Association Between Serum Homocysteine Levels and Severity of Diabetic Kidney Disease in 489 Patients with Type 2 Diabetes Mellitus: A Single-Center Study

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Background:
This study from a single center aimed to investigate the association between serum homocysteine (Hcy) levels and severity of diabetic kidney disease (DKD) in 489 patients with type 2 diabetes mellitus (T2DM).

Material/Methods:
A total of 1163 patients with T2DM, including 674 T2DM without kidney disease (T2DM group) and 489 T2DM with DKD (DKD group), were evaluated. The DKD group was subdivided according to the chronic kidney disease (CKD) and albuminuria staging criteria in “Kidney Disease: Improving Global Outcomes 2012 clinical practice guideline” to quantify the severity of DKD: CKD stage 1 (n=164), CKD stage 2 (n=103), CKD stage 3 (n=95), CKD stage 4 (n=71), and CKD stage 5 (n=56) and stage A1 (n=57), stage A2 (n=250), and stage A3 (n=182), respectively. Peripheral blood was collected after 8 hours of fasting to test Hcy levels.

Results:
In CKD stages, Hcy levels gradually increased with increasing DKD severity (CKD stage 1 to 5); while in albuminuria stages, Hcy levels did not gradually increase with increasing DKD severity (stage A1 to A3). Hcy was an independent risk factor for CKD stages 2-5 (P<0.05), and had no effect on the albuminuria stages (P>0.05), while it may indirectly affect the occurrence of albuminuria stages through its impact on estimated glomerular filtration rate.

Conclusions:
The relationship between Hcy level and DKD severity was related to the different staging methods used. Hcy was an independent risk factor for CKD stages but not albuminuria stages.

Keywords: Diabetic Kidney Disease • Diabetic Nephropathy • Type 2 Diabetes Mellitus • Homocysteine • Hyperhomocysteinemia

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Background

Diabetic kidney disease (DKD) is a progressive renal injury clinically characterized by increased albuminuria and decreased renal function (decreased glomerular filtration rate [GFR]) [1]. DKD is one of the most common chronic complications of diabetes and is also the leading cause of end-stage renal disease (ESRD) [2]. In type 2 diabetes mellitus (T2DM), DKD usually occurs in patients with more than 10 years of disease course, its onset is relatively insidious, and often accompanied by retinopathy [3]. The diagnosis and staging of DKD is based on albuminuria and GFR in diabetes patients [4,5]. DKD staging is a method for quantifying the severity of DKD [1]. According to the different indicators used for staging, DKD staging methods include chronic kidney disease (CKD) staging [6] and albuminuria staging (urine albumin/creatinine ratio [UACR]-based staging) [4,5].

Homocysteine (Hcy) is a non-protein-forming sulfur-containing amino acid that is located at the intersection of two metabolic pathways, remethylation, and transsulfuration [6]. As food intake may affect the results, fasting for 8-12 h is recommended before testing the concentration of homocysteine [7]. The normal Hcy level in humans is 5-15 µmol/L [8]. When it exceeds 15 µmol/L, it is called hyperhomocysteinemia (HHcy) [7]. It has been shown that HHcy was an independent risk factor for neurodegenerative [9] and cardiovascular diseases [10]. Increased Hcy levels can be observed in patients with heart failure [11], cognitive impairment [12], and renal insufficiency, especially those with ESRD [13].

Current studies on the relationship between Hcy and DKD severity are inconsistent [14-17]. Most studies are based on the albuminuria staging method [14,15,17], and there are few studies based on the CKD staging method [16]. Different staging methods were used in the above studies, so we hypothesized that the inconsistency of previous studies may be related to the different staging methods used to assess the severity of DKD. Therefore, this study from a single center aimed to investigate the association between serum Hcy levels and severity of DKD in 489 patients with T2DM using 2 different staging methods.

Material and Methods

Ethics Statement

Patient consent was waived due to the retrospective design. This study was approved by the ethics review boards of our hospital, in compliance with the principles of the Declaration of Helsinki.

