Observational Study

Medicine®

OPEN

Relationship between plasma total homocysteine and the severity of renal function in Chinese patients with type 2 diabetes mellitus aged ≥75 years

Ning Ma, MM[a,b], Ning Xu, BS[b], Dong Yin, BS[b], Weiwei Liu, MM[b], Mengping Wu, MM[b], Xingbo Cheng, MD, PhD[a,*]

Abstract

We aimed to investigate the relationship between total homocysteine (tHcy) levels in the plasma and renal function severity in patients with type 2 diabetes mellitus (T2DM) aged ≥75 years.

We included 221 patients with T2DM aged ≥60 years (59 aged ≥75 years).

tHcy levels among the 4 groups of patients aged ≥60 years significantly differed, but not in those aged ≥75 years. tHcy levels in patients aged ≥60 years were negatively correlated with the estimated glomerular filtration rate. The area under the receiver operating characteristic curve of tHcy for predicting diabetic kidney disease (DKD) was 0.636. Fasting c-peptide and creatinine were independently associated with tHcy levels in patients aged ≥60 years, whereas insulin and creatinine were independently associated with tHcy levels in those aged ≥75 years.

tHcy concentrations were elevated in T2DM and can potentially serve as a risk factor for DKD, but it is not an ideal biomarker.

Abbreviations: AUC = crude area under the ROC curve, BUN = blood urea nitrogen, CKD = chronic kidney disease, DBP = diastolic blood pressure, DKD = diabetic kidney disease, DN = diabetic neuropathy, eGFR = estimated glomerular filtration rate, HC = hip circumference, HOMA = homeostatic model assessment, HOMA-IR = homeostatic model assessment index of insulin resistance, QUICKI = quantitative insulin check index, ROC = receiver operating characteristic, SBP = systolic blood pressure, sUA = serum uric acid, T2DM = type 2 diabetes mellitus, tHcy = homocysteine, UAE = urinary albumin excretion, UmALB/Cr = microalbumin/Cr ratio, WC = waist circumference, WHR = waist-to-hip ratio.

Keywords: diabetic kidney disease, homocysteine, hyperhomocysteinemia, older patients, type 2 diabetes mellitus

1. Introduction

Type 2 diabetes mellitus (T2DM) is a serious public health issue, and is now the third-leading cause of disease-related mortality worldwide. In 2017, approximately 451 million adults aged between 18 and 99 years suffer from diabetes, and this number is predicted to increase to 629 million by 2045.[1] Macrovascular and microvascular complications are primary causes of T2DM-related mortality.[2] Diabetic kidney disease (DKD) is a microvascular complication that is highly destructive and a commonly observed complication of T2DM and a major cause of end-stage renal disease. The overall median prevalence of chronic kidney disease (CKD) in patients with T2DM is 10.8% (range, 10.2%–11.3%); presently, there are an estimated 119.5 (range, 112.9–125.0) million patients with CKD in China.[3]

Various biomarkers have been identified for the diagnosis and prognosis of diabetic nephropathy[4] including high-sensitive C-reactive protein, C-peptide, and total bilirubin. In patients with T2DM, these biomarkers indicate microvascular damage[5] and are reported to be associated with increased microangiopathy.[6] Plasma total homocysteine (tHcy) is elevated in patients with T2DM[7] especially among those with microalbuminuria.[8] Hyperhomocysteinemia, occurring in approximately 85% patients with CKD because of altered renal metabolism and impaired renal excretion,[9] is a risk factor for diabetic retinopathy as well as for cardiovascular disease, and it has become a topic of research.[10]

Several studies have reported an association between increased plasma tHcy levels and DKD; however, few have focused on the relationship between tHcy and diabetic nephropathy risk in patients with T2DM aged ≥75 years. Therefore, the present study aimed to summarize the present data for providing more robust

Editor: Liang-Jun Yan.

This work was supported by Jiangsu Provincial Commission of Health and Family Planning (Grant NO. Z2018021) and Lianyungang Commission Health Foundation (Grant NO. zd1802).

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Endocrinology and Metabolism, First Affiliated Hospital of Soochow University, [b] Department of Endocrinology and Metabolism, Lianyungang No 1 People's Hospital, Jiangsu, China.

[*] Correspondence: Xingbo Cheng, Department of Endocrinology and Metabolism, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China (e-mail: 20174132022@stu.suda.edu.cn)

How to cite this article: Ma N, Xu N, Yin D, Liu W, Wu M, Cheng X. Relationship between plasma total homocysteine and the severity of renal function in Chinese patients with type 2 diabetes mellitus aged ≥75 years. Medicine 2020;99:27 (e20737).

Received: 26 February 2020 / Received in final form: 14 May 2020 / Accepted: 15 May 2020

http://dx.doi.org/10.1097/MD.0000000000020737

evidences of the potential relationship between plasma tHcy levels and the risk for DKD in older patients with T2DM.

2. Methods

2.1. Study participants and study design

A total of 221 Chinese patients with T2DM aged ≥60 years (101 males and 120 females) were included. Of the total, 59 were aged ≥75 years (30 males and 29 females). All patients received treatment at the Department of Endocrinology and Metabolism at our hospital from July 2019 to December 2019. T2DM was diagnosed according to the following criteria, which was recommended by the 2014 guidelines of the American Diabetes Association[11] fasting glucose level of ≥7.0 mmol/L, glycated hemoglobin concentration of ≥6.5% or postload plasma glucose concentration of ≥11.1 mmol/L (2 hours, 75 g oral glucose tolerance test). Patients were divided into 4 groups as follows: group A (microalbumin/Cr ratio [UmALB/Cr] < 30 mg/g), group B (30 mg/g ≤ UmALB/Cr ≤ 300 mg/g), group C (UmALB/Cr > 300 mg/g), or group D (Cr > 97 or > 73 μmol/L for males vs females, respectively). Each participant was asked to answer a detailed questionnaire regarding drug use, disease history, smoking history, and alcohol consumption. The exclusion criteria were as follows: age of < 60 years; prior diagnosis of type 1 diabetes, Cushing syndrome, thyroid disorders, or cancer; systemic corticosteroid treatment, lipid-lowering drugs, or hypertension medications; acute or chronic viral hepatitis; liver, renal, or heart failure; infection or inflammation and pregnancy. Because the cohort comprised older patients with T2DM, chronic diseases such as hypertension and coronary heart disease were not regarded as exclusion criteria. The study protocol was approved by the local Medical Ethics Committee, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. All patients were provided an explanation of the study, and each patient provided written informed consent.

