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Original Article

Influence of renal function on the association between homocysteine level and risk of ischemic stroke

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Abstract: We examined whether the association between total homocysteine (tHCY) and risk of ischemic stroke (IS) varies depending on renal function to gain insight into why tHCY-lowering vitamins do not reduce the incidence of cardiovascular disease in clinical trials. We analyzed data from 542 IS patients with large artery atherosclerosis (LAA) or small artery occlusion (SAO) after stratification by estimated glomerular filtration rate (eGFR) to evaluate renal function. We found that tHCY level was positively associated with the occurrence of IS in both LAA (OR: 1.159, 95% CI: 1.074-1.252, *P*<0.001) and SAO (OR: 1.143, 95% CI: 1.064-1.228, *P*<0.001) patients and in LAA (OR: 1.135, 95% CI: 1.047-1.230, *P*=0.002) and SAO (OR: 1.159, 95% CI: 1.060-1.268, *P*=0.001) subgroups with normal renal function but not in LAA or SAO subgroups with renal insufficiency. eGFR level was positively associated with IS in LAA (OR: 1.022, 95% CI: 1.010-1.034, *P*<0.001) and SAO (OR: 1.024, 1.012-1.037, *P*<0.001) subgroups with normal renal function but was negatively associated with IS in LAA (OR: 0.875, 95% CI: 0.829-0.925, *P*<0.001) and SAO (OR: 0.890, 95% CI: 0.850-0.932, *P*<0.001) subgroups with renal insufficiency. Folic acid level was negatively associated with IS in LAA (OR: 0.734, 95% CI: 0.606-0.889, *P*=0.002) and SAO (OR: 0.861, 95% CI: 0.767-0.967, *P*=0.012) subgroups with renal insufficiency. Therefore, renal function as evaluated by eGFR exerts a significant influence on the association between tHCY and risk of IS.

Keywords: Renal function, estimated glomerular filtration rate, MDRD equation, homocysteine, ischemic stroke

Introduction

Elevated plasma total homocysteine (tHCY) is an independent risk factor for cardiovascular disease (CVD) [1-3]. However, clinical trials report that supplementation of HCY metabolism-related vitamins (HMRVs) to reduce tHCY fails to decrease the incidence of CVD [4-7]. Although subgroup analysis in some studies and meta-analyses show that HMRV supplementation has beneficial effects on the secondary prevention of ischemic stroke (IS) [5, 7, 8], the relationship between HMRVs and IS risk is not completely understood [10]. In addition, folic acid supplementation as the basis of antihypertensive therapy has been shown to reduce the risk of a first attack of IS [9].

Renal insufficiency (RI) is also an independent risk factor for CVD [11, 12], and elevated tHCY increases the risk of CVD in RI patients [13-15]. Although RI may increase tHCY and the risk of IS, the associations among these factors remain to be clarified. Therefore, we examined the impact of renal function on the association between tHCY and risk of IS to gain insight into why tHCY-lowering interventions by HMRVs fail to reduce the incidence of CVD.

Materials and methods

Study subjects

Potential study subjects included all patients admitted to our hospital between October 2012 and March 2016 who met the diagnostic criteria of IS [16]. A total of 542 IS patients with macro- or micro-vascular disease were selected, and their renal function and levels of folic acid, vitamin B_{12}, and tHCY were measured. A

Table 1. Comparison of baseline characteristics between controls and IS patient subtypes