Inclusion and Exclusion Criteria

The inclusion criteria are as follows: 1) Hospitalized T2DM patients in the Endocrinology Department of the Second Affiliated Hospital of Nanchang University from January 2017 to July 2021; 2) aged 18 years or older; 3) with complete clinical data required for the study. The exclusion criteria were as follows: 1) patients with special disease states, such as diabetic ketoacidosis, hyperosmolar hyperglycemic state, infection, pregnancy, shock; 2) with primary kidney disease and/or renal artery stenosis; 3) with a duration of hypertension longer than that of diabetes mellitus; 4) with abnormal folic acid and vitamin B12 levels or those taking folic acid, vitamin B12, and vitamin B6 that may affect the Hcy level; 5) with only 1 record of UACR of ≥30 mg/g and/or eGFR of <60 mL/min/1.73 m² or those with an interval between 2 abnormal results of less than 3 months.

Study Population

This study was a single-center, cross-sectional study which retrospectively collected the data of 1163 patients with T2DM who were hospitalized in the Endocrinology Department of the Second Affiliated Hospital of Nanchang University from January 2017 to July 2021, including 674 T2DM without kidney disease (T2DM group), eGFR of ≥90 mL/min/1.73 m² and UACR of <30 mg/g) and 489 T2DM with DKD (DKD group, eGFR of <60 mL/min/1.73 m² and/or UACR of ≥30 mg/g). T2DM was diagnosed based on the 1999 WHO diagnostic criteria [18], the cut-off plasma glucose values for diagnosing diabetes are as follows: fasting plasma glucose ≥7.0 mmol/L; random plasma glucose ≥11.1 mmol/L; plasma glucose 2-h post-glucose load (75 g) ≥11.1 mmol/L. The diagnosis of DKD was based on the criteria jointly recommended by the American Diabetes Association and the National Kidney Foundation in 2014 [4,5], that is, CKD caused by diabetes mellitus, defined as a GFR lower than 60 mL/min/1.73 m² and/or UACR higher than 30 mg/g for more than 3 months. The DKD group was subdivided according to the CKD and albuminuria staging criteria in "Kidney Disease: Improving Global Outcomes 2012 clinical practice guideline" [19] to quantify the severity of DKD: CKD stage 1 (n=164, eGFR of ≥90 mL/min/1.73 m² and UACR of ≥30 mg/g), CKD stage 2 (n=103, eGFR of 60-89 mL/min/1.73 m² and UACR of ≥30 mg/g), CKD stage 3 (n=95, eGFR of 30-59 mL/min/1.73 m²), CKD stage 4 (n=71, eGFR of 15-29 mL/min/1.73 m²), and CKD stage 5 (n=56, eGFR of <15 mL/min/1.73 m² or need for a dialysis) and stage A1 (n=57, UACR of <30 mg/g and eGFR of ≥60 mL/min/1.73 m²), stage A2 (n=250, UACR of 30-300 mg/g), and stage A3 (n=182, UACR of >300 mg/g), respectively.

General Data and Clinical Indicators

We collected and compared general data including age, gender, body mass index (BMI). Compared with nondiabetic patients,
diabetic patients showed a higher prevalence of hypertension [20], also, hypertension is an important risk factor for CKD [21], therefore, we collected blood pressure data of patients, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and history of hypertension. DKD usually occurs in patients with more than 10 years of disease course, which is important information for the diagnosis and progression of DKD, so we collected the disease course data of patients. In diabetic patients, glucose metabolic disorder is often accompanied by lipid metabolic disorders, this metabolic dysregulation is associated with the development of DKD [22]. Therefore, we collected data on glucolipid metabolism in patients including fasting plasma glucose (FPG), fasting C peptide (FCP), glycosylated hemoglobin A1c (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. eGFR and UACR are the basis for DKD staging, so data on eGFR and UACR were collected. Serum uric acid (SUA) is a predictor of worsening kidney function [23], so we also collected data on SUA. Folic acid and vitamin B12 are important factors affecting Hcy levels, so we collected data on Hcy, the detection rate of HHcy, as well as folic acid and vitamin B12. FPG was measured using hexokinase/glucose-6-phosphate dehydrogenase method (Beckman Coulter, Suzhou, China), FCP was measured using chemiluminescent method (CobaseE601, Germany), HbA1c was measured using high performance liquid chromatography assay (Bio-Rad, USA), TC was measured using enzymatic colorimetric method (Beckman Coulter, Suzhou, China), TG was measured using GPO-POD method (Beckman Coulter, Suzhou, China), HDL and LDL were measured by direct homogeneous assay methods using detergents (Beckman Coulter, Suzhou, China), SUA was measured using uricase method (BECMAN AU5800, USA), UACR was measured using odds ratios (Biosystems, USA), vitamin B12 was determined by time-of-flight mass spectrometry (ABSCIEX 4500MD, USA), Hcy was determined by enzymatic methods using test kits (AUSA Co., Ltd., Shenzhen, China). eGFR was calculated according to the modified GFR estimating equation for Chinese adults with chronic kidney disease: eGFR modification of diet in renal disease (MDRD)=186×(serum creatinine in mg/dl)^-1.154×(age in years)^-0.203×0.742 (if female)×1.233 [24]. Blood samples were collected after an 8- to 12-hour fast.