2.2. Patients' basic clinical characteristics

Anthropometric data including weight, height, waist circumference (WC), and hip circumference (HC) were collected by nurses. The waist-to-hip ratio (WHR) was calculated by dividing WC by HC. Before measuring the blood pressure, each patient was instructed to rest for at least 10 minutes while seated. Blood pressure was measured in triplicates using an electronic sphygmomanometer (Omron Corporation, Kyoto, Japan), and the average value of the 3 measurements was used for analysis.

2.3. Biochemical measurements

After overnight fasting (≥12 hours), blood samples were collected between 07:00 and 08:00 h and separated by centrifugation. Fasting glucose, fasting insulin, fasting c-peptide, blood urea nitrogen (BUN), Cr, cystatin-c, serum uric acid (sUA), alpha-fetoprotein, carbohydrate antigen 19-9, carcinoembryonic antigen, neuron-specific enolase, D-dimer, serum lipids (triglycerides, total cholesterol, and low- and high-density lipoprotein cholesterol), plasma tHcy, and insulin levels were measured using the Beckman Coulter AU5800 Clinical Chemistry analyzer (Beckman Coulter, Inc, Brea, CA). Using a high-performance liquid chromatography system (Beckman Coulter, Inc), glycated hemoglobin concentrations were assessed.

2.4. Insulin secretion and insulin sensitivity or resistance indices

As an index of insulin resistance (homeostatic model assessment index of insulin resistance [HOMA-IR]), homeostasis model assessment was done to evaluate insulin resistance status, using the formula HOMA-IR = (fasting glucose [mmol/L] × fasting insulin [mIU/L]/22.5).[12] The following formula was used to calculate homeostatic model assessment (HOMA) of β-cell function as an index of insulin secretion:[12,13] HOMA-β = fasting insulin (mIU/L) × 20/(fasting glucose [mmol/L] − 3.5). The quantitative insulin check index (QUICKI) was calculated as an index of insulin sensitivity using the formula[13] QUICKI = 1/[log10 fasting glucose (mg/dL) + log10 fasting insulin (mIU/L)].

2.5. Classification of renal dysfunction

The UmALB/Cr ratio and serum levels of BUN, Cr, and cystatin-c were determined using the Beckman Coulter AU5800 Clinical Chemistry analyzer (Beckman Coulter, Inc). The estimated glomerular filtration rate (eGFR) was used to evaluate renal function according to the Chronic Kidney Disease Epidemiology Collaboration equation.[14]

2.6. Measurements of plasma tHcy concentrations

Plasma tHcy concentrations were measured by the enzymatic cycling method using a commercial kit (Beckman Coulter, Inc) in accordance with the manufacturer's instructions. For human tHcy measurements, the assay has a sensitivity of <10.0 μmol/L with intra- and interassay coefficients of variation of <10% and <5%, respectively.

2.7. Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows version 22.0 (IBM Corporation, Armonk, NY). Normal distribution of the data was tested using Kolmogorov–Smirnov test. Normally distributed data and data with skewed distributions are presented as mean ± standard deviation and median and interquartile range, respectively. Categorical data were compared with the χ^2 test. The Kruskal–Wallis H test or one-way analysis of variance was performed to identify differences in continuous variables among the 4 groups. For multiple testing among groups, one-way analysis of variance or the Kruskal–Wallis H test was used followed by Bonferroni correction. Bivariate correlation analysis was performed to identify correlations between tHcy levels and other variables. For the identification of independent factors related with tHcy, multiple stepwise regression analysis was performed while controlling for covariates. Non-normally distributed data were log-transformed (e.g., log-tHcy) before correlation analysis as well as multiple stepwise regression analysis. Plasma tHcy concentrations for the detection of DKD were derived from receiver operating characteristic (ROC) curves. A 2-sided probability (P) value of <.05 was considered to be significant. For power analysis, according to the plasma tHcy concentrations and number of study participants in the 4 groups, the effect size was 0.636.

3. Results

3.1. Characteristics of the study participants

The clinical characteristics of the 221 study participants (≥60 years) are presented in Table 1, whereas those of the

Table 1

General clinical and laboratory parameters of older Chinese patients with T2DM aged ≥60 y.