	Control (n=164)	LAA (n=294)	Test Value	*P* value	SAO (n=248)	Test Value	*P* value
Age	62.74±14.02	71.53±11.55	-6.286	<0.001	68.62±11.40	-4.226	<0.001
Gender	81 (49.39%)	183 (62.24%)	7.125	0.008	143 (57.66%)	2.722	0.099
Smoking	30 (18.29%)	83 (28.23%)	5.595	0.018	64 (25.81%)	3.165	0.075
TIA/Stroke	--	47 (15.99%)	--	--	48 (19.35%)	--	--
HP	76 (46.34%)	249 (84.69%)	75.146	<0.001	207 (83.47%)	63.267	<0.001
DM	26 (15.85%)	106 (36.05%)	20.941	<0.001	102 (41.13%)	29.448	<0.001
CVD	5 (3.05%)	37 (12.59%)	11.494	0.001	17 (6.85%)	2.829	0.093
SBP	137.29±17.54	154.82±23.43	-7.985	<0.001	152.87±22.54	-7.102	<0.001
DBP	79.38±10.71	82.26±13.40	-2.121	<0.001	83.73±13.15	3.775	<0.001
FPG	5.08 (4.73, 5.62)	6.01 (5.17, 7.64)	-6.582	<0.001	5.45 (4.83, 7.29)	-4.018	<0.001
LDL-C	2.99±0.78	3.02±1.00	0.381	0.703	3.08±0.94	0.963	0.336
eGFR	113.37 (90.71, 131.37)	106.15 (86.92, 128.55)	-1.888	0.059	112.26 (87.61, 128.29)	-1.145	0.157
FA	7.90 (6.00, 11.65)	6.20 (4.60, 9.70)	-3.782	<0.001	6.70 (4.30, 10.10)	-3.315	0.001
$VitB_{12}$	319.50 (223.00, 436.00)	293.00 (203.00, 400.00)	-2.151	0.031	272.00 (191.00, 375.00)	-2.564	0.010
tHCY	14.85 (12.60, 16.80)	17.0 (14.70, 20.40)	-5.642	<0.001	17.30 (14.20, 21.50)	-5.169	<0.001

HP, hypertension; DM, diabetes mellitus; CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; FA, folic acid; $VitB_{12}$, vitamin B_{12}; tHCY, total homocysteine.

total of 164 elderly patients who underwent health examination in our hospital during the same period served as a control group. Patients with thyroid disease, brain tumor, encephalitis, brain trauma, or severe multiple organ dysfunction were excluded.

Clinical information collection

Patient clinical information was collected including gender, age, smoking status, hypertension, diabetes, transient ischemic attack, and previous stroke. Patients who were current smokers or had ceased smoking within the last 5 years were considered smokers.

Imaging and patient classification

IS patients underwent cranial magnetic resonance imaging (MRI) and carotid color ultrasonography within 72 h of admission. Cranial MRI was applied with regular T_1, T_2, diffusion-weighted imaging, and magnetic resonance angiography (MRA) or time-of-flight (tof)-MRA. Cranial computed tomography, computed tomography angiography, or digital subtraction angiography was performed for patients who could not undergo MRI. Carotid color ultrasonography was performed by two experienced physicians, and a third physician confirmed the results in cases of disagreement.

Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification [17] was performed based on clinical and imaging information. Patients were classified into large artery atherosclerosis (LAA), small artery occlusion (SAO), cardioembolism, stroke of other determined etiology, or stroke of undetermined etiology groups. A modified Modification of Diet in Renal Disease Study (MDRD) equation was used to calculate estimated glomerular filtration rate (eGFR) [18]. LAA and SAO patients were analyzed and further stratified into normal renal function (NR; nLAA and nSAO) and RI (iLAA and iSAO) subgroups depending on their eGFR level. Normal renal function was defined as eGFR ≥90 ml/min/1.73 m^2; otherwise, patients were considered RI.

Blood testing

Fasting venous blood samples were collected within 24 h of examination. A HITACHI 7600 Biochemical Analyzer was used to measure creatinine, fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL-C), and tHCY. Folic acid and vitamin B_{12} levels were measured using radioimmunoassay.

Statistical analysis

SPSS 19.0 software was used for statistical analysis. Continuous variables that were normally distributed are reported as mean ± standard deviation and compared using *t*-tests. Continuous variables that were not normally distributed are reported as median and compared using Mann-Whitney U tests. Frequency

Table 2. Comparison of baseline characteristics between controls and IS patient subgroups with normal renal function