### Statistical Analysis

SPSS software (IBM SPSS Statistics, version 21.0) was used for the statistical analysis. Data were presented as means±standard deviations for normally distributed parameters and as medians (25%; 75%) for log-normally distributed parameters. The independent sample t test was used to compare the differences between the indicators in the T2DM group and the DKD group. The Dunnett method for one-way analysis of variance was used to compare each observation group with the control group. Count data were presented as n (%), and the chi-square test was used for comparison between the groups. Pearson correlation analysis was used for bivariate data with normal distribution and Spearman correlation analysis for bivariate data with non-normal distribution or categorical data. Since the data in this study did not meet the requirements of the parallel test of ordered logistic regression, multiple logistic regression was used to adjust for the confounding factors that affect the CKD and albuminuria stages. Unless the test level was adjusted, the difference was considered statistically significant at P<0.05.

### Results

A total of 1163 patients were evaluated including 674 patients in the T2DM group and 489 patients in the DKD group (Table 1). We first compared the data in the DKD group and T2DM group, then, comparisons were performed between each CKD/ACR stage and the T2DM group. In the comparison of each CKD stage with the T2DM group, it was found that the eGFR levels gradually decreased from the T2DM group to CKD stage 5, while the corresponding Hcy levels gradually increased, that is, Hcy and eGFR showed an opposite trend (Table 1, Figure 1A). In the comparison of each albuminuria stage with the T2DM group, it was found that UACR gradually increased from the T2DM group to albuminuria stage A3, but the corresponding Hcy levels did not increase in a similar trend (Table 2), while Hcy and eGFR also showed an opposite trend in albuminuria stages (Table 2, Figure 1B).

The correlation analysis showed that the Hcy was positively correlated with UACR (r=0.299, P<0.001) and negatively correlated with eGFR (r=-0.581, P<0.001), the correlation coefficient between Hcy and eGFR (|r|=0.581) was higher than that between Hcy and UACR (|r|=0.299) (Table 3). The correlation scatter plot showed that the Hcy was negatively correlated with the eGFR (P<0.0001) and positively correlated with the UACR (P<0.0001); however, the linear relationship between the Hcy and eGFR was more significant (Figure 2). The CKD stages and albuminuria stages were both positively correlated with Hcy (P<0.001), while the correlation coefficient between Hcy and CKD stages (|r|=0.487) was higher than that between Hcy and albuminuria stages (|r|=0.389) (Table 3).

The multiple logistic regression analysis of CKD stages revealed that when no confounding factors were adjusted, the Hcy was found as a risk factor for DKD and CKD stage 2-5 (P<0.001). After adjusting for Model 1 and Model 2, Hcy remained a risk factor for DKD and CKD stages 2-5 (P<0.05), and its influence (OR value) gradually increased from CKD stage 2 to CKD stage 5. Therefore, Hcy was considered an independent risk factor for DKD and CKD stages 2-5 among the patients with...
Table 1. Comparison of the general data and clinical indicators between the CKD stage and T2DM group.

