suggested that age did not modulate the association between tHcy and type 2 diabetes in the hypertensive subjects. Negative associations were observed between smoking, alcohol drinking, salt intake and type 2 diabetes; because this was a retrospective study, subjects with type 2 diabetes who were included were not new patients but had been diagnosed with diabetes for years and were managed by the community health service centers. On the advice of doctors, they had changed their lifestyles, for example by quitting smoking and drinking, and controlling their salt intake.

An elevated homocysteine level has been reported to be associated with nephropathy in patients with diabetes,[21] while Ndrepepa et al suggested that plasma homocysteine levels were increased in patients with type 2 diabetes primarily due to impaired renal function.[22] In a study of Pima Indians, the significant association between homocysteine and mortality from type 2 diabetes was confounded by renal disease, with little confounding by vitamin B-12 or folate concentrations.[23] In our study, because the level of creatinine differed significantly between cases and controls in a multivariable analysis, we used model 2 to adjust for its potential effect, and excluded subjects with renal failure. The positive association between tHcy and type 2 diabetes in the hypertensive women subjects remained significant, and we thus believe that the significant association was not confounded by renal status. The differences among studies may be due to the heterogeneity in study design and differences in the patients included.

Currently, the specific mechanisms of hyperhomocysteinemia on the risk of type 2 diabetes are not clear. Several assumptions have been put forward. Homocysteinemia may motivate insulin resistance and β-cell dysfunction through adverse metabolic effects, ultimately contributing to the etiology of type 2 diabetes and its related complications.[24,25] The promotion of glucose-induced oxidative stress on endothelial cells is another possible explanation for the presumably increased susceptibility of subjects with diabetes to raised levels of tHcy. This hypothesis was confirmed in an animal model which demonstrated that tHcy-induced endothelial dysfunction is more liable to occur in the presence than in the absence of diabetes.[26] Moreover, homocysteine can stimulate oxidative stress and inhibit nitric oxide formation, and hyperhomocysteinemia may play a role in the atherogenic effects observed in type 2 diabetes by promoting platelet hyperactivity.[27]

The main strength of our study was its community-based design, the standardized and comprehensive protocol for data collection, the rigorous methodology, the availability of a large sample size, and abundant information on covariates. We excluded patients undergoing insulin therapy, because the levels of tHcy were relatively higher in these subjects.[17] Our study first supplemented the literature on the relationship between tHcy and type 2 diabetes in hypertensive subjects by suggesting that there is a women gender-specific association, which may have clinical implications. In future prospective studies, we will take the actual estrogen measurements of hypertensive women to confirm the observed differences in gender.

This study has some limitations, one of which is the cross-sectional nature of the examination. Second, the detection of an independent effect of tHcy on the risk of type 2 diabetes in hypertensive subjects remains difficult due to the many factors that can affect tHcy. Although we considered many clinical and lifestyle variables, the changes in tHcy might be derived from a combination of these factors, and residual confounding may have affected our findings. Clarification of these results by well-designed longitudinal studies including more potentially confounding factors will be necessary to elucidate the significance of tHcy in type 2 diabetes with hypertension, and provide a definitive potential solution for the management of this disease. Moreover, we did not examine the blood samples for dietary B vitamins, which are the most important dietary factors in tHcy metabolism.

In conclusion, within a large cross-sectional study of more than 5,000 hypertensive patients, the elevation of tHcy was independently and gender-specifically related to type 2 diabetes in the hypertensive women subjects. These observations not only provided implicit evidence linking elevated tHcy to the development of type 2 diabetes but also led to the suggestion that lowering tHcy may prevent type 2 diabetes in hypertensive women. However, this conclusion is only applicable to the Chinese population, especially to people in southern China. More importantly, when we separated tHcy into hyperhomocysteinemic (≥15 μmol/L) and normal (<15 μmol/L) levels, no significant results were observed.

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AUTHOR DISCLOSURES

All of the authors had full access to the data and participated in the concept, design, and drafting of this manuscript. The authors declare that they have no conflicts of interest. This work has not been published elsewhere.

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Original Article

Elevated total plasma homocysteine levels are associated with type 2 diabetes in women with hypertension

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升高的血浆同型半胱氨酸水平在女性高血压患者中与2型糖尿病的关系

背景与目的：在高血压患者中针对同型半胱氨酸水平与2型糖尿病之间关系的研究较少。**方法与研究设计：**该研究对深圳市南山区60个社区的5935名原发性高血压患者进行了调查。病例组为高血压患者伴发糖尿病，对照组为高血压患者无糖尿病。采用标准方法对其人口学特征及生化指标进行调查。采用多变量分析方法分析同型半胱氨酸水平与2型糖尿病的关系。**结果：**该研究一共纳入5241例对照（男/女：2716/2625）与594例病例（男/女：291/303）。在女性高血压患者中，同型半胱氨酸水平与2型糖尿病之间呈剂量反应关系。在控制了相应的混杂因素之后，同型半胱氨酸水平每升高 5 μmol/L，在原始模型中，比值比及95%可信区间为 1.11（1.07-1.16），在模型 1 中，比值比及 95%可信区间为 1.05 (1.01-1.11)，在模型 2 中，比值比及 95%可信区间为 1.07 (1.02-1.13)。在高血压患者中，未发现高 Hcy 血症分级(≥15 μmol/L vs <15 μmol/L)与 2 型糖尿病存在关联。**结论：**当将同型半胱氨酸水平按照四分位数进行分级时，女性高血压患者同型半胱氨酸水平与2型糖尿病之间存在关联。然而并未发现高Hcy血症分级(≥15 μmol/L vs <15 μmol/L)与2型糖尿病存在关联。

关键词：同型半胱氨酸、糖尿病、高血压、交互、以人群为基础的研究