	Control (n=164)	nLAA (n=211)	Test Value	*P* value	nSAO (n=172)	Test Value	*P* value
Age	62.74±14.02	69.75±11.57	-4.492	<0.001	66.69±1.39	-2.522	0.012
Gender	81 (49.39%)	80 (62.09%)	6.052	0.014	96 (55.81%)	1.390	0.238
Smoking	30 (18.29%)	40 (18.96%)	0.027	0.870	46 (26.74%)	3.426	0.064
TIA/Stroke	--	26 (12.32%)	--	--	31 (18.02%)	--	--
HP	76 (46.34%)	185 (87.68%)	74.520	<0.001	143 (83.14%)	50.088	<0.001
DM	26 (15.85%)	81 (38.39%)	22.980	<0.001	77 (44.77%)	33.016	<0.001
CVD	5 (3.05%)	27 (12.80%)	11.233	0.001	8 (4.65%)	0.580	0.446
SBP	137.79±17.24	155.54±23.03	-7.730	<0.001	153.27±21.12	-6.913	<0.001
DBP	79.38±10.71	83.67±12.61	-3.014	0.003	84.41±12.13	4.135	<0.001
FPG	5.08 (4.73, 5.62)	25.97 (5.17, 7.55)	-6.766	<0.001	5.58 (4.91, 7.62)	-5.165	<0.001
LDL-C	2.99±0.78	3.08±0.92	-0.446	0.656	3.09±0.88	1.172	0.242
eGFR	113.37 (90.71, 131.37)	119.37 (103.29, 137.01)	-3.028	0.002	122.62 (107.14, 137.87)	-3.703	<0.001
FA	7.90 (6.00, 11.65)	6.70 (4.70, 9.90)	-2.937	0.003	7.25 (4.33, 10.38)	-2.434	0.004
$VitB_{12}$	319.50 (223.00, 436.00)	307.00 (203.00, 412.00)	-1.592	0.111	268.00 (193.25, 381.00)	-2.088	0.037
tHCY	14.85 (12.60, 16.80)	16.00 (14.00, 18.50)	-3.727	<0.001	16.10 (13.60, 19.28)	-2.897	0.004

HP, hypertension; DM, diabetes mellitus; CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; FA, folic acid; $VitB_{12}$, vitamin B_{12}; tHCY, total homocysteine.

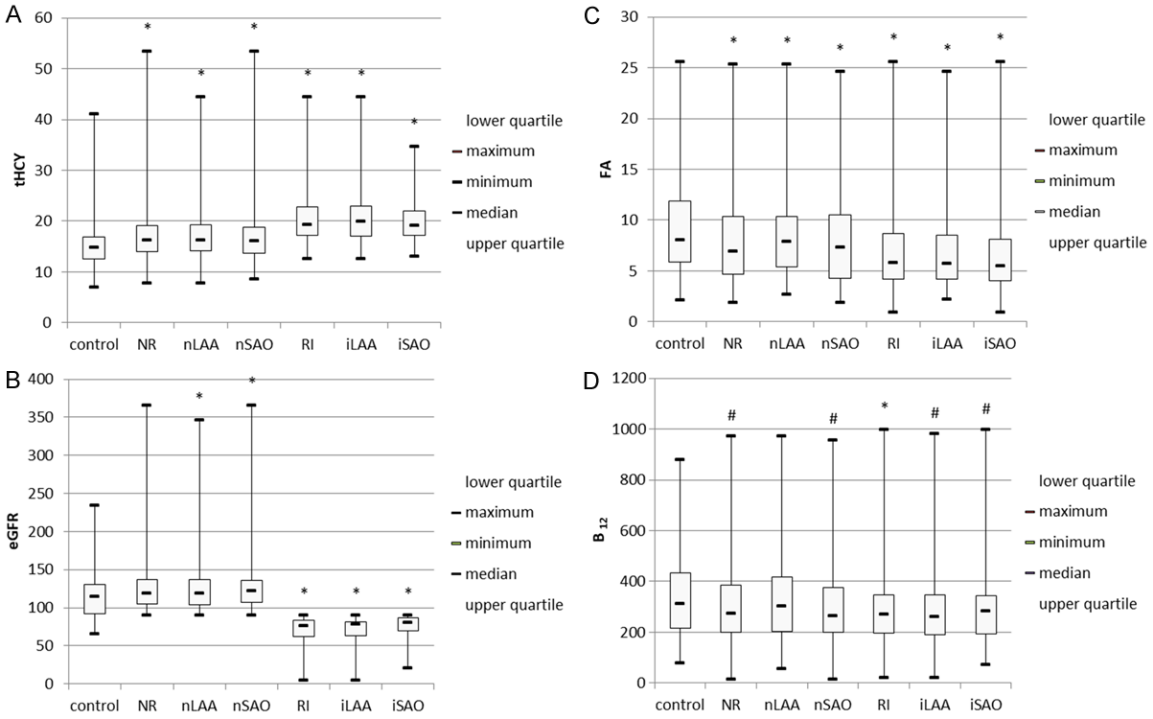

Figure 1. Comparison of tHCY, eGFR, folic acid, and vitamin B_{12} levels in controls and subgroups of IS patients with different renal function. A. tHCY level was higher in IS patients with NR or RI than in controls. B. eGFR was higher in nLAA and nSAO patients but was lower in IS patients with RI compared with controls. C. Folic acid level was lower in IS patients with NR or RI than in controls. D. Vitamin B_{12} level was lower in IS patients with NR or RI than in controls, except for the nLAA subgroup. Compared with control group, #P<0.05, *P<0.01.

data are expressed as percentage and compared using χ^2 tests. Logistic regression was performed to determine independent risk factors in IS subtypes and renal function subgroups. A P-value <0.05 was considered statistically significant.