Variable	A	B	C	D	*P* value
N	95	32	49	45	
Sex (M/F)	38/57	20/12	25/24	18/27	.11
Age (y)*	68.51 ± 6.08	72.00 ± 7.86	68.49 ± 6.07	72.44 ± 6.82	.001
BMI (kg/m^2)†	25 (23.07–27.7)	25.6 (23.62–27.85)	26.1 (22.7–28.17)	25.79 (23.9–29)	.763
WC (cm)*	93.37 ± 9.89	96.74 ± 8.69	89.20 ± 7.94	93.77 ± 8.32	.008
WHR*	0.94 ± 0.07	0.95 ± 0.07	0.93 ± 0.05	0.95 ± 0.07	.549
SBP (mm Hg)*	134.44 ± 15.39	143.56 ± 16.31	148.90 ± 17.01	147.49 ± 17.63	.000
DBP (mm Hg)*	78.14 ± 9.17	78.38 ± 9.57	79.80 ± 9.90	80.24 ± 12.60	.621
Duration of DM (mo)	144.12 ± 87.46	164.80 ± 110.03	221.02 ± 129.47	225.33 ± 113.06	.000
Fasting glucose (mmol/L)†	8.07(5.47–10.94)	11 (8.39–12.89)	9.49 (6.46–13.47)	8.44 (5.93–10.74)	.008
Fasting insulin (mIU/L)†	9.78 (5.32–15.84)	10.29 (5.89–18.01)	9.77 (5.45–18.18)	5.58 (4.72–11.23)	.304
Fasting c-peptide (pmol/L)*	622.4 ± 418.7	823.54 ± 467.1	643.2 ± 391.5	816.8 ± 578.5	.035
HbA1c (%)*	8.77 ± 1.91	9.20 ± 2.83	9.17 ± 1.80	8.66 ± 1.83	.495
Creatinine†	54.7 (47.2–68.6)	64.1 (51.9–83.3)	63.6 (50.3–84.2)	132.4 (101.5–182.7)	.000
Blood urea nitrogen†	6.06 (4.77–7.33)	6.85 (5.4–9.26)	6.32 (5.24–7.51)	11.4 (9.03–14.28)	.000
Cystatin-c†	0.94 (0.79–1.17)	1.05 (0.81–1.26)	1.14 (0.96–1.28)	2.05 (1.68–2.43)	.000
UmALB/Cr	9.47 ± 6.97	98.64 ± 74.64	585.02 ± 322.00	648.97 ± 545.93	.000
TC (mmol/L)*	4.42 ± 1.62	4.22 ± 1.72	4.60 ± 1.64	4.84 ± 1.55	.379
TG (mmol/L)†	1.48 (1.03–2.21)	1.92 (1.13–3.06)	1.67 (1.19–2.26)	1.84 (1.36–3.47)	.080
LDL-C (mmol/L)*	2.61 ± 1.41	2.42 ± 0.98	2.63 ± 0.96	2.80 ± 1.10	.629
HDL-C (mmol/L)*	1.08 ± 0.27	0.94 ± 0.27	1.04 ± 0.21	0.99 ± 0.23	.040
THcy	8.40 ± 4.25	10.98 ± 6.64	9.68 ± 4.50	16.23 ± 8.94	.000
Serum uric acid	283.62 ± 72.46	326.04 ± 107.93	327.51 ± 92.35	423.42 ± 92.32	.000
CA199	22.59 ± 37.42	21.22 ± 12.70	21.44 ± 14.18	2 4.79 ± 27.83	.952
D-Dimer†	88.0 (61.5–159.5)	98 (74–150)	131.5 (83.75–373)	198 (129–357)	.000
eGFR	95.48 ± 23.50	81.96 ± 36.13	93.72 ± 28.22	54.04 ± 26.19	.000
HOMA-IR†	3.78 (1.71–5.47)	3.95 (3.13–8.4)	5.66 (2.16–10.17)	2.27 (1.32–5.39)	.065
HOMA-β†	18.96 (9.21–37.08)	21.94 (5.77–30.61)	18.5 (8.35–58.65)	15.08 (5.68–35.37)	.791
QUICKI†	0.52 (0.48–0.63)	0.51 (0.44–0.54)	0.47 (0.42–0.59)	0.58 (0.48–0.67)	.065

Group A (the normal albuminuria group, UmALB/Cr < 30 mg/g).

Group B (microalbuminuria group, 30 mg/g ≤ UmALB/Cr ≤ 300 mg/g).

Group C (macroalbuminuria group, UmALB/Cr > 300 mg/g).

Group D (renal insufficiency group, Cr > 97 μmol/L [males], >73 μmol/L [females]).

BMI = body mass index, DBP = diastolic blood pressure, QUICKI = Quantitative Insulin Check Index, SBP = systolic blood pressure, WC = waist circumference, WHR = waist-hip ratio, T2DM = type 2 diabetes mellitus.

The enumerated data were compared with the χ^2 test.

* Normally distributed data were compared with the independent sample one-way ANOVA and are presented as the mean ± SD.

† Data with skewed distribution were compared with the Kruskal–Wallis H test and are presented as the median (IQR).

59 older patients (≥75 years) are presented in Table 2. We observed that sex ratio and mean body mass index between the patients aged ≥60 and ≥75 years were similar. As expected, according to the UmALB/Cr ratio, tHcy, systolic blood pressure (SBP), WC, HC, T2DM duration, fasting glucose, sUA, D-dimer, eGFR, ($P < .01$), fasting c-peptide and high-density lipoprotein cholesterol ($P < .05$) increased in patients aged ≥60 years. Among the older patients (≥75 years), T2DM duration, sUA ($P < .01$), and fasting c-peptide ($P < .05$) relatively increased. Among the patients aged ≥75 years, no significant differences were noted in plasma tHcy levels among the groups ($P = .334$).

3.2. Plasma tHcy concentrations

Plasma tHcy levels significantly increased in patients aged ≥60 years (Fig. 1A). Significant differences were noted in UmALB/Cr ratios among groups A to D (8.40 ± 4.25, 10.98 ± 6.64, 9.68 ± 4.50, and 16.23 ± 8.94, respectively, $P = .000$). However, in older patients aged ≥75 years, no significant differences were noted in plasma tHcy levels ($P = .334$; Fig. 1B).