| Items                  | T2DM | DKD group | CKD stage 1 | CKD stage 2 | CKD stage 3 | CKD stage 4 | CKD stage 5 |
|------------------------|------|-----------|-------------|-------------|-------------|-------------|-------------|
| N                      | 674  | 489       | 164         | 103         | 95          | 71          | 56          |
| Sex (M/F)              | 394/280 | 303/186  | 104/60      | 60/43       | 63/32       | 38/33       | 38/18       |
| Age (year)             | 56.80±11.67 | 62.79±12.37*** | 58.68±13.22 | 64.55±10.61*** | 64.62±11.26*** | 64.94±12.11*** | 65.70±12.29*** |
| BMI (kg/m²)            | 24.18±3.42 | 24.38±3.70 | 24.24±4.05  | 24.29±3.52  | 24.28±3.47  | 24.19±3.40  | 25.32±3.69  |
| SBP (mmHg)             | 129.13±18.89 | 137.80±23.67*** | 132.59±21.25 | 139.99±20.90*** | 136.16±23.96*** | 141.55±27.65*** | 147.05±25.86*** |
| DBP (mmHg)             | 76.44±9.33 | 79.49±11.39*** | 78.83±10.01* | 80.72±11.02*** | 78.95±11.37  | 77.77±12.43 | 82.30±13.94*** |
| FPG (mmol/L)           | 9.45±4.03 | 9.08±4.57  | 11.04±4.92*** | 9.31±4.40   | 8.73±4.16   | 6.55±2.51*** | 6.76±3.77*** |
| FCP (ng/ml)            | 1.83±0.71 | 2.28±1.43  | 1.79±1.61   | 2.12±1.33   | 2.36±1.65   | 3.18±1.99   | 3.88±2.39   |
| HbA1c (%)              | 9.00±2.40 | 8.75±2.34  | 10.00±2.21*** | 8.70±2.05   | 8.44±2.20   | 7.92±2.16** | 6.77±1.58*** |
| TC (mmol/L)            | 4.96±1.16 | 4.86±1.47  | 5.19±1.38   | 5.14±1.24   | 4.68±1.74   | 4.49±1.55*  | 4.19±1.11*** |
| TG (mmol/L)            | 1.42±0.67 | 1.60±1.12  | 1.64±1.09   | 1.62±1.05   | 1.63±1.09   | 1.33±1.11   | 1.65±1.28   |
| HDL (mmol/L)           | 1.13±0.30 | 1.06±0.32*** | 1.09±0.30  | 1.14±0.35   | 1.03±0.30   | 1.02±0.38** | 0.95±0.24*** |
| LDL (mmol/L)           | 2.97±0.91 | 2.86±1.08  | 3.15±1.03   | 3.06±0.89   | 2.70±1.22   | 2.56±1.12** | 2.29±0.84*** |
| eGFR (ml/min/1.73 m²)  | 119.57±24.22 | 70.03±43.98*** | 120.85±23.87 | 75.11±8.97*** | 47.53±8.76*** | 22.24±3.79*** | 10.64±3.31*** |
| SUA (µmol/L)           | 313.29±86.34 | 397.39±133.34*** | 322.55±88.07 | 377.82±101.03*** | 431.69±105.74*** | 464.81±128.32*** | 508.87±194.39*** |
| UACR (mg/g)            | 6.35±3.83 | 137.21±41.69*** | 76.72±42.68  | 143.56±53.49*** | 104.12±193.49*** | 421.31±205.63*** | 1128.98±282.24*** |
| Hcy (µmol/L)           | 11.50±4.16 | 16.80±8.99*** | 11.59±3.39 | 13.97±4.10*** | 17.47±6.27*** | 22.25±9.03*** | 29.17±13.27*** |
| FA (ng/ml)             | 14.25±4.51 | 12.07±4.97*** | 13.70±4.54 | 12.48±4.90*** | 11.41±4.57*** | 10.02±4.70*** | 10.28±5.62*** |
| VitB12 (pg/ml)         | 467.48±153.55 | 458.86±168.34*** | 477.52±160.06 | 445.38±151.32*** | 443.16±161.03*** | 495.03±205.48*** | 409.75±170.42*** |
| Duration of diabetes (year) | 5.00±1.00 | 10.00±5.00*** | 7.00±1.00 | 10.00±5.00*** | 10.00±5.00*** | 11.00±5.00*** | 12.50±5.00*** |
The multiple logistic regression analysis of albuminuria stages showed that when no confounding factors were adjusted, Hcy was a risk factor for DKD and stage A1-A3 (P<0.001). Since the eGFR was an important factor affecting the UACR, Model 1 was adjusted for other general data and clinical indicators and revealed that Hcy was still a risk factor for DKD and stage A1-A3 (P<0.001). However, after adjusting for eGFR, the Hcy did not affect the albuminuria stages (P>0.05). Therefore, we speculated that Hcy may indirectly affect the occurrence of albuminuria stages through its influence on eGFR (Table 5).