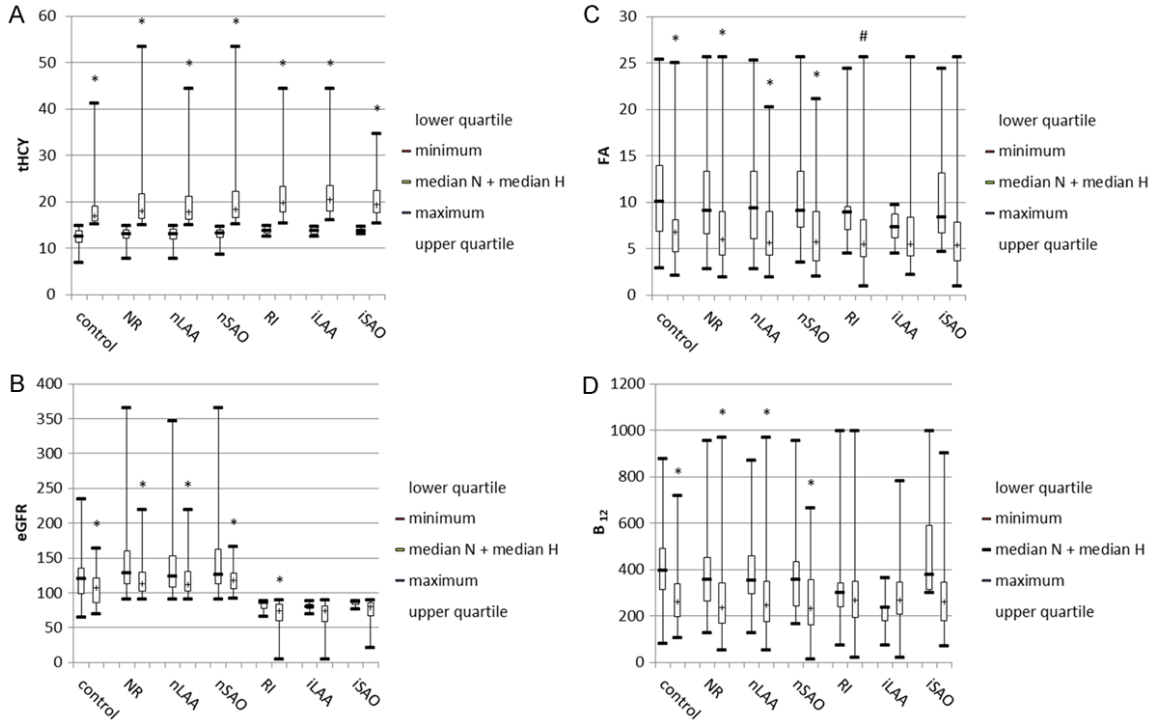

Figure 2. Variation in eGFR, folic acid, and vitamin B_{12} levels with tHCY level in controls and subgroups of IS patients with different renal function. A. tHCY level was lower in normal tHCY patients (NHCY) or controls than in hyperhomocysteinemic patients (HHCY) or controls. B-D. eGFR and levels of folic acid and vitamin B_{12} were lower in HHCY subgroups than in NHCY subgroups of controls or IS patients with normal renal function. By contrast, eGFR and levels of folic acid and vitamin B_{12} were similar between NHCY and HHCY subgroups in IS patients with RI. Median N, median for NHCY subgroups; median H, median for HHCY subgroups. Compared with NHCY group, #P<0.05, *P<0.01.

Results

Baseline data comparison between controls and IS patients

Compared with controls, both LAA and SAO subtypes of IS patients were older, had higher incidences of hypertensionand diabetes, and exhibited elevated systolic pressure, diastolic pressure, fasting plasma glucose, and tHCY as well as decreased folic acidand vitamin B_{12} levels (**Table 1**). The proportions of male patients, smokers, and patients with CVD historywere higher in the LAA subtype than in the control group. No significant differences in LDL-C or eGFR were found between IS patients and controls.