3.3. Correlations between plasma tHcy concentrations and the clinical parameters

Bivariate correlation analysis indicated that plasma tHcy levels (log-transformed) in patients aged ≥60 years were positively correlated with log-transformed diastolic blood pressure (DBP) ($r = 0.197$, $P = .006$), WHR ($r = 0.178$, $P = .018$), fasting c-peptide ($r = 0.242$, $P = .001$), Cr ($r = 0.474$, $P = .000$), BUN ($r = 0.201$, $P = .005$), UmALB/Cr ($r = 0.262$, $P = .000$), and sUA ($r = 0.341$, $P = .000$), but it negatively correlated with log-eGFR ($r = -0.370$, $P = .000$; Table 3). Correlation analysis indicated that plasma tHcy concentrations (log-transformed) positively correlated with log-Cr ($r = 0.317$, $P = .016$), BUN ($r = 0.136$, $P = .312$), cystatin-c ($r = 0.309$, $P = .041$), and D-dimer ($r = 0.389$, $P = .003$; Table 4). Plasma tHcy levels (log-transformed) negatively correlated with log-eGFR ($r = -0.304$, $P = .021$). The clinical parameters (T2DM duration, WHR, DBP, fasting c-peptide, UmALB/Cr, Cr, BUN, sUA, and eGFR) of each category are shown in Table 5. After adjusting for covariates associated with tHcy, WHR, DBP, fasting c-peptide, UmALB/Cr, Cr, BUN, sUA, and eGFR were eventually excluded from the logistic regression model (Table 5).

Table 2

General clinical and laboratory parameters of older Chinese patients with T2DM aged ≥75 y.

Variable	A	B	C	D	*P* value
N	16	11	9	23	—
Sex (M/F)	6/10	6/5	5/4	13/10	.644
Age (y)*	77.50 ± 2.66	80.27 ± 3.69	78.22 ± 2.64	78.22 ± 2.98	.132
BMI (kg/m^2)†	24.47 (21.7–27.7)	26.68 (23.17–28.09)	22.84 (21.3–27.1)	25.68 (24.06–29.0)	.482
WC (cm)*	93.33 ± 10.55	95.67 ± 8.59	85.88 ± 6.77	94.91 ± 6.65	.056
WHR*	0.95 ± 0.07	0.94 ± 0.07	0.92 ± 0.06	0.96 ± 0.07	.522
SBP (mm Hg)*	136.31 ± 16.23	145.18 ± 15.50	149.78 ± 17.20	145.26 ± 11.27	.118
DBP (mm Hg)*	75.00 ± 7.07	76.36 ± 9.30	80.22 ± 8.83	75.43 ± 10.14	.531
Duration of DM (mo)	153.94 ± 28.88	141.18 ± 27.95	299.33 ± 47.80	271.30 ± 24.99	.001
Fasting glucose (mmol/L)†	8.91 ± 3.16	11.26 ± 3.28	14.62 ± 10. 91	9.07 ± 3.66	.054
Fasting insulin (mIU/L)†	10.54 (4.55–13.51)	16.93 (11.17–23.46)	5.8 (4.66–19.29)	5 (4.65–9.4)	.134
Fasting c-peptide (pmol/L)*	592.17 ± 297.85	1070.29 ± 541.41	576.89 ± 377.62	867.87 ± 481.83	.033
HbA1c (%)*	9.13 ± 2.10	9.74 ± 1.88	9.00 ± 2.13	8.82 ± 1.69	.666
Creatinine†	67.4 (57.7–73.4)	74.1 (51.2–85.8)	71.9 (51.15–80.7)	153.1 (104.3–188.35)	.000
Blood urea nitrogen†	6.47 (6.06–8.7)	7.41 (5.43–9.66)	6.71 (4.99–9.9)	12.59 (10.3–14.83)	.000
Cystatin-c†	1.11 ± .26	1.27 ± .28	1.13 ± .27	2.27 ± .65	.000
TC (mmol/L)*	4.01 ± 1.08	3.92 ± 1.14	3.85 ± 1.35	4.67 ± 1.50	.251
TG (mmol/L)†	1.51 (1.03–3.43)	1.54 (0.97–2.89)	1.84 (1.02–1.98)	1.84 (1.32–3.56)	.550
LDL-C (mmol/L)*	2.29 ± 0.74	2.29 ± 0.77	2.15 ± 1.00	2.60 ± 0.85	.482
HDL-C (mmol/L)*	1.04 ± 0.29	0.88 ± 0.21	1.12 ± 0.31	0.96 ± 0.25	.227
Serum uric acid	319.27 ± 65.78	339.49 ± 100.77	331.06 ± 83.73	426.41 ± 80.71	.001
CA199	22.12 ± 18.05	21.12 ± 10.16	17.88 ± 9.66	18.76 ± 8.90	.812
D-Dimer†	143 (117–181)	180 (117.25–261.25)	138 (80–548)	284.5 (145.75–722.5)	.176
eGFR	34.66 ± 19.52	60.25 ± 26.19	80.21 ± 15.09	82.49 ± 25.72	.000
THcy	9.42 ± 4.90	12.53 ± 8.80	9.28 ± 5.27	13.55 ± 8.98	.334
HOMA-IR†	3.32 (1.77–4.73)	7.58 (4.79–9.03)	5.52 (3.96–7.50)	2.13 (1.55–4.12)	.521
HOMA-β†	21.61 (8.82–45.3)	23.61 (14.42–51.13)	4.35 (0.56–76.04)	9.6 (5.68–33.36)	.315
QUICKI†	0.55 ± 0.11	0.46 ± 0.05	0.48 ± 0.03	0.60 ± 0.15	.062

Group A (the normal albuminuria group, UmALB/Cr < 30 mg/g).

Group B (microalbuminuria group, 30 mg/g ≤ UmALB/Cr ≤ 300 mg/g).

Group C (macroalbuminuria group, UmALB/Cr > 300 mg/g).

Group D (renal insufficiency group, Cr > 97 μmol/L [males], >73 μmol/L [females]).