**Discussion**

In the present study, we investigated the association between serum Hcy levels and severity of DKD in 489 patients with T2DM using 2 different staging methods. We found that in CKD stages (eGFR-based staging), Hcy levels gradually increased with increasing DKD severity (CKD stage 1 to CKD stage 5); while in albuminuria stages (UACR-based staging), Hcy levels did not gradually increase with increasing DKD severity (stage A1 to A3), but an opposite trend between Hcy and eGFR was observed. Correlation analysis showed that Hcy was more closely related to eGFR and CKD stages than to UACR and albuminuria stages. Finally, the multiple logistic regression analysis revealed that Hcy was an independent risk factor for CKD stage 2-5 and may indirectly affect the occurrence of albuminuria stages through its influence on eGFR.

The current studies of the Hcy levels and DKD severity are mostly based on albuminuria staging. A meta-analysis involving seven studies divided the study population into three groups: T2DM group (T2DM without DKD), microalbumin group, and macroalbuminuria group [14]. The analysis showed that the Hcy level of >15 µmol/L is associated with higher risk of DKD (P<0.05; **P<0.01; ***P<0.001; * P<0.005).

**Figure 1.** The opposite trend between Hcy and eGFR in CKD stages (A) and albuminuria stages (B) (GraphPad Prism 9, GraphPad Software). Hcy – homocysteine; eGFR – estimated glomerular filtration rate.
Table 2. Comparison of the general data and clinical indicators between the albuminuria stage and T2DM group.

| Items              | T2DM       | Stage A1 | Stage A2 | Stage A3 |
|--------------------|------------|----------|----------|----------|
| N                  | 674        | 57       | 250      | 182      |
| Sex (M/F)          | 394/280    | 151/99   | 119/62   |
| Age (year)         | 56.80±11.67| 69.16±10.34***| 61.7±13.23***| 63.0±11.06***|
| BMI (kg/m²)        | 24.18±3.42 | 23.61±3.21 | 24.45±3.87 | 24.51±3.59 |
| SBP (mmHg)         | 129.13±18.89| 127.89±17.72 | 131.7±21.13 | 149.26±24.18***|
| DBP (mmHg)         | 76.44±9.33 | 75.3±10.45 | 78.06±10.18 | 82.77±12.40***|
| FPG (mmol/L)       | 9.45±4.03  | 7.95±3.72  | 9.84±6.62  | 8.40±4.56  |
| Hcy (µmol/L)       | 11.50±4.16 | 20.14±8.98***| 14.36±7.11***| 19.10±10.11***|

Data are presented as means±standard deviations for normally distributed parameters, medians (25%; 75%) for log-normally distributed parameters, or n (%) for count data. T2DM – type 2 diabetes mellitus; DKD – diabetic kidney disease; N – number; M – male; F – female; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FPG – fasting plasma glucose; FCP – fasting C peptide; HbA1c – glycosylated hemoglobin A1c; TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; eGFR – estimated glomerular filtration rate; SUA – serum uric acid; UACR – urinary albumin/creatinine ratio; Hcy – homocysteine; FA – folic acid; VitB12 – vitamin B12; HHcy – hyperhomocysteinemia; Hcy level of >15 µmol/L.

* P<0.05; ** P<0.01; *** P<0.001; # P<0.0083.
Hcy levels in different albuminuria stages. Based on this information and previous studies, we found that the reason for the inconsistent results of previous studies may be the inconsistent grouping criteria used and/or the eGFR/creatinine levels vary in different groups [17,26]. There are relatively few studies that explored the correlation between the Hcy level and CKD stages. Pastore et al evaluated a total of 178 subjects and found that the Hcy level of the patients with DKD gradually increased from CKD stage 1 to CKD stage 4 [16], which is consistent with our results [16].