Baseline data comparison between controls and IS patients with normal renal function

Compared with controls, both nLAA and nSAO subgroups of IS patients with normal renal function were older, had higher incidences of hypertensionand diabetes, and exhibited elevated systolic pressure, diastolic pressure, fasting plasma glucose, eGFR and tHCY as well as decreased folic acid level (**Table 2**, **Figure 1A-D**). The proportions of male patients and patients with CVD history were higher in the nLAA group than in the control group. Vitamin B_{12} level was lower in the nSAO group than in the control group. No significant differences in smoking status or LDL-C were found between IS patients and controls. After grouping by tHCY level, higher tHCY and lower eGFR, folic acid, and vitamin B_{12} levels were found in IS patients of normal renal function with hyperhomocysteinemia compared with normal tHCY levels (**Figure 2A-D**).

Baseline data comparison between controls and IS patients with RI

Compared with controls, both iLAA and iSAO subgroups of IS patients with RI were older, were more likely to be male, had higher incidences of hypertension, diabetes, and CVD,

Table 3. Comparison of baseline characteristics between controls and IS patient subgroups with RI

	Control (n=164)	iLAA (n=83)	Test Value	*P* value	iSAO (n=76)	Test Value	*P* value
Age	62.74±14.02	76.33±9.98	-7.182	<0.001	76 73.09±10.21	-5.634	<0.001
Gender	81 (49.39%)	52 (62.65%)	3.899	0.048	49 (64.47%)	4.759	0.029
Smoking	30 (18.29%)	43 (51.81%)	29.731	<0.001	18 (23.68%)	0.944	0.331
TIA/Stroke	–	21 (25.30%)	–	–	17 (22.37%)	–	–
HP	76 (46.34%)	74 (89.16%)	42.360	<0.001	64 (84.21%)	30.641	<0.001
DM	26 (15.85%)	29 (34.94%)	11.598	0.001	25 (32.89%)	9.012	0.003
CVD	5 (3.05%)	10 (12.05%)	7.825	0.005	9 (11.84%)	5.797	0.016
SBP	137.79±17.24	153.32±24.47	-5.346	<0.001	151.67±25.39	-4.462	<0.001
DBP	79.38±10.71	79.00±14.10	-0.179	0.858	82.01±15.28	-0.971	0.332
FPG	5.08 (4.73, 5.62)	6.15 (5.17, 8.02)	-4.274	<0.001	5.29 (4.75, 6.17)	-0.522	0.602
LDL-C	2.99±0.78	2.96±1.16	-1.045	0.296	3.01±1.03	-0.416	0.678
eGFR	113.37 (90.71, 131.37)	74.32 (55.30, 81.79)	-10.975	<0.001	78.38 (70.81, 86.65)	-10.055	<0.001
FA	1537.9 (6.00, 11.65)	5.75 (4.22, 8.48)	-3.865	<0.001	5.45 (3.83, 8.45)	-3.578	<0.001
$VitB_{12}$	319.50 (223.00, 436.00)	261.50 (194.00, 363.00)	-2.340	0.019	289.50 (186.30, 349.80)	-2.065	0.039
tHCY	14.85 (12.60, 16.80)	19.70 (16.80, 23.50)	-7.140	<0.001	18.90 (17.10, 23.40)	-7.105	<0.001

HP, hypertension; DM, diabetes mellitus; CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; FA, folic acid; $VitB_{12}$, vitamin B_{12}; tHCY, total homocysteine.

and exhibited elevated systolic pressure and tHCY as well as decreased eGFR, folic acid, and vitamin B_{12} levels (**Table 3**, **Figure 1A-D**). The proportion of patients who were smokers and level of fasting plasma glucose was higher in the iLAA group than in the control group. No significant differences in diastolic pressure or LDL-C were found between IS patients and controls. After grouping by HCY level, higher HCY was found in IS patients with hyperhomocysteinemia compared with normal tHCY levels (**Figure 2A-D**). Differences in eGFR and folic acid level of RI patients between hyperhomocysteinemic and normal homocysteinemic groups disappeared after classification into iLAA and iSAO subgroups. No differences in vitamin B_{12} level were found in RI patients between hyperhomocysteinemic and normal homocysteinemic groups.