BMI = body mass index, DBP = diastolic blood pressure, QUICKI = Quantitative Insulin Check Index, SBP = systolic blood pressure, T2DM = type 2 diabetes mellitus, WC = waist circumference, WHR = waist-hip ratio.

The enumerated data were compared with the χ^2 test.

* Normally distributed data were compared with the independent sample one-way ANOVA and are presented as the mean ± SD.

† Data with skewed distribution were compared with the Kruskal–Wallis H test and are presented as the median (IQR).

WHR, DBP, fasting c-peptide, UmALB/Cr, Cr, BUN, sUA, and eGFR were identified as independent factors associated with plasma tHcy concentrations in patients aged ≥60 years (P = .018,.006,.001,.000,.000,.005,.000, and.000, respectively). After adjusting for covariates associated with tHcy, DBP, fasting insulin, Cr, fasting c-peptide, total cholesterol, low-density lipoprotein cholesterol, D-dimer, and eGFR were removed from the logistic regression model (Table 6). DBP, fasting insulin, Cr,

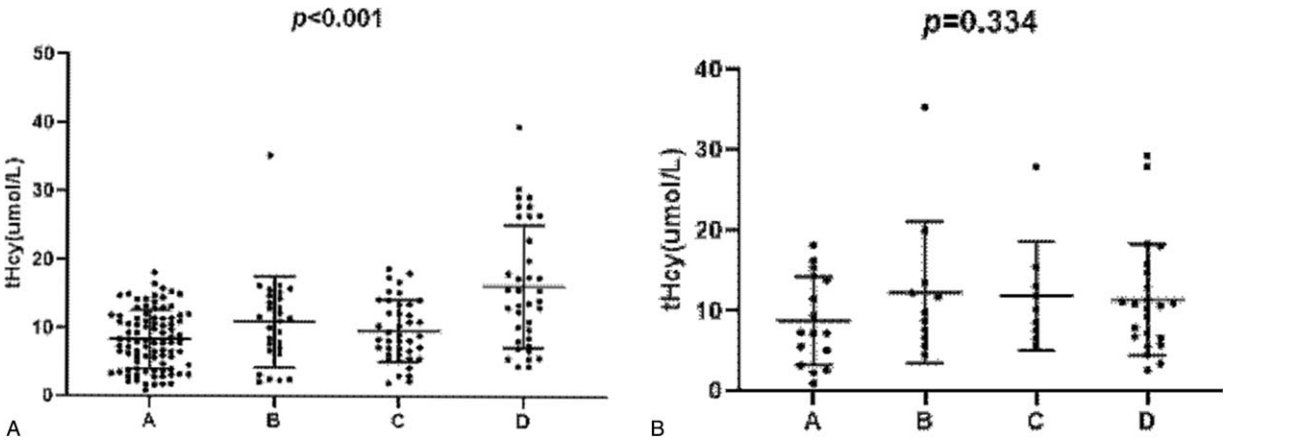

Figure 1. A, Median (IQR) plasma tHcy levels in Chinese T2DM patients aged ≥60 years. B, Plasma total homocysteine levels (median (IQR) marked) in older than 75 years of Chinese older patients with T2DM. T2DM = type 2 diabetes mellitus.

Table 3

Bivariate correlation between plasma tHcy levels and other variables (≥60 y).

THcy	r	P
Sex (M/F)	−0.085	.238
Age (y)	0.112	.117
BMI (kg/m^2)	−0.026	.720
WC (cm)	0.128	.091
WHR	0.178*	.018
SBP (mm Hg)	0.133	.063
DBP (mm Hg)	0.197†	.006
Duration of DM (mo)	0.090	.213
Fasting glucose (mmol/L)	0.038	.603
Fasting insulin (mIU/L)	−0.008	.928
Fasting c-peptide (pmol/L)	0.242†	.001
HbA1c (%)	−0.018	.806
Creatinine	0.474†	.000
Blood urea nitrogen	0.201†	.005
Cystatin-c	−0.019	.811
UmALB/Cr	0.262†	.000
TG (mmol/L)	0.026	.718
TC (mmol/L)	0.114	.116
LDL-C (mmol/L)	0.101	.164
HDL-C (mmol/L)	−0.002	.974
Serum uric acid	0.341†	.000
CA199	0.049	.516
D-Dimer	0.128	.085
eGFR	−0.370†	.000
HOMA-IR	−0.044	.607
HOMA-β	0.020	.818
QUICKI	0.024	.783

BMI = body mass index, DBP = diastolic blood pressure, QUICKI = Quantitative Insulin Check Index, SBP = systolic blood pressure, tHcy = homocysteine, WC = waist circumference, WHR = waist-hip ratio.
* $P < .05$.
† $P < .01$.

fasting c-peptide, total cholesterol, low-density lipoprotein cholesterol, D-dimer, and eGFR were identified as independent factors associated with plasma tHcy concentrations in patients aged ≥75 years ($P = .037$,.033,.018,.028,.017,.002, and.021, respectively). After adjusting for covariates associated with tHcy, fasting c-peptide and Cr were removed from the multiple stepwise regression model (Table 7). Fasting c-peptide and Cr were identified as independent factors associated with plasma tHcy concentrations in patients with T2DM aged ≥60 years ($P = .031$ and.000, respectively). After adjusting for covariates associated with tHcy, fasting insulin and Cr were removed from the multiple stepwise regression model. Fasting insulin and Cr were identified

Table 4

Bivariate correlation between plasma tHcy levels and other variables (≥75 y).

THcy	r	P
Fasting c-peptide (pmol/L)	0.017	.905
Creatinine	0.317*	.016
Blood urea nitrogen	0.136	.312
Cystatin-c	0.309*	.041
eGFR	−0.304*	.021
D-Dimer	0.389†	.003

tHcy = homocysteine.
* $P < .05$.
† $P < .01$.