A study from China based on a large sample of pathological diagnosis of DKD in T2DM patients reported that Hcy is one of the causes and a strong predictor of DKD in patients with

Table 3. Hcy was more closely related to eGFR and CKD stages than to UACR and albuminuria stages.

|                      | Hcy                          | CKD stages                          | Albuminuria stages                          |
|----------------------|------------------------------|-------------------------------------|----------------------------------------------|
| Age                  | r=0.261, P<0.001***          | r=0.264, P<0.001***                 | r=0.214, P<0.001***                          |
| BMI                  | r=0.036, P=0.224             | r=0.035, P=0.238                    | r=0.035, P=0.239                             |
| SBP                  | r=0.148, P<0.001***          | r=0.216, P<0.001***                 | r=0.253, P<0.001***                          |
| DBP                  | r=0.065, P=0.027             | r=0.132, P<0.001***                 | r=0.174, P<0.001***                          |
| FPG                  | r=-0.167, P<0.001***         | r=-0.157, P<0.001***                | r=-0.093, P=0.002**                          |
| FCP                  | r=0.262, P<0.001***          | r=0.252, P<0.001***                 | r=0.164, P<0.001***                          |
| HbA1c                | r=-0.199, P<0.001***         | r=-0.136, P<0.001***                | r=-0.061, P=0.039*                           |
| TC                   | r=-0.171, P<0.001***         | r=-0.108, P<0.001***                | r=-0.043, P=0.142                           |
| TG                   | r=-0.025, P=0.399            | r=0.078, P=0.008**                  | r=0.077, P=0.009**                           |
| HDL                  | r=-0.159, P<0.001***         | r=-0.143, P<0.001***                | r=-0.082, P=0.120                           |
| LDL                  | r=-0.173, P<0.001***         | r=-0.132, P<0.001***                | r=-0.105, P<0.001***                         |
| eGFR                 | r=-0.581, P<0.001***         | r=-0.587, P<0.001***                | r=-0.571, P<0.001***                         |
| SUA                  | r=0.356, P<0.001***          | r=0.423, P<0.001**                  | r=0.352, P<0.001***                          |
| UACR                 | r=0.299, P<0.001***          | r=0.757, P<0.001***                 | –                                             |
| Hcy                  | –                            | r=0.487, P<0.001***                 | r=0.389, P<0.001***                          |
| FA                   | r=-0.320, P<0.001***         | r=-0.287, P<0.001***                | r=-0.249, P<0.001***                         |
| VitB12               | r=-0.123, P<0.001***         | r=0.052, P=0.079                    | r=0.055, P=0.060                            |
| Duration of diabetes | r=0.231, P=0.001***          | r=0.358, P<0.001***                 | r=0.326, P<0.001***                          |

CKD – chronic kidney disease; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FPG – fasting plasma glucose; FCP – fasting C peptide; HbA1c – glycosylated hemoglobin A1c; TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; eGFR – estimated glomerular filtration rate; SUA – serum uric acid; UACR – urinary albumin/creatinine ratio; Hcy – homocysteine; FA – folic acid; VitB12 – vitamin B12; * P<0.05; ** P<0.01; *** P<0.001.

Figure 2. Compared with UACR (B), Hcy has a better linear correlation with eGFR (A) (GraphPad Prism 9, GraphPad Software).

Hcy – homocysteine; eGFR – estimated glomerular filtration rate; UACR – urinary albumin/creatinine ratio.
T2DM [27], similar phenomenon was also reported in another recent literature [28]. The circulating blood Hcy level increases by 5 µmol/L, and the probability of DKD is increased by approximately 4 times [27]. Zhang et al found that after an intervention to reduce the Hcy level in the blood circulation, the progression of DKD was alleviated [29]. In the current study, we also found that the Hcy was an independent risk factor for CKD stage 2-5 among the patients with T2DM (P<0.001).

Before adjusting for eGFR, Hcy was a risk factor for stage A1-A3 (P<0.001), however, after adjusting for eGFR, the Hcy was no more a risk factor for the albuminuria stages (P>0.05).

Based on the above information, we speculated that Hcy may indirectly affect the occurrence of albuminuria stages through its influence on eGFR. Therefore, a healthy diet, regular exercise [30], management of blood glucose [31] and blood pressure, as well as the administration of drugs (such as folic acid and vitamin B12) that can reduce Hcy levels are very important to slow the development of DKD in T2DM patients [29].