Logistic regression analysis to determine risk factors of IS

Logistic regression analysis was performed within IS subtypes and renal function subgroups with IS as a dependent variable and risk factors (P<0.2) as independent variables. Forward logistic regression method was used to select variables (inclusion, P<0.05; exclusion, P>0.10). After adjusting for other variables, eGFR was positively associated with the occurrence of IS in nLAA and nSAO subgroups but was negatively associated with IS in iLAA and iSAO subgroups (**Table 4**). After adjusting for other variables, tHCY was positively associated with the occurrence of IS in LAA and SAO subtypes. Subgroup analysis showed that tHCY was positively associated with the occurrence of IS in nLAA and nSAO subgroups but not in iLAA or iSAO subgroups. Folic acid was negatively correlated with the occurrence of IS in iLAA and iSAO subgroups.

Discussion

Although epidemiological studies show that elevated tHCY increases the risk of CVD, clinical trials show that HMRV supplementation to reduce tHCY fails to lower the incidence of CVD [4-7], compelling investigators to reconsider this relationship. However, HMRV supplementation for reducing tHCY has been found to decrease the occurrence of IS in some trials [19], although contradictory results among studies present a great challenge to researchers investigating the association between tHCY and IS [20].

RI induces the up-regulation of tHCY and increases the incidence of and mortality from CVD [11, 12, 21]. Creatinine and eGFR are independent risk factors for increased tHCY in both healthy controls and patients [22, 23]. The comorbidity of chronic kidney disease in patients with elevated tHCY is 5.76 times that in patients without elevated tHCY, suggesting that chronic kidney disease might play a crucial role in hyperhomocysteinemia [22]. Compared with patients with an eGFR >60 ml/1.73 m^2/min, CVD risk is 1.4, 2.0, 2.8, and 3.4 times

Table 4. Logistic regression for IS risk factors within IS patient subtypes and renal function subgroups

	LAA (n=294)			nLAA (n=211)			iLAA (n=83)			SAO (n=248)			nSAO (n=172)			iSAO (n=76)		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Age	1.033	1.006~1.060	0.017	1.031	1.002~ 1.061	0.036	–	–	–	–	–	–	–	–	–	–	–	–
TIA/Stroke	9.733	2.121~44.662	0.003	9.599	1.858~49.587	0.007	–	–	–	11.775	2.637~52.585	0.001	15.776	3.048~81.645	0.001	6.535	1.183~36.107	0.031
HP	12.08	5.860~24.900	<0.001	8.163	3.980~16.650	<0.001	77.91	10.890~ 557.150	<0.001	–	–	–	12.47	5.520~28.200	<0.001	174.14	17.990~ 1685.820	<0.001
DM	–	–	–	1.260	1.080~1.47	0.004	–	–	–	10.830	5.23 22.41	<0.001	1.200	1.050~1.380	0.009	–	–	–
SBP	1.028	1.013~1.043	<0.001	1.030	1.013~1.047	<0.001	1.038	1.007~1.069	0.014	1.031	1.017~1.045	<0.001	1.043	1.026~1.061	<0.001	–	–	–
FPG	1.314	1.120~1.542	0.001	1.274	1.087~1.494	0.003	1.287	1.042~1.589	0.019	1.174	1.032~1.335	0.014	1.173	1.022~1.347	0.023	–	–	–
eGFR	1.009	1.000~1.019	0.055	1.022	1.010~1.034	<0.001	0.875	0.829~0.925	<0.001	–	–	–	1.024	1.012~1.037	<0.001	0.890	0.850~0.932	<0.001
FA	–	–	–	–	–	–	0.734	0.606~0.889	0.002	–	–	–	–	–	–	0.861	0.767~0.967	0.012
tHCY	1.159	1.074~1.252	<0.001	1.135	1.047~1.230	0.002	–	–	–	1.143	1.064~1.228	<0.001	1.159	1.060~1.268	0.001	–	–	–

HP, hypertension; DM, diabetes mellitus; SBP, systolic blood pressure; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; FA, folic acid; tHCY, total homocysteine.

higher in patients with an eGFR of 45-59, 30-44, 15-29, and <15 ml/1.73 m^2/min, respectively [12]. A meta-analysis shows that lower eGFR is significantly correlated with an increased incidence of IS [24]. Impaired renal function in chronic kidney disease is more closely related to IS in female patients and that occurring in the posterior circulation [25, 26]. Increased hemorrhagic transformation and poor prognosis have been reported in IS patients with RI [27-30]. Although the fact that RI increases tHCY and CVD incidence and mortality is widely accepted, the influence of RI on the relationship between risk factors and CVD has not received much attention.