Table 5

Logistic regression analysis: factors associated with plasma tHcy levels in older Chinese T2DM patient aged ≥60 y.

Independent factors	β (unstandardized coefficient)	Std. error	t	P value
WHR*	18.173	7.623	2.384	.018
DBP (mm Hg)*	0.128	0.046	2.794	.006
Fasting c-peptide (pmol/L)*	0.003	0.001	3.298	.001
UmALB/Cr	0.004	0.001	3.764	.000
Creatinine	0.067	0.009	7.425	.000
Blood urea nitrogen	0.158	0.056	2.821	.005
Serum uric aci	0.022	0.004	5.002	.000
eGFR	−0.083	0.015	−5.541	.000

DBP = diastolic blood pressure, T2DM = type 2 diabetes mellitus, tHcy = homocysteine, WHR = waist-hip ratio.
* These variables were log-transformed before analysis.
† $P < .05$.
‡ $P < .01$.

as independent factors of patients aged ≥75 years ($P = .028$ and .002, respectively, Table 8).

3.4. Plasma tHcy concentrations and DKD

The crude area under the ROC curve (AUC) of tHcy to predict the presence of DKD was 0.636 [95% confidence interval = 0.545–0.727], suggesting that plasma tHcy is a potential biomarker of DKD (Fig. 2).

4. Discussion

Older patients with T2DM are a rapidly emerging population presenting important clinical challenges. Individuals in this diverse group can widely differ in physical and pathological statuses that can increase the risks of complications and negatively impact glycaemic control, disease severity as well as the quality of life. Diabetic nephropathy risk is increased in older patients with T2DM. DKD is a critical complication associated with T2DM with an increasing prevalence worldwide.[15] The proportion of older patients with T2DM and DKD is predicted to increase from 15% to 25%.[16] Diabetic nephropathy is the primary complication associated with T2DM, leading to kidney failure.[17] DKD is defined as a progressive increase in urinary albumin excretion (UAE), which leads to a decrease in glomerular

Table 6

Logistic regression analysis: factors associated with plasma tHcy levels in older Chinese T2DM patient aged ≥75 y.

Independent factors	β (unstandardized coefficient)	Std. error	t	P value
DBP (mm Hg)*	0.261	0.116	2.245	.029†
Fasting insulin (mIU/L)	−0.152	0.070	−2.165	.037†
Fasting c-peptide (pmol/L)*	0.005	0.002	2.198	.033†
Creatinine	0.043	0.017	2.452	.018†
TC (mmol/L)*	1.721	0.759	2.267	.028†
LDL-C (mmol/L)*	2.989	1.208	2.474	.017†
D-Dimer	0.005	0.002	3.224	.002‡
eGFR	−0.075	0.032	−2.367	.021†

DBP = diastolic blood pressure, tHcy = homocysteine, T2DM = type 2 diabetes mellitus.
* These variables were log-transformed before analysis.
† $P < .05$.
‡ $P < .01$.

Table 7

Multiple stepwise regression analysis: independent factors associated with plasma tHcy levels in Chinese patients with T2DM (≥60 y).

Independent factors	β (unstandardized coefficient)	Std. error	*t*	*P* value
Fasting c-peptide (pmol/L)	0.062	0.001	2.177	.031*
Creatinine	0.002	0.009	6.592	.000†

tHcy = homocysteine, T2DM = type 2 diabetes mellitus.
* $P < .05$.
† $P < .01$.

Table 8

Multiple stepwise regression analysis: independent factors associated with plasma tHcy levels in Chinese patients with T2DM (≥75 y).

Independent factors	β (unstandardized coefficient)	Std. error	*t*	*P* value
Fasting insulin (mIU/L)‡	−1.191	0.080	−2.381	.028
Creatinine‡	0.076	0.022	3.520	.002

T2DM = type 2 diabetes mellitus, tHcy = homocysteine.
* $P < .05$.
† $P < .01$.

filtration and eventually renal failure.[18] DKD is also the most important cause of end-stage renal disease, which is an independent risk factor for cardiovascular disease as well as increased mortality. Furthermore, 30% patients with T2DM develop renal disease.[19,20] UmALB/Cr, Cr, urea, eGFR, UAE, and cystatin-c are important biomarkers of renal injury. Of these, UAE is used for the diagnosis and prognosis of DKD because it helps in the early detection of renal parenchymal injury.[21,22] Cystatin-c is another important biomarker of renal injury, which is a low-molecular-weight protein synthesized by nucleated cells, and functions in the regulation of cysteine proteases. Furthermore, cystatin-c has been reported as a very promising marker for the early detection of end-stage renal disease in patients with T2DM.[22,23]

Older patients with T2DM have high rates of premature death, functional disability, and coexisting illnesses such as hypertension, coronary heart disease, and stroke compared with those without T2DM. Cho et al[24] reported that hyperthermia is associated with increased microalbuminuria risk in patients with T2DM, which is in accordance with the concept that hyperthermia has an etiological role in the pathogenesis of diabetic neuropathy (DN). Abbasi et al[25] proposed that early identification of risks associated with T2DM and microvascular complications helps in the introduction of preventive interventions to stop or to delay disease onset that is crucial in decreasing morbidity and mortality in patients with T2DM in addition to microvascular complications.