This is the first study to explore the relationship between the Hcy level and DKD severity using 2 staging methods. However, this study also has the following limitations. First, it was a

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**Table 4.** Hcy was an independent risk factor for CKD stages 2-5 among the patients with T2DM.

|                | Crude OR (95% CI) | Adjusted OR (95% CI) |
|----------------|-------------------|----------------------|
|                | Model 1           | Model 2              |
| T2DM           | 1                 | 1                    |
| DKD group      | 1.196 (1.160, 1.234)*** | 1.175 (1.138, 1.214)*** | 1.136 (1.071, 1.204)*** |
| CKD stage 1    | 1.008 (0.958, 1.062) | 0.983 (0.930,1.040)  | 0.991 (0.904, 1.086)  |
| CKD stage 2    | 1.181 (1.122, 1.243)*** | 1.147 (1.085,1.213)*** | 1.090 (1.006, 1.181)* |
| CKD stage 3    | 1.328 (1.265, 1.395)*** | 1.303 (1.238, 1.372)*** | 1.187 (1.107, 1.271)** |
| CKD stage 4    | 1.427 (1.354, 1.504)*** | 1.416 (1.341, 1.495)*** | 1.262 (1.170, 1.362)*** |
| CKD stage 5    | 1.494 (1.414, 1.578)*** | 1.480 (1.397, 1.567)*** | 1.356 (1.250, 1.471)*** |

**Model 1:** adjusted for sex, age, and BMI; **model 2:** adjusted for model 1 variables+ SBP, DBP, FPG level, FCP level, HbA1c level, TC level, TG level, HDL level, LDL level, SUA level, UACR, folic acid level, vitamin B12 level, duration of diabetes mellitus, and history of hypertension; Hcy – homocysteine; OR – odds ratio; CI – confidence interval; T2DM – type 2 diabetes mellitus; DKD – diabetic kidney disease; CKD – chronic kidney disease; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FPG – fasting plasma glucose; FCP – fasting C peptide; HbA1c – glycated hemoglobin A1c; TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; SUA – serum uric acid; UACR – urinary albumin/creatinine ratio; * P<0.05; ** P<0.01; *** P<0.001.

**Table 5.** Hcy was not an independent risk factor for albuminuria stages among the patients with T2DM.

|                | Crude OR (95% CI) | Adjusted OR (95% CI) |
|----------------|-------------------|----------------------|
|                | Model 1           | Model 2              |
| T2DM           | 1                 | 1                    |
| DKD group      | 1.196 (1.160, 1.234)*** | 1.104 (1.066, 1.144)*** | 1.021 (0.984, 1.060)  |
| Stage A1       | 1.251 (1.203, 1.302)*** | 1.104 (1.052, 1.158)*** | 0.964 (0.909, 1.022)  |
| Stage A2       | 1.155 (1.116, 1.195)*** | 1.084 (1.044, 1.126)*** | 1.024 (0.985, 1.065)  |
| Stage A3       | 1.242 (1.199, 1.286)*** | 1.126 (1.081, 1.172)*** | 0.994 (0.945, 1.044)  |

**Model 1:** Adjusted for sex, age, SBP, DBP, FPG level, FCP level, HbA1c level, TC level, TG level, HDL level, LDL level, SUA level, folic acid level, vitamin B12 level, duration of diabetes mellitus, and history of hypertension; **model 2:** Adjusted for model 1 variables+ eGFR; Hcy, homocysteine; OR – odds ratio; CI – confidence interval; T2DM – type 2 diabetes mellitus; DKD – diabetic kidney disease; SBP – systolic blood pressure; DBP – diastolic blood pressure; FPG – fasting plasma glucose; FCP – fasting C peptide; HbA1c – glycated hemoglobin A1c; TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; eGFR – estimated glomerular filtration rate; SUA – serum uric acid; * P<0.05; ** P<0.01; *** P<0.001.
cross-sectional study, making it difficult to draw the causal relationship between the Hcy level and DKD severity; second, the use of various drugs was not included in the analysis; third, the different sample sizes of each group may have partially affected the results of the study; lastly, the total sample size of the study was relatively small, which may not be able to adequately reflect other problems.

Conclusions

The relationship between Hcy level and DKD severity was related to the different staging methods used. Hcy was an independent risk factor for CKD stages but not albuminuria stages.

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Declaration of Figures’ Authenticity

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