We found that elevated tHCY was associated with the occurrence of IS in patients with normal renal function, consistent with previous findings that elevated tHCY is a risk factor for CVD [1-3]. Higher tHCY accompanied by lower eGFR and vitamin levels is in line with previous observations that impaired renal function or vitamin deficiency are determinant factors for elevated tHCY in certain populations [14, 23, 31]. Therefore, the effect of HMRV supplementation on the relationship between tHCY and IS risk deserves more attention. We previously found that tHCY is not related to risk of IS when HMRV level is normal, whereas elevated tHCY or decreased vitamin B_{12} levels are independently associated with increased risk of IS when HMRV level is deficient, suggesting that HMRVs impact the association between elevated tHCY and increased IS incidence in patients with normal renal function [32].

In patients with normal renal function, we found that increased eGFR was positively associated with the occurrence of IS, which might be caused by low creatinine levels. Normal eGFR was also accompanied by slightly higher tHCY and lower levels of folic acid and vitamin B_{12} in patients. Elderly Chinese individuals prefer a vegetarian diet and may have inadequate protein intake or malabsorption, leading to low levels of creatinine and vitamin B_{12}, elevated eGFR, and thus increased morbidity from stroke. A study of Taiwanese individuals shows that subclinical malnutrition resulting from a vegetarian diet may offset its benefits in reducing cardiovascular risk [33]. Also, a renal high filtration state as measured by eGFR significantly exacerbates atherosclerosis in Chinese individuals [34]. Such findings are consistent with those from the present study, suggesting that a high renal filtration state as assessed by eGFR might also increase the risk of CVD. Therefore, patients may benefit from lifestyle modifications that improve renal function.

In patients with RI, we found no relationship between tHCY and IS, but decreased eGFR and folic acid level were associated with increased risk of IS. These results are consistent with studies reporting that supplementation of folic acid reduces the incidence of stroke [9, 19]. More specifically, the China Stoke Primary Prevention Trials showed that supplementation of folic acid in RI patients can delay declines in renal function and reduce the incidence of stroke and CVD [9, 36]. RI or Folic acid deficiency increases tHCY and the risk of CVD, which can alter the nature of the relationship between tHCY and CVD [23, 37]. Our finding that decreased eGFR or deficiency in folic acid, but not increased tHCY, was related to the occurrence of IS suggests that increased tHCY in RI patients might be a biomarker of folic acid deficiency or metabolite-excreting disorder resulting from renal function injury. Hence, accurate evaluation of renal function or HMRV levels may be helpful for understanding the contradictory results of previous studies on the correlation between tHCY and IS risk.

We also found that lower eGFR was associated with a higher risk of stroke in patients with RI. Also, decreased eGFR was accompanied by increased tHCY and lower levels of folic acid and vitamin B_{12}. Reduced renal elimination, inhibition of crucial enzymes in the methionine cycle by uremic toxins, and low level of vitamins contribute to elevated tHCY [35]. RI reduces the excretion of tHCY [38]; inhibits the production of cystathionine-γ-lyase; activates Akt, PKC, and NF-kappaB signaling pathways [39-41]; induces an inflammatory response [42-44]; exacerbates atherosclerosis [45]; and disrupts the blood-brain barrier [46]; thereby increasing the risk of CVD [47].

The kidney is an important excretory organ that modulates the concentrations of various metabolites [48, 49]. Therefore, renal function may affect the relationship between risk factors and CVD [50]. We advise considering renal function status when evaluating associations

between risk factors and CVD to allow more accurate interpretation of those relationships.

This study has some limitations. The reduction in eGFR in patients with RI was mild, as our patient population did not include those with uremia or dialysis. eGFR evaluated by MDRD equation may vary between populations, which may create bias between control and patient groups [51-53]. Hence, the reliability of our results must be strengthened by further stratifying analysis of eGFR after increasing sample size [50].

In conclusion, this study is the first to report the influence of renal function as measured by eGFR on the association between plasma tHCY and risk of IS. Further studies are warranted to clarify the impact of RI on the relationship between elevated tHCY and CVD.

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Disclosure of conflict of interest

None.

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