Therefore, the present study described the relationship among plasma tHcy levels, different biochemical measurements, and biomarkers to assess the association between plasma tHcy and renal disease in older Chinese patients with T2DM. However, few studies have investigated these potential associations. Several biomarkers such as C-reactive protein, adiponectin, and gamma-glutamyl transpeptidase, are associated with an increased risk of developing T2DM.[26] Wang et al reported that increased plasma tHcy levels correlated with a greater risk of renal failure, which is characterized by a rapid decrease in eGFR. Moreover, plasma tHcy is significantly associated with decreased eGFR after

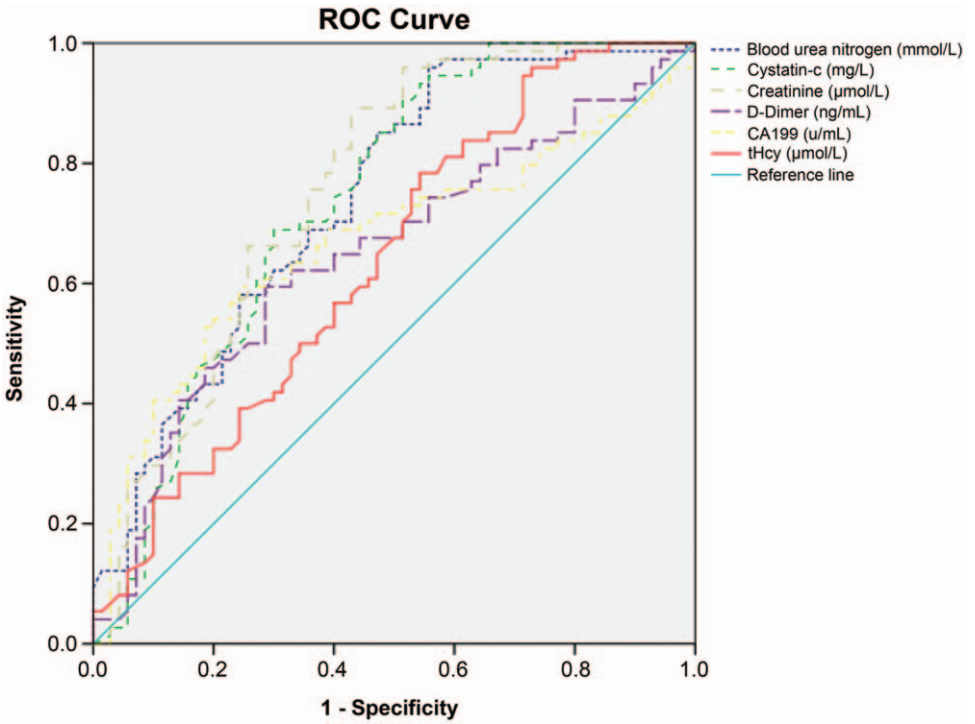

Figure 2. The crude AUC of the ROC curve of plasma total homocysteine for predicting the presence of DKD. AUC = crude area under the ROC curve, DKD = diabetic kidney disease, ROC = receiver operating characteristic.

controlling for other factors that promote disease progression. Plasma tHcy acts as an independent risk factor as well as an early predictor of the progression of DN in patients with T2DM.[27] Deebukkhum et al[28] found that decreased eGFR associated with increased tHcy levels may present a higher risk for future DN. The left ventricular mass index, carotid intima media thickness, and Cr level positively correlated with plasma tHcy levels. Therefore, plasma tHcy might be a useful predictor of end-organ damage like cardiac, carotid, and renal diseases in patients newly diagnosed with T2DM.[29] A previous study found that hypertension and plasma tHcy and triglyceride levels significantly associated with an increased DKD risk and have been used as a risk prediction model with good test performance and discriminatory capacity. Their observations suggest that increased plasma tHcy levels are associated with an increased DKD risk in Chinese diabetic patients.[30]

There were 3 crucial findings in the current study. First, according to the UmALB/Cr in Chinese patients aged ≥60 years, plasma tHcy levels, and SBP, WC, T2DM duration, fasting glucose, sUA, D-Dimer, and eGFR were statistically significant in each group ($P < .01$), although no significant difference was noted in plasma tHcy levels among Chinese patients with T2DM aged ≥75 years ($P = .334$); however, significant differences were noted among T2DM duration, sUA ($P < .01$), and fasting c-peptide ($P < .05$). Second, in this study, plasma tHcy concentrations in patients with T2DM aged ≥60 years positively correlated with log-DBP, fasting c-peptide, Cr, BUN, UmALB/Cr, and sUA, but negatively correlated with log-eGFR. Meanwhile, the results of multiple stepwise regression analysis suggested that fasting c-peptide and Cr independently associated with plasma tHcy in patients with T2DM aged ≥60 years, whereas fasting insulin and Cr levels were independently associated in those aged ≥75 years. Lastly, in this study, the crude AUC of plasma tHcy for prediction of the presence of DKD was 0.636 (95% confidence interval = 0.545–0.727), suggesting that plasma tHcy is predictive of the risk for DKD. Unfortunately, the number of older patients with T2DM aged ≥75 years participating in this study was relatively less. Hence, a larger population is needed to confirm the cut-off value of plasma tHcy for predicting DKD, unlike other predictors, such as Cr and BUN.

This hospital-based observational study is the first to provide clinical evidence on the role of circulating tHcy in developing DKD. However, the present study has several limitations that need be addressed. First, the study is based on a cross-sectional design and the cohort was relatively small. Therefore, these findings need be confirmed in other ethnicities and in larger prospective cohort studies. Second, as an observational study, a cause-and-effect relationship could not be confirmed between plasma tHcy and physiopathologic DKD mechanism. Both in vitro and in vivo studies are necessary to elucidate the underlying mechanisms. Third, because of the study design and limited funds, we were only able to determine the plasma tHcy concentrations in DKD. Other data for the prediction of DKD were not available. Therefore, further studies are required.

5. Conclusion

Plasma tHcy concentrations are relatively elevated in older patients with DKD, especially those aged ≥75 years. Our data suggest that tHcy might serve as a biomarker for the development of DKD in older patients, but it is not ideal for predicting T2DM. Nonetheless, this study is the first to report plasma tHcy as a marker of DKD in older Chinese patients with T2DM, especially those aged ≥75 years. These findings provide new clinical insights into the roles of tHcy in DKD pathogenesis.

Acknowledgments

The authors express their sincere thanks to all the volunteers and nurses who offered help in the study.

Author contributions

Conceptualization: Ning Ma, Xingbo Cheng.
Data curation: Ning Ma, Weiwei Liu, Mengping Wu.
Formal analysis: Weiwei Liu.
Investigation: Ning Ma, Ning Xu, Dong Yin.
Methodology: Ning Ma, Weiwei Liu, Mengping Wu, Xingbo Cheng.
Project administration: Ning Ma, Ning Xu, Dong Yin, Weiwei Liu, Xingbo Cheng.
Supervision: Ning Ma, Ning Xu, Dong Yin, Weiwei Liu, Xingbo Cheng.
Writing – original draft: Ning Ma, Ning Xu, Dong Yin, Weiwei Liu, Xingbo Cheng.
Writing – review & editing: Ning Ma, Xingbo Cheng.

References

[1] Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271–81.
[2] Chang HY, Singh S, Mansour O, et al. Association between sodium-glucose cotransporter 2 inhibitors and lower extremity amputation among patients with type 2 diabetes. JAMA Intern Med 2018;178:1190–8.
[3] Zhang L, Wang F, Wang L, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. Lancet 2012;379:815–22.
[4] Zheng N, Shi X, Chen X, et al. Associations between inflammatory markers, hemostatic markers and microvascular complications in 182 Chinese patients with type 2 diabetes mellitus. Lab Med 2015;46:214–20.
[5] Ho H, Cheung CY, Sabanayagam C, et al. Retinopathy signs improved prediction and reclassification of cardiovascular disease risk in diabetes: a prospective cohort study. Sci Rep 2017;7:41492.
[6] Toth PP, Simko RJ, Palli SR, et al. The impact of serum lipids on risk for microangiopathy in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2012;11:109.
[7] Elias A, Eng S. Homocysteine concentrations in patients with diabetes mellitus—relationship to microvascular and macrovascular disease. Review. Diabetes Obes Metab 2005;7:117–21.
[8] Wotherspoon F, Laight DW, Browne DL, et al. Plasma homocysteine, oxidative stress and endothelial function in patients with Type 1 diabetes mellitus and microalbuminuria. Diabet Med 2006;23:1350–6.
[9] Wu CC, Zheng CM, Lin YF, et al. Role of homocysteine in end-stage renal disease. Clin Biochem 2012;45:1286–94.
[10] Chico A, Pérez A, Córdoba A, et al. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia 1998;41:684–93.
[11] Association AD. Standards of medical care in diabetes—2014. Diabetes Care 2014;37(suppl 1):S14–80.
[12] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[13] Seltzer HS, Allen EW, Herron AL, et al. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 1967;46:323–35.
[14] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
[15] Mahmoodnia L, Aghadavod E, Beigrezaei S, et al. An update on diabetic kidney disease, oxidative stress and antioxidant agents. J Renal Inj Prev 2017;6:153–7.

[16] Dunning T, Sinclair A, Colagiuri S. New IDF Guideline for managing type 2 diabetes in older people. Diabetes Res Clin Pract 2014;103: 538–40.
[17] Zhang X, Cui X, Li F, et al. Association between diabetes mellitus with metabolic syndrome and diabetic microangiopathy. Exp Ther Med 2014;8:1867–73.
[18] Association AD. 9 microvascular complications and foot care. Diabetes Care 2016;39(suppl 1):S72–80.
[19] Karnib HH, Ziyadeh FN. The cardiorenal syndrome in diabetes mellitus. Diabetes Res Clin Pract 2010;89:201–8.
[20] Gross JL, Silveiro SP, Canani LH, et al. [Diabetic nephropathy and cardiac disease]. Arq Bras Endocrinol Metabol 2007;51:244–56.
[21] Cirillo M. Evaluation of glomerular filtration rate and of albuminuria/ proteinuria. J Nephrol 2010;23:125–32.
[22] Murussi M, Murussi N, Campagnolo N, et al. [Early detection of diabetic nephropathy]. Arq Bras Endocrinol Metabol 2008;52:442–51.
[23] Massey D. Commentary: clinical diagnostic use of cystatin C. J Clin Lab Anal 2004;18:55–60.
[24] Cho EH, Kim EH, Kim WG, et al. Homocysteine as a risk factor for development of microalbuminuria in type 2 diabetes. Korean Diabetes J 2010;34:200–6.
[25] Abbasi A, Peelen LM, Corpeleijn E, et al. Prediction models for risk of developing type 2 diabetes: systematic literature search and independent external validation study. BMJ 2012;345:e5900.
[26] Abbasi A, Sahlqvist AS, Lotta L, et al. A systematic review of biomarkers and risk of incident type 2 diabetes: an overview of epidemiological, prediction and aetiological research literature. PLoS One 2016;11: e0163721.
[27] Wang H, Cui K, Xu K, et al. Association between plasma homocysteine and progression of early nephropathy in type 2 diabetic patients. Int J Clin Exp Med 2015;8:11174–80.
[28] Deebukkhum S, Pingmuangkaew P, Tangvarasittichai O, et al. Estimated creatinine clearance, homocysteine and high sensitivity-C-reactive protein levels determination for early prediction of nephropathy and atherosclerosis risk in type 2 diabetic patients. Indian J Clin Biochem 2012;27:239–45.
[29] Kundi H, Kiziltunc E, Ates I, et al. Association between plasma homocysteine levels and end-organ damage in newly diagnosed type 2 diabetes mellitus patients. Endocr Res 2017;42:36–41.
[30] Ma L, Liu Q, Jiang Y, et al. Genetically elevated circulating homocysteine concentrations increase the risk of diabetic kidney disease in Chinese diabetic patients. J Cell Mol Med 2019;23:2794